Effects of phytobiotic feed additives on growth traits, blood biochemistry, and meat characteristics of broiler chickens exposed to *Salmonella typhimurium*

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ABSTRACT Because of concerns over the use of antibiotics in poultry feed, this study was designed to determine the effectiveness of phytobiotic supplementation as an alternative to antibiotic use based on growth performance and meat characteristics of broilers exposed to *Salmonella typhimurium*. The effects of an antibiotic and 3 phytobiotic feed additives (**PFA**), Mix-Oil Mint (**MO$_{mint}$**), Mix-Oil Liquid (**MO$_{liq}$**), and Sangrovit Extra (**Sang$_{ext}$**), were compared. At day of age, 280 Ross chicks were randomly allocated into 6 treatments. At 15 d, all chicks except negative control were exposed to *S. typhimurium*. The offered 6 diets were as follows: T1, negative control; T2, infected with *S. typhimurium*; T3, infected + avilamycin (0.1 g/kg); T4, infected + MO$_{mint}$ (0.2 g/kg); T5, infected + plant extract in liquid form MO$_{liq}$ (0.25 mL/L); and T6, infected + Sang$_{ext}$ (0.15 g/kg). During the cumulative starter period, PFA improved performance over that of the control, and the food conversion ratio (**FCR**) was lower for T3 and T5 compared with T1 ($P < 0.05$). During the cumulative finisher period (15–35 d), a lower body weight gain ($P < 0.01$) was observed in T2. T1 had the best FCR and production efficiency factor, but they were not significantly different from those of T3, T4, and T6 ($P < 0.001$). At 35 d, T1 and T4 had a higher breast percentage as compared with those of T2 ($P < 0.05$). Blood glucose decreased significantly ($P > 0.05$) in T2 and T5 compared with that in T1 and T4. Alanine transaminase concentration decreased significantly ($P < 0.01$) in T4 and T5 compared with that in T1, T2, and T3. Treatments had significant effects on breast temperature and pH ($P < 0.001$). A significant decrease in the myofibril fragmentation index occurred in T1 and T6. Hardness and chewiness were influenced by treatments ($P < 0.05$). In conclusion, dietary supplementation with PFA could effectively compare with that of antibiotic avilamycin in the maintenance of growth performance and improvement in meat characteristics of broilers challenged with *S. typhimurium*.

Key words: broiler, meat characteristic, performance, phytobiotic, *Salmonella typhimurium*

INTRODUCTION

Salmonellosis is an expensive disease in poultry production because it can severely decrease performance; weight gain can decrease by 24%, and the food conversion ratio (**FCR**) can increase by 12% in broilers (Chalghoumi et al., 2009; Aljumaah et al., 2020). At a young age, chicks are susceptible to exposure to infection with *Salmonella* from different sources. It was estimated that more than 200 *Salmonella* serovars could colonize the gastrointestinal tract of chickens (Gast, 2007). One infectious serotype in chickens is *Salmonella typhimurium*, which can cause Salmonellosis in humans (Gopinath et al., 2012; Park et al., 2013). Prophylactic measures, such as antibiotics and vaccinations, are used to control this infection (Bajpai et al., 2012).

Removal of antimicrobial growth promoters (**AGP**) in poultry diets has increased the incidence of pathogenic infections, consequently, having a negative effect on the output of poultry production. The current trend of searching for available alternatives has increased; among possible alternatives, phytobiotic feed additives (**PFA**) have shown encouraging effects on poultry output (Jang et al., 2007; Amad et al., 2011; Gheisar et al., 2015;

Abudabos et al., 2018). Phytobiotics or PFA have been traditionally used because of their pharmacological effects. It has been suggested that herbs, spices, and extracted oils can motivate feed intake, improve antioxidant status, increase the secretion of endogenous enzymes, and display antibacterial effects (Panda et al., 2006; Lee et al., 2015; Kim et al., 2016; Gheisar and Kim, 2018). Moreover, it has been stated that PFA change the fluidity and permeability of the cell membrane; consequently, the absorption of nutrients is enhanced (Amad et al., 2011). Yakhkeshi et al. (2011) showed that feeding poultry diets containing AGP alternatives alleviated the negative effects of removing AGP from the feed. Herbs and plant extract supplementation of broiler feed improved carcass quality and breast muscle yield (Saeed et al., 2018, 2019, 2020). Moreover, it was reported that various PFA improved the antioxidant status of the meat (Keokamnerd et al., 2008; Kim et al., 2009; Lee et al., 2015). A study by Juskiewicz et al. (2011) demonstrated that PFA changed the fatty acid composition of broiler breast meat. Similarly, Zdunczyk et al. (2010) reported that PFA increased the sum of polyunsaturated fatty acids compared with that of the control.

Recently, several studies have documented inconsistent results of PFA supplementation of broiler feed (Lee et al. 2003, 2015; Zdunczyk et al., 2010; Amad et al. 2011). The inconsistency in results could be caused by the bacterial challenge, time of the challenge, and/or type of bacteria used in the experiment (Amad et al., 2011; Lee et al., 2015; Abudabos et al., 2016; Saleh et al., 2018). Limited studies have been conducted to determine the effect of PFA on meat characteristics postchallenge with *S. typhimurium*. The scarcity and inconsistency of research inspired us to conduct the current research to test the hypothesis that PFA could positively affect growth performance and meat characteristics of broiler chickens experimentally exposed to *S. typhimurium*, thereby supporting PFA as an alternate to AGP in broiler diet.

MATERIALS AND METHODS

Mix-Oil Mint (**MO_mint**), Mix-Oil Liquid (**MO_liq**), and Sangrovit Extra (**Sang_Ext**) are commercially available PFA, and during the current experiment, their effects were examined *in vivo* and compared with those of an AGP, avilamycin. Mix-Oil is available in 2 forms, powder and liquid, and consists of a mixture of 7 plant extracts: oregano, eucalyptus, thyme, garlic, lemon, rosemary, and sweet orange (Animal Wellness Products, Oakland, NE). Sangrovit Extra is an herbal extract, and it consists of various nutritional acids and 4 alkaloids obtained from plume poppy (*Macleaya cordata*). It was obtained from Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Experimental Design and Treatments

A total of 280-day-old broiler chicks (Ross 308) with comparable body weight were selected and randomly

Table 1. Ingredients and calculated nutrients of broiler starter and finisher diets.

Ingredient	Starter (1–15 d)[¥] %	Finisher (15–35 d)[¥] %
Yellow corn	57.20	62.79
Soybean meal	30.00	25.90
Corn gluten meal	6.00	4.00
Choline chloride	0.05	0.00
Corn oil	2.18	3.54
Dicalcium phosphate	2.30	2.00
Limestone	0.70	0.57
Salt	0.40	0.30
DL-methionine	0.18	0.135
Lysine-HCL	0.32	0.22
Threonine	0.10	0.11
Vitamin-Mineral premix[1]	0.50	0.50
Total	100	100
Calculated analysis		
ME, kcal/kg	3,000	3,150
Crude protein, %	23.0	20.0
Available phosphorus,%	0.48	0.42
Calcium, %	0.96	0.85
D. Lysine, %	1.28	1.10
Sulfur amino acids, %	0.85	0.85
Threonine, %	0.86	0.60

For the starter period: T1, Control group; T2, control + avilamycin at the rate of 0.10 g/kg; T3, control + plant extract in dried form at the rate of 0.20 g/kg; T4, control + plant extract in liquid form at the rate of 0.25 mL/L; and T5, control + Sangrovit Extra at the rate of 0.15 g/kg. For the finisher period: T1, control; T2, infected with *S. typhimurium*; T3, infected + avilamycin at the rate of 0.1 g/kg; T4, infected + plant extract in dried form at the rate of 0.02 g/kg; T5, infected + plant extract in liquid form at the rate of 0.25 mL/L; and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg.

[1]Vitamin-mineral premix contains the following per kg: vitamin A, 12,000,000 IU; vitamin D3, 5,000,000 IU; vitamin E, 80,000 IU; vitamin K3, 3,200 mg; vitamin B1, 3,200 mg; vitamin B_2, 8,600 mg; vitamin B_3, 65,000 mg; pantothenic acid, 20,000 mg; vitamin B_6, 4,300 mg; biotin 220 mg; antioxidants (BHA + BHT), 50,000 mg; B_9, 2,200 mg; B_{12}, 17 mg; copper, 16,000 mg; iodine, 1,250 mg; iron, 20,000 mg; manganese, 120,000 mg; selenium, 300 mg, and zinc, 110,000 mg.

allocated to different treatments. Upon arrival, the chicks were tested for *Salmonella* spp., and the absence of infection was confirmed. The experiment was conducted in temperature controlled rooms, wherein the temperature was gradually reduced from 33°C ± 0.5°C during the first week to 22°C ± 0.3°C toward the end of the experiment. Starter (1–15 d) and finisher (15–35 d) diets were formulated according to the recommendation of the Ross 308 guide and NRC. (1994) (Table 1). During the starter period, the chicks received one of the following treatments: T1, control; T2, control + avilamycin (AGP) at the rate of 0.10 g/kg; T3, control + dry plant extract (MO_mint) at the rate of 0.20 g/kg; T4, control + liquid plant extract (MO_Liq) at the rate of 0.25 mL/L; and T5, control + Sangrovit Extra (Sang_Ext) at the rate of 0.15 g/kg. During the finisher period, another treatment was added to serve as a positive control, and the chicks received 1 of the following 6 treatments: T1, negative control; T2, positive control (infected with *S. typhimurium*); T3, T2 + 0.10 g/kg avilamycin (AGP); T4, T2 + 0.20 g/kg plant extract in dried form (MO_mint); T5, T2 + 0.25 mL/L plant extract in liquid form (MO_Liq); and T6, T2 + 0.15 g/kg Sangrovit Extra (Sang_Ext). The plant extract in liquid form (MO_Liq) was prepared in 50 L plastic barrel, whereas

Table 2. Effects of dietary treatment on growth performance in broilers (1–15 d).

	Treatment					SEM	P-value
	T1 control	T2 AGP	T3 MO$_{mint}$	T4 MO$_{Liq}$	T5 Sang$_{Ext}$		
FI (g)	529.2	536.5	527.7	543.6	533.5	6.49	NS
BWG (g)	419.8	433.1	436.7	437.2	442.3	7.89	NS
FCR (g/g)	1.262[a]	1.239[a,b]	1.212[b]	1.244[a,b]	1.207[b]	0.015	0.05
PEF	239.5	257.3	259.6	258.7	261.7	9.21	NS

[a-b]Means within the same row with different superscripts differ ($P < 0.05$).

T1, Control group; T2, control + avilamycin at the rate of 0.10 g/kg (AGP); T3, control + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$) T4, control + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T5, control + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Abbreviations: BWG, Body weight gain per bird; FCR, Feed conversion ratio; FI, feed intake per bird; PEF, performance efficiency factor = (Livability $\times$ live weigh (kg)/Age in days $\times$ FCR $\times$ 100).

avilamycin (AGP), dry plant extract (MO$_{mint}$), and Sangrovit Extra (Sang$_{Ext}$) were mixed in the feed. A vertical mixer with a 200 kg capacity was used for the homogenization of the feed treatments for 16 min. Micronutrients with the appropriate additive were premixed and incorporated in the diets.

Challenge Inoculum Preparation

At 15 d, birds in T1 were orally gavaged with 1 mL water, whereas birds from all other treatments were inoculated with 1 mL (3×10^9 CFU) of *S. typhimurium* (ATCC# 14,028). The inocula were maintained at $-50°C$ in 13% glycerol broth. Before inoculation, the preparation was activated using tryptone soy agar and 5% sheep blood (Oxoid, CM 129) for 24 h at 37°C. Inocula were prepared by suspending fresh culture of *S. typhimurium* in sterile normal saline and adjusting the solution to reach the appropriate concentration. To confirm the purity of the strain, it was plated on X.L.D. Agar (Oxoid CM0469). The inocula were transferred to the rearing facility in sterile containers under ice. Viable cell counts were performed before and after the inoculation, according to Marcq et al. (2011).

Performance and Meat Measurements

Feed intake (**FI**) and body weight gain (**BWG**) were measured by weighing all chickens in the cage at 5 d intervals; the FCR and production efficiency factor (**PEF**) were calculated. At 15 and 35 d of age, 1 bird per replicate was sampled. The carcasses were defeathered and dissected, and the visceral organs were removed and weighed. At 35 d, breasts were used for quality measurements as previously described (Al-Owaimer et al., 2014). Breast core temperature was measured in duplicates at 15 min postmortem (Temperature $_{15\ min}$) with a portable digital thermocouple (EcoScan Temp JKT; Thermo Scientific, Waltham, MA). The pH was measured in duplicates at 15 min (pH $_{15min}$) and 24 h (pH $_{24h}$) postmortem by placing a pH probe 2.0 cm below the pectoralis muscle (pH 211; Hanna Instruments, Woonsocket, RI). The L* (lightness), a* (redness), and b* (yellowness) color values were determined at 15 min and 24 h postmortem using a Chroma meter (CR-400; Konica Minolta, Tokyo, Japan). Cooking loss (**CL**) of the *pectoralis major* muscle samples were determined by cooking samples on a commercial indoor countertop grill until an internal temperature of 70°C was reached. The internal temperature was determined by inserting a thermocouple thermometer probe (Ecoscan Temp JKT; Thermo Scientific) into the geometric center of the muscle. The percentage of CL was calculated as (initial weight − final weight)/(initial weight) $\times$ 100. The water-holding capacity (**WHC**) was determined by collecting 2 g samples from the cranial side of the breast fillets. Samples were placed between 2 pieces of filter paper, and 2 pieces of Plexiglas and left under a 10 kg weight for

Table 3. Effects of dietary treatment on carcass dissection of male broilers (15 d).

	Treatment					SEM	P-value
	T1 control	T2 AGP	T3 MO$_{mint}$	T4 MO$_{Liq}$	T5 Sang$_{Ext}$		
Dressing percentage (%)	60.3	60.6	60.6	59.8	59.9	0.67	NS
Fat (%)	1.10	0.77	1.20	1.20	1.00	0.14	NS
Liver (%)	2.51[b]	2.60[a,b]	2.91[a]	2.79[b]	2.83[a]	0.11	0.05
Gizzard (%)	4.00[a]	3.39[b]	4.05[a]	3.82[a,b]	3.75[a,b]	0.16	0.05
Heart (%)	0.61	0.62	0.59	0.63	0.55	0.04	NS
Small intestine (%)	6.39[a,b]	6.66[a]	6.03[b,c]	5.89[c]	5.67[c]	0.19	0.01
Ceca (%)	0.97	1.12	0.88	0.85	0.79	0.09	NS
Thymus (%)	0.37	0.34	0.43	0.29	0.39	0.05	NS
Spleen (%)	0.08	0.07	0.09	0.11	0.089	0.011	NS
Bursa (%)	0.19	0.18	0.22	0.19	0.17	0.019	NS

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, Control group; T2, control + avilamycin at the rate of 0.10 g/kg (AGP); T3, control + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$) T4, control + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T5, control + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Table 4. Effects of dietary treatment on cumulative growth performance in broilers (15–35 d).

	Treatment						SEM	P-value
	T1 negative control	T2 positive control	T3 AGP	T4 MO$_{mint}$	T5 MO$_{Liq}$	T6 Sang$_{Ext}$		
FI (g)	2,389.6	2,331.9	2,446.1	2,387.9	2,406.9	2,386.4	33.86	NS
BWG (g)	1,660.1[a]	1,475.1[b]	1,649.2[a]	1,602.8[a]	1,608.9[a]	1,600.8[a]	32.63	0.01
FCR (g/g)	1.442[c]	1.586[a]	1.484[b,c]	1.491[b,c]	1.498[b]	1.491[b,c]	0.017	0.001
PEF	426.2[a]	350.3[c]	409.4[a,b]	396.6[a,b]	388.4[b]	402.2[a,b]	11.12	0.001
Mortality (%)	1.3	8.9	2.1	2.4	3.7	2.1	2.42	NS

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, control (negative); T2, infected with *S. typhimurium* (positive control); T3, infected + avilamycin at the rate of 0.10 g/kg (AGP); T4, infected + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$); T5, infected + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Abbreviations: BWG, Body weight gain per bird; FCR, Feed conversion ratio; FI, feed intake per bird; PEF, performance efficiency factor = (Livability × live weigh (kg)/Age in days × FCR × 100).

5 min. Samples were weighed, and WHC was calculated as the difference between the initial and final weights (Wilhelm et al., 2010).

The myofibril fragmentation index (**MFI**) was determined using a 4 g minced sample. Samples were homogenized with 40 mL of a cold isolating MFI buffer, and the absorbance of 0.5 mg/mL solution was determined at 540 nm, and the MFI was calculated. For shear force (**SF**) determination, 3 round cores (1.27 cm in diameter) were removed from each cooked muscle sample parallel to the longitudinal orientation of the muscle fibers. Shear force was determined as the maximum force (kg) perpendicular to the fibers using a Texture Analyzer (TA-HD; Stable Micro Systems, Godalming, UK) equipped with a Warner-Bratzler attachment.

The texture profile analysis (**TPA**) is an instrumental measurement of the sensory qualities of chicken breasts that mimics the conditions to which food is subjected to inside a mouth. The cooked samples were used to determine TPA using a Texture Analyzer (TA.HD; Stable Micro Systems) equipped with a compression platen attachment.

Each sample underwent 2 cycles of 80% compression. Hardness was determined as the maximum force needed to compress the sample. Cohesiveness was calculated as the ratio of the total energy required for the first and second compression. Springiness was determined as the ability of a sample to recover its original form after removing of the compressing force, and chewiness was calculated by the multiplication of all previous factors (springiness × hardness × cohesiveness).

Blood Biochemical Measurement

At the end of the experimental period (35 d), a 2 mL blood sample was taken from 1 bird per replicate. The blood sample was centrifuged at 2,000 rpm for 15 min. The serum was stored $-20°C$. The amounts of serum protein, globulin, albumin, glucose, cholesterol, and triglycerides were determined using commercial kits (M di Europa GmbH Wittekamp 30, Hannover, Germany). In addition, the amounts of the following serum enzymes were determined using the UV/kinetic method: alanine transaminase (**ALT**), aspartate aminotransferase (**AST**), and alkaline phosphatase.

Statistical Analysis and Model

Five treatments (starter period) and 6 treatments (finisher period) were replicated 8 times in a randomized complete block design. Data were analyzed with general linear models performed using the Statistical Analysis System (SAS, 2003). Differences between means for measurements showing significant differences in the analysis of variance were tested using the Tukey test. The means ± SEM are presented, and differences are

Table 5. Effect of dietary treatment on carcass dissection of male broilers (35 d).

	Treatment						SEM	P-value
	T1 negative control	T2 positive control	T3 AGP	T4 MO$_{mint}$	T5 MO$_{Liq}$	T6 Sang$_{Ext}$		
Dressing percentage (%)	63.4	62.2	62.2	63.5	62.7	63.4	0.71	NS
Breast (%)	26.1[a]	24.0[b]	25.0[a,b]	26.0[a]	25.6[a]	25.7[a]	0.42	0.05
Leg (%)	18.3[b]	18.3[b]	18.3[b]	19.4[a]	18.4[b]	19.0[a,b]	0.28	0.05
Fat (%)	1.58	1.29	1.62	1.42	1.57	1.27	0.16	NS
Liver (%)	1.88	2.15	2.10	2.24	2.00	2.17	0.11	NS
Gizzard (%)	2.71	2.52	2.50	2.63	2.33	2.48	0.12	NS
Small intestine (%)	4.31	4.40	4.39	4.80	4.90	4.79	0.16	NS
Thymus (%)	0.45	0.47	0.46	0.38	0.35	0.47	0.04	NS
Spleen (%)	0.099	0.109	0.102	0.095	0.107	0.101	0.01	NS
Bursa (%)	0.159	0.168	0.147	0.159	0.141	0.159	0.02	NS

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, control (negative); T2, infected with *S. typhimurium* (positive control); T3, infected + avilamycin at the rate of 0.10 g/kg (AGP); T4, infected + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$); T5, infected + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Table 6. Effects of treatments on blood biochemical parameters and liver enzymes of male broiler chickens at 35 d.

	Treatment						SEM	P-value
	T1 negative control	T2 positive control	T3 AGP	T4 MO$_{mint}$	T5 MO$_{Liq}$	T6 Sang$_{Ext}$		
Protein (g/dL)	2.71	2.28	2.54	2.68	2.85	2.92	0.19	NS
Albumin (g/dL)	1.26	1.28	1.19	1.24	1.25	1.10	0.07	NS
Globulin (g/dL)	1.45	1.00	1.35	1.44	1.61	1.82	0.20	NS
Glucose (mg/dL)	216.1[a]	173.6[c]	194.1[a,b,c]	213.3[a,b]	174.2[c]	187.8[b,c]	9.56	0.01
Cholesterol (mg/dL)	100.6	135.4	98.5	102.6	103.6	92.4	11.9	NS
Triglycerides (mg/dL)	62.9[a]	66.8[a]	43.9[b]	45.9[b]	45.7[b]	41.3[b]	4.6	0.001
ALT (U/L)	11.3[a]	11.4[a]	11.8[a]	8.3[b,c]	7.22[c]	10.1[a,b]	0.95	0.01
AST (U/L)	223.9	232.5	283.3	266.2	281.3	237.4	18.54	NS

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, control (negative); T2, infected with *S. typhimurium* (positive control); T3, infected + avilamycin at the rate of 0.10 g/kg (AGP); T4, infected + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$); T5, infected + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase.

considered statistically significant at $P < 0.05$. The following model was used for the experiment: $Y_{hi} = \mu + \theta_h + \tau_i + \varepsilon_{hi}$; $\varepsilon_{hi} \sim N(o, \sigma2)$; ε_{hi} was the independent variable, where Y_{hi} was the random variable, representing the response for treatment $_i$ observed in block $_h$, μ is a constant (the overall mean), θh is the (additive) effect of the $_h$th block ($_h = 1, 2,..., 8$), τ_i is the (additive) effect of the $_i$th treatment ($_i = 1, 2,..., 5$ for starter period; $_i = 1, 2,..., 6$ for finisher period), and ε_{hi} is the random error for the $_i$th treatment in the $_h$th block.

RESULTS

Table 2 shows the results for FI, BWG, FCR, and PEF for the cumulative starter period (1–15 d). Differences in FI, BWG, and PEF were not significant ($P > 0.05$). However, FCR was lower for MO$_{mint}$ and Sang$_{Ext}$ compared with the control ($P < 0.05$), but they were not significantly different from that of AGP and MO$_{Liq}$.

During the starter period, the liver percentage was affected by treatment ($P < 0.05$), and birds receiving MO$_{mint}$ and Sang$_{Ext}$ had a higher liver percentage than those that received control and MO$_{Liq}$ (Table 3). The gizzard percentage was higher for MO$_{mint}$ and control compared with AGP ($P < 0.05$). The small intestine

percentage was lower for MO$_{mint}$, MO$_{Liq}$, and Sang$_{Ext}$ compared with AGP ($P < 0.01$).

The cumulative finisher period performance (15–35 d) when the bacterial challenge was applied is presented in Table 4. A lower BWG ($P < 0.01$) was achieved by positive control compared with all other treatments. Significantly lower FCR and PEF were associated with positive control compared with that of all other treatments. Birds receiving negative control had the best FCR and PEF, but they were not significantly different from those of AGP, MO$_{mint}$, and Sang$_{Ext}$ ($P < 0.001$). However, MO$_{Liq}$ had a higher FCR and PEF compared with that of positive control but were not significantly different from those of AGP, MO$_{mint}$, and Sang$_{Ext}$.

At 35 d, breast and leg percentages were affected by treatment ($P < 0.05$), and birds that received negative control, and MO$_{mint}$ had a higher breast percentage compared with those received positive control (Table 5). However, the leg percentage was higher for MO$_{mint}$ compared that of negative control, positive control, AGP, and MO$_{Liq}$ ($P < 0.05$).

The effects of treatments on blood parameters of broilers at 35 d are given in Table 6. Protein, albumin, globulin, cholesterol, and AST were not affected by treatment ($P > 0.01$). Blood glucose decreased significantly ($P > 0.05$) in positive control and MO$_{Liq}$ compared with negative control and MO$_{mint}$. However, triglycerides increased in positive control compared

Table 7. pH, temperature, and color parameters of breast fillets of male broilers (35 d).

	Treatment						SEM	P-value
	T1 negative control	T2 positive control	T3 AGP	T4 MO$_{mint}$	T5 MO$_{Liq}$	T6 Sang$_{Ext}$		
Temperature $_{15\,min}$	25.9[a,b]	26.5[a]	23.9[c]	25.8[a,b]	25.2[b]	25.9[a,b]	0.29	0.001
pH $_{15min}$	6.66[a]	6.48[b]	6.40[b,c]	6.34[c,d]	6.33[c,d]	6.29[d]	0.03	0.001
pH $_{24hr}$	6.23[a]	6.10[b]	6.06[b]	6.10[b]	6.05[b]	6.04[b]	0.026	0.001
L* $_{15min}$	36.56	33.72	34.76	34.78	33.76	33.52	1.03	NS
a* $_{15min}$	5.40[c]	6.39[b,c]	6.29[b,c]	6.56[b,c]	8.07[a]	7.10[a,b]	0.48	0.01
b* $_{15min}$	6.45	6.00	6.48	5.67	6.39	5.28	0.37	NS
L* $_{24hr}$	38.37	36.39	34.91	35.67	34.34	35.14	1.07	NS
a* $_{24hr}$	4.96[b]	6.99[a]	6.42[a]	6.54[a]	7.63[a]	7.38[a]	0.47	0.004
b* $_{24hr}$	7.05	7.61	7.29	7.34	7.06	7.14	0.42	NS

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, control (negative); T2, infected with *S. typhimurium* (positive control); T3, infected + avilamycin at the rate of 0.10 g/kg (AGP); T4, infected + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$); T5, infected + plant extract in liquid form at the rate of 0.25 mL/L (MO$_{Liq}$); and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Table 8. Physical properties and texture profile analysis of breast fillets of male broilers measured at 35 d.

	Treatment							
	T1 negative control	T2 positive control	T3 AGP	T4 MO$_{mint}$	T5 MO$_{Liq}$	T6 Sang$_{Ext}$	SEM	*P*-value
CL, %	28.19	29.30	25.64	29.11	31.33	29.43	2.23	NS
WHC, %	28.47	30.74	27.80	27.29	31.39	28.56	1.56	NS
MFI	72.67[c]	90.94[a]	87.24[a,b]	90.35[a]	87.11[a,b]	78.89[b,c]	3.95	0.013
SF, kgf	1.09	1.18	1.26	1.21	1.46	1.48	0.11	NS
Hardness, kg	0.96[a]	0.77[a]	0.77[a]	0.83[a]	0.48[b]	0.82[a]	0.098	0.05
Springiness	0.59	0.59	0.59	0.62	0.63	0.60	0.017	NS
Cohesiveness	0.46	0.48	0.46	0.48	0.48	0.49	0.009	NS
Chewiness	2.49[b]	2.08[a,b]	2.01[b]	2.41[a,b]	2.41[a,b]	2.32[a]	0.234	0.05

[a-c]Means within the same row with different superscripts differ ($P < 0.05$).

T1, control (negative); T2, infected with *S. typhimurium* (positive control); T3, infected + avilamycin at the rate of 0.10 g/kg (AGP); T4, infected + plant extract in dried form at the rate of 0.20 g/kg (MO$_{Liq}$); T5, infected + plant extract in liquid form at the rate of 0.25 ml/l (MO$_{Liq}$); and T6, infected + Sangrovit Extra at the rate of 0.15 g/kg (Sang$_{Ext}$).

Abbreviations: AGP, antimicrobial growth promoter; CL, cooking loss; MFI, myofibril fragmentation index; SF, shearing force; WHC, water-holding capacity.

with that of all other treatments ($P < 0.001$), and ALT concentration decreased significantly ($P < 0.01$) in MO$_{mint}$ and MO$_{Liq}$ compared with that of negative control, positive control, and AGP.

Treatments had significant effects on breast temperature, pH$_{15min}$, and pH$_{24h}$ ($P < 0.001$). Positive control had a higher temperature compared with that of AGP and MO$_{Liq}$; however, negative control had higher pH$_{15min}$ and pH$_{24h}$, compared with that of all other treatments (Table 7). As shown in Table 7, no differences ($P > 0.05$) were observed in L*$_{15min}$, b*$_{15min}$, L*$_{24hr}$, and b*$_{24hr}$ (lightness and yellowness); conversely, there was a strong difference ($P < 0.01$) in a*$_{15min}$ and a*$_{24hr}$ (redness) between breast fillets of birds fed different treatments. Cooking loss, WHC, and SF indexes are shown in Table 8. No differences ($P > 0.05$) in CL, WHC, and SF were observed among birds receiving different treatments. However, a significant decrease ($P < 0.05$) occurred in MFI in negative control and Sang$_{Ext}$. The TPA measurements are shown in Table 8. Springiness and cohesiveness were not affected by treatment, whereas there were significant effects on hardness and chewiness ($P < 0.05$).

DISCUSSION

The results of the present study illustrated that feeding PFA improved FCR in unchallenged chicks during the starter period. It has been reported that PFA improved growth performance during bacterial infection (Abudabos et al. 2016, 2018) and without the infection (Amad et al., 2011; Lee et al., 2015; Saleh et al., 2018). A substantial number of studies stated that various plant extracts improved digestion, absorption, and bacterial diversity in the gut (Amad et al., 2011; Saleh et al., 2018). Jamroz et al. (2006) suggested that PFA supplementation of a broiler's diet stimulated the secretion of mucus in the intestine. Performance improvements could be associated with the presence of different important alkaloids, which produce positive effects on broiler health. Sanguinarine is a well-known herbal product with excellent biological properties (Abudabos et al., 2016). In addition, it influences gastric motility, the fermentation

process, and gut histomorphology (Jankowski et al., 2009). Gheisar et al. (2015) reported that supplementing broiler diet with a PFA led to a 3.9 and 3.4% improvement in BWG and FCR, respectively.

The commercial phytobiotic Sangrovit contains at least 1.5% sanguinarine (C$_{20}$H$_{14}$NO$_4$) a quaternary benzo[c]phenanthridine alkaloid, extracted from *M. cordata* plants (Lin et al., 2018), which is recognized by The European Food Safety Authority for use in the animal production industry as a feed additive (Vienna et al., 2007). Previous investigations reported that sanguinarine supplementation can improve protein retention by decreasing intestinal decarboxylation of aromatic amino acids by inhibiting L-amino acid decarboxylase (Vieira et al., 2008) in addition to a broad range of pharmacological properties such as anti-inflammatory antifungal, antimicrobial, and anticancer (Weerasingh et al., 2001; Ding et al., 2002). Other studies have also suggested that sanguinarine supplementation enhances feed intake by modulating the tryptophan serotonin pathway (Roth and Mellor, 2001). A recent investigation has shown that dietary supplementation of sanguinarine significantly altered gut microbiota resulting in a decrease of Bacteroidetes and increase of Firmicutes, improved growth performance, enhanced mucosal morphology, reduced TNF-α and IL-4 concentrations in the jejunum mucosal, and lowered glucose, uric acid, and urea nitrogen levels in broiler chickens (Liu et al., 2020).

The oil blend MixOil (Animal Wellness Products, Nebraska) is formed of a blend of 7 plant extracts including *Eucalyptus saligna*, *Allium sativum*, *Citrus Limonium*, *Origanum vulgare*, *Rosmarinus officinalis*, *Citrus aurantium*, and *Thymus vulgaris*, several of these components have been reported previously to have antimicrobial properties against many enteric pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *S. typhimurium* (Lopez et al., 2005; Mith et al., 2014; Aljumaah et al., 2019). It is suggested that essential oils hydrophobicity plays a key role in promoting the penetration of the phospholipids layer of bacterial mitochondrial and cell membranes, resulting in leakage of critical cell components and ions leading to cell death (Prabuseenivasan et al., 2006).

In the present study, production parameters declined in the *Salmonella*-infected birds; however, these parameters were much improved when they were treated with different natural PFA. The infected group of birds given the basal unsupplemented diet (T2) had significantly reduced BWG, FCR, and PEF compared with the infected birds supplemented with PFA. Decreased growth is a prominent symptom of Salmonellosis and the cause of major performance losses in poultry production (Chalghoumi et al., 2009). In addition, the production performance in the birds fed with natural additives (T4 and T6) was parallel to the birds fed with the AGP. Similar results were obtained by Windisch et al. (2009) who analyzed literature data for the effects of PFA on poultry. Overall, PFA improved FCR by 3.4% and reduced FI by 2.1% in broilers.

The intestinal weight at 15 d was higher in the AGP and control group compared with PFA groups. This is in agreement with the results of Lee et al. (2015). Lower intestinal weight in these groups may result in better digestion and absorption. The average breast muscle and leg percentages of broilers in the PFA groups in the finisher period (T4, T5, and T6) were higher by 7 and 3%, respectively, compared with the positive control group (T2). Similarly, Zdunczyk et al. (2010) also reported significantly increased breast muscle weights of broilers fed a diet with Sangrovit.

Meat pH is an important variable that occurs during rigor mortis and can affect the texture, color, and WHC. Meat pH after 15 min postmortem is a good indicator of the characteristics of the meat (Mehaffey et al., 2006). Generally, a rapid decline in breast meat pH can lead to protein denaturation, which may cause pale color and low WHC. The data presented herein were in the range of those of Corzo et al. (2009) who reported that pH_{15min} for broiler breast meat was between 6.3 and 6.6. In this experiment, the highest pH_{15min} and pH_{24hr} were obtained in the unchallenged control group and were 6.66 and 6.23, respectively, which suggested that the bacterial challenge may have negatively affected the pH_{15min} of breast muscle.

Meat color is an indicator of meat quality. There was a strong treatment effect on redness (a^*_{15min} and a^*_{15min}). The negative control group had the lowest redness values, which was in agreement with the results of Al-Owaimer et al. (2014). The MFI represents damage to myofibrils caused by homogenization, and it is correlated with other indices for muscles, such as SF and tenderness (Olson et al., 1976). In this study, lower MFI occurred for the negative control group and Sangrovit Extra, and it could be concluded that the PFA provided myofibrils of the muscle protection from damage.

It was speculated that essential oil components and various plant-derived components may have hypocholesterolemic effects in broiler chickens (Lee et al., 2003; Yakhkeshi et al., 2011). In the current study, we did not observe PFA-mediated hypocholesterolemic effects; however, PFA significantly reduced triglycerides compared with that of the control. The determination of serum enzymes (ALT and AST) is often used to detect hepatic health and reflect the degree of hepatocellular damage and leakage (Jaensch, 2000). The activity of the ALT enzyme decreased for PFA groups, which could indicate that supplementation with PFA had a positive effect on chicken health. In conclusion, dietary supplementation with different PFA could be used effectively relative to that of an antibiotic for the maintenance of the growth performance and improvement of meat characteristics of broilers challenged with *S. typhimurium*.

ACKNOWLEDGMENTS

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