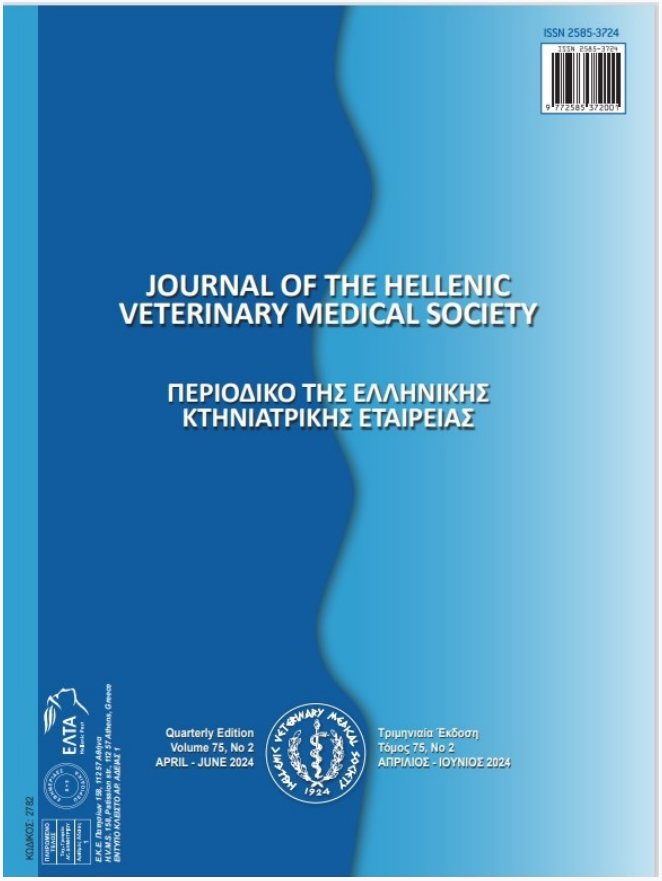
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 2 (2024)



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doi: 10.12681/jhvms.34751

To cite this article:

Gumus, R., Ercan, N., Özbilgin, A., Moğulkoç, M., & İmik, H. (2024). The effect of dietary supplementation with natural antioxidants on growth performance, antioxidant capacity and intestinal microbial counts of broiler. *Journal of the Hellenic Veterinary Medical Society*, *75*(2), 7441−7450. https://doi.org/10.12681/jhvms.34751

J HELLENIC VET MED SOC 2024, 75 (2): 7441-7450
ΠΕΚΕ 2024, 75 (2): 7441-7450

Research article
Ερευνητικό άρθρο

The effect of dietary supplementation with natural antioxidants on growth performance, antioxidant capacity and intestinal microbial counts of broiler

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ABSTRACT: This study was carried out to determine the effects of different doses of curcumin and resveratrol added to the diet of broilers on growth performance, antioxidant metabolism and intestinal microflora. A total of 200 male broilers at the age of 0 days were used in the study. Groups of the study was designed in 5 groups as Control (CONT), CRM_{250}, CRM_{500}, RSV_{250} and RSV_{500}, and 0, 250 mg kg^{-1} curcumin, 500 mg kg^{-1} curcumin, 250 mg kg^{-1} resveratrol and 500 mg kg^{-1} resveratrol were added to diets of the groups, respectively. Body weight (BW), average daily weight gain (DWG) and feed conversion ratio (FCR) values were found to be statistically similar in all groups ($P > 0.05$) at the end of the study. Feed consumption (FI) values are decreased in the CRM_{250} and CRM_{500} groups 28-35 between days, the CRM_{500} group in 0-42 between days ($P < 0.05$). It was determined that glutathione peroxidase (GPx) activity in serum was significantly increased in the CRM_{250} group ($P < 0.05$), and superoxide dismutase (SOD) activity in thigh tissue was significantly increased in the CRM_{500} group ($P < 0.01$). *Escherichia coli* (*E. coli*) colonization in the intestinal flora was significantly reduced in the RSV_{500} group ($P < 0.01$). As a result, according to the findings obtained, it was seen that the additives applied did not have a negative effect on performance parameters, also had curative effects on antioxidant metabolism and intestinal microflora.

***Keywords*:** Antioxidant; Curcumin; Intestinal flora; Growth performance; Resveratrol.

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Date of initial submission: 21-06-2023
Date of acceptance: 17-01-2024

INTRODUCTION

Broiler meat is a popular product among animal foods in terms of both price and taste and it is widely consumed around the world (Khan et al., 2019). On the other hand, broiler meat is highly susceptible to oxidation as it contains high levels of polyunsaturated fatty acids (PUFA) and essential amino acids. Lipid oxidation causes bitterness in the meat (De Lima Júnior et al., 2013) and has a positive relationship with protein oxidation (Wang et al., 2018). In a study conducted in ducks, it was reported that adding curcumin to the diet increased antioxidant capacity by inhibiting lipid and protein oxidation in their meat (Jin et al., 2020). Antioxidative capacity of muscle is associated with meat quality, and it has been reported that adding some foods with antioxidant function to the diet improves the quality of poultry meat (Gumus et al., 2018; Zhang et al., 2018).

Resveratrol is a polyphenol compound naturally found in a variety of plants, including grapes, strawberries, and peanuts (Berman et al., 2017). Previous research has shown that resveratrol exhibits a strong antioxidant capacity to effectively scavenge free radicals and reactive oxygen species (ROS) (Xia et al., 2017; Zhang et al., 2017a). It has been reported that resveratrol induces antioxidant capacity to increase serum GPx, total SOD and catalase (CAT) activities in chickens under heat stress (Liu et al., 2014). Similarly, Zhang et al. (2017a) reported that dietary resveratrol supplementation increased total antioxidant capacity and CAT activity in muscles. It has been reported that it regulates meat quality and energy metabolism, as it improves the antioxidant capacity of meat tissue in many stress (heat, etc.) conditions, especially transport stress (Ma et al., 2010; Zhang et al., 2017a). It has also been reported that resveratrol improves daily weight gain in broilers (He et al., 2019) and feed conversion ratio in quails (Ölmez et al., 2020).

Turmeric (*Curcuma longa*), a member of the *Zingiberacae* family, is a perennial herb and its roots and stems are used as spice and medicine (Durrani et al., 2006). The curcumin contain approximately 77 % diferuloylmethane (curcumin); 18% of them contain demethoxycurcumin, and 5% of them contain bisdemethoxycurcumin (Bener et al., 2016). Curcumin isolated from the roots and stems of turmeric has a wide range of biological activities, including antioxidant (Karami et al., 2011), antibacterial, antifungal, antiprotozoal, antiviral, anticoccidial and anti-inflammatory properties (Durrani et al., 2006; Liu et al., 2014; Wang et al., 2015). In studies on broiler chickens, it has been reported that when turmeric is added to the diet, body weight gain increases and feed conversion ratio improves (Durrani et al., 2006; Samarasinghe et al., 2003). It has been reported that the antioxidant capacity of the body is improved in ducks supplemented with curcumin in their diet (Jin et al., 2021a). It has also been reported that curcumin, added to the diet of laying hens under heat stress, improves antioxidant capacity by increasing SOD, CAT and GPx activities (Nawab et al., 2019).

The aim of this study was to examine and evaluate the effects of resveratrol and curcumin supplementations on growth performance, antioxidant capacity, and intestinal flora of broilers. This study offers insights into the application of resveratrol and curcumin in livestock and poultry farming and the food industry.

MATERIALS AND METHODS

Ethical approval

All the experimental procedures were approved by the Local Ethics Board for Animal Experiments of Sivas Cumhuriyet University (Decision Number: 2020/307).

Birds, diet and experimental design

The study was conducted in the Faculty of Veterinary Medicine in Cumhuriyet University. 200 day-old male broiler chickens (*Ross 308*) were randomly allocated to one CONT group and four experimental groups (CRM_{250}, CRM_{500}, RSV_{250} and RSV_{500}). Each group has 4 replicates of 10 broilers each one. The animals were housed in 20 four-storey cages measuring 120×80×60 cm. The CONT group received a basal diet, the other groups CRM_{250}, CRM_{500}, RSV_{250} and RSV_{500} were given a basal diet added with 250 mg kg^{-1} curcumin, 500 mg kg^{-1} curcumin, 250 mg kg^{-1} resveratrol and 500 mg kg^{-1} resveratrol, respectively (Table 1). The doses of curcumin (Jin et al., 2021a) and resveratrol (Liu et al., 2014) were determined as reported in previous studies. Curcumin ($C_{21}H_{20}O_6$, cas no: 458-37-7, purity grade 95.11 %, Chem-Impex Int. Company, Wood Dale, IL, USA) was obtained from the market. Resveratrol ($C_{14}H_{12}O_3$, cas no: 501-36-0, purity grade 99.13 %, Chem-Impex Int. Company, Wood Dale, IL, USA) was obtained from the market. The ambient temperature was gradually decreased from 33 °C in first week to 22 °C on day 14 and was then kept constant afterwards. The lighting program

Table 1. Ingredients and nutrient composition of broiler starter and grower diets in the study.

Ingredients, %	Starter (0 to 21 d)	Grower (21 to 42 d)
Maize	55.87	56.90
Soybean meal 44% HP	32.52	28.60
Gluten meal 60% HP	5.51	5.84
Soybean oil	2.00	4.82
L-Lizin HCl	0.24	0.16
DL-Methionine	0.13	0.02
Limestone*	1.24	1.15
Dicalcium phosphate	1.69	1.69
Choline chloride	0.20	0.22
Salt	0.30	0.30
Vit-Min. Premix**	0.30	0.30
Total	100	100
Nutrient content		
Crude protein, %	22.50	19.55
Metabolic energy (kcal kg^{-1})	2960	3150
Crude fibre, %	3.12	3.18
Calcium, %	0.98	0.91
Phosphorous, %	0.54	0.51

*Curcumin and resveratrol have replaced limestone in the same amount in the groups which CRM_{250}, CRM_{500}, RSV_{250} and RSV_{500} have been included.

**Supplied per kg of diet: vitamin A: 10000 IU; vitamin D3: 3500 IU; vitamin E: 60 mg; vitamin K3: 3 mg; vitamin B12: 0.1 mg; Thiamine: 3 mg; Riboflavin: 6 mg; niacin. 40 mg; Pyridoxine: 5 mg; Pantothenic acid: 11 mg; Folic acid: 1 mg; Biotin: 0.15 mg; Cholin chloride: 500 mg; Etoxycoin: 150 mg; Fe: 60 mg; Zn: 60 mg; Mn:100 mg; Cu: 10 mg; I: 1.6 mg and Se: 0.15 mg

applied was a continuous 23 h light. The diets used in the experiment were formulated according to the recommendations of NRC (1994) and their chemical analysis was performed according to AOAC (2005) (Table 1). Feed and water were supplied ad libitum.

Performance parameters

The animals were weighed on days 0, 7, 14, 21, 28, 35 and 42 of the experiment; total Body weight (BW) and average daily weight gain (DWG) were calculated. Feed intake (FI) (g/bird/day) was calculated as the difference between feed provided at the beginning and the heavy leftovers at the end of each period. Feed conversion ratio (FCR) was calculated as the difference between total feed supplied and left over divided by total weight gain. Mortality was recorded on a daily basis and used to adjust the total number of broiler chickens to determine the total FI per bird and FCR.

Collection of blood, thigh and liver tissues and intestinal contents

The animals included in the study were slaughtered at a slaughterhouse located at a distance of 100 m to the pens in which they were raised. Therefore, transport stress was eliminated. It was ensured that both the slaughterhouse and the materials used for the slaughter of the animals were aseptic. Prior to slaughter, the knives used for the slaughter of each animal were washed in alcohol and passed through a flame for the sterilisation of the outer surface. At the end of the fattening period, a total of 40 broilers, comprising 8 animals from each group (two broilers per repetition), were slaughtered. Prior to slaughter, the broilers were fasted for 10 hr. The slaughtered animals were bled for 120 s. The blood samples, collected in volumes of 5 mL into tubes without anti-coagulant (Becton Dickinson Co. USA), were centrifuged at +4 °C and 1792 g for 10 min in a cooled centrifuge (Hettich 38R, Hettich Zentrifugen, Tuttlingen, Germany). The harvested serum samples were stored at -80 °C until use. From slaughtered animals thigh and liver tissues were collected, homogenized, frozen in liquid nitrogen at -80°C, and stored until biochemical analyses.

The intestinal contents from the cecum were obtained for microbial count.

Tissue homogenization and antioxidant analyses

Activities of SOD (BT LAB Cat. No. E0295Ch), CAT (BT LAB Cat. No. E0118Ch) and GPx (BT LAB Cat. No. E0298Ch), with MDA (BT LAB Cat. No. E0171Ch) levels in tissue and serum samples were determined using the commercial kits on a microplate reader (Thermo Multiscan) in accordance with kit procedures. SOD, CAT and GPx activities as ng ml^{-1}, while MDA levels were calculated as mmol ml^{-1} in

response to the absorbances of the samples.

Analyses of intestinal microflora

On day 42, eight broilers from each groups (two broilers per repetition) were slaughtered, and their intestinal tracts were immediately removed. For the isolation and enumeration of intestinal microflora, one gram of caecal content from each broilers was aseptically collected and homogenized with 9 mL of 0.1 % peptone water. Serial 10-fold dilutions were made in sterile peptone water from 10-1 to 10-6 and 0.1 ml from last three dilutions were plated in duplicate onto respective selective medias.

Escherichia coli counts were performed on Tryptone Bile X-Glucuronide (TBX) agar and incubated for 24 hours at 37 °C. Enterobacteriaceae and coliforms were grown on Violet Red Bile Glucose agar (VRBG, Oxoid CM485) and Violet Red Bile agar (VRB, Oxoid CM107) respectively, using the pour plate technique and enumerated after 24-48 hours of incubation at 37 ºC.

Tryptose Sulfite Cycloserine Agar (TSC Agar) Base (Merck 1.11972) was utilized for the Clostridium count. The plates were incubated for 24 h at 45°C under anaerobic conditions, anaerobic indicator (Mitsubishi) was included to monitor the atmospheric condition.

Petri dishes observed 30 to 300 colonies were counted using a colony counter (Jin et al., 1996). The microbial counts were expressed as log10 cfu per gram of caecal contents.

Statistical analyses

The data obtained was assessed using the SPSS 20 software (SPSS, 2011). Differences between the groups were determined with the one-way analysis of variance (ANOVA) test and Duncan's post-test. The data were expressed as mean±standard error of mean (SEM). The significance among groups was accepted as $P < 0.05$, $P < 0.01$.

RESULTS

Performance parameters

The average body weight (BW) performance parameters, average daily weight gain (DWG), average daily feed intake (FI) and feed conversion ratio (FCR) values is given in Table 2. It was found that the BW, DWG and FCR values were statistically similar in all groups and all times ($P > 0.05$). The average FI values decreased at 28-35 days in CRM_{250} and CRM_{500} groups. But during all the peridod decreased only in the CRM_{500} group ($P < 0.05$). (Table 2).

Antioxidative Parameters

Values of parameters in serum related to antioxidant metabolism are given in Figure 1. It was found that serum CAT and SOD activities and MDA levels were similar in all groups ($P > 0.05$), while GPx activity increased significantly in the CRM_{250} group ($P < 0.01$) (Figure 1).

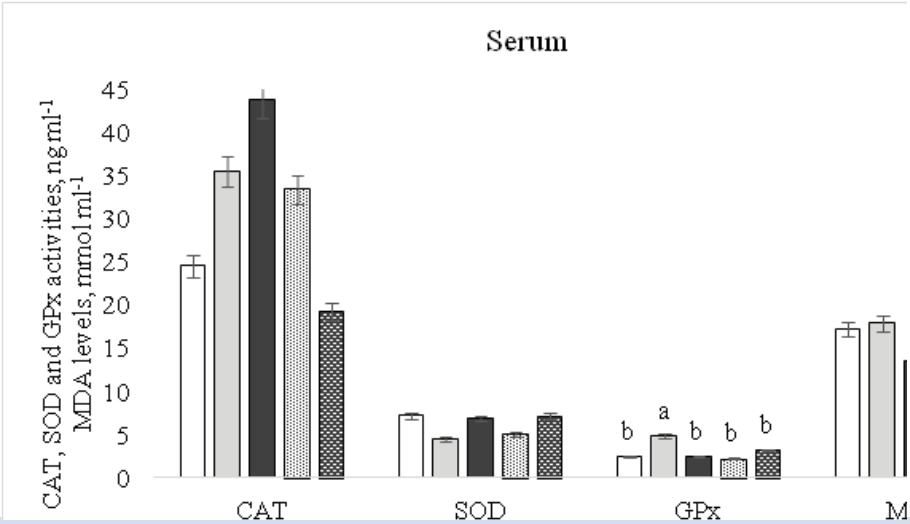

Figure 1. Effects of diets supplemented with curcumin and resveratrol on antioxidant parameters in serum of broiler chickens. All values are given as mean ± SEM, (n=8). a-b - Within a panel, bars labeled with different letters significantly differ ($P < 0.01$). CONT: basal diet alone, CRM_{250}: basal diet + 250 mg kg^{-1} of curcumin, CRM_{500}: basal diet + 500 mg kg^{-1} of curcumin, RSV_{250}: basal diet + 250 mg kg^{-1} of resveratrol and RSV_{500}: basal diet + 500 mg kg^{-1} of resveratrol.. SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase, MDA: Malondialdehyde.

Table 2. Effects of diets supplemented with curcumin and resveratrol on performance parameters of broiler chickens.

Parameters	Days	Groups[1]					*P*-value
		CONT	CRM_{250}	CRM_{500}	RSV_{250}	RSV_{500}	
BW, (g)	0	41.2±0.31	42.0±0.59	42.6±0.47	41.3±0.31	41.8±0.48	0.236
	7	148.6±1.07	143.6±4.37	147.1±1.79	146.7±2.40	143.6±3.82	0.692
	14	383.0±7.58	387.2±7.27	385.3±2.18	390.2±10.70	369.7±6.03	0.375
	21	701.4±10.59	702.0±3.41	700.5±1.29	707.2±5.76	685.7±6.25	0.236
	28	1118.1±35.23	1058.0±7.68	1048.3±8.60	1124.8±20.92	1076.0±31.77	0.150
	35	1728.2±49.94	1602.1±29.48	1603.2±24.04	1698.8±20.96	1671.6±69.80	0.218
	42	2378.8±48.81	2199.3±29.70	2219.7±34.81	2415.8±78.04	2384.5±68.01	0.053
DWG, (g)	0-7	15.4±0.13	14.5±0.54	14.9±0.27	15.1±0.30	14.6±0.52	0.532
	7-14	33.5±0.97	34.8±0.66	34.0±0.53	34.8±1.20	32.3±0.44	0.232
	14-21	45.5±0.57	45.0±1.51	45.0±0.43	45.3±0.90	45.1±0.22	0.992
	21-28	59.5±3.54	50.9±0.78	49.7±1.41	59.7±2.30	55.8±4.13	0.076
	28-35	87.2±2.72	77.7±5.28	79.3±2.47	82.0±2.92	85.1±6.29	0.520
	35-42	92.9±1.36	85.3±2.03	88.1±4.98	102.4±10.57	101.8±0.48	0.150
	0-42	55.7±1.17	51.4±0.70	51.8±0.83	56.5±1.85	55.8±1.61	0.051
FI, g/d	0-7	16.0±0.21	16.2±0.70	14.8±1.35	16.2±0.37	14.8±0.32	0.425
	7-14	44.3±1.20	45.9±0.70	45.5±0.75	45.9±1.62	42.9±0.88	0.282
	14-21	68.9±1.39	69.9±0.56	69.4±0.50	69.9±0.05	67.6±1.11	0.353
	21-28	89.8±2.86	83.6±1.26	83.6±1.78	92.5±1.36	88.1±3.28	0.072
	28-35	147.3±4.47^{a}	135.6±2.82^{bc}	131.9±2.12^{c}	149.6±1.34^{a}	143.8±1.77^{ab}	0.004
	35-42	169.0±4.83	163.9±1.48	153.6±4.20	174.4±9.24	173.4±1.15	0.087
	0-42	89.2±2.47^{ab}	85.8±0.39^{bc}	83.1±0.71^{c}	91.5±2.15^{a}	88.4±1.15^{ab}	0.032
FCR	0-7	1.04±0.01	1.11±0.02	0.99±0.09	1.06±0.00	1.02±0.02	0.311
	7-14	1.32±0.01	1.32±0.01	1.34±0.01	1.32±0.01	1.33±0.01	0.427
	14-21	1.51±0.02	1.56±0.04	1.54±0.03	1.55±0.03	1.50±0.03	0.617
	21-28	1.51±0.04	1.64±0.00	1.68±0.03	1.55±0.04	1.59±0.07	0.080
	28-35	1.69±0.06	1.77±0.16	1.67±0.07	1.83±0.08	1.71±0.02	0.823
	35-42	1.82±0.05	1.92±0.06	1.75±0.09	1.72±0.10	1.70±0.02	0.253
	0-42	1.60±0.02	1.67±0.03	1.60±0.01	1.62±0.03	1.59±0.03	0.212

All values are given as mean ± SEM, (n=40). [a-b-c]: The superscript letters in the same line indicate a statistical difference ($P < 0.05$, $P < 0.01$).

[1]CONT: basal diet alone, CRM_{250}: basal diet + 250 mg kg^{-1} of curcumin, CRM_{500}: basal diet + 500 mg kg^{-1} of curcumin, RSV_{250}: basal diet + 250 mg kg^{-1} of resveratrol and RSV_{500}: basal diet + 500 mg kg^{-1} of resveratrol. BW: Body weight, DWG: Daily weight gain, FI: Feed intake, FCR: Feed conversion rate.

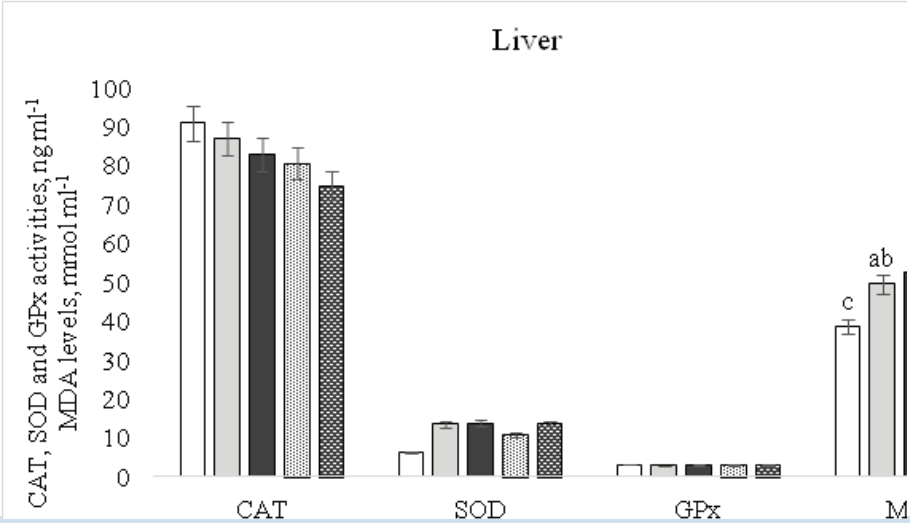

Figure 2. Effects of diets supplemented with curcumin and resveratrol on antioxidant parameters in liver of broiler chickens. All values are given as mean ± SEM, (n=8). a-b-c - Within a panel, bars labeled with different letters significantly differ ($P < 0.01$). CONT: basal diet alone, CRM_{250}: basal diet + 250 mg kg^{-1} of curcumin, CRM_{500}: basal diet + 500 mg kg^{-1} of curcumin, RSV_{250}: basal diet + 250 mg kg^{-1} of resveratrol and RSV_{500}: basal diet + 500 mg kg^{-1} of resveratrol.. SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase, MDA: Malondialdehyde.

It was determined that CAT, SOD and GPx activities in liver tissue were similar in all groups ($P > 0.05$), while MDA level increased significantly in $CRM_{250,}$ CRM_{500} and RSV500 groups ($P < 0.05$) (Figure 2).

It was determined that CAT, SOD and GPx activities in thigh tissue were similar in all groups ($P > 0.05$), while that the MDA levels were significantly increased in the CRM_{500} group ($P < 0.01$) (Figure 3).

Intestinal Flora

In the examinations performed on the intestinal flora, it was determined that the number of *E. coli* decreased significantly in the RSV_{500} group ($P < 0.01$) and was similar in the other groups ($P > 0.05$) (Table 3). It was determined that there was no difference between the groups in terms of numbers of *Clostridium spp.* and *Coliform spp* ($P > 0.05$), and the number of *Enterobacteriaceae* increased significantly in the RSV_{250}, RSV_{500} and CRM_{250} groups compared to the Control group ($P < 0.01$) (Table 3).

DISCUSSION

Natural products with antioxidant and antibacterial effects are widely used as feed additives in the animal feed industry due to their potential beneficial effects, including feed preservation and improvement of animal health. These additives protect many products, especially foods, from degeneration and provide a more durable form. People consume many products containing these antioxidants. Therefore, we conducted this study to demonstrate the beneficial effects of curcumin and resveratrol separately added to the diet in broilers.

The biological properties of curcumin, obtained from turmeric stems and root, have made it a natural substitute for antibiotics in animal feed. Several studies have been conducted to evaluate the effects of curcumin on the growth performance of broilers (Abou-Elkhair et al., 2014; Nayaka et al., 2013; Olu-

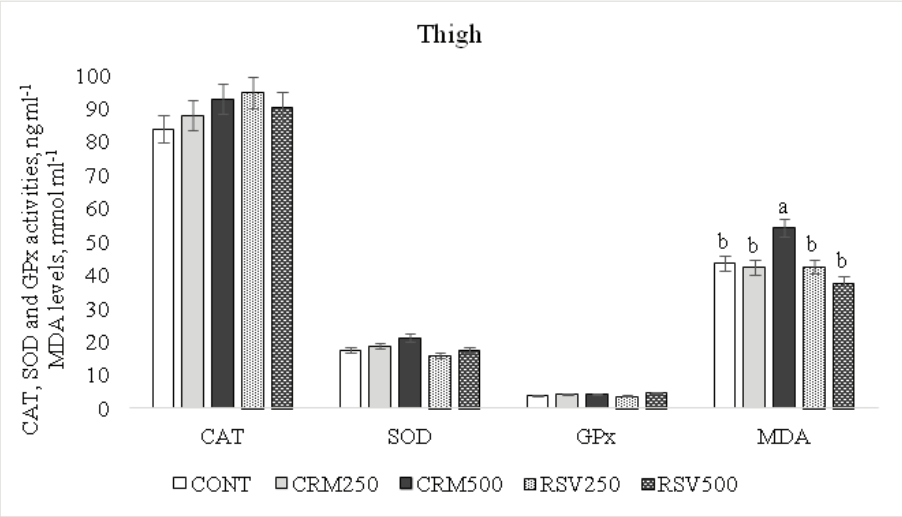

Figure 3. Effects of diets supplemented with curcumin and resveratrol on antioxidant parameters in thigh of broiler chickens. All values are given as mean ± SEM, (n=8). a-b - Within a panel, bars labeled with different letters significantly differ ($P < 0.01$). CONT: basal diet alone, CRM_{250}: basal diet + 250 mg kg^{-1} of curcumin, CRM_{500}: basal diet + 500 mg kg^{-1} of curcumin, RSV_{250}: basal diet + 250 mg kg^{-1} of resveratrol and RSV_{500}: basal diet + 500 mg kg^{-1} of resveratrol.. SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase, MDA: Malondialdehyde.

Table 3. Effects of diets supplemented with curcumin and resveratrol on intestinal mikroflora of broiler, log10 cfu g^{-1}

Parameters	Groups[1]					*P*-value
	CONT	CRM_{250}	CRM_{500}	RSV_{250}	RSV_{500}	
Escherichia coli	5.17±0.04^{a}	5.03±0.12^{a}	5.24±0.07^{a}	5.05±0.12^{a}	4.01±0.08^{b}	0.000
Clostridium spp.	5.23±0.37	4.32±0.12	4.56±0.24	4.74±0.15	4.80±0.07	0.079
Coliform spp.	3.856±0.21	4.93±0.13	4.23±0.18	4.61±0.28	4.43±0.29	0.051
Enterobacteriaceae	3.88±0.15^{c}	4.78±0.17^{ab}	4.30±0.20^{bc}	5.16±0.08^{a}	4.51±0.25^{b}	0.000

All values are given as mean ± SEM, (n=8). $^{a\text{-}b\text{-}c}$: The superscript letters in the same line indicate a statistical difference ($P < 0.05$, $P < 0.01$). [1]CONT: basal diet alone, CRM_{250}: basal diet + 250 mg kg^{-1} of curcumin, CRM_{500}: basal diet + 500 mg kg^{-1} of curcumin, RSV_{250}: basal diet + 250 mg kg^{-1} of resveratrol and RSV_{500}: basal diet + 500 mg kg^{-1} of resveratrol.

kosi and Dono, 2014); however, the results of these studies have been inconsistent. In a study with broilers was reported that 100, 200 and 300 mg kg^{-1} doses of turmeric extract added to the diet in the 2-12 week fattening trial had no effect on BW and DGW, but only 300 mg kg^{-1} dose had a positive effect on average daily FI and FCR (Wang et al., 2015). In a study conducted in broilers, it was reported that curcumin at a dose of 50 mg kg^{-1} added to the diet did not affect BW, DWG, FI and FCR at the end of the 44-day fattening trial (Galli et al., 2020a). In another study, it was stated that the addition of 100 mg kg^{-1} curcumin to the diet in broilers undergoing a 42-day fattening trial did not affect BW, DWG, and FCR, but reduced FI (Galli et al., 2020b). Unlike these studies, it has been reported that 100 mg kg^{-1} curcumin supplementation in the diet in broilers under heat stress increased the final BW values, average DWG and average daily FI, and improved the FCR value (Salah et al., 2021). It was also stated that the addition of resveratrol at doses of 200, 350 and 500 mg kg^{-1} to the diet in broilers exposed to heat stress did not affect the average daily FI and FCR, while the doses of 350 and 500 mg kg^{-1} increased the average DWG (He et al., 2019). In a study conducted in ducks, it was reported that curcumin supplementation at doses of 300, 400 and 500 mg kg^{-1} added to the diet significantly increased BW, DWG and FI, but did not affect FCR (Jin et al., 2021a). In a study conducted in broilers, it was reported that dietary curcumin did not affect BW and FI at the end of the 5-week fattening trial (Abd El-Hack et al., 2021). In another study conducted in broilers, it was reported that only 5 g kg^{-1} dose of turmeric powder at doses of 2.5, 5 and 10 g kg^{-1} added to the diet increased DWG, decreased FI and improved FCR at 35-days age (Durrani et al., 2006). It has also been reported that 400 mg kg^{-1} resveratrol added to the diet in broilers subjected to heat stress improves final BW and DWG, but does not affect FCR (Wang et al., 2021). In a study conducted in quails, it was reported that 100, 200 and 400 mg kg^{-1} resveratrol additives added to the diet did not affect DWG at the end of the 0-5 week fattening period, 100 mg kg^{-1} dose reduced FI and improved FCR (Ölmez et al., 2020). Nm et al. (2018) reported in their study in broilers that the addition of 1% curcumin led to a 10% decrease in FCR and an improvement in weight gain. Considering the results obtained in the current study, BW, DWG and FCR values were similar in all groups, and the mean daily FI value was according to the average 0-42 of the days, it was observed that it decreased in the CRM_{500} group. Regarding growth performance, the results obtained in both the current study and the studies in the literature differ. It is thought that these differences may be related to the dosage of the additives used in the studies, the breed of the animals and other nutrients in the diet.

It is known that many nutrients with antioxidant activity are beneficial on health, yield and meat quality when added to the diet. For example, adding curcumin (Salah et al., 2021) and resveratrol (Zhang et al., 2018) to the diet of broilers can improve the quality of animal products by improving the antioxidant status of muscle. Reactive oxygen species (ROS) are produced as an inevitable byproduct of animal metabolism. Many stress factors can lead to an excessive accumulation of ROS production, leading to oxidative stress, protein and lipid peroxidation, and cellular damage. Resveratrol can inhibit NADPH oxidase-mediated ROS production by downregulating the expression and activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and reduces mitochondrial superoxide formation through stimulation of mitochondrial biogenesis (Xia et al., 2017). In addition, resveratrol can increase the expression of various antioxidant enzymes and decrease MDA content (Xia et al., 2017; Zhang et al., 2017a). Studies have reported that adding antioxidants such as curcumin (Jin et al., 2020) and resveratrol to the diet inhibits lipid and protein oxidation (Yang et al., 2022). In this study, it was determined that serum MDA levels were similar in all groups, and the MDA level increased significantly in the liver of the RSV_{500}, CRM_{250} and CRM_{500} groups and in the thigh tissue of the CRM_{500} group. It has been reported that 350 and 500 mg/kg doses of resveratrol added to the diet in broilers exposed to heat stress reduce the amount of MDA in the serum (He et al., 2019), while the 400 mg/kg dose does not affect the amount of MDA in the jejunum (Wang et al., 2021). Also, it was stated that 400 mg kg^{-1} dose of resveratrol added to the diet did not affect the amount of MDA in the breast meat of broilers (Zhang et al., 2018), while 100, 200 and 400 mg kg^{-1} doses reduced the MDA level in the serum of quails (Ölmez et al., 2020). In a study conducted in broilers, it was reported that the addition of 100 mg kg^{-1} curcumin to the diet significantly reduced the MDA level of the breast and thigh tissues of animals subjected to heat stress (Salah et al., 2021). In another study conducted in broilers, it was stated that turmeric extract at doses of 100, 200 and 300 mg kg^{-1} added to the diet decreased the serum MDA level at the ages of

5 and 8 weeks (Wang et al., 2015). In addition to these findings, it was stated that 4 g kg^{-1} turmeric added to the diet in broilers did not affect the amount of MDA in thigh meat (Kyakma et al., 2022), and 50 mg kg^{-1} curcumin did not affect the amount of MDA and ROS in the serum (Galli et al., 2020a).

Antioxidant enzymes such as SOD, GPx and CAT are important components of the antioxidant system. GPx, SOD and CAT are the first line of cell defense against free radicals and reactive oxygen species (ROS) and are indispensable in the defense strategy of antioxidants in the body (Ighodaro and Akinloye, 2018). The higher the antioxidant enzyme activity (SOD, GPx and CAT), the stronger the body's antioxidant capacity (Zhang et al., 2017a). In poultry studies, dietary resveratrol supplementation has been reported to improve the antioxidant status of the animal body and/or product (Liu et al., 2014; Zhang et al., 2018). In the current study, it was observed that CAT, SOD and GPx activities in liver, were similar in all groups, serum GPx activity in the C_{250} group increased. Studies have shown that 500 mg kg^{-1} resveratrol added to the diet of ducks increases SOD, CAT and GPx activities in breast meat (Jin et al., 2021b), and that 100, 200, and 300 mg kg^{-1} turmeric extract added to the diet in broilers increases superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in serum at 5 and 8 weeks of age (Wang et al., 2015). Similarly, resveratrol at doses of 350 and 500 mg kg^{-1} added to the diet in broilers subjected to heat stress has been reported to increase SOD, CAT and GPx activities in serum (He et al., 2019). It has also been reported that 50 mg kg^{-1} dose of curcumin added to the broiler diet increases SOD activity in serum (Galli et al., 2020a), and 100 mg kg^{-1} dose increases SOD activity in breast meat (Galli et al., 2020b). In addition, it was stated that 400 mg kg^{-1} resveratrol added to the diet increased CAT activity in broiler breast meat (Zhang et al., 2018), and curcumin added to the diet of ducks at doses of 300, 400 and 500 mg kg^{-1} significantly increased GPx activity in breast meat (Jin et al., 2021a). It has also been reported that 400 mg kg^{-1} resveratrol added to the diet in broilers subjected to heat stress increases GPx activity in the jejunum (Wang et al., 2021). It has been reported that did not affect the total SOD and GPx activities of 400 mg kg^{-1} resveratrol added to the diet in breast meat (Zhang et al., 2018) and the 4 g kg^{-1} turmeric dose did not affect SOD activity in thigh meat (Kyakma et al., 2022), while curcumin at a dose of 50 mg kg^{-1} reduces GPx activity in serum at the studies conducted in broilers (Galli et al., 2020a). Similarly, it has been reported that 300, 400 and 500 mg kg^{-1} doses of curcumin added to the diet of ducks in breast meat (Jin et al., 2021a) did not affect the CAT and SOD activities in the jejunum with 400 mg kg^{-1} resveratrol added to the diet of heat stressed broilers (Wang et al., 2021).

The intestinal microbial ecosystem plays an important role in animal nutrition, physiology and immune defense mechanisms. Any disruption in the microbial ecosystem creates an opportunity for pathogenic bacteria to colonize and cause bacterial infections (Fouhse et al., 2016). Therefore, stabilization of the intestinal microflora is critical for intestinal health, barrier function and nutrient absorption (Patra et al., 2019). Among the leading causes of poor performance in poultry farming are bacteria such as *E. coli, Clostridium spp., Eimeria spp., and Salmonella spp.*, which can be controlled using antimicrobial and coccidiostatic additives added to feeds (Galli et al., 2020a). Colibacillosis caused by *E. coli* is an important intestinal disease in the poultry industry as it increases mortality and decreases growth performance (Kumar et al., 2004). *E. coli* causes immunological stress by damaging the intestines of poultry and produces lipopolysaccharide, which can affect the physiological and pathological processes of birds and interfere with their normal functions (Munyaka et al., 2012). Therefore, feed supplements and additives are used to control pathogens and improve zootechnical indices (Nunes et al., 2012). However, antibiotics and coccidiostatics have been banned in many countries as they cause undesirable effects, including accumulation in tissues and bacterial resistance (Costa et al., 2011). It has been determined that curcumin has antibacterial activity by decreasing the GTPase activity of FtsZ protofilaments, which has been determined to be important for bacterial cytokinesis as well as bacterial cell division and viability (Rai et al., 2008). In this study, 500 mg kg^{-1} resveratrol added to the broiler diet reduced the colonization of *E. coli* from pathogens in the cecum, and the additives used were found to be *Clostridium spp.* was found to have a decreasing tendency. Similar to these results, in a study conducted in broilers, it was reported that supplementation of 300 and 600 mg kg^{-1} resveratrol to the diet reduced intestinal E. coli colonization in broilers on days 24 and 42 (Mohebodini et al., 2019). Zhang et al. (2017b) reported that 400 mg kg^{-1} resveratrol added to the diet of heat stressed broilers reduced *E. coli* populations in jejunal digestion. In another study in broilers, the total bacterial count was found to be similar in the

stool analysis of the group that added 100 mg kg^{-1} curcumin to their diet and the control group that added flavomycin (20 mg kg^{-1}) and diclazuril (200 mg kg^{-1}) to their diet (Galli et al., 2020b). Again, polyphenols from some fruits have shown potential to inhibit various pathogens such as *E. coli* and *Salmonella* enteritidis (Al-Zoreky, 2009). Contrary to this information, it was stated that 50 mg kg^{-1} curcumin added to the diet of broilers did not affect the number of *E. coli* and total bacteria in feces (Galli et al., 2020a). Although it is generally accepted that polyphenols can cause bacterial population changes in the intestinal tract and maintain the balance between beneficial and pathogenic bacterial numbers (Viveros et al. 2011), the mechanisms are not yet clear.

CONCLUSIONS

As a result, it was observed that curcumin and resveratrol additives added to the broiler diet did not have a significant effect on performance parameters but had positive effects on antioxidant metabolism and intestinal microflora. Therefore, it can be said that adding curcumin and resveratrol to the broiler diet would be appropriate for raising healthy animals.

FUNDING SOURCES

The study was supported with the grant project (the Project number: V-104) by the Scientific Research Project Fund of Sivas Cumhuriyet University, Sivas Province, Turkey.

AUTHORS' CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [R. Gümüş], [N. Ercan], [A. Özbilgin], [M. N. Moğulkoç] and [H. İmik]. The first draft of the manuscript was written by [R. Gümüş] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

Abd El-Hack ME, El-Saadony MT, Swelum AA, Arif M, Abo Ghanima MM, Shukry M, El-Tarabily KA (2021). Curcumin, the active substance of turmeric: its effects on health and ways to improve its bioavailability. J Sci Food Agric 101(14):5747-5762.

Abou-Elkhair R, Ahmed HA, Selim S (2014). Effects of black pepper (Piper nigrum), turmeric powder (Curcuma longa) and coriander seeds (Coriandrum sativum) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. Asian-Australas J Anim Sci 27(6):847-854.

Al-Zoreky NS (2009). Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int J Food Microbiol 134(3):244-248.

AOAC (2005). Official Methods of Analysis of AOAC International (18 ed.). USA: AOAC International.

Bener M, Özyürek M, Güçlü K, Apak R (2016). Optimization of microwave-assisted extraction of curcumin from Curcuma longa L.(Turmeric) and evaluation of antioxidant activity in multi-test systems. Rec Nat Prod 10(5):542-554.

Berman AY, Motechin RA, Wiesenfeld MY, Holz MK (2017). The therapeutic potential of resveratrol: a review of clinical trials. NPJ Precision Onc 1(1):1-9.

Costa LB, Almeida VV, Berenchtein B, Tse MLP, Andrade AC, Miyada VS (2011). Phytobiotic additives and sodium butyrate as alternatives to antibiotics for weanling pigs. Arch Zootec 60(231):733-744.

De Lima Júnior DM, Rangel ADN, Urbano SA, Moreno GMB (2013). Lipid oxidation and lamb meat quality. Acta Vet Bras 7(1):14-28.

Durrani FR, Ismail M, Sultan A, Suhail SM, Chand N, Durrani Z (2006). Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler chicks. J Agric Biol Sci 1(2):9-11.

Fouhse JM, Zijlstra RT, Willing BP (2016). The role of gut microbiota in the health and disease of pigs. Anim Front 6(3):30-36.

Galli GM, Gerbet RR, Griss LG, Fortuoso BF, Petrolli TG, Boiago MM, Da Silva AS (2020a). Combination of herbal components (curcumin, carvacrol, thymol, cinnamaldehyde) in broiler chicken feed: Impacts on response parameters, performance, fatty acid profiles, meat quality and control of coccidia and bacteria. Microb Pathog 139:103916.

Galli GM, Griss LG, Boiago MM, Petrolli TG, Glombowsky P, Bissacotti BF, Silva AS (2020b). Effects of curcumin and yucca extract addition in feed of broilers on microorganism control (anticoccidial and antibacterial), health, performance and meat quality. Res Vet Sci 132:156-166.

Gumus R, Gelen SU, Koseoglu S, Ozkanlar S, Ceylan ZG, Imik H (2018). The effects of fucoxanthin dietary inclusion on the growth performance, antioxidant metabolism and meat quality of broilers. Braz J Poultry Sci 20(3):487-496.

He S, Li S, Arowolo MA, Yu Q, Chen F, Hu R, He J (2019). Effect of resveratrol on growth performance, rectal temperature and serum parameters of yellow-feather broilers under heat stress. Anim Sci J 90(3):401-411.

Ighodaro OM, Akinloye OA (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 54(4):287-293.

Jin LZ, Ho YW, Abdullah N, Jalaudin S (1996). Influence of dried Bacillus substillis and lactobacilli cultures on intestinal microflora and performance in broilers. Asian-Australas J Anim Sci 9(4):397-404.

Jin S, Pang Q, Liu R, Yang H, Liu F, Wang M, Shan A (2020). Dietary curcumin decreased lipid oxidation and enhanced the myofibrillar protein structure of the duck (Anas Platyrhynchos) breast muscle when subjected to storage. LWT-Food Sci Technol 133:109986.

Jin S, Yang H, Liu F, Pang Q, Shan A, Feng X (2021a). Effect of dietary curcumin supplementation on duck growth performance, antioxidant capacity and breast meat quality. Foods 10(12):2981.

Jin S, Pang Q, Yang H, Diao X, Shan A, Feng X (2021b). Effects of di-

etary resveratrol supplementation on the chemical composition, oxidative stability and meat quality of ducks (Anas platyrhynchos). Food Chem, 130263.

Karami M, Alimon AR, Sazili AQ, Goh YM, Ivan M (2011). Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of longissimus muscle in Kacang goat with aging time. Meat Sci 88(81):102-108.

Khan MA, Ali S, Yang H, Kamboh AA, Ahmad Z, Tume RK, Zhou G (2019). Improvement of color, texture and food safety of ready-to-eat high pressure-heat treated duck breast. Food Chem 277:646-654.

Kumar A, Jindal N, Shukla CL, Asrani RK, Ledoux DR, Rottinghaus GE (2004). Pathological changes in broiler chickens fed ochratoxin A and inoculated with Escherichia coli. Avian Pathol 33(4):413-417.

Kyakma SS, Tella TK, Sanwo KA (2022). Some meat quality parameters of broiler chickens fed diets containing different additives. Niger J Anim Prod 49(2):33-45.

Liu LL, He JH, Xie HB, Yang YS, Li JC, Zou Y (2014). Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens. Poult Sci 93(1):54-62.

Ma X, Lin Y, Jiang Z, Zheng C, Zhou G, Yu D, Chen F (2010). Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs. Amino Acids 38(1):95-102.

Mohebodini H, Jazi V, Bakhshalinejad R, Shabani A, Ashayerizadeh A (2019). Effect of dietary resveratrol supplementation on growth performance, immune response, serum biochemical indices, cecal microflora, and intestinal morphology of broiler chickens challenged with Escherichia coli. Livest Sci 229:13-21.

Munyaka PM, Tactacan G, Jing M, House JD, Rodriguez-Lecompte JC (2012). Immunomodulation in young laying hens by dietary folic acid and acute immune responses after challenge with Escherichia coli lipopolysaccharide. Poult Sci 91(10):2454-2463.

Nawab A, Li G, Liu W, Lan R, Wu J, Zhao Y, An L (2019). Effect of dietary curcumin on the antioxidant status of laying hens under high-temperature condition. J Therm Biol 86:102449.

Nayaka HS, Umakantha B, Ruban SW, Murthy HNN, Narayanaswamy HD (2013). Performance and hematological parameters of broilers fed neem, turmeric, vitamin e and their combinations. Emir J Food Agric 483-488.

Nm J, Joseph A, Maliakel B, Im K (2018). Dietary addition of a standardized extract of turmeric (TurmaFEEDTM) improves growth performance and carcass quality of broilers. J Anim Sci Technol 60(1):1-9.

NRC (1994). National research council. Nutrient requirements of poultry. 9th rev.ed. National Academy Press, Washington.

Nunes JO, Bertechini AG, Brito JÁGD, Fassani ÉJ, Mesquita FR, Makiyama L, Meneghetti C (2012). Evaluation of the use of probiotic (Bacillus subtilis C-3102) as additive to improve performance in broiler chicken diets. Rev Bras Zootec 41(11):2374-2378.

Olukosi OA, Dono ND (2014). Modification of digesta pH and intestinal morphology with the use of benzoic acid or phytobiotics and the effects on broiler chicken growth performance and energy and nutrient utilization. J Anim Sci 92(9):3945-3953. 2014.

Ölmez M, Şahin T, Makav M, Karadağoğlu Ö (2020). Effect of resveratrol supplemented to japanese quail (coturnix coturnix japonica) rations on performance and some biochemical parameters. Kafkas Univ Vet Fak Derg 26(6):807-812.

Patra AK, Amasheh S, Aschenbach JR (2019). Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds-a comprehensive review. Crit Rev Food Sci Nutr 59(20):3237-3266.

Rai D, Singh JK, Roy N, Panda D (2008). Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J 410(1):147-155.

Salah AS, Ahmed-Farid OA, Nassan MA, El-Tarabany MS (2021). Dietary curcumin improves energy metabolism, brain monoamines, carcass traits, muscle oxidative stability and fatty acid profile in heat-stressed broiler chickens. Antioxidants 10(8):1265.

Samarasinghe K, Wenk C, Silva KFST, Gunasekera JMDM (2003). Turmeric (Curcuma longa) root powder and mannanoligosaccharides as alternatives to antibiotics in broiler chicken diets. Asian-Australas J Anim Sci 16(10):1495-1500.

SPSS (2011). Statistical Packages for the Social Sciences, 20 ed. IBM Inc., Chicago.

Xia N, Daiber A, Förstermann U, Li H (2017). Antioxidant effects of resveratrol in the cardiovascular system. Br J Pharmacol 174(12):1633-1646.

Viveros A, Chamorro S, Pizarro M, Arija I, Centeno C, Brenes A (2011). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult Sci 90(3):566-578.

Wang D, Huang H, Zhou L, Li W, Zhou H, Hou G, Hu L (2015). Effects of dietary supplementation with turmeric rhizome extract on growth performance, carcass characteristics, antioxidant capability, and meat quality of Wenchang broiler chickens. Ital J Anim Sci 14(3):3870.

Wang Z, He Z, Gan X, Li H (2018). Interrelationship among ferrous myoglobin, lipid and protein oxidations in rabbit meat during refrigerated and superchilled storage. Meat Sci 146:131-139.

Wang C, Zhao F, Li Z, Jin X, Chen X, Geng Z, Zhang C (2021). Effects of resveratrol on growth performance, intestinal development, and antioxidant status of broilers under heat stress. Animals 2021;11(5):1427.

Yang H, Wang Y, Jin S, Pang Q, Shan A, Feng X (2022). Dietary resveratrol alleviated lipopolysaccharide-induced ileitis through Nrf2 and NF-κB signalling pathways in ducks (Anas platyrhynchos). J Anim Physiol Anim Nutr 106:1306-1320.

Zhang C, Wang L, Zhao XH, Chen XY, Yang L, Geng ZY (2017a). Dietary resveratrol supplementation prevents transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status. Poult Sci 96(7):2219-2225.

Zhang C, Zhao XH, Yang L, Chen XY, Jiang RS, Jin SH, Geng ZY (2017b). Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poult Sci 96(12):4325-4332.

Zhang C, Yang L, Zhao X, Chen X, Wang L, Geng Z (2018). Effect of dietary resveratrol supplementation on meat quality, muscle antioxidative capacity and mitochondrial biogenesis of broilers. J Sci Food Agric 98(3):1216-1221.