



Effects of Using Plant Extracts and Liquid Mineral on Growth Performance, Organ Weight and Meat Quality of Broiler Chickens

Muhammad Ammar Dilawar^{1,2}, Hong Seok Mun¹, Myeong Gil Jeong¹, Eun Ju Yang³, Hyeoung Seog Park⁴ and Chul Ju Yang^{1,2,*}

¹Animal Nutrition and Feed Science Laboratory, Department of Animal Science and Technology, Suncheon National University, 255 Jungang-ro, Suncheon, Jeollanam-do 57922, Republic of Korea

²Interdisciplinary Program in IT-Bio Convergence System (BK21 Plus), Suncheon National University, 255 Jungang-ro, Suncheon, Jeollanam-do 57922, Republic of Korea

³Food Research Centre, Jeonnam Bio Industry Foundation, Naju 58275, Republic of Korea

⁴EFC Company, Gwangyang 57714, Republic of Korea

ABSTRACT

A 35-day trial investigated the influence of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) extracts and liquid mineral (LM) in drinking water, on the growth performance, breast and thigh meat yield, digestive organ weights and meat quality characteristics in broilers. A total of 210 broilers were divided into the following treatment groups: (i) control, (ii) T1 (0.05% 4 MA: 1 GT + 0.01% LM), (iii) T2 (0.05% 4 MA: 1 GT + 0.05% LM), (iv) T3 (0.05% 4 MA: 1 GT + 0.1% LM), (v) T4 (0.1% 4 MA: 1 GT + 0.01% LM), (vi) T5 (0.1% 4 MA: 1 GT + 0.05% LM) and (vii) T6 (0.1% 4 MA: 1 GT + 0.1% LM). Significantly improved weight gain ($P < 0.05$) and FCR was observed in T2 relative to the control and other treatment groups. Breast and thigh meat yields were not influenced by the treatments. However, the weight of small intestine was significantly reduced ($P < 0.05$) in birds receiving dietary treatment T4 as compared with the control. On an average, meat TBARS value was decreased ($P < 0.05$) in all supplemented groups (except T1 and T2) and the lowest ($P < 0.05$) meat microbiological count was seen in T4. These results indicate that certain ratios of plant extracts and liquid minerals can be used to enhance the performance and meat quality in poultry.

Article Information

Received 26 February 2020

Revised 23 May 2020

Accepted 13 February 2021

Available online 04 August 2021

Authors' Contribution

CJY, HSM and HSP designed the study. MAD, MGJ and EJY performed the experiment. MAD, EJY and HSM analyzed the data. CJY and HSP supervised the study. MAD and HSM wrote the manuscript. MAD and CJY proofread the manuscript.

Key words

Geranium thunbergii, Growth performance, Liquid mineral, Meat quality, *Mentha arvensis*.

INTRODUCTION

To improve animal health and productivity, antibiotic feed additives (AFAs) have been widely used in poultry feed for decades. Despite their beneficial contribution to the industry, the unregulated and prolonged use of AFAs has resulted in drug resistant strains of poultry pathogens. As the global consumption of chicken is increasing at a steady rate of 3.6% annually, it is imperative to develop effective antibiotic alternatives without compromising the productivity (Suresh *et al.*, 2017). In this context, the use of phyto-genic feed additives (PFAs) in poultry feed has gained interest among the manufacturers and consumers. PFAs are known to have antioxidative, antimicrobial and immunomodulatory properties, and have also been shown to improve nutrient absorption and digestion in the gut

(Ahmed *et al.*, 2013). Their ability to increase poultry productivity is suggested to include mechanisms such as stimulation of intestinal mucosa, an increase in the digestive secretions, promotion of beneficial microorganism in the gut and inducing morphological changes in crypt size and villi in the intestine (Diaz-Sanchez *et al.*, 2015).

The poultry meat is susceptible to oxidative reactions due to the higher composition of unsaturated muscle lipids. The oxidative processes have a unfavorable impact on animal growth, performance and food quality (Estévez, 2015), there is a growing need to control these reactions by using antioxidant products. Of these, the natural antioxidants derived from plants and specific inorganic elements are of great interest among the industry and consumers (Fellenberg and Speisky, 2006). *Mentha arvensis* (MA) belongs to the family of Lamiaceae, which is used in season foods, as a household remedy and for industrial purposes. Recent researches have confirmed that the extract of MA possesses antioxidant, anti-inflammatory, anti-allergic and antimicrobial properties

* Corresponding author: yangcj@scnu.ac.kr
0030-9923/2021/0001-0001 \$ 9.00/0

Copyright 2021 Zoological Society of Pakistan

and have positive impact on growth performance in poultry (Biswas *et al.*, 2014; Dilawar *et al.*, 2019). It is also reported by Chanwitheesuk *et al.* (2005) that among 43 plant extracts evaluated, MA contains the highest vitamin E (0.0294 mg%) and total xanthophyll (26.5 mg%), which is linked to higher antioxidant activity of this plant. *Geranium thunbergii* (GT) is a perennial plant that belongs to the Geraniaceae family and is found in Korea, Japan and China. It is reported to possess anti-inflammatory, anti-mutagenic and anti-oxidant properties (Sung *et al.*, 2011). Among the 20 plants compared, GT showed the highest antioxidant potential; the gallic acid and total phenolic contents of this plant were 2312 mg/100g and 104 mg Gallic Acid Equivalent (GAE)/g, respectively (Kim *et al.*, 2010).

Minerals are important for a wide range of physiological processes in all animals. It is suggested that certain minerals in the diets of poultry exert beneficial effects on meat quality and growth (Shastak and Rodehutsord, 2015). However, excess dietary level of some minerals could interfere with the availability and metabolism of other minerals. Major problems regarding mineral requirement in animals are environmental concerns, if they exceed the physiological requirements (Carlson *et al.*, 1999). Additionally, the density of minerals differs from the other ingredients used in poultry feed. Consequently, there is difficulty in uniform mixing of these micro-ingredients in the feed, and each animal may not get the optimum level of minerals required for production. Sericite and clay mineral supplements have generally been included in animal diet to provide better flowability and pellet quality while preventing contamination by mycotoxins (Fowler *et al.*, 2015). Hence, supplementing minerals in drinking water could be an alternative approach that ensures uniform intake by each bird. Although numerous studies have reported the beneficial effects on the broiler's performance and meat quality by supplementing the diets with plant extracts and minerals but the optimal combination of plant extracts and liquid minerals and their addition in drinking water, has not yet been extensively studied. The present study, therefore, aimed to supplement liquid minerals and plant extracts in drinking water and determine if they have an impact on growth performance, carcass yield, internal organ weights, thiobarbituric acid reactive substances (TBARS) value, microbial count and pH of poultry meat.

MATERIALS AND METHODS

Plant extract and liquid mineral preparation

Plant extracts of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) were prepared by drying the leaves, followed by grinding using a blender (Wong *et*

al., 2006). Both plant extracts were prepared separately, using 100 g of dried ground leaves in 5 L of distilled water. The mixture was allowed to stand at normal room temperature for 2 h in the dark, with occasional agitation. The procedure was repeated for the extraction of 2 kg dried sample of each plant and adjusted to 1° Brix with sterile water. Each extract was filtered through Whatman No. 1 filter paper and stored at room temperature without any further treatment. Combination treatments were prepared in the poultry house prior to feeding, and added to drinking water.

The liquid mineral (LM) used in the experiment was provided by the Korea Food Ingredients Association (Daejeon, South Korea). The minerals were extracted from sericite and yellow clay. Mineral extracts for broilers were prepared by adding 5 g of citric acid, 5 g of malic acid and 20 L of water to 1 kg of mineral material mixed with sericite and yellow clay. After 24 h of extraction, extracts were filtered through a 1 µm filter bag. The liquid mineral contained (in ppm) K 3,109.11, Mg 643.61, Na 3,510.72, Pb 0.13, Cd 0.20, As 0.16, Hg undetermined, F 5.03. Combination treatments were mixed in the poultry house prior to feeding, and added to drinking water.

Animal care

All experimental procedures used in this study were authorized by the Animal Care and Use Committee of Sunchon National University, South Korea.

Birds and experimental design

Totally, 210 one-day old male "Ross" broiler chicks purchased from a commercial hatchery were weighed and randomly allocated into 7 treatment groups, having 5 replicates with 6 birds each (7 treatments x 5 replicates x 6 birds). The chicks were housed and reared in a closed, ventilated, and caged experimental broiler house (100 cm long x 80 cm wide x 40 cm high/cage). A linear feeder at the front for *ad libitum* feed intake and a nipple drinker at the back were provided in the cage for free access to water. Every morning, equal amounts of water were supplied to each replicate cage, to ensure uniform intake throughout the entire study. Temperature was maintained at 33°C from d 1 to d 7, gradually decreased to 24°C at a rate of 3°C per week, and then kept at 24°C until the end of the experiment. The relative humidity was maintained at 50% all over the experiment. The light schedule and stocking density were similar to the guidelines set in the Ross Broiler Management Handbook 2018.

The ingredients, calculated nutrient composition, vitamin and mineral content of the experimental basal diets are presented in Table I. The dietary treatments are summarized in Table II.

Table I.- Feed ingredients and analyzed chemical composition of the broiler diets.

Broiler diets	Starter	Finisher
Ingredients (% , as-fed basis)		
Corn	50.00	56.00
Soybean meal	37.00	28.84
Corn gluten meal	0.50	1.00
Wheat-10%	6.00	7.00
Limestone-Small	2.03	1.92
Salt-Proc	0.25	0.25
DCP-18%	0.40	0.46
L-Lys sulfate 70%	0.30	0.18
Minemix	0.20	0.20
Vitamix	0.05	0.05
L-Threonine-98%	-	0.01
MHA-Liquid	0.26	0.29
Sunphase5000FTU	0.01	0.01
Soybean oil	3.00	3.80
Total	100.00	100.00
Calculated composition (% DM)		
ME (kcal/kg)	3,090.97	3,207.91
Crude protein (%)	22.04	19.00
Crude fat (%)	5.35	6.23
Crude ash (%)	5.71	5.26
Crude fibre (%)	2.59	2.39
Ca (%)	1.09	1.04
Phosphorus (%)	0.45	0.43
Lysine (%)	1.34	1.07
Methionine (%)	0.57	0.55

Each kilogram contains: vitamin A, 12,061.00 IU (starter) and 12,122.00 IU (finisher); vitamin D₃, 3000.00 IU; vitamin E, 28.06 ppm (starter) and 29.35 ppm (finisher); vitamin K, 2.10 ppm (starter) and 2.11 ppm (finisher); choline chloride, 1,329.10 ppm (starter) and 1,146.20 ppm (finisher); copper, 73.02 ppm (starter) and 72.07 ppm (finisher); manganese, 77.92 ppm (starter) and 75.80 ppm (finisher); zinc, 73.75 ppm (starter) and 71.57 ppm (finisher); iodine, 0.94 ppm; selenium, 0.30 ppm; iron, 148.63 ppm (starter) and 4141.21 ppm (finisher).

Table II.- Treatment groups and the percentage of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) extract and liquid mineral (LM) used in the study.

Treatments		LM	4 MA:1 GT
0% 4 MA: 1GT + 0% LM	Control	-	-
0.05% 4 MA: 1 GT + 0.01% LM	T1	0.01%	0.05%
0.05% 4 MA: 1 GT + 0.05% LM	T2	0.05%	0.05%
0.05% 4 MA: 1 GT + 0.1% LM	T3	0.1%	0.05%
0.1% 4 MA: 1 GT + 0.01% LM	T4	0.01%	0.1%
0.1% 4 MA: 1 GT + 0.05% LM	T5	0.05%	0.1%
0.1% 4 MA: 1 GT + 0.1% LM	T6	0.1%	0.1%

K 3,109.11, Mg 643.61, Na 3,510.72, Pb 0.13, Cd 0.20, As 0.16, Hg undetermined, F 5.03

Growth performance measurement

For 5 weeks, the chicks were monitored daily and body weight gain (BWG) was measured every week. Feed intake (FI) was also determined weekly by deducting the residual feed from the total feed provided to the broilers, and the feed conversion ratio (FCR) was calculated subsequently for each treatment.

Thigh and breast meat yield, internal organ weights and sampling procedures

At 35 d of age, 3 broilers from each replicate (15 birds per treatment) were selected randomly; a sharp knife was used for cutting the neck, followed by hanging the birds for three min bleeding time and then subjected to carcass processing. The thigh and breast meat were separated from the carcass and weighed.

The meat samples were ground with the help of a meat grinder and stored at 4°C for oxidative stability (TBARS value) and pH analysis. The gizzard, proventriculus, pancreas, liver without the gallbladder, and small and large intestine were removed and weighed. The gastrointestinal tract was weighed after the removal of contents.

Oxidative stability and pH analysis of meat

After refrigerating meat samples, the TBARS value of the meat samples were determined at 0, 1, 2 and 3 weeks after storage, applying a previously described method (Ahmed *et al.*, 2015a). Briefly, about 4 g meat was mixed into a 10 ml solution consisting of 20% trichloroacetic acid (TCA) in 2M phosphoric acid and 10 ml of distilled water. The mixture was homogenized using a homogenizer (Ultra-Turrax T-25 Basic, IKA Werke, GMBH and Co. KG, Staufen, Germany) at maximum speed for 1.5 min. The mixture was filtered through a Hyundai Micro No. 60 (Hyundai Micro Co., Ltd.) filter paper. An equal amount of the filtrate (2 ml) and 2-thiobarbituric acid (98% 0.005 M 4,6-dihydroxy-2-mercaptopyrimidine prepared in distilled water) were heated in a shaking water bath at 80°C for 30 min. After cooling, a VIS-Spectrophotometer (Libra S22, Biochrom Ltd. Cambridge, England) was used to measure the absorbance at 530 nm after cooling. The TBARS value was expressed as milligrams of malondialdehyde (MDA) per 100 g of meat.

The pH was determined by mixing 1 g of meat sample with 9 ml of distilled water for 1.5 min in a homogenizer. The pH values of samples were measured using a standardized electrode attached to digital pH meter (Docu-pH+ meter, Sartorius, USA).

Meat microbiological count

About 25 g of meat samples (3 replicates) from each treatment group were taken from the thigh meat

and were examined for microorganisms. Samples were homogenized with 225 mL of 0.85% (w/v) NaCl solution, thereby obtaining a 1:10 dilution of the sample; subsequently, 10-fold serial dilutions (10^{-2} to 10^{-13}) were prepared using 0.85% NaCl solution. Using a sterile micro-pipette, 20 μ l sample from each serial dilution was transferred and spread on Tryptic soy agar plates (Becton, Dickinson and Company, Sparks, MD 21152 USA) using a sterilized triangle spreader for microbial enumeration. For each dilution, duplicate plates were incubated at 37°C for 48 h, and colonies were immediately counted after appropriate incubation time. The microbial number was then calculated as follows: multiplied value = no. of colonies $\times 10^n \times (100/20)$; where n=dilution value. The log (multiplied value) was determined after calculating the multiplied value, and the calculated log value of microbial count was expressed as log₁₀CFU/g.

Statistical analysis

Statistical analyses were performed using the SAS Statistical Package Program (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA) to determine if variables differed between groups. The BWG, feed intake, FCR, organ weights and TBARS, pH and microbial count of meat were compared between groups by one-way ANOVA and subsequent Duncan's Multiple Range Test. Probability values of less than 0.05 ($P < 0.05$) are considered significant.

RESULTS AND DISCUSSION

Growth performance, meat yield and internal organs weight

The initial body weight of birds did not differ ($P > 0.05$) between the dietary treatments (Table III). From 0-5 weeks, broilers supplemented with the T2 treatment had a greater ($P < 0.05$) BWG (1658 g) as compared to the control (1534 g). FCR was also significantly improved ($P < 0.05$) in the T2 group (1.62) compared with birds fed with the control diet (1.72). The mean thigh and breast meat yield and the weight of organs percentage relative to the body weight are shown in Tables IV and V. The meat yield showed no significant difference between the plant extracts and liquid mineral supplemented groups and control group ($P > 0.05$). Similarly, no differences were observed for weight of organs and relative weight of organs, except weight of the small intestine, which was significantly decreased in birds receiving T4 and T5 diets relative to the control ($P < 0.05$).

Several studies have shown that the supplementation of plant extracts in broiler diets can either increase, decrease or have no significant effect on productivity (Agah *et al.*, 2019). In the current study, plant extract supplementation did not improve BWG and FI during the starter phase (0-3 weeks). This may be due the low digestive enzyme secretion capacity in young birds (Khattak *et al.*, 2014).

Table III.- Effect of plant extracts and liquid mineral combination on growth performance of broilers.

Performance parameter	Control (n=30)	T1 (n=30)	T2 (n=30)	T3 (n=30)	T4 (n=30)	T5 (n=30)	T6 (n=30)	SEM	P-value
0 to 3 weeks									
Initial wt (g)	39	39	39	39	39	39	39	0.14	0.52
Final wt (g)	763	788	788	748	760	788	773	20.29	0.79
Weight gain	724	749	749	709	721	749	734	20.26	0.79
Feed intake	936	970	960	912	922	971	937	30.51	0.79
FCR	1.29	1.30	1.28	1.28	1.28	1.30	1.28	0.02	0.99
4 to 5 weeks									
Initial wt (g)	763	788	788	748	760	788	773	20.29	0.79
Final wt (g)	1,573 ^{bc}	1,630 ^{ab}	1,698 ^a	1,460 ^c	1,644 ^{ab}	1,507 ^c	1,570 ^{bc}	33.83	0.001
Weight gain	810 ^{bc}	842 ^{abc}	910 ^a	712 ^d	884 ^{ab}	719 ^d	798 ^c	24.53	0.0001
Feed intake	1,695 ^{ab}	1,792 ^a	1,724 ^{ab}	1,600 ^c	1,738 ^a	1,602 ^c	1,664 ^{abc}	36.01	0.02
FCR	2.09 ^{ab}	2.16 ^{ab}	1.90 ^c	2.26 ^{abc}	1.97 ^{bc}	2.24 ^{abc}	2.09 ^{ab}	0.07	0.001
0 to 5 weeks									
Final wt (g)	1,573 ^{bc}	1,630 ^{ab}	1,698 ^a	1,460 ^c	1,644 ^{ab}	1,507 ^c	1,570 ^{bc}	33.83	0.001
Weight gain	1,534 ^{bc}	1,590 ^{ab}	1,658 ^a	1,421 ^c	1,605 ^{ab}	1,468 ^c	1,531 ^{bc}	33.37	0.001
Feed intake	2,631	2,762	2,685	2,513	2,661	2,573	2,601	58.10	0.18
FCR	1.72 ^{ab}	1.75 ^{ab}	1.62 ^c	1.77 ^a	1.66 ^{bc}	1.75 ^{ab}	1.70 ^{ab}	0.02	0.03

^{a,b,c} Values with different superscripts in the same row differ significantly ($P < 0.05$). n, number of birds. For details of treatments see Table II.

Table IV.- Effect of plant extracts and liquid mineral combination on the meat yield and internal organ weight of broilers.

Performance parameter	Control (n=15)	T1 (n=15)	T2 (n=15)	T3 (n=15)	T4 (n=15)	T5 (n=15)	T6 (n=15)	SEM	P-value
Breast meat	355.67	309.20	326.80	292.00	312.00	299.60	289.60	22.96	0.76
Thigh meat	249.00	218.40	232.40	230.00	206.40	215.20	200.40	11.64	0.40
Body organ weight (g)									
Crop	4.87	5.36	4.39	3.32	4.50	3.68	4.41	0.56	0.23
Proventriculus	5.67	5.19	4.97	5.28	4.85	4.87	5.48	0.37	0.61
Gizzard	27.97	23.70	22.34	21.79	22.80	19.59	24.20	1.60	0.07
Liver	25.33	22.76	26.34	23.98	23.29	21.25	23.17	1.25	0.15
Pancreas	3.11	3.19	2.93	2.88	2.82	2.81	2.98	0.24	0.91
Heart	6.60	6.24	6.07	6.78	5.32	5.14	6.32	0.52	0.38
Gallbladder	2.15	2.07	1.85	1.60	1.79	1.76	1.67	0.23	0.70
Bursa	1.90	1.72	1.30	1.04	1.59	1.15	1.35	0.21	0.21
Small intestine	33.15 ^a	30.54 ^{ab}	30.20 ^{ab}	33.02 ^a	24.32 ^c	27.24 ^{bc}	29.44 ^{abc}	1.62	0.01
Colon	2.16	2.39	2.19	1.31	1.97	1.76	1.54	0.30	0.41
Cecum	6.94	7.65	7.17	5.81	5.04	3.95	6.68	0.89	0.14
Kidney	8.50	6.76	7.43	6.51	7.04	6.62	7.06	0.63	0.39
Spleen	1.04	1.03	0.58	0.80	0.89	0.75	0.86	0.09	0.08

^{a,b,c} Values with different superscripts in the same row differ significantly (P<0.05). n, number of birds. For details of treatments see Table II.

Table V.- Effect of plant extracts and liquid mineral combination on the relative organ weight of broilers (%).

Organ	Control (n=15)	T1 (n=15)	T2 (n=15)	T3 (n=15)	T4 (n=15)	T5 (n=15)	T6 (n=15)	SEM	P-value
Crop	0.31	0.33	0.26	0.23	0.27	0.25	0.29	0.01	0.15
Proventriculus	0.36	0.32	0.29	0.36	0.29	0.32	0.35	0.01	0.73
Gizzard	1.78	1.46	1.32	1.49	1.39	1.30	1.54	0.14	0.05
Liver	1.61	1.40	1.55	1.64	1.41	1.41	1.47	0.15	0.08
Pancreas	0.20	0.20	0.17	0.19	0.17	0.18	0.19	0.02	0.10
Heart	0.42	0.38	0.36	0.46	0.32	0.34	0.40	0.04	0.30
Gallbladder	0.14	0.13	0.11	0.10	0.11	0.12	0.10	0.003	0.60
Bursa	0.12	0.11	0.08	0.07	0.09	0.07	0.08	0.006	0.17
Small intestine	2.10 ^a	1.87 ^{ab}	1.78 ^{ab}	2.07 ^a	1.48 ^c	1.81 ^{bc}	1.87 ^{abc}	0.16	0.01
Colon	0.14	0.15	0.13	0.09	0.12	0.11	0.10	0.003	0.30
Cecum	0.44	0.47	0.42	0.39	0.31	0.27	0.43	0.05	0.06
Kidney	0.54	0.42	0.44	0.45	0.43	0.43	0.40	0.03	0.45
Spleen	0.06	0.06	0.03	0.05	0.05	0.04	0.05	0.001	0.07

^{a,b,c} Values with different superscripts in the same row differ significantly (P<0.05). n, number of birds. For details of treatments see Table II.

In contrast, during finishing period (4-5 weeks), inclusion of plant and mineral extracts in water significantly improved the BWG in T2 while a decrease was observed in T3 and T5 relative to the control. It is well reported that feeding of plant extracts has the ability to increase the feed intake by enhancing the palatability and flavor of feed (Upadhaya and Kim, 2017). Furthermore, the influence of phyto-genic feed additives on improved growth performance could be explained as their beneficial impact on the intestinal tract by increasing the activity of digestive enzymes, as well as the

antioxidant activity of active compounds present in plant extracts, improved utilization and absorption of nutrients, and inhibition of harmful bacteria (Windisch *et al.*, 2008). Khattak *et al.* (2014) also reported the same positive results on broiler performance after supplementing the feeds with extracts from basil, laurel, caraway, oregano, lemon, sage, tea and thyme. In contrast, the physiological difference in the alimentary tract and the dose of phyto-genic extracts supplemented in the water used in this study might have caused differences in the results of T3 and T5 group during

4-5 weeks. It was reported by Schone *et al.* (2006) that supplementation with fennel or caraway oil significantly reduces the feed intake in growing pigs. The increased percentage of plant extracts in T3 and T5 during finishing phase may have decreased the palatability due to the excess flavor in water, thereby resulting in reduced FI and BWG. Considering the previous reports that mineral level of diets has no or very little impact on growth performance in pigs and poultry (Burkett *et al.*, 2009), we therefore deduce that only plant extracts are responsible for the increased or decreased FI and BWG in our trial.

Breast and thigh meat yield did not differ significantly between treatment groups and the control (Table IV). Similar results were obtained by Carlos *et al.* (2014) subsequent to supplementation of plant extracts derived from oregano, chili and cinnamon. However, these results

differ from the experiment of Jamroz *et al.* (2005), who reported an increase in the breast yield in broilers fed diets with a mixture of plant extracts. In terms of weight and relative weight of different body organs, no parameters were influenced except weight of the small intestine (Table V). The weight of intestine of birds receiving treatments T4 and T5 were significantly lower than the birds fed with control diet. Shargh *et al.* (2012) also found a lower ileum weight in birds receiving garlic. They proposed that the weight of intestine reduces due to a reduction in muscular thickness, which in turn increases the nutrient absorption. In the present study, the lowest small intestine weight was found in the supplemented groups, which may therefore be linked with increased nutrient absorption and finally increased body weight.

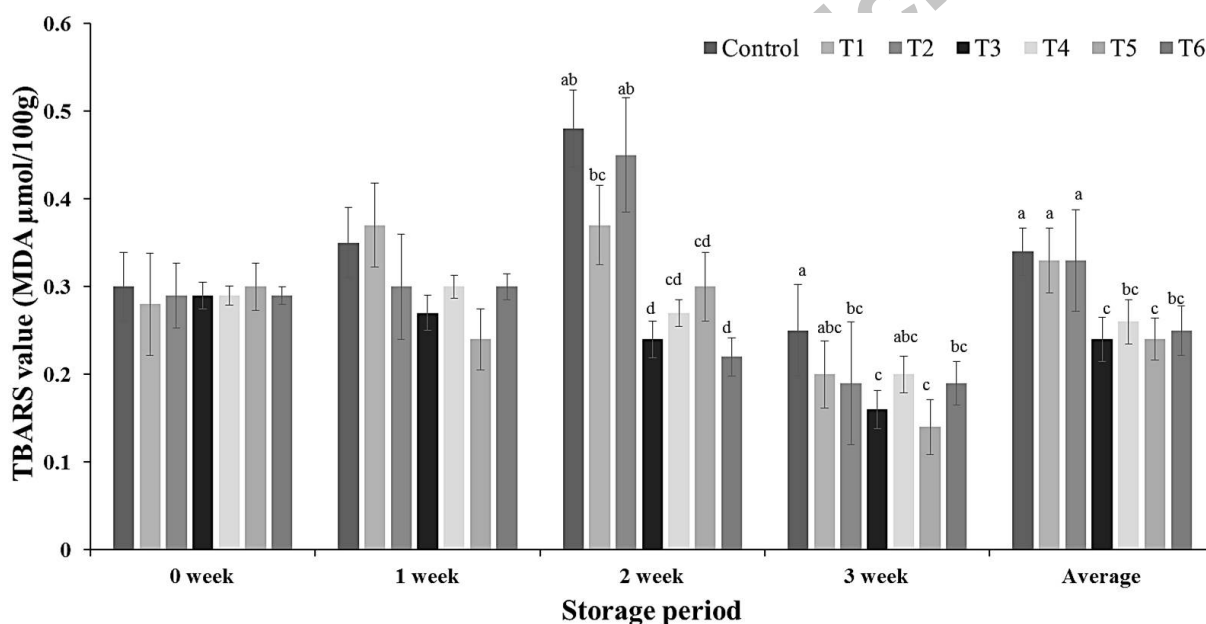


Fig. 1. Effects of plant extracts and liquid mineral combination on chicken meat TBARS value. ^{a,b,c} Values with different superscripts in the same row differ significantly ($P < 0.05$). n, number of birds (n=15). For details of treatments see Table II.

Table VI.- Effects of plant extracts and liquid mineral combination on broiler meat pH.

Storage period	Control (n=15)	T1 (n=15)	T2 (n=15)	T3 (n=15)	T4 (n=15)	T5 (n=15)	T6 (n=15)	SEM	P-value
0 week	6.33	6.44	6.31	6.51	6.35	6.48	6.41	0.06	0.23
1 week	6.51	6.64	6.38	6.52	6.34	6.41	6.36	0.06	0.08
2 weeks	6.20	6.32	6.19	6.38	6.30	6.34	6.34	0.05	0.15
3 weeks	6.41	6.59	6.51	6.66	6.65	6.75	6.56	0.07	0.05
Average	6.37	6.50	6.35	6.52	6.41	6.49	6.42	0.05	0.29

For details of treatments see Table II.

Oxidative stability and pH of meat

The effects of plant extracts and liquid minerals on the TBARS value of broiler meat are presented in Figure 1. The dietary treatments had no significant effect on the oxidative stability values of thigh meat after 1 week of storage ($P>0.05$). However, after 3 weeks of storage, significantly lower ($P<0.05$) TBARS values were seen in the meat of broilers fed diets with inclusion of plant extracts and liquid minerals except T1 and T4. Furthermore, the pH of meat remained unaffected ($P>0.05$) in all treatments, after 0, 1, 2 and 3 weeks of storage (Table VI).

The shelf life of packed meat is short due to microbial proliferation and rapid oxidation of lipids. Free radicals are produced as a result of lipid oxidation, which subsequently result in the production of unpleasant color and flavors in meat. Phospholipids are more prone to oxidation. Since the contents of phospholipids are higher in poultry meat, it is considered to be more prone to oxidation among the meat products (Puvača *et al.*, 2015). In the present study, supplementing the diet of birds with different levels and ratios of plant extracts and liquid minerals significantly decreased the oxidation of lipids, as demonstrated by the lower TBARS values of broiler meat. It is well documented that polyphenolic compounds present in plant extracts have the ability to act as antioxidants by scavenging free radicals and protecting lipids from oxidative reactions (Ahmed *et al.*, 2015b). Several plant components like polyphenols, tannins and flavonoids are present in MA and GT, which are known to have antioxidant properties (Biswas *et al.*, 2014). The antioxidant capacities of different plants like thymol and carvacrol (Luna *et al.*, 2010), pomegranate

(*Punica granatum* L.) (Ahmed *et al.*, 2015a) and oregano (Forte *et al.*, 2018) are reported to reduce the oxidative deterioration of fat in poultry meat, thereby decreasing malondialdehyde (MDA) production. The reduction of TBARS values in this experiment can be explained by the fact that tannins and flavonoids like quercetin, which are present in MA and GT, have the ability to scavenge superoxide free radicals and retard lipid oxidation. Likewise, Ghazaghi *et al.* (2014) reported the improved oxidative stability and meat quality in Japanese quail due to supplementation with *Mentha* species. Additionally, MA and GT are reported to possess high amounts of total phenolics and DPPH free radical scavenging ability, which subsequently increases the antioxidant capacity of plant extracts and reduces the TBARS values (Wong *et al.*, 2006; Yang *et al.*, 2010).

One critical factor in determining meat quality is postmortem pH following muscle glycolysis (Janisch *et al.*, 2012). In this study, the pH of meat remains unaffected by diet. These results are in agreement with other reports where supplementation with plant extracts or minerals did not modify the pH of poultry meat (Jung *et al.*, 2010).

Meat microbiological count

The microbial count of broiler meat for dietary treatments is presented in Figure 2. On an average and after 0 weeks of storage, the meat microbiological count was decreased ($P<0.05$) for the T4 group as compared to the control and other treatments. After 1 and 3 weeks of storage, the microbial count of meat showed no significant differences between the dietary treatments ($P>0.05$).

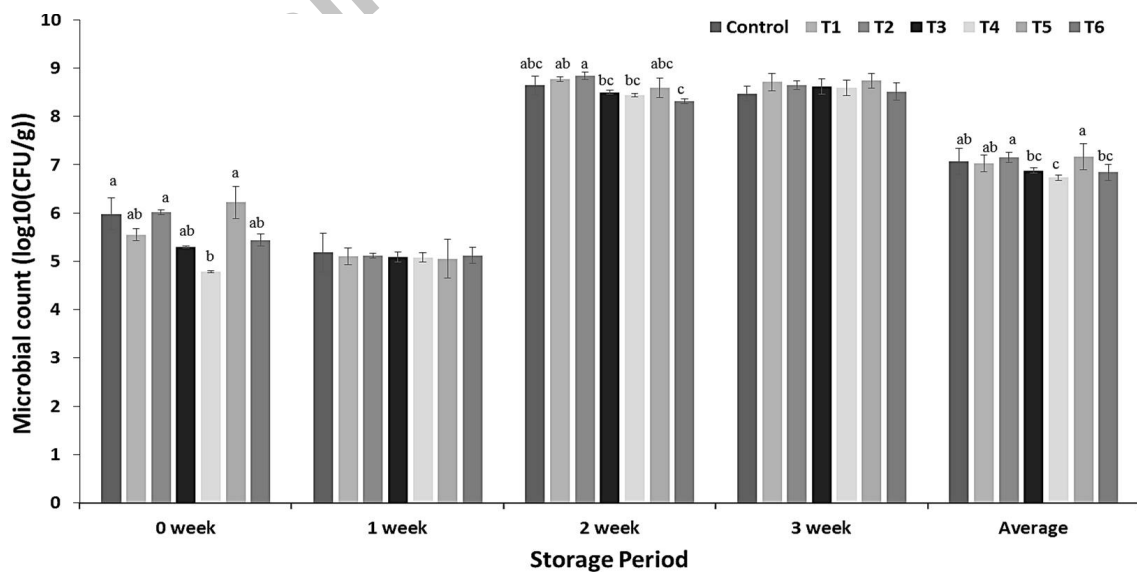


Fig. 2. Effects of plant extracts and liquid mineral combination on chicken meat microbial value. ^{a,b,c} Values with different superscripts in the same row differ significantly ($P<0.05$). n, number of birds (n=15). For details of treatments see Table II.

The antimicrobial activity of plant extracts against food borne organisms is due to the presence of phenolic substances such as thymol, carvacrol, citronellol and eugenol (Gheisar and Kim, 2018). Broilers fed T4 diet were found to have a lower meat microbial count (at 0 week and on average). The antimicrobial activity of menthol present in *M. arvensis* against various microorganisms has been previously reported (Singh *et al.*, 2015), whereas the antibacterial activity of *G. thunbergii* is reported by Bigos *et al.* (2012). Elimination of microorganisms from poultry before processing improves the shelf life of processed feed. Therefore, feed supplementation with plant extracts is generally used to inhibit bacterial growth. The phenolic compounds of MA and GT have the ability to enter the cell membrane of bacteria, leading to disruption of the membrane, and ultimately reducing the final microbial count (Upadhaya and Kim, 2017). The phenolics present in both plant extracts have proven activity against both Gram-positive and Gram-negative bacteria (Brenes and Roura, 2010), which may be the reason of reduced microbial count. Interestingly, antimicrobial action of plant extracts is known to improve the microbial hygiene of carcass. Aksit *et al.* (2006) reported the beneficial effect of adding 0.1% *Origanum onites* to the feed (15g/kg of commercial product), on the microbial load of total bacterial count of carcass or specific food-borne pathogens (*e.g.* Salmonella). In addition, different laboratory studies have shown that the effects of essential oils obtained from *Mentha* species inhibit the growth of various pathogenic bacteria (Khempaka *et al.*, 2013).

CONCLUSION

Results from the present study demonstrate that the growth performance and FCR in broilers can be improved by feeding T2. The antioxidative potential and microbiological count of meat can be improved by T4 without compromising growth performance. Further research is required to determine ideal ratio for industrial applications.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Trade, Industry and Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Encouragement Program for the Industries of the Economic Cooperation Region (R0005793). This work was supported by the BK21 plus program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea (5199990214660).

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

- Agah, M.J., Mirakzehi, M.T. and Saleh, H., 2019. Effects of olive leaf extract (*Olea europea* L.) on growth performance, blood metabolites and antioxidant activities in broiler chicken under heat stress. *J. Pl. Anim. Sci.*, **29**: 657–666.
- Ahmed, S.T., Hossain, M.E., Kim, G.E., Hwang, J.A., Ji, H. and Yang, C.J., 2013. Effects of resveratrol and essential oils on growth performance, immunity, digestibility and fecal microbial shedding in challenged piglets. *Asian-Aust. J. Anim. Sci.*, **26**: 683–690. <https://doi.org/10.5713/ajas.2012.12683>
- Ahmed, S.T., Islam, M.M., Mun, H.S., Bostami, A.B.M.R. and Yang, C.J., 2015a. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.). *Fd. Chem.*, **188**: 481–488. <https://doi.org/10.1016/j.foodchem.2015.04.140>
- Ahmed, S.T., Kim, G., Islam, M.M., Mun, H.S., Bostami, A.B.M.R. and Yang, C.J., 2015b. Effects of dietary chlorine dioxide on growth performance, intestinal and excreta microbiology, and odorous gas emissions from broiler excreta. *J. appl. Poult. Res.*, **24**: 502–510. <https://doi.org/10.3382/jap/pfv058>
- Aksit, M., Göksoy, E., Kök, F., Özdemir, D. and Özdoğan, M., 2006. The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses. *Eur. Poult. Sci.*, **70**: 168–173.
- Bigos, M., Wasiela, M., Kalemba, D. and Sienkiewicz, M., 2012. Antimicrobial activity of Geranium oil against clinical strains of *Staphylococcus aureus*. *Molecules*, **17**: 10276–10291. <https://doi.org/10.3390/molecules170910276>
- Biswas, N.N., Saha, S. and Ali, M.K., 2014. Antioxidant, antimicrobial, cytotoxic extract of *Mentha arvensis* L. and analgesic activities of ethanolic extracts of *Mentha arvensis* L. *Asian-Pacific J. trop. Biomed.*, **4**: 792–797. <https://doi.org/10.12980/APJTB.4.2014C1298>
- Brenes, A. and Roura, E., 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Anim. Feed Sci. Tech.*, **158**: 1–14. <https://doi.org/10.1016/j.anifeedsci.2010.03.007>
- Burkett, L.J., Stalder, K.J., Powers, W.J., Bregendahl, K., Pearce, J.L., Baas, T.J., Bailey, T. and Shafer, B.L., 2009. Effect of inorganic and organic trace

- mineral supplementation on the performance, carcass characteristics, and fecal mineral excretion of phase-fed, grow-finish swine. *Asian-Aust. J. Anim. Sci.*, **22**: 1279-1287. <https://doi.org/10.5713/ajas.2009.70091>
- Carlos, T.C.F., Barbosa, L., Polycarpo, G., Afonso, E., Utimi, N., Bortolato, C., Araújo, C. and Araújo, L.F., 2014. Performance and carcass yield of broilers supplemented with plant extract during the finisher phase. *Braz. J. Poult. Sci.*, **16**: 443–448. <https://doi.org/10.1590/1516-635X1604443-448>
- Carlson, M.S., Hill, G.M. and Link, J.E., 1999. Early and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide : Effect on metallothionein and mineral concentrations. *J. Anim. Sci.*, **77**: 1199–1207. <https://doi.org/10.2527/1999.7751199x>
- Chanwitheesuk, A., Teerawutgulrag, A. and Rakariyatham, N., 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Fd. Chem.*, **92**: 491–497. <https://doi.org/10.1016/j.foodchem.2004.07.035>
- Diaz-Sanchez, S., Souza, D.D., Biswas, D. and Hanning, I., 2015. Botanical alternatives to antibiotics for use in organic poultry production. *Poult. Sci.*, **94**: 1419–1430. <https://doi.org/10.3382/ps/pev014>
- Dilawar, M.A., Saturno, J.F.L., Mun, H.S., Kim, D.H., Jeong, M.J. and Yang, C.J., 2019. Influence of two plant extracts on broiler performance, oxidative stability of meat and odorous gas emission from excreta. *Annu. Anim. Sci.*, **19**: 1099-1113. <https://doi.org/10.2478/aoas-2019-0046>
- Estévez, M., 2015. Oxidative damage to poultry: From farm to fork. *Poult. Sci.*, **94**: 1368–1378. <https://doi.org/10.3382/ps/pev094>
- Fellenberg, M.A. and Speisky, H., 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. *Worlds Poult. Sci. J.*, **62**: 53–70. <https://doi.org/10.1079/WPS200584>
- Forte, C., Branciarri, R., Pacetti, D., Miraglia, D., Ranucci, D., Acuti, G., Balzano, M., Frega, N.G. and Trabalza-Marinucci, M., 2018. Dietary oregano (*Origanum vulgare* L.) aqueous extract improves oxidative stability and consumer acceptance of meat enriched with CLA and n-3 PUFA in broilers. *Poult. Sci.*, **97**: 1774–1785. <https://doi.org/10.3382/ps/pex452>
- Fowler, J., Li, W. and Bailey, C., 2015. Effects of a calcium bentonite clay in diets containing aflatoxin when measuring liver residues of aflatoxin B1 in starter broiler chicks. *Toxins*, **7**: 3455–3464. <https://doi.org/10.3390/toxins7093455>
- Ghazaghi, M., Mehri, M. and Bagherzadeh-Kasmani, F., 2014. Effects of dietary *Mentha spicata* on performance, blood metabolites, meat quality and microbial ecosystem of small intestine in growing Japanese quail. *Anim. Feed Sci. Tech.*, **194**: 89–98. <https://doi.org/10.1016/j.anifeedsci.2014.04.014>
- Gheisar, M.M. and Kim, I.H., 2018. Phytobiotics in poultry and swine nutrition – a review. *Ital. J. Anim. Sci.*, **17**: 92–99. <https://doi.org/10.1080/1828051X.2017.1350120>
- Jamroz, D., Wiliczekiewicz, A., Wertelecki, T., Orda, J. and Skorupi, J., 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. *Br. Poult. Sci.*, **46**: 37–41. <https://doi.org/10.1080/00071660500191056>
- Janisch, S., Wicke, M. and Krischek, C., 2012. Mitochondrial respiratory and antioxidative enzyme activities in turkey meat. *Animal*, **6**: 346–352. <https://doi.org/10.1017/S1751731111001649>
- Jung, S., Ho, J., Kim, B., Yun, H., Kruk, Z.A. and Jo, C., 2010. Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. *Meat Sci.*, **86**: 520–526. <https://doi.org/10.1016/j.meatsci.2010.06.007>
- Khattak, F., Ronchi, A., Castelli, P. and Sparks, K., 2014. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. *Poult. Sci.*, **93**: 132–137. <https://doi.org/10.3382/ps.2013-03387>
- Khempaka, S., Pudpila, U. and Molee, W., 2013. Effect of dried peppermint (*Mentha cordifolia*) on growth performance, nutrient digestibility, carcass traits, antioxidant properties, and ammonia production in broilers. *J. appl. Poult. Res.*, **22**: 904–912. <https://doi.org/10.3382/japr.2013-00813>
- Kim, M., Park, J. and Lim, S., 2010. Antioxidant activity and cell toxicity of pressurised liquid extracts from 20 selected plant species in Jeju, Korea. *Fd. Chem.*, **122**: 546–552. <https://doi.org/10.1016/j.foodchem.2010.03.007>
- Luna, A., Lábaque, M.C., Zygadlo, J.A. and Marin, R.H., 2010. Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. *Poult. Sci.*, **89**: 366–370. <https://doi.org/10.3382/ps.2009-00130>
- Puvača, N., Kostadinović, L., Popović, S., Lević, J., Ljubojević, D., Tufarelli, V., Jovanović, R., Tasić, T., Ikonić, P. and Lukač, D., 2015. Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum*, *Piper nigrum*, *Capsicum*

- annuum*). *Anim. Prod. Sci.*, **56**: 1920–1927. <https://doi.org/10.1071/AN15115>
- Schone, F., Vetter, A., Hartung, H., Bergmann, H., Biertumpfel, A., Richter, G., Muller, S. and Breitschuh, G., 2006. Effects of essential oils from fennel (*Foeniculi aetheroleum*) caraway (*Carvi aetheroleum*) in pigs. *J. Anim. Physiol. Nutr.*, **90**: 500–510. <https://doi.org/10.1111/j.1439-0396.2006.00632.x>
- Shargh, M.S., Dastar, B., Zerehdaran, S., Khomeiri, M. and Moradi, A., 2012. Effects of using plant extracts and a probiotic on performance, intestinal morphology, and microflora population in broilers. *J. appl. Poult. Res.*, **21**: 201–208. <https://doi.org/10.3382/japr.2010-00145>
- Shastak, Y. and Rodehutsord, M., 2015. A review of the role of magnesium in poultry nutrition. *Worlds Poult. Sci. J.*, **71**: 125–138. <https://doi.org/10.1017/S0043933915000112>
- Singh, R., Shushni, M.A.M. and Belkheir, A., 2015. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arab J. Chem.*, **8**: 322–328. <https://doi.org/10.1016/j.arabjc.2011.01.019>
- Sung, Y.Y., Yoon, T., Yang, W.K., Kim, S.J. and Kim, H.K., 2011. Anti-obesity effects of *Geranium thunbergii* extract via improvement of lipid metabolism in high-fat diet-induced obese mice. *Mol. Med. Rep.*, **4**: 1107–1113. <https://doi.org/10.1055/s-0031-1282702>
- Suresh, G., Das, R.K., Brar, S.K., Rouissi, T., Ramirez, A.A., Chorfi, Y. and Godbout, S., 2017. Alternatives to antibiotics in poultry feed: Molecular perspectives. *Crit. Rev. Micro.*, **44**: 318–355. <https://doi.org/10.1080/1040841X.2017.1373062>
- Upadhaya, S.D. and Kim, I.H., 2017. Efficacy of phytogetic feed additive on performance, production and health status of monogastric animals - A review. *Ann. Anim. Sci.*, **17**: 929–948. <https://doi.org/10.1515/aoas-2016-0079>
- Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A., 2008. Use of phytogetic products as feed additives for swine and poultry. *J. Anim. Sci.*, **86**: 140–148. <https://doi.org/10.2527/jas.2007-0459>
- Wong, S.P., Leong, L.P. and Koh, J.H.W., 2006. Antioxidant activities of aqueous extracts of selected plants. *Fd. Chem.*, **99**: 775–783. <https://doi.org/10.1016/j.foodchem.2005.07.058>
- Yang, Y., Li, J., Zu, Y., Fu, Y., Luo, M., Wu, N. and Liu, X., 2010. Optimisation of microwave-assisted enzymatic extraction of corilagin and geraniin from *Geranium sibiricum* Linne and evaluation of antioxidant activity. *Fd. Chem.*, **122**: 373–380. <https://doi.org/10.1016/j.foodchem.2010.02.061>