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INFLUENCE OF TWO PLANT EXTRACTS ON BROILER PERFORMANCE, OXIDATIVE STABILITY OF MEAT AND ODOROUS GAS EMISSIONS FROM EXCRETA

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Abstract

A feeding trial was conducted to evaluate the effects of plant extracts derived from *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) on broiler performance, carcass yield, digestive organ weights, meat quality and odorous gas emissions from the excreta. A total of 210 one-day-old broiler chicks were randomly assigned to one of the following dietary treatments for five weeks: (1) control, (2) 0.1% MA, (3) 0.05% GT, (4) 0.1% GT and 0.1% mixed preparation with varying ratios such as (5) 0.1% 1MA:1GT, (6) 0.1% 1MA:4GT and (7) 0.1% 4MA:1GT. The weight gain (0–3 weeks) was significantly (P<0.01) increased in response to dietary inclusion of plant extracts; however, no significant differences were observed in breast and thigh meat yield (P>0.05). Additionally, no differences were observed in proventriculus, gizzard, pancreas, small and large intestine weight. When compared with the control group, birds fed plant extracts had the lowest meat TBARS value after 0 and 3 weeks of storage (P<0.05). Moreover, the pH value was significantly lower (P<0.05) in the plant extract supplemented groups at 0 weeks. The faecal H_2S emissions were significantly reduced at 0 h and 6 h in all supplemented groups relative to the control (P<0.05). Similarly, NH_3 emissions were reduced at 0 h and 6 h for all supplemented groups except the 0.1% 1MA:1GT group (P<0.05). Overall, the results of the present study indicate that plant extracts supplementation can be used to improve performance and meat quality of broiler chickens while reducing the emission of harmful gases from the excreta.

Key words: *Mentha arvensis, Geranium thunbergii*, meat quality, odorous gas emission, broiler

In the poultry industry, antibiotics have been widely used for therapeutic and prophylactic purposes, as well as to improve the growth rate and feed conversion efficiency (Huyghebaert et al., 2011). However, excessive use of dietary antibiotics has resulted in the development of various drug-resistant bacteria, drug residues in the body tissue and organs and imbalance of normal microflora (Awad et al., 2009). Conversely, the removal of antibiotic growth promoters (AGPs) has compromised

animal performance and led to an increase in the incidence of certain animal diseases (Suresh et al., 2017). This has led to searches for bioactive agents that could serve as alternatives to antibiotics. As a result, investigations of phytogenics and their effects as feed additives are gaining importance because of their antimicrobial activity and stimulatory effects on the digestive system (Symeon et al., 2014). The mechanism of action for most of the herbs in the poultry as growth promoters include modification of the intestinal microbiota, increased digestibility, increased absorption of nutrients, increased nitrogen absorption, boosted immune response, modification of the histomorphology of the gastrointestinal tract and protection against oxidative reactions (Kumar et al., 2014).

Phytogenic feed additives (often known as botanicals or phytobiotics) are plant-derived compounds that are incorporated into the diets of animals to improve the productivity of livestock through improvement of feed digestibility and acceptance, of growth performance and of the quality characteristics of the derived products (Windisch et al., 2008). Phytobiotics as individual compounds or in mixed preparations can provide many beneficial effects including stimulation of feed intake, improvement of endogenous digestive enzyme secretion and activation of the immune system (Hashemi and Davoodi, 2011). They also possess anti-bacterial, anthelmintic, antiviral, anti-inflammatory, antioxidant and coccidiostatic properties (Rahimi et al., 2011). Various spices have long been used in food products to improve their flavour and oxidative stability (Applegate et al., 2010). In recent decades, the natural antioxidants found in plants have been extensively studied and aromatic plants and spices have been shown to be effective at reducing lipid peroxidation processes (Brenes and Roura, 2010; Džinić et al., 2015).

Mentha arvensis (MA), which is also known as "Pakha" in Korea, belongs to the Lamiaceae family. This plant has been reported to possess different active constituents that are responsible for its phytochemical properties and helps in the treatment of indigestion, hypertension, diarrhoea and ailments of the liver and spleen. This plant also possesses terpenes such as neomenthol, isomenthol, d-menthone and p-cymene, as well as flavonoids, vitamin K, thymol and eugenol (Biswas et al., 2014). MA yields 40–50% menthol, which possesses antibacterial, antioxidant and antiseptic effects (Schuhmacher et al., 2003). Another plant of interest is *Geranium thunbergii* (GT), which is commonly called "Hyeoncho" in Korea. This plant belongs to the Geraniaceae family and is a perennial plant commonly found in Korea, China, and Japan. GT has been reported to have anti-inflammatory, anti-mutagenic, anti-oxidative, anti-hypertensive, anti-bacterial and anti-fungal effects. In addition to citronellol and isomenthone, which are the main components of this plant, its primary tannin is geraniin, that possesses anti-bacterial, anti-hypertensive and anti-fungal properties (Okuda and Ito, 2011; Sung et al., 2011).

Despite the beneficial properties of *M. arvensis* and *G. thunbergii*, limited research regarding the effects of their application on animal nutrition has been performed. Therefore, the present study was conducted to investigate whether supplementation with *M. arvensis* and *G. thunbergii* extracts alone or in combination at different ratios in drinking water would impact broiler performance, meat quality and yield, digestive organ weight and odorous gas emissions from excreta.

Material and methods

Plant extract preparation and composition

M. arvensis and *G. thunbergii* were purchased from a local merchant (Bonghwa, Korea). The leaves were dried and ground using a domestic blender according to the method described by Wong et al. (2006), after which they were kept in a refrigerator at 4°C until extraction. Extracts of both plants were prepared separately by adding 100 g of the dried ground leaves to 5 L of distilled water and allowing the mixture to stand at room temperature for 2 h in the dark, with occasional agitation. The same procedure was repeated for the extraction of 2 kg of dried leaves of each plant, after which the samples were adjusted to 1° Brix with sterilized water. Each extract was then filtered through Whatman No. 1 filter paper and stored without further treatment. Combination treatments were prepared in the poultry house prior to addition to the drinking water.

Animal care

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

Birds and housing

A total of 210 one-day-old male "Ross" broiler chicks were purchased from a commercial hatchery and used in this study. Broilers were housed and reared in a closed, ventilated, and caged experimental broiler house (100 cm long × 80 cm wide × 40 cm high/cage) with a stocking density of 800 cm^2/bird at Sunchon National University. A linear feeder at the front and a nipple drinker at the back were provided in the cage for *ad libitum* feed intake and free access to water (with dietary treatment inclusion) throughout the experiment. Extracts of the test plants were incorporated into the water supply of the birds, which was provided via nipple drinkers. The temperature of the poultry house was maintained at 33°C for d 1 to 7, then gradually decreased to 24°C at a rate of 3°C per week. The 24°C temperature was maintained until the end of the experiment and the relative humidity was maintained at around 50%.

Dietary treatments

The ingredients, chemical composition and vitamin and mineral content of the experimental basal diets are shown in Table 1. Commercially available broiler diets used as basal diets were prepared with the same batch of ingredients for the starter (0 to 21 d) and finisher (22 to 35 d) periods. Birds were weighed and randomly allocated into seven treatment groups having five replicates with six birds each (7 treatments × 5 replicates × 6 birds). The dietary treatments are summarized in Table 2.

Growth performance measurement

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

Table 1. Feed ingredients and chemical composition of broiler diets

	Starter	Finisher
Ingredient (%, 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 D_3 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 2. Treatment groups and ratio of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) extract used in the study

Treatment	Abbreviation	Ratio	
		MA	GT
Control	Control	–	–
0.1% *Mentha arvensis* extract	0.1% MA	1	–
0.05% *Geranium thunbergii* extract	0.05% GT	–	1
0.1% *Geranium thunbergii* extract	0.1% GT	–	1
0.1% *M. arvensis*: 1 *G. thunbergii* extract	0.1% 1MA:1GT	1	1
0.1% *M. arvensis*: 4 *G. thunbergii* extract	0.1% 1MA:4GT	1	4
0.1% *M. arvensis*: 1 *G. thunbergii* extract	0.1% 4MA:4GT	4	1

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

At 35 d of age, three broilers from each replicate (15 birds per treatment) were selected at random and slaughtered by cutting their jugular veins. The breast and thigh meat were then excised from the carcass and weighed after removing the bones, connective tissue and skin.

The thigh meat samples were ground using 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 excised and weighed separately. The gastrointestinal tract was weighed after removal of its contents.

Meat oxidative stability and meat pH analysis

After refrigerating the meat samples at 4°C, the thiobarbituric acid reactive substances (TBARS) values of meat samples were determined at 0, 1, 2 and 3 weeks after storage using the method described by Ahmed et al. (2015 a). Briefly, about 4 g of meat was mixed into a 10 ml solution containing 20% trichloroacetic acid (TCA) in 2M phosphoric acid and 10 ml of distilled water. The mixture was then homogenized using a homogenizer (Ultra-Turrax T-25 Basic, IKA Werke, GMBH & CO. KG, Staufen, Germany) at full speed for 1.5 min. Next, the mixture was filtered through Hyundai Micro No. 60 (Hyundai Micro Co., Ltd.) filter paper. Equal amounts of the filtrate (2 ml) and 2-thiobarbituric acid (98% 4,6-dihydroxy-2-mercaptopyrimidine, 0.005M in DW) were heated in a shaking water bath for 30 min. After being cooled, 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 kilogram of meat.

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

Odorous gas emissions from excreta

About 500 g of faeces from the bottom tray of each replicate cage were collected, homogenized and put into plastic zipper bags (d 35 of the experiment). These bags were then placed in 2-litre plastic boxes in triplicate to measure the emission of hydrogen sulfide (H_2S), ammonia (NH_3), and sulfur dioxide (SO_2) from the excreta of birds. The boxes were covered with a lid containing two holes, one of which that was used to insert a tube with a cap to measure gas emissions and another that was sealed with an Advantec® membrane filter (pore size 1.0 µm, Toyo Roshi Kaisha Ltd., Otowa, Tokyo, Japan) to facilitate insertion of fresh air to offset the negative pressure created during drawing of the headspace air. Following sampling at 0 h, the samples were allowed to ferment at room temperature (average 27°C), with subsequent samples collected at 6, 12 and 24 h. A Gastec (model AP-20) gas sampling pump (Gastec Corp., Kitagawa, Japan) and a Gastec detector tube (3LA, 3M for NH_3; 4LB, 4LK for H_2S; 5LA for SO_2) were used to measure the emissions of odorous gases. The tube was open during measurement and 100 mL of headspace air was

collected from approximately 2.0 cm above the sample surface. The concentration of odorous gases was expressed as ppm/100 mL.

Statistical analysis

Statistical analyses were conducted with General Linear Models (GLM) using the SAS Statistical Package Program 9.0 (SAS, 2005). Duncan's multiple range test was used to examine differences among treatments. The probability level of $P<0.05$ was considered significant.

Results

Growth performance

The initial body weight (BW) of chicks did not differ ($P>0.05$) among treatments (Table 3). From week 0 to 3, birds supplemented with plant extracts had an improved BWG and FI compared to the control group ($P<0.05$). In addition, birds fed with 0.05% GT and 4MA:1GT had a greater ($P<0.05$) BWG (2374 g and 2351 g) than the control (2161 g) throughout the experiment (week 0 to 5). Furthermore, dietary inclusion with 4MA:1GT resulted in a numerically better FCR (1.42) than the control (1.48). Overall, supplemented groups had a greater final BW and increased FI in relation to the non-supplemented group ($P<0.05$).

Thigh and breast meat yields and internal organ weights

Breast and thigh meat yield did not differ significantly among treatments ($P>0.05$); however, the inclusion of 4MA:1GT resulted in better yield than the control (i.e., 457 g vs 400 g for breast meat and 273.60 g vs. 271.17 g for thigh meat, respectively; Table 4).

The means of the weights of organs for the different groups are presented in Table 4. The weights of the proventriculus, gizzard, liver, pancreas, small intestine and large intestine did not differ significantly among the dietary treatments and the control ($P>0.05$).

Oxidative stability

The TBARS values of the meat samples are presented in Table 5. After 0 and 3 weeks of storage, there was a significant ($P<0.05$) decrease in the TBARS value because of supplementation with the plant extracts. After 3 weeks of storage, the highest TBARS value was calculated in the control (1.02). However, on average, dietary plants supplementation had no significant effects ($P>0.05$) on the TBARS values of poultry meat.

Meat pH

As shown in Table 6, significant differences were observed in the meat pH at week 0 ($P<0.05$). After 3 weeks of storage, the highest pH value was found in 0.1% MA (6.38) and the lowest in 0.1% 4MA:1GT (6.16). However, no significant difference was observed among treatments in meat pH during storage ($P>0.05$).

Table 3. Effects of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) extract on broiler growth performance

Performance parameter	Control (n=30)	MA	GT		0.10% MA:GT			SEM	P-value
		0.1% (n=30)	0.05% (n=30)	0.1% (n=30)	1:1 (n=30)	1:4 (n=30)	4:1 (n=30)		
0 to 3 weeks									
initial wt (g)	45	45	44	44	45	44	45	0.23	0.60
final wt (g)	845 d	949 abc	965 ab	922 bc	909 c	938 abc	975a	15.16	<0.0001
weight gain	801 d	904 abc	921 ab	878 bc	865 bc	894 abc	930 a	15.11	<0.0001
feed intake	1088 c	1211 ab	1312 a	1215 ab	1180 bc	1199 b	1180 bc	29.80	0.01
FCR	1.36	1.34	1.43	1.38	1.37	1.34	1.27	0.03	0.0616
4 to 5 weeks									
initial wt (g)	845 d	949 abc	956 ab	922 bc	909 c	938 abc	975 a	15.16	<0.0001
final wt (g)	2206 c	2300 abc	2419 a	2292 abc	2332 abc	2277 bc	2396 ab	39.26	0.02
weight gain	1361 ab	1351 ab	1453 a	1371 ab	1422 ab	1340 b	1421 ab	31.13	0.17
feed intake	2103 b	2120 b	2280 a	2165 ab	2207 ab	2116 b	2169 ab	45.87	0.15
FCR	1.55	1.57	1.57	1.58	1.55	1.58	1.53	0.02	0.68
0 to 5 weeks									
final wt (g)	2206 c	2300 abc	2419 a	2292 abc	2332 abc	2277 bc	2396 ab	39.26	0.02
weight gain	2161 c	2255 abc	2374 a	2248 abc	2287 abc	2233 bc	2351 ab	39.18	0.02
feed intake	3191 b	3330 b	3592 a	3381 b	3387 b	3315 b	3350 b	62.10	0.01
FCR	1.48	1.48	1.52	1.50	1.48	1.48	1.42	0.02	0.08

a, b, c, d – within the same row, mean values with different letters are significantly different ($P<0.05$). n = number of birds.

Table 4. Effects of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) extract on breast and thigh meat yield and digestive organ weights

Performance parameter	Control (n=15)	MA	GT		0.1% MA:GT			SEM	P-value
		0.1% (n=15)	0.1% (n=15)	0.5% (n=15)	1:1 (n=15)	1:4 (n=15)	4:1 (n=15)		
Meat yield (g)									
breast meat	400.17	376.20	435.60	474.40	456.60	441.80	457.00	24.32	0.204
thigh meat	271.17	277.60	272.80	262.80	263.00	269.20	273.60	10.90	0.957
Digestive organ weights (g)									
liver	40.53 ab	43.24 a	36.27 abc	33.14 bc	35.93 abc	29.88 c	34.37 bc	2.09	0.01
pancreas	3.82	4.91	3.60	3.38	3.89	3.34	3.79	0.32	0.07
proventriculus	7.08	8.23	7.59	7.50	8.16	6.93	9.63	0.65	0.27
gizzard	28.78	29.41	24.43	24.92	26.94	21.05	24.33	2.06	0.14
small intestine	46.72	41.02	47.85	43.40	44.09	41.14	46.34	3.21	0.70
colon	3.79	5.71	4.97	3.51	4.32	3.58	4.14	0.1	0.72

a, b, c – within the same row, mean values with different letters are significantly different ($P<0.05$). n = number of birds.

Table 5. Effects of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) on TBARS values of broiler meat during refrigerated storage (mg of MDA/kg of meat)

Item	Control (n=15)	MA	GT		0.1% MA:GT			SEM	P-value
		0.1% (n=15)	0.05% (n=15)	0.1% (n=15)	1:1 (n=15)	1:4 (n=15)	4:1 (n=15)		
Storage period									
0 week	0.36 a	0.34 b	0.31 c	0.32 c	0.31 c	0.31 c	0.30 c	0.004	<0.0001
1 week	0.16 b	0.20 b	0.19 b	0.16 b	0.16 b	0.25 b	0.48 a	0.027	<0.0001
2 week	0.37	0.33	0.36	0.31	0.36	0.35	0.36	0.046	0.99
3 week	1.02 a	0.54 b	0.63 b	0.60 b	0.70 b	0.54 b	0.54 b	0.092	0.01
Average	0.48	0.35	0.37	0.35	0.39	0.36	0.42	0.033	0.102

a, b, c – within the same row, mean values with different letters are significantly different (P<0.05). n = number of birds.

Table 6. Effects of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) on pH of broiler meat during refrigerated storage

Item	Control (n=15)	MA	GT		0.1% MA:GT			SEM	P-value
		0.1% (n=15)	0.05% (n=15)	0.1% (n=15)	1:1 (n=15)	1:4 (n=15)	4:1 (n=15)		
Storage period									
0 week	5.81 a	4.88 b	5.29 ab	4.81 b	5.11 b	4.89 b	5.33 ab	0.18	0.01
1 week	6.21	6.08	6.16	6.17	6.24	6.24	6.04	0.07	0.46
2 week	5.88	5.79	5.86	6.08	6.09	5.60	5.92	0.09	0.21
3 week	6.28	6.38	6.23	6.23	6.32	6.28	6.16	0.05	0.23
Average	6.04	5.78	5.88	5.82	5.94	5.75	5.60	0.07	0.14

a, b, c – within the same row, mean values with different superscripts are significantly different (P<0.05). n = number of birds.

Table 7. Effects of *Mentha arvensis* (MA) and *Geranium thunbergii* (GT) on odorous gas emissions from broiler's excreta (ppm)

Items	Control (n=5)	MA	GT		0.1% MA:GT			SEM	P-value
		0.1% (n=5)	0.05% (n=5)	0.1% (n=5)	1:1 (n=5)	1:4 (n=5)	4:1 (n=5)		
NH_3									
0 hour	37.33 b	28.67 bc	20.67 bc	25.33 bc	58.00 a	24.00 bc	14.00 c	5.524	0.003
6 hour	183.33 a	146.67 ab	143.33 ab	110.00 bc	190.00 a	110.00 bc	70.00 c	13.772	0.001
12 hour	196.67 abc	213.33 ab	163.33 abc	220.00 a	213.33 ab	126.67 bc	120.00 c	23.352	0.082
24 hour	193.33	170.00	183.33	250.00	226.67	173.33	186.67	20.395	0.258
Average	160.67 ab	149.33 abc	139.00 abc	162.00 a	167.00 a	114.00 bc	105.33c	12.687	0.047
SO_2									
0 hour	0.60 bc	0.87 a	0.67 bc	0.73 ab	0.60 bc	0.73 ab	0.53 c	0.048	0.016
6 hour	0.13 b	0.00b	0.10 b	0.00 b	0.27 a	0.06 b	0.00 b	0.032	0.005
12 hour	0.06	0.00	0.00	0.00	0.09	0.00	0.00	0.015	0.193
24 hour	0.06	0.00	0.00	0.00	0.07	0.00	0.00	0.013	0.255
Average	1.09	0.71	0.73	0.76	0.73	0.68	0.60	0.082	0.411
H_2S									
0 hour	0.33 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.17 ab	0.048	0.010
6 hour	1.53 a	0.00 b	0.30 b	0.00 b	0.53 b	0.00 b	0.00 b	0.150	0.005
12 hour	0.00	0.00	0.00	0.00	0.57	0.00	0.00	0.081	0.463
24 hour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000
Average	0.49 a	0.00 b	0.05 b	0.00 b	0.24 ab	0.00 b	0.03 b	0.046	0.015

a, b, c – within the same row, mean values with different letters are significantly different ($P<0.05$). n = number of replicate cages.

Odorous gas emissions from broiler excreta

The amounts of different gases emitted from broiler's excreta are presented in Table 7. On average, dietary supplementation with 0.1% 4MA:1GT resulted in significantly lower ($P<0.05$) emission of NH_3 from broiler excreta relative to the control. Similarly, the average emission of H_2S from excreta was decreased by the inclusion of plant extracts ($P<0.05$). Furthermore, the emission of SO_2 at 0-hour was significantly lower in the 0.1% 4MA:1GT group than in the other groups. However, on average, there was no significant difference among treatments, although 0.1% 4MA:1GT showed the best results.

Discussion

Feed additives in poultry diets are intended not only to provide growth promoting activity, but also to regulate various functions of the body. Phytogenic feed additives are plant-derived products that can improve poultry growth performance. New commercial additives derived from plants include plant extracts and their purified constituents. These products have various advantages over antibiotics as they are residue free and recognized as safe by the food industry (Varel, 2001).

Improvement in the growth performance and feed efficiency of broiler chickens fed phytogenic extracts (Gheisar et al., 2015; Jamroz et al., 2003; Windisch et al., 2008) is a result of the synergistic effects among complex active molecules present in phytobiotics (Hashemi and Davoodi, 2011). In the present study, treatment with the two plant extracts led to increased weight gain (0–3 weeks). These findings are in line with those of previous studies that showed supplementation of feed and drinking water with different plant extracts improved the performance of broilers and reduced the prevalence of pathogens (Forte et al., 2018; Junior et al., 2014). On the other hand, sometimes no positive effects of different phytobiotics have been shown in trials conducted with broiler chickens (Lewis et al., 2003; Symeon et al., 2014; Jang et al., 2007). In the present study, supplementation with plant extracts led to an increase in weight gain (5–10%) and feed intake (3.8–12.56%) relative to the control. The main reasons for this may have been 1) improved feed digestibility after inclusion of plant extracts (Hernández et al., 2004), 2) increased endogenous secretions, bile acid and pancreatic juice in response to the treatments (Cross et al., 2007). The results of the present experiment indicate that the weights of the proventriculus, gizzard, pancreas, and small and large intestine were not affected by the dietary treatments. Similarly, Figen et al. (2011) found no difference in the relative weights of these organs in broilers fed oregano and garlic essential oil. These results are also in agreement with those of an experiment conducted by Hernández et al. (2004) who found no significant difference in organ weight in response to dietary supplementation with plant extracts.

The combination of two plant extracts and their different ratios and percentages used in this experimental trial had varying effects on growth performance. While analyzing the effects of various herbs and oils on broiler growth performance, Cross

et al. (2007) concluded that their performance was determined by the quantity and quality of various active compounds present in each plant extract. Some important factors that determine the effectiveness of phytobiotics are the time of harvesting, age of the plant, methods of extraction, genetic variations of the plant, plant parts and physical properties, dosage level used and synergism or antagonism with other ingredients used in the diet formulation. The variability of these parameters results in the observed discrepancies in the existing literature (Yang et al., 2009). In addition, there are some intrinsic and extrinsic factors that determine the efficacy of dietary phytobiotics such as the nutritional status of birds, composition of the diet, and health status of birds and their environment (Giannenas et al., 2003).

The shelf life of meat depends on the oxidation of lipids and proteins. In the present study, the TBARS value and pH of meat were measured because they dramatically affect its shelf life. TBARS measurement serves as an indicator of lipid peroxidation that determines the levels of thiobarbituric acid reactive substances. In our experiment, supplementation of the diets of birds with different levels of *Mentha arvensis* and *Geranium thunbergii* reduced the oxidation of lipids as indicated by the lower TBARS values of broiler thigh meat after 3 weeks of storage. A similar reduction in TBARS value was observed after dietary supplementation of broiler diets with 2% dried peppermint (Khempaka et al., 2013). From a chemical point of view, the two herbal plants used in this experiment contain phenolic and flavonoid compounds that possess strong antioxidant activities. Chanwitheesuk et al. (2005) and Kim et al. (2010) reported the presence of natural antioxidants in both plants, which are resposible for the observed antioxidant activity in the current experiment. The phenolics, flavonoids and tannins of the two plants may act in the same way as these compounds have the capacity to exert intense antioxidant activity by scavenging free radicals, converting them into stable products and terminating oxidative reactions (Ahmed et al., 2015 a). In addition, Singh et al. (2015) and Yang et al. (2010) also reported high phenolic contents and increased DPPH and ABTS radical scavenging capacity of *M. arvensis* and *G. thunbergii,* which are strongly correlated with the reduced meat TBARS value observed in this trial.

Meat pH is considered important to prediction of the spoilage of meat during storage. Specially, elevated pH is directly linked to meat spoilage and results in proliferation of harmful microorganisms. Hence, it is important that meat reaches a low pH as soon as possible to ensure good shelf life and avoid spoilage (Savell et al., 2005). Dietary supplementation with the two plant extracts significantly reduced the pH values after 0 weeks of storage. After 3 weeks of storage, the lowest pH was obtained in the 0.1% 4MA:1GT group, indicating reduced spoilage of meat by microorganisms. Consistent with these findings, positive influences of different plants and their extracts on meat quality have been observed by Savković et al. (2008).

The emission of harmful gases such as NH_3 from poultry houses has detrimental effects on the environment, animal industry and health and safety of people working on farms. It is recommended that the level of NH_3 in a poultry house does not exceed 25 ppm. The main cause of NH_3 production is the enzymatic or biological degradation of faecal uric acid (Atapattu et al., 2017). In the present study, dietary supplementation with the tested plant extracts significantly ($P<0.05$) reduced NH_3

production (7–34.4%). Several studies have reported a reduction in ammonia level because of the inclusion of phytobiotics. For example, dried peppermint (0.5–2%) led to a 32.93% reduction in ammonia production (Suganya et al., 2016). In the present study, there was an increase in NH_3 emissions after 24 h, which can be explained by the fact that the pH of excreta is an important factor influencing NH_3 emissions from excreta of animals. Indeed, Ahmed et al. (2015 b) reported an increase in the pH of excreta with time that contributed to the conversion of ammonia-nitrogen to NH_3, allowing emission of ammonia into the atmosphere.

On average, the emission rates of sulfuric odorous gases (H_2S and SO_2) were lower from broiler excreta in the plant extract supplemented groups than the control. Ushida et al. (2003) reported that production of volatile sulfur by anaerobic bacteria was a result of dissimilatory sulfate reduction and metabolism of sulfur-containing amino acids. In the present study, the emission of volatile sulfur gases (H_2S and SO_2) was reduced in broiler excreta as a response to dietary supplementation with plant extracts. This can be explained as a result of a reduction in the concentration of sulfur reducing bacteria caused by the antimicrobial activity of *M. arvensis* and *G. thunbergii* (Biswas et al., 2014). Previous studies (Varel, 2001, 2002) have also suggested that the addition of essential oils from plants could result in a reduction in the total anaerobic bacteria, faecal coliforms and faecal coliforms contributing to the control of short-chain fatty acids, L-lactate and gases production in stored waste.

Conclusion

For the last decade, the use of natural feed additives in animal and human nutrition has been encouraged. In the present study, treatment with 0.1% 4MA:1GT led to a significantly increased BWG and showed a numerically better FCR. All other treatments showed better results but 4MA:1GT produced promising effects. This combination had potent antioxidant properties, improved the shelf life of meat and reduced ammonia production from excreta. Therefore, this product might be a promising alternative to antibiotic growth promoters in poultry.

Conflict of interest

We certify that there is no conflict of interest with any organization regarding the material discussed in the manuscript.

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