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Protective Effect of *Melaleuca alternifolia* Essential Oil on Broiler Growth Performance, Intestinal Morphology, Gut Microflora, and Gut Redox Status: An Alternative Growth Promoter to Antibiotics

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ZXF, HLH, LHY, ZLP conducted investigation of the research work. ZXF and HLH did statistical analyses, software, formal analysis, writing review, editing type face, and data curation. LHY and ZLP contributed in review and editing of the manuscript, visualization and validation. ZXF and ZLP helped in conceptualization, methodology, resources, supervision, project administration, funding acquisition, and review and editing of the manuscript. All authors reviewed and approved the manuscript.

Key words
***Melaleuca alternifolia*, Broilers, Intestine health, Antioxidant activity, Antimicrobial activity**

ABSTRACT

The effects of dietary *Melaleuca alternifolia* essential oil supplementation were investigated as an alternative to antibiotics on growth performance, intestinal health, and oxidative status in broilers. A total of 1960 yellow-skinned 1-day-old female broilers with an initial body weight of 38 g were randomly assigned to one of four treatment groups (seven replicate pens per treatment, 70 broilers per pen). Broilers consumed the basal diet free from antibiotics (control), or the basal diet supplemented with either 20 mg virginiamycin/kg, or 25 or 50 mg *M. alternifolia* essential oil/kg for 75 days. The *M. alternifolia* essential oil groups and the antibiotic group decreased the feed/gain (F/G) ratio of broilers from 1 to 25 days and from 1 to 75 days of age ($P < 0.05$) when compared with the control. The F/G ratios were similar between the antibiotic and *M. alternifolia* essential oil groups. The *M. alternifolia* essential oil and antibiotic groups showed decreased ($P < 0.05$) endotoxin and diamine oxidase levels in serum and increased ($P < 0.05$) villus height in the ileum and caecum. These results showed that the intestinal health of the broilers was improved by *M. alternifolia* essential oil or antibiotic supplements. The *M. alternifolia* essential oil and antibiotic groups had smaller ($P < 0.05$) *Escherichia coli* populations and lower redox levels in the broiler intestine than the control. Our results show that *M. alternifolia* essential oil promotes broiler intestinal health, probably through modulating intestinal bacteria and redox status. Dietary supplementation with *M. alternifolia* essential oil as an alternative to antibiotics could be used to achieve beneficial growth performance in broilers.

INTRODUCTION

The poultry industry uses antibiotics to improve meat production through increased feed conversion, growth rate promotion, and disease prevention (Castanon, 2007). Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth, as well as to achieve improved intestinal health (Dibner and Richards, 2005). However, owing to the development of bacteria strains that are resistant to antibiotics, these practices have now been brought into question (Mehdi *et al*., 2018). European Union countries were the first to ban antibiotic growth promoters (Huyghebaert *et al*., 2011). In China, restrictions have also been placed on the use of in-feed antibiotics (Clark *et al*., 2012). Therefore, there is an urgent need to find alternative treatments with fewer side effects.

In the past decade, research into essential (volatile) oils has received increased attention from both industrial and academic sectors because of the growing interest in green consumerism, and the need for alternative techniques to assure the quality and safety of perishable foods (Burt, 2004). Evidence has also emerged that essential oils could be used as alternatives to antibiotics in broilers (Aristimunha *et al*., 2016; Skoufos *et al*., 2016; Adaszyńska-Skwirzyńska and Szezerbinska, 2017). *Melaleuca alternifolia*, which belongs to the Myrtaceae family (Siddique *et al*., 2015), is described as a scrubland species, and is found throughout South America, western India, and Australia (Felipe *et al*., 2017). In recent years, *M. alternifolia* has been gradually introduced into southern China. The essential oil of *M. alternifolia* is obtained by steam or hydro-distillation

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from *M. alternifolia* biomass as secondary metabolites. It is a volatile oil, rich in monoterpenes, and has a strong characteristic odor (Brophy *et al*., 1989; Leleach et ai., 2000). The volatile compounds that comprise these oils are known to exert different biological actions, such as anti-microbial and anti-oxidative activities (Adaszyńska-Skwirzyńska and Szezerbinska, 2017). However, the efficacy of *M. alternifolia* essential oil as a replacement for antibiotic growth promoters in broiler chickens has not been reported.

The present study was conducted to assess the effectiveness of using *M. alternifolia* essential oil as an alternative to a growth-promoting antibiotic (virginiamycin) on growth performance in female broiler chicks. Traits such as intestinal integrity, intestinal oxidative status, and intestinal microbiota were determined to study possible modes of action for a potentially enhanced performance.

MATERIALS AND METHODS

Animals, diets, and treatments

A total of 1960 yellow-skinned female broilers were selected from the same farm, based on body weight (BW), genetic background, and health status. Each broiler (35 g BW) was randomly assigned to one of four dietary treatments from blocks designed to balance initial BW across treatments. Each treatment had seven replicate pens of 70 broilers per pen (2.5 × 4 m). The dietary treatments were: Control group, basal diet without any antibiotic; Antibiotic group, basal diet supplemented with 20 mg of virginiamycin per kilogram; *M. alternifolia* essential oil groups, basal diet supplemented with 25 mg or 50 mg of *M. alternifolia* essential oil per kilogram (replacement for antibiotic). The temperature of the room was maintained at 32–34°C for the first 3 days, which was then reduced by 2–3°C per week to a final temperature of 20°C. Broilers had *ad libitum* access to feed and water throughout the 75-day feeding experiment. The composition of the control group diet is shown in Table I. At 25, 55, and 75 days of age, broilers were weighed after a 12-h feed deprivation, and feed consumption was recorded to calculate the average daily feed intake (ADFI), the average daily gain (ADG), and the feed: gain ratio (F/G).

Sample collection

One broiler per pen (total of seven broilers per treatment) was harvested for intestinal morphology, intestinal microbiota, and intestinal oxidative status. Samples of the ileum and caecum were removed from the same segment and then rinsed with ice-cold physiological saline. One section was snap-frozen in liquid nitrogen and then stored at −80°C until further analysis. Other sections of intestine (1 cm) were kept in 4% neutral buffered formalin for gut morphological analysis. Blood samples were collected by beaker during exsanguination and was then quickly separated into five tubes. A 10-ml blood sample was placed on ice immediately and subsequently centrifuged at 1300 *g* at 4°C for 15 min to obtain serum. The serum samples were stored at −80°C for subsequent analysis. The digesta samples were immediately removed from the ileum and caecum of each broiler and stored at −80°C until further analysis.

Table I. Composition and nutrient level of the basal diet.

Items	1-25 days	26-55 days	56-75 days
Ingredients			
Corn	634.38	691.85	706.79
Soybean meal	206.95	135.46	115.88
Cottonseed meal	60.00	60.00	60.00
Corn gluten meal	50.00	50.00	50.00
Limestone	15.70	14.40	14.20
Dicalcium phosphate	9.30	10.3	8.60
Soybean oil	8.10	19.00	27.50
L-Lysine	5.13	6.92	6.21
[a]Premix compound	4.40	4.40	4.40
NaCl	3.50	3.50	3.50
DL-Methionine	1.28	2.4	1.54
Threonine	0.46	1.27	0.98
Choline chloride	0.80	0.50	0.40
Total batch	1000	1000	1000
Calculated nutrient levels			
Crude protein (%)	20.50	18.00	17.00
Calcium (%)	0.90	0.85	0.80
Available phosphorus (%)	0.301	0.31	0.280
AME (kcal/kg)	2900	3030	3100
Lysine (%)	1.05	0.97	0.88
Met+Cys (%)	0.71	0.76	0.65
Threonine (%)	0.65	0.63	0.57
Arginine (%)	1.22	1.02	0.95
Tryptophan (%)	0.18	0.14	0.13

a Premix compound provided per kg of diet: retinol, 3.0 mg; cholecalciferol, 0.045 mg; tocopherol, 20mg; menadione, 3.5 mg; riboflavin, 8.0 mg; niacin, 35 mg; D-pantothenic acid, 10 mg; cobalamin, 0.015 mg; biotin, 0.18mg; folacin, 1.2mg; thiamine, 2.0 mg; pyridoxine, 3.5 mg; 8.0 mg of Cu from $CuSO_4.5H_2O$; 80 mg of Zn from $ZnSO_4.H_2O$; 100 mg of Mn from $MnSO_4.H_2O$; 60 mg of Fe from $FeSO_4.H_2O$; 0.7 mg of I from KI; 0.3 mg of Se from Na_2SeO_3.

Gut morphological analysis

The digestive tract was removed and the ileum and caecum fixed in 10% phosphate-buffered formalin. The samples were sectioned at 5 mm thickness and stained with haemotoxylin and eosin. Villus height, villus width, and villus crypt depth were measured on the stained sections using a light microscope fitted with an image analyser (Image Pro Plus 6.0; Media Cybernetics, Bethesda, MD, USA). Twenty villi and crypts were measured for each segment.

Measurement of serum endotoxin and diamine oxidase levels

Serum endotoxin and diamine oxidase (DAO) levels were measured using the double-antibody sandwich enzyme-linked immunosorbent assay kit ELISA (R & D Systems, Minneapolis, MN, USA). Immunological detection of the endotoxin and DAO levels were performed according to the manufacturer's instruction.

Extraction of microbial DNA from gastrointestinal tract digesta

Total DNA of ileum and caecum digesta was extracted and purified from gastrointestinal tract digesta using a QIAampDNAstoolkit(Qiagen,Hilden,Germany)according to the manufacturer's instructions. DNA concentration was determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA obtained from the intestinal luminal content was used as the template to analyse intestinal bacteria. Primers *Escherichia coli* F: 5` CATGCCGCGTGTATGAAGAA3`; R: 5`CGGGTAACGTCAATGAGCAAA3` and *Lactobacillus* F: 5`GCAGCAGTAGGGAATCTTCCA3`; R: 5`GCATTYCACCGCTACACATG3` used in this study were either synthesized according to our previous protocols or designed with Primer 5.0 according to broiler gene sequences. Real-time polymerase chain reaction was performed according to a previous study (Zou *et al*., 2016). The relative expression of genes in the treatment groups was normalized based on the values of the control group.

Serum and intestine redox status measurements

Serum and intestine thiobarbituric acid reactive substances (TBARS), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px) levels were assayed using the commercial kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). TBARS was analysed based on the reaction with 2-thiobarbituric acid (TBA). The resulting pink product was measured spectrophotometrically at 535 nm, and the TBARS concentration was expressed as nmol malondialdehyde (MDA) equivalents/mg protein. SOD activity was measured by using its ability to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide ions generated by the xanthine/xanthine oxidase system. The extent of NBT reduction was assayed at 560 nm. One unit of SOD was defined as the activity that inhibited the reaction by 50%. The SOD activity was expressed as U/mg protein. GSH-Px activity was determined based on quantifying the rate of oxidation of GSH to glutathione disulfide by H_2O_2 and catalyzed by GSH-Px. GSH reacts with 5,5′-dithiobis-*p*-nitrobenzoic acid (DTNB) to produce yellow coloured 5-thio-2-nitrobenzoic acid (TNB), which can be quantified spectrophotometrically at 412 nm. One unit of GSH-Px was defined as the enzyme amount that reduced the level of GSH by 1.0 µmol/L in the reaction system in 1 min. GSH-Px activity was calculated and expressed as U/mg protein. Protein concentrations of supernatant fractions were quantified with a commercial kit at 562 nm (BCA Protein Assay, Beyotime, China).

Statistical analysis

All results are expressed as data were analyzed by ANOVA procedures of SAS (v 8.2, SAS Inst., Inc., Cary, NC.). Significant differences between treatment means were determined by Duncan's Multiple Range Test method (Duncan, 1955). All the values were represented as means ± standard error of the mean (SEM) and those at $p < 0.05$ were considered significant. Different letters denote significant differences between groups.

RESULTS

Growth performance

The growth performance of broilers fed with different treatments is summarized in Table II. The *M. alternifolia* essential oil group and the antibiotic group decreased the F/G ratio of broilers from 1 to 25 days and from 1 to 75 days of age ($P < 0.05$) when compared with the Control group, and the value was similar between the antibiotic group and the *M. alternifolia* essential oil group ($P > 0.05$). In addition, compared with the control group, the antibiotic and *M. alternifolia* essential oil groups showed greater ADGs from 26 to 55 days and from 1 to 75 days of age, although only the change in the Antibiotic group was significant ($P < 0.05$). No difference in ADFI was observed among the four treatments ($P > 0.05$).

Gut morphology

The intestinal morphological indices, including villus height, crypt depth, and villus width, were calculated and are shown in Table III. The results indicated that the ileum and caecum villus height in control broilers was decreased significantly compared with that in antibiotic

group broilers ($P < 0.05$). The *M. alternifolia* essential oil group showed increased villus height in ileum compared with the control group ($P < 0.05$). The value was similar between the different doses of the *M. alternifolia* essential oil groups ($P > 0.05$).

Table II. Growth performance of broilers from 1 to 75 days of age.

Items	Control group	Antibiotic group	*M. ahemifolia* essential oil group		SEM	P value
			25mg/kg	50mg/kg		
ADFI (kg day^{-1})						
1-25 days	24.3	24.5	23.9	24.4	0.17	0.64
26-55 days	56.1	58.6	57.7	57.2	0.43	0.16
56-75 days	93.0	92.0	90.4	91.3	0.75	0.62
1-75 days	55.7	56.5	55.5	55.7	0.30	0.65
ADG (kg day^{-1})						
1-25 days	13.1	13.6	13.2	13.5	0.12	0.34
26-55 days	18.4^{b}	19.7^{a}	19.3^{ab}	19.2^{ab}	0.17	0.02
56-75 days	25.4	25.6	24.8	25.4	0.26	0.69
1-75 days	18.7^{b}	19.4^{a}	18.9^{ab}	19.1^{ab}	0.09	0.05
F/G						
1-25 days	1.85^{a}	1.80^{b}	1.81^{b}	1.80^{b}	0.01	0.02
26-55 days	3.05	2.97	3.00	2.99	0.02	0.62
56-75 days	3.66	3.59	3.65	3.60	0.01	0.15
1-75 days	2.98^{a}	2.90^{b}	2.93^{b}	2.91^{b}	0.01	< 0.01

ADG, average daily gain; ADFI, average daily feed intake; F/G, feed intake/gain; Control group, basal die without any antibiotic; Antibiotic group, basal diet supplemented with 20 mg virginiamycin/kg; *M. Ahemifolia* essential oil group, basal diet supplemented with 25 mg and 50 mg *M. alternifolia* essential oil/kg replacement for antibiotic. SEM, standard error of means (n=7). ^{a,b} Letters within a row denote statistical differences between means.

Endotoxin and diamine oxidase

The endotoxin and DAO levels in broiler serum are shown in Table IV. The *M. alternifolia* essential oil group and Antibiotic group showed decreased endotoxin and DAO in broiler serum when compared with the Control group ($P < 0.05$). The levels were similar for the Antibiotic and *M. alternifolia* essential oil groups ($P > 0.05$).

Intestinal microbiota

The microbial populations in the different intestinal tracts are shown in Table V. Although no difference was observed in *Lactobacillus* populations between the treatments ($P > 0.05$), the *Escherichia coli* populations were decreased in the *M. alternifolia* essential oil and antibiotic groups compared with the Control group ($P < 0.05$).

Table III. Gut morphology in the ileum and caecum of broilers.

Items	Control group	Antibiotic group	*M. ahemifolia* essential oil group		SEM	P value
			25mg/kg	50mg/kg		
Ileum						
Villous height (μm)	262.20^{c}	363.12^{a}	322.50^{b}	328.58^{b}	9.50	< 0.01
Villous width (μm)	52.62	64.52	66.86	61.32	2.56	0.22
Crypt depth (μm)	243.75	239.32	262.47	259.90	12.67	0.90
Caecum						
Villous height (μm)	87.01^{b}	113.06^{a}	99.58^{ab}	100.75^{ab}	3.49	< 0.01
Villous width (μm)	42.13	42.03	39.69	39.91	1.78	0.94
Crypt depth (μm)	50.89	52.05	51.94	54.34	1.78	0.93

SEM, standard error of means (n=7). ^{a,b} Letters within a row denote statistical differences between means.

Table IV. Endotoxin and diamine oxidase levels in the broiler serum.

Items	Control group	Antibiotic group	*M. Ahemifolia* essential oil group		SEM	P value
			25mg/kg	50mg/kg		
Endotoxin (EU/ml)	4.83^{a}	2.38^{b}	2.77^{b}	2.74^{b}	0.28	<0.01
DAO (U/L)	10.88^{a}	5.22^{b}	7.31^{b}	5.96^{b}	0.57	<0.01

DAO, diamine oxidase; SEM, standard error of means (n=7). ^{a,b} Letters within a row denote statistical differences between means.

Table V. Major microbiota in different regions of the broiler intestinal tract.

Items	Control group	Antibiotic group	*M. ahemifolia* essential oil group		SEM	P value
			25mg/kg	50mg/kg		
Escherichia coli						
Ileum	1.00^{a}	0.73^{b}	0.74^{b}	0.73^{b}	0.03	< 0.01
Caecum	1.00^{a}	0.74^{b}	0.82^{b}	0.73^{b}	0.03	< 0.01
Lactobacillus						
Ileum	1.00	0.87	0.95	0.97	0.03	0.44
Caecum	1.00	1.01	1.00	0.99	0.02	0.96

SEM, standard error of means (n=7). The treatment group was normalized based on the values of the control group. ^{a,b} Letters within a row denote statistical differences between means.

Table VI. Oxidative status in the broiler serum and intestine.

Items	Control group	Antibiotic group	*M. ahemifolia* essential oil group		SEM	P value
			25 mg/kg	50 mg/kg		
Serum						
TBARS (nmol ml^{-1})	9.38^{a}	6.79^{b}	6.12^{bc}	4.49^{c}	0.40	< 0.01
T-SOD (U ml^{-1})	257.33^{c}	272.02^{bc}	297.53^{ab}	330.10^{a}	4.06	< 0.01
GPx (U)	2523.41^{b}	2938.39^{a}	3051.04^{a}	3213.01^{a}	38.09	< 0.01
Ileum						
TBARS (nmol mgprot^{-1})	5.69^{a}	3.38^{b}	1.95^{b}	1.32^{b}	0.46	< 0.01
T-SOD (U mgprot^{-1})	228.74^{c}	313.77^{b}	419.06^{a}	431.82^{a}	21.03	< 0.01
GPx (U)	40.50^{a}	61.96^{ab}	77.38^{a}	85.62^{a}	5.90	0.02
Caecum						
TBARS (nmol mgprot^{-1})	47.79^{a}	28.18^{b}	15.76^{c}	13.13^{c}	3.65	< 0.01
T-SOD (U mgprot^{-1})	521.47^{b}	631.90^{ab}	596.08^{ab}	693.73^{a}	22.02	0.03
GPx (U)	40.00^{b}	105.90^{ab}	114.11^{ab}	132.75^{a}	13.90	0.08

TBARS, thiobarbituric acid reactive substances; T-SOD, total superoxide dismutase; GPx, glutathione peroxidase. SEM, standard error of means (n=7). $^{a, b, c}$ Letters within a row denote statistical differences between means.

Oxidative status

Table VI shows the redox status of the serum, ileum, and caecum of the broilers. Compared with the control group, the *M. alternifolia* essential oil and Antibiotic groups had lower TBARS levels in serum, ileum, and caecum ($P < 0.05$). Furthermore, the TBARS levels in serum and caecum for the *M. alternifolia* essential oil group were lower than those of the antibiotic group ($P < 0.05$). GSH-Px and T-SOD activities were higher in the 50 mg/kg *M. alternifolia* essential oil group than the control group for serum, ileum, and caecum ($P < 0.05$). In serum and ileum, the GSH-Px activity was higher in the 50 mg/kg *M. alternifolia* essential oil group than in the antibiotic group ($P < 0.05$). T-SOD activity in the serum and GSH-Px activity in the caecum were higher in the antibiotic group ($P < 0.05$) than in the control group.

DISCUSSION

Antibiotics are used to fight bacterial infections, but their widespread use has led to the emergence of resistant bacteria. This leaves scientists worried about the dangers to human and animal health posed by resistant bacteria (Mehdi *et al*., 2018). Some strategies can be borrowed to reduce the use of antibiotics in chicken farms. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters (Mehdi *et al*., 2018). Essential oils are aromatic oily liquids obtained from plant materials, and their effects on digestive physiology, microbiology of the gut, growth promotion, and antibacterial action have been reviewed (Burt *et al*., 2004; Franz *et al*., 2010). *M. alternifolia* essential oil is steam-distilled from the biomass of the native Australian tea tree. It contains more than 100 components, the majority being monoterpene or sesquiterpene hydrocarbons and their alcohols (Carson *et al*., 2006). In a previous study, we identified 14 components representing 92.87% of the *M. alternifolia* oil. Terpinene-4-ol (31.11%), γ-terpinene (25.30%), and α-terpinene (12.70%) were the major constituents, followed by 1,8-cineole (6.83%), *p*-cymene (4.23%), terpinolene (4.03%), limonene (2.50%), α-terpineol (2.35%), aromadendrene (1.75%), and δ-cadinene (1.41%) (Zhang *et al*., 2018). Furthermore, it was also observed that *M. alternifolia* oil has significant antimicrobial and antioxidative activities *in vitro* (Zhang *et al*., 2018). Extending this observation to a living system, Liu *et al*. (2017) have demonstrated that the essential oil of *M. alternifolia*, when administered *in vivo*, significantly enhance broiler growth, modulate immune responses, and improve intestinal morphology. Their findings suggest that adding 50 mg/kg of this essential oil to broiler basal diets is beneficial. Moreover, it has been deduced that a diet supplemented with 300 mg/kg of *M. alternifolia* essential oil not only elevates the growth performance and meat quality of broilers but also bolsters their cellular immunity (Ghanima *et al*., 2021). However, the efficacy of *M. alternifolia* essential oil as a replacement for antibiotic growth promoters in broiler chickens has not been reported. The present study showed that the F/G ratios at 1 to 25 days and 1 to 75 days were reduced after supplementation with the *M. alternifolia* essential oil when compared with the Control group. Nevertheless, for broiler performance,

the product achieved similar effects to antibiotics. Thus, our study showed that *M. alternifolia* essential oil induces positive effects on the growth performance of broilers. We found that *M. alternifolia* essential oil can act as alternative growth promoter in broilers.

Interestingly, the F/G ratio was reduced in the *M. alternifolia* essential oil group with no change in the ADFI at 1–75 days. It was also important to determine whether the health of the digestive tract was improved and further heightened nutrient utilization (Liu *et al*., 2017). The function of the intestinal barrier can be assessed by many indexes, such as serum endotoxin level and intestine morphology (Vente *et al*., 2003; Forsyth *et al*., 2011; Suzuki, 2013). In the present study, the heights of villi in the ileum and caecum of the broilers were increased after treatment with *M. alternifolia* essential oil or antibiotics. The endotoxin and DAO levels in broiler serum decreased significantly after treatment with *M. alternifolia* essential oil or antibiotics. These results agreed with previous findings, which demonstrated that a feed supplement of plant essential oil increases the ileum villus height and decreases the serum endotoxin level in broilers. Our results indicate that *M. alternifolia* essential oil supplementation is a promising approach for protecting the intestinal health in broilers in a similar way to antibiotics.

The intestinal microbiota plays a critical role in the maintenance of mucosal homeostasis (Ivanov and Littman, 2011). A greater population of *E. coli* can affect the intestinal mucosa because the organism releases toxins, resulting in an intimate interaction between the microbiota and the host enterocytes (Sartor, 2008; Finamore *et al*., 2014). The abundance and composition of intestinal bacteria can be easily affected by various dietary factors (Maslowski and Mackay, 2011). In the present study, the dietary consumption of *M. alternifolia* essential oil decreased the populations of *E. coli* in the ileum and caecum. *Melaleuca alternifolia* essential oil is also documented to inhibit the proliferation of *E. coli in vitro* and *in vivo* (Tsao *et al*., 2010; Zhang *et al*., 2018). Reactive oxygen species (ROS) play an important role in the pathogenesis of gastrointestinal injury and dysfunction in broilers (Zhu *et al*., 2012). The enhanced oxidative status in the intestine induced by the inclusion of antibiotic would result from its antimicrobial ability, which was supported by the simultaneously improved immunity and intestinal microflora population (Chen *et al*., 2018). Any imbalance between the production of these molecules and their safe disposal may culminate in oxidative stress (Young *et al*., 2005; Onmaz *et al*., 2011). Excessive levels of ROS damage cellular proteins, including cytoskeletal proteins, and ultimately disrupt the gastrointestinal health (Bhattacharyya *et al*., 2014). *Melaleuca alternifolia* essential oil is known to have antioxidant activity *in vitro*. MDA is the end product of lipoperoxidation and is considered an excellent indicator of oxidative stress (Ho *et al*., 2013). Therefore, it was also observed that *M. alternifolia* essential oil was beneficial for decreasing TBARS levels in the intestine and serum of broilers. The beneficial effect of *M. alternifolia* essential oil supplementation on the antioxidant status in this study may be related to the antioxidant characteristics of its components. Furthermore, reported that essential oil protects against H_2O_2-induced IPEC-J2 cell damage by inducing Nrf2 and related antioxidant enzymes (Zou *et al*., 2016). The activities of the antioxidative enzymes, T-SOD and GSH-Px, are known to contribute to sustaining a delicate oxidative balance in biological tissues exposed to oxidative conditions (Halliwell, 1994). In the present study, dietary supplementation with *M. alternifolia* essential oil reduced the production of lipid peroxidation products and increased the activities of GSH-Px and T-SOD in the serum and intestinal tract. These findings suggest that *M. alternifolia* essential oil improved the redox status of broilers by increasing the activity of antioxidant enzymes and reducing peroxidation products.

CONCLUSION

The results of the study indicate that *M. alternifolia* essential oil exhibits antibacterial and antioxidative properties that make it an alternative to antibiotics as a growth promoter in broilers. The present data indicate that dietary supplementation with *M. alternifolia* essential oil can promote the intestinal health in broilers without using antibiotics. The protective effects of *M. alternifolia* essential oil on the intestine are associated with decreased populations of *E. coli* and lower intestinal oxidative stress.

DECLARATIONS

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IRB approval

This research was conducted in accordance with

ethics committee procedures of animal experiments.

Ethical statement

This research was conducted in accordance with ethics committee procedures of animal experiments.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adaszyńska-Skwirzyńska, M. and Szczerbińska, D., 2017. Use of essential oils in broiler chicken production. A review. *Annls Anim. Sci*., **17**: 317-335. https://doi.org/10.1515/aoas-2016-0046

Aristimunha, P.C., Rosa, A.P., Boemo, L.S., Garcez, D.C., Rosa, D.P. and Londero, A., 2016. A blend of benzoic acid and essential oil compounds as an alternative to antibiotic growth promoters in broiler diets. *J. appl. Poult. Res*., **25**: 1-9. https://doi.org/10.3382/japr/pfw015

Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S.E., 2014. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol. Rev*., 94: 329-354. https://doi.org/10.1152/physrev.00040.2012

Brophy, J.J., Davies, N.W., Southwell, I.A., Stiff, I.A. and Williams, L.R., 1989. Gas chromatographic quality control for oil of Melaleuca terpinen-4-ol type (Australian tea tree). *J. Agric. Fd. Chem*., **37**: 1330-1335. https://doi.org/10.1021/jf00089a027

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods. A review. *Int. J. Fd. Microbiol*., **94**: 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Carson, C.F., Hammer, K.A. and Riley, T.V., 2006. *Melaleuca alternifolia* (Tea tree) oil: A review of antimicrobial and other medicinal properties. *Clin. Microbiol. Rev*., **19**: 50-62. https://doi.org/10.1128/CMR.19.1.50-62.2006

Castanon, J.I., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci*., **86**: 2466-2471. https://doi.org/10.3382/ps.2007-00249

Chen, Y., Wen, C. and Zhou, Y., 2018. Dietary synbiotic incorporation as an alternative to antibiotic improves growth performance, intestinal morphology, immunity and antioxidant capacity of broilers. *J. Sci. Fd. Agric*., **98**: 3343-3350. https://doi.org/10.1002/jsfa.8838

Clark, S., Daly, R., Jordan, E., Lee, J., Mathew, A. and Ebner, P., 2012. Extension education symposium: The future of biosecurity and antimicrobial use in livestock production in the United States and the role of extension. *J. Anim. Sci*., **90**: 2861-2872. https://doi.org/10.2527/jas.2011-4739

Dibner, J.J. and Richards, J.D., 2005. Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci*., **84**: 634-643. https://doi.org/10.1093/ps/84.4.634

Felipe, L.O., Júnior, W., Araújo, K.C. and Fabrino, D.L., 2017. Lactoferrin, chitosan and *Melaleuca alternifolia*-natural products that show promise in candidiasis treatment. *Braz. J. Microbiol*., **49**: 212-219. https://doi.org/10.1016/j.bjm.2017.05.008

Finamore, A., Roselli, M., Imbinto, A., Seeboth, J., Oswald, I.P. and Mengheri, E., 2014. *Lactobacillus amylovorus* inhibits the TLR4 inflammatory signaling triggered by enterotoxigenic *Escherichia coli* via modulation of the negative regulators and involvement of TLR2 in intestinal Caco-2 cells and pig explants. *PLoS One*, **9**: e94891. https://doi.org/10.1371/journal.pone.0094891

Forsyth, C.B., Shannon, K.M., Kordower, J.H., Voigt, R.M., Shaikh, M. and Jaglin, J.A., 2011. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS One*, **6**: 1-10. https://doi.org/10.1371/journal.pone.0028032

Franz, C., Baser, K. and Windisch, W., 2010. Essential oils and aromatic plants in animal feeding–a European perspective. A review. *Flav. Fragr. J*., **25**: 327-340. https://doi.org/10.1002/ffj.1967

Ghanima, M.M.A., Swelum, A.A. and Shukry, M., 2011. Impacts of tea tree or lemongrass essential oils supplementation on growth, immunity, carcass traits, and blood biochemical parameters of broilers reared under different stocking densities. *Poult. Sci*., **100**: 101443. https://doi.org/10.1016/j.psj.2021.101443

Halliwell, B., 1994. Free radicals and antioxidants: A personal view. *Nutr. Rev*., **52**: 253-265. https://doi.org/10.1111/j.1753-4887.1994.tb01453.x

Ho, E., Galougahi, K.K., Liu, C.C., Bhindi, R. and Figtree, G.A., 2013. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol*., **1**: 483-491. https://doi.org/10.1016/j.redox.2013.07.006

Huyghebaert, G., Ducatelle, R. and Van, I.F., 2011. An update on alternatives to antimicrobial growth promoters for broilers. *Vet. J*., **187**: 182-188. https://doi.org/10.1016/j.tvjl.2010.03.003

Ivanov, I.I. and Littman, D.R., 2011. Modulation of

immune homeostasis by commensal bacteria. *Curr. Opin. Microbiol*., **14**: 106-114. https://doi.org/10.1016/j.mib.2010.12.003

Leleach, D.N., Lea, D., Lee, L.S., Henry, R.J. and Baverstock, P.R., 2000. Natural variation in the essential oil content of *Melaleuca alternifolia* Cheel (Myrtaceae). *Biochem. Syst. Ecol*., **28**: 367-382. https://doi.org/10.1016/S0305-1978(99)00071-X

Maslowski, K.M. and Mackay, C.R., 2011. Diet, gut microbiota and immune responses. *Nat. Immunol*., **12**: 5-9. https://doi.org/10.1038/ni0111-5

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Gayatri, S. and Rouissi, T., 2018. Use of antibiotics in broiler production: Global impacts and alternatives. *Anim. Nutr*., **4**: 170-178. https://doi.org/10.1016/j.aninu.2018.03.002

Onmaz, A., Hoven, R.V., Gunes, V., Cinar, M. and Kucuk, O., 2011. Oxidative stress in horses after a 12-h transport period. *Rec. Med. Vet*., **162**: 213-217.

Sartor, R.B., 2008. Microbial influences in inflammatory bowel diseases. *Gastroenterology*, **134**: 577-594. https://doi.org/10.1053/j.gastro.2007.11.059

Siddique, S., Perveen, Z., Nawaz, S., Shahzad, K. and Ali, Z., 2015. Chemical composition and antimicrobial activities of essential oils of six species from Family Myrtaceae. *J. Essent. Oil Bear. Pl*., **18**: 950-956. https://doi.org/10.1080/0972060X.2014.935020

Skoufos, I., Giannenas, I., Tontis, D., Bartzanas, T., Kittas, C. and Panagakis, P., 2016. Effects of oregano essential oil and attapulgite on growth performance, intestinal microbiota and morphometry in broilers. *S. Afr. J. Anim. Sci*., **46**: 77-88. https://doi.org/10.4314/sajas.v46i1.10

Suzuki, T., 2013. Regulation of intestinal epithelial permeability by tight junctions. *Cell. Mol. Life Sci*.,**70**: 631-659. https://doi.org/10.1007/s00018-012-1070-x

Tsao, N., Kuo, C.F., Lei, H.Y., Lu, S.L. and Huang, K.J., 2010. Inhibition of group A streptococcal infection by *Melaleuca alternifolia* (tea tree) oil concentrate in the murine model. *J. appl. Microbiol*., **108**: 936-944. https://doi.org/10.1111/j.1365-2672.2009.04487.x

Vente-Spreeuwenberg, M., Verdonk, J., Beynen, A. and Verstegen, M., 2003. Interrelationship between gut morphology and faeces consistency in newly weaned piglets. *Anim. Sci*., **77**: 85-94. https://doi.org/10.1017/S1357729800053686

Liu, Y., Yang, X., Xin, H., Chen, S., Yang, C., Duan, X.Y., 2017. Effects of a protected inclusion of organic acids and essential oils as antibiotic growth promoter alternative on growth performance, intestinal morphology and gut microflora in broilers. *Anim. Sci. J*., **88**: 1414. https://doi.org/10.1111/asj.12782

Young, J.F., Rosenvold, K., Stagsted, J., Nielsen, J.H. and Andersen, H.J., 2005. Significance of vitamin E supplementation, dietary content of polyunsaturated fatty acids, and preslaughter stress on oxidative status in pig as reflected in cell integrity and antioxidative enzyme activities in porcine muscle. *J. Agric. Fd. Chem*., **54**: 745-749. https://doi.org/10.1021/jf0490652

Zhang, X.F., Guo, Y.J., Guo, L.Y., Jiang, H. and Ji, Q.H., 2018. *In vitro* evaluation of antioxidant and antimicrobial activities of melaleuca alternifolia essential oil. *BioMed. Res. Int*., pp. 1-8. https://doi.org/10.1155/2018/2396109

Zhu, L., Zhao, K., Chen, X. and Xu, J., 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. *J. Anim. Sci*., **90**: 2581-2589. https://doi.org/10.2527/jas.2012-4444

Zou, Y., Wang, J., Peng, J. and Wei, H., 2016. Oregano essential oil induces SOD1 and GSH expression through Nrf2 activation and alleviates hydrogen peroxide-induced oxidative damage in IPEC-J2 cells. *Oxid. Med. Cell Longev*., **5987183**. https://doi.org/10.1155/2016/5987183