Effects of phytogetic additives on meat quality traits in broiler chickens¹

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ABSTRACT: Phytogetics have been reported to improve growth performances in farm animals and are thereby considered as potential key solutions for antibiotic-free livestock nutrition. Yet, their effects on meat quality are still not well defined; therefore, the aim of this study was to determine the effects of 5 experimental phytogetic additives (3 dietary and 2 water supplements) on growth and meat quality in broilers. One-day-old broiler chicks ($n = 576$) were assigned to 48 floor pens and divided into 6 treatments (Control, AV/HGP/16 premix [AVHGP], Superliv concentrate premix [SCP], bacteriostatic herbal growth promotor [BHGP], AV/SSL/12 [AVSSL], and Superliv Gold [SG]) in a complete randomized design (8 pens/treatment with 12 birds/pen, and 96 birds/group). Feed intake and BW were recorded, and birds were processed at 42 d to evaluate carcass traits. Breast muscle tissues were excised to determine stress- and antioxidant-related genes

expression. Both AVSSL- and SG-treated broilers produced heavier ($P < 0.05$) slaughter weights compared with the control-fed broilers, whereas AVSSL supplementation decreased ($P < 0.05$) fat pad size and increased ($P < 0.05$) breast weights compared with the control-fed broilers. Although pH and a^* values remained unchanged, L^* was decreased ($P < 0.05$) in all treatment and b^* was reduced ($P < 0.05$) in SG when compared with controls. The trained sensory panelists detected more ($P < 0.05$) green herb flavor in the breast meat from AVHGP than SCP, SG, and control birds. The expression of superoxide dismutase 2, extracellular signal-regulated kinase 1/2, and JNK gene was upregulated in AVHGP and BHGP compared with the control ($P < 0.05$). Together, these results indicated that phytogetic additives might improve meat quality of broilers through modulation of stress- and antioxidant-related pathways.

Key words: antioxidation, gene expression, meat quality, phytogetics, sensory

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INTRODUCTION

Broiler production in the United States, and worldwide, is more efficient and cost-effective now than 20 yr ago because of advances in genetic

selection and on-farm housing/environmental and nutritional management. Today, a broiler averaging over 2.80 kg of BW can be sent to market in 42 d, whereas, in 1995, a bird was sent to market at the same age averaged only 2 kg (National Chicken Council, 2017). High-yielding, fast-growing feed-efficient broilers are beneficial to help meeting the growing demands for poultry product, but may be to the detriment of meat quality. With rapid growth rate, metabolic muscle myopathies have developed, including woody breast and white striping, as well as issues with water-holding

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capacity, tenderness, and fat oxidation (Wu et al., 1994; Smith et al., 2002; Lyon et al., 2004). There is, therefore, a critical need for novel effective strategies (nutrition, management, and/or selection) to prevent, or reduce, the incidence of these metabolic disorders and improve meat quality.

Restriction on the use of direct-feed antibiotics in many countries has fueled the interest in alternative products like phytogenics (a group of natural products) that have been the focus of several studies in recent years. These plant-derived products have been reported to have several beneficial effects from antimicrobial and antioxidant activities to improvement of gut health and growth performance (Bazargani-Gilani et al., 2014; Olnood et al., 2015; Yang et al., 2015). For instance, oregano and other herbal-based products have been included in the diet to help alleviate coccidiosis issues and improve the gut health of the birds (Mohiti-Asli and Ghanaatparast-Rashti, 2015). A recent field trial study has shown that phytogenic (Superliv)-supplemented diet increase BW and feed conversion ratio (FCR) in broilers (Amitav et al., 2013, 2015; Sudhir et al., 2016). However, whether these phytogenic additives improve meat quality remains unknown.

The present study aimed, therefore, to determine the effects of 3 phytogenic feed additives and 2 phytogenic water additives, including AV/HGP/16, Superliv concentrate premix, AGP premix (bacteriostatic herbal growth promotor with essential oils), AV/SSL/12, and Superliv Gold, respectively, on the carcass parameters and meat quality traits in commercial modern broilers.

MATERIALS AND METHODS

The present study was conducted in accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institutes of Health and the protocol was approved by the University of Arkansas Animal Care and Use Committee (protocol no. 16084).

Animals, Diets, and Experimental Design

A total of 576-d-old male broiler chicks (Cobb 500 by-products, Cobb-Vantress, Inc., Siloam Spring, AR) with an average BW of 41 ± 0.4 g were allotted randomly to 48 pens (12 birds/pen), and 8 pens were assigned randomly to 1 of 6 treatments: basal starter, grower, and finisher diet (CTRL; Table 1), basal diet supplemented with 0.55 g/kg of diet of either a bacteriostatic herbal growth

Table 1. Composition of basal diets

Ingredient, %	Starter		
	(0 to 14 d)	(15 to 28 d)	Finisher (29 to 42 d)
Corn	59.36	64.22	66.48
Soybean meal	32.85	27.70	25.25
MBM, 50%	2.50	2.50	2.50
Poultry oil	2.01	2.65	3.14
Sodium chloride	0.38	0.31	0.31
Sodium bicarbonate	0.00	0.05	0.05
Limestone	0.80	0.74	0.70
Dicalcium phosphate	1.13	1.00	0.85
Vitamin premix ¹	0.10	0.10	0.10
Mineral premix ²	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10
Selenium premix 0.06%	0.02	0.02	0.02
Santoquin	0.02	0.02	0.02
L-Lys HCl	0.17	0.14	0.10
DL-Met	0.30	0.24	0.21
L-Thr	0.11	0.06	0.05
Copper chloride	0.02	0.02	.
Xylanase	0.01	0.01	0.01
Phytase	0.02	0.02	0.02

¹Premix supplied 3,181.5 IU vitamin A; 2,272.7 IU vitamin D3; 22.73 vitamin E; 0.005 mg vitamin B12; 0.62 mg menadione; 2.73 mg riboflavin; 4.09 mg d-panthothenic acid; 15.91 mg niacin; 0.36 mg folic acid; 1.14 mg pyridoxine; 0.64 mg thiamine; and 0.034 mg biotin per kg of diet.

²Premix supplied 55-mg Ca, 100-mg Mn, 27-mg Mg, 100-mg Zn, 50-mg Fe, 10-mg Cu, and 1-mg I per kg of diet.

promotor with essential oils (BHGP), AV/HGP/16 premix (AVHGP), or Superliv concentrate premix (SCP); or fed CTRL diet with either AV/SSL/12 (AVSSL), and Superliv Gold (SG) added to water at 2, 4, and 7 mL/100 birds/d during the starter, grower, and finisher phases, respectively. The composition of these phytogenic additives are proprietary to Ayurved Ltd. (Kaushambi, Ghaziabad, India), but are apolyherbal formulations of pre-standardized and tested herbs. Birds had ad libitum access to feed (Choretime feeders; Georgia Poultry, Newton Grove, NC) and clean water (Ziggity water system, Georgia Poultry, Newton Grove, NC) at all times, and ambient temperature was reduced gradually from 32 to 25 °C during the first 21 d of the trial, whereas relative humidity (RH, ~20%) and a light:dark cycle of 20-h light:4-h dark were maintained throughout the 42-d feeding trial.

Birds were processed at 42 d of age using a commercial inline system at the University of Arkansas Pilot Processing Plant (Fayetteville, AR). Birds were electrically stunned (11 V, 11 mA for 11 s), exsanguinated, scaled at 53.8 °C for 2 min, and picked using a commercial, inline defeatherer (Foodcraft Model 3; Baker international, MI, USA). After manual evisceration and rinsing, carcasses were prechilled at

12 °C for 15 min and chilled for 90 min at 1 °C in immersion chilling tanks with manual agitation at 15-min regular intervals. Slaughter weight, prechill, and postchill carcasses, as well as abdominal fat weights were recorded, and following a 4-h chill at 4 °C, breasts and tenders were removed and weighed before breast fillets were stored overnight in Ziploc bags (17.7 × 12.7 cm, SC Johnson Inc., Racine, WI) at 4 °C. At the end of the 24-h chilling period, each breast fillet was removed from bags, blotted dry on paper towels, and reweighed to calculate drip loss according to the procedure of [Woelfel et al. \(2002\)](#). Drip loss was calculated as the difference between initial and final weights and expressed as a percentage of the initial breast fillet weight. Following drip loss measurement, instrumental (L^* , a^* , and b^*) color was measured on the ventral side of the right breast fillet from 3 readings with a Minolta colorimeter (CR-400; Konica Minolta Sensing Inc., Sakai Osaka, Japan; size 102 (W) × 217 (H) × 63 (D) mm) using illuminant D65 and a 2.54-cm aperture. In addition, ultimate muscle pH was measured with a temperature-compensating pH meter (Testo 205; Testo Inc., West Chester, PA) inserted into the cranial region of the right breast lobe with averaged 3 measurements per each sample.

Thiobarbituric Acid-Reactive Substances

Thiobarbituric acid-reactive substances (TBARS) were determined according to the procedure of [Witte et al. \(1970\)](#) as modified by [Kuntapanit \(1978\)](#). Briefly, right breast fillet from 3 birds/pen were cut into equal-dimensional sections, packaged in Whirlpak bags (7.5 × 18.5 cm; Sigma-Aldrich, St. Louis, MO), stored at 4 °C, and samples from each bird were removed at 1-, 4-, 7-, and 10-d postprocessing, and subsequently frozen at −80 °C until analysis. Samples were tempered at −20 °C for 24 h before knife-minced, and 2 g of mince breast fillet were mixed with 8 mL of 50 mM phosphate buffer (pH 7.0) and 2 mL of 30% trichloroacetic acid, homogenized (VWR 250 Homogenizer; VWR, Radnor, PA) for 20 to 30 s, and the homogenate was filtered through Whatman filter paper (grade 4; Sigma-Aldrich, St. Louis, MO). A 2-mL aliquot of filtrate was mixed with 2 mL of 0.02 M 2-thiobarbituric acid, placed in a 100 °C water bath for 20 min, and subsequently placed into an ice-water bath for 15 min. Absorbance read at 533 nm on a spectrophotometer (Synergy HT multi-mode microplate reader, BioTek, Winooski, VT) was multiplied by 12.21 to calculate mg of malonaldehyde (MDA)/kg of breast meat.

Sensory Panel

Frozen breast fillets were thawed overnight at 4 °C before being cooked in a convection oven preheated to 176 °C on aluminum-lined and covered pans with raised wire racks to an internal endpoint temperature of 76 °C monitored with a handled digital thermometer. Cooked fillets were cut into 2.54 × 2.54 cm × fillet thickness cubes. Duplicate samples were served warm to a 9-person professional meat descriptive panel (University of Arkansas Food Science Department's Sensory Service Center, Fayetteville, AR) in 2 sessions. The descriptive panel was initially trained according to the Spectrum method ([Meilgaard et al., 2007](#)), and panelists had more than 7,500 h of sensory experience, especially with poultry and other meat products. A presampling orientation session was performed to refine particular sensory attribute definitions similar to that described by [Lyon and Lyon \(1998\)](#) and [Cavitt et al. \(2004\)](#). Each panelist evaluated samples presented to them in a random order, and evaluated aromas (cooked chicken, brothy, chicken fat, blood serum/metallic, green herb, wet feathers, and barnyard), basic tastes (salt, sweet, sour, and bitter), flavors (cooked chicken, brothy, chicken fat, organy, blood serum, metallic, green herb, cardboard, wet feathers, and barnyard), feeling factors (metallic and astringency), and texture (moisture release). All sensory attributes and texture characteristics were scored to the nearest 0.5 on a scale ranging from 0 (least intense) to 15 (most intense).

RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

Total RNA were extracted from chicken left-beast muscle tissues by Trizol reagent (Life Technologies, Grand Island, NY) according to manufacturer's recommendations. After DNase treatment and purification, total RNA concentrations were determined for each sample by Take 3 Micro-Volume Plate using Synergy HT multimode microplate reader (BioTek, Winooski, VT), and RNA integrity and quality were assessed by both OD_{260}/OD_{280} nm absorption ratio (>1.9) and by using 1% agarose gel electrophoresis. For cDNA synthesis, total RNA (1 µg) was reverse transcribed using qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD) in a 20-µL total reaction. The reverse transcription reaction was performed at 42 °C for 30 min followed by an incubation at 85 °C for 5 min. Real-time quantitative PCR

(Applied Biosystems 7500 Real-Time PCR system) was performed using 5 μ L of 10 $\times$ -diluted cDNA, 0.5 μ M of each forward and reverse specific primer, and SYBR Green Master Mix (ThermoFisher Scientific, Rockford, IL) in a total 20- μ L reaction as previously described by [Lassiter et al. \(2015\)](#) and [Flees et al. \(2017\)](#). Oligonucleotide primers specific for chicken superoxide dismutase 1 and 2 (**SOD1/2**), glutathione peroxidase 1 (**GPX1**), heat shock protein 70 (**HSP-70**), extracellular signal-regulated kinase 1 and 2 (**ERK1/2**), c-jun N-terminal kinase 1 (**JNK1**), nuclear factor erythroid 2-related factor (NRF2), and r18S as housekeeping gene were summarized in [Table 2](#). Relative expressions of target genes were determined by the $2^{-\Delta\Delta C_t}$ method ([Schmittgen and Livak, 2008](#)). Samples extracted from CTRL-fed broilers were used as a calibrator.

Total Antioxidant Activity

Antioxidant properties of the phytogenic additives were determined by measuring radical-scavenging ability using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) colorimetric assay as previously described by [Loizzo et al. \(2010\)](#). Briefly, phytogenic extracts were diluted 20:1 using methanol and centrifuged (6037 g) for 5 min. In a 48-well plate, 0.2 mL of sample supernatant was added to 0.2 mL DPPH (0.1 mM in methanol). After shaking for 30 min in dark conditions,

plates were read in microplate reader (BioTek Instruments, Winooski, VT) at 517 nm. All samples and blanks were read in triplicate, and scavenging effect (%) was determined using the following formula:

$$\text{Scavenging effect (\%)} = \left(\frac{[Ab_{517\text{blank}} - Ab_{517\text{sample}}]}{Ab_{517\text{blank}}} \right) \times 100.$$

Statistical Analysis

Data were analyzed as a completely randomized design, with pen as the experimental unit. The ANOVA was generated using the mixed models procedure of SAS (SAS Institute, Inc., Cary, NC), with phytogenic supplement as the lone fixed effect in the model. In the ANOVA of the sensory data, panelist nested within session was included as a random effect in the model. Least square means were computed and separated with the pairwise *t*-tests (PDIF option of SAS) when a significant ($P < 0.05$) *F*-test was noted. Gene expression and TBARS data were analyzed by 1- and 2-way ANOVA, respectively, and means for gene expression and TBARS were compared using Student–Newman–Keuls (**SNK**) and Tukey's multiple comparison tests, respectively, in GraphPad Prism (Version 7.0, Graph Pad Software, La Jolla, CA).

Table 2. Oligonucleotide primers for real-time qPCR

Gene ¹	Accession number ²	Primer sequence (5' → 3')	Orientation	Product size (bp)
SOD1	NM_205064	TGGCTTCCATGTGCATGAAT	Forward	58
		AGCACCTGCGCTGGTACAC	Reverse	
SOD2	NM_204211	GCTGGAGCCCCACATCAGT	Forward	61
		GGTGCGGTGGTGTGTTGCT	Reverse	
GPX1	NM_001277853	TCCCCTGCAACCAATTCG	Forward	57
		AGCGCAGGATCTCCTCGTT	Reverse	
HSP70	J02579	GGGAGAGGGTTGGGCTAGAG	Forward	55
		TTGCCTCCTGCCCAATCA	Reverse	
ERK1	NM_204150	CGGACCATGATCACACAGGAT	Forward	63
		CAGGAGCCCTGTACCAACGT	Reverse	
ERK2	AY033635	CGGACCATGATCACACAGGAT	Forward	63
		CAGGAGCCCTGTACCAACGT	Reverse	
JNK1	NM_205095	GCCGATGATCAGCCAGGAT	Forward	62
		GGCCCAATGGAAGCAAGAG	Reverse	
r18S	AF173612	TCCCCTCCCGTTACTTGGAT	Forward	60
		GCGCTCGTCGGCATGTA	Reverse	
NRF2	NM_001030646	GGCCAACGTCCGAAGTGAT	Forward	55
		CCATGACACCCGCTGCTT	Reverse	

¹ERK1 and ERK2 = extracellular signal-regulated kinase 1 and 2, respectively; GPX1 = glutathione peroxidase 1; HSP70 = heat shock protein 70; JNK1 = c-jun N-terminal kinase 1; SOD1 and SOD2 = superoxide dismutase 1 and 2, respectively; r18S = ribosomal 18S; NRF2 = nuclear factor (erythroid-derived 2)-like 2.

²Accession number refers to Genbank (NCBI).

RESULTS

Productive Data and Carcass Characteristics

Birds drinking water supplemented with AVSSL and SG had heavier ($P < 0.05$) slaughter weights than all other birds, and produced heavier ($P < 0.05$) hot (HCW) and chilled (CCOG) carcass weights than birds consuming diets supplemented with AVHGP, SCP, and BHGP (Table 3). Supplementing broiler water with AVSSL and SG resulted in decreased ($P < 0.05$) fat pad percentages and increased ($P < 0.05$) breast weights compared with the untreated (control) birds. The other additives (AVHGP, SCP, and BHGP) did not elicit any significant changes in fat pad size and breast weights compared with the control group (Table 3).

Meat Color and pH

Breast pH after 24-h postmortem did not differ ($P > 0.05$) among the treatment groups; however, fillets from AVSSL-supplemented birds had the greatest ($P < 0.05$) drip loss percentages, whereas drip losses were greater ($P < 0.05$) in fillets of birds

fed CTRL, AVHGP, and SCP than either broilers eating BHGP-supplemented diets or drinking SG-supplemented water (Table 4).

Even though redness (a^*) values of breast fillets did not differ ($P > 0.05$) among experimental groups, fillets from CTRL- and AVHGP-fed birds were lighter (greater L^* values, $P < 0.05$) in color than fillets from broilers supplemented with SCP, BHGP, AVSSL, and SG (Table 4). In addition, fillets from birds drinking SG had greater ($P < 0.05$) b^* values (indicative of a more yellow color) than breast fillets from all other experimental groups (Table 4).

TBARS and Total Antioxidant Activity

The DPPH analysis demonstrated that all phyto-genic additives used in this study had a greater total antioxidant activity compared with the CTRL-fed broilers (Figure 1). Across all treatment groups, TBARS values were greater ($P < 0.01$) after 4, 7, and 10 d of refrigerated storage than initial (day 0) TBARS values, and there was a tendency ($P = 0.07$) for TBARS values to be reduced 37.0%, 28.0%, 13.3%, and 19.2% in breast samples from birds supplemented with SC, AVSSL, BHGP, and

Table 3. Productive and carcass characteristics

Parameters ¹	Experimental groups ²					
	C	AVHGP	SCP	BHGP	AVSSL	SG
SW (g)	2263.7 ± 34 ^b	2214.8 ± 32.1 ^b	2228.9 ± 36.4 ^b	2243.9 ± 34 ^b	2383.8 ± 34 ^a	2361.8 ± 34 ^a
HCW (g)	1630.5 ± 26.2 ^{ab}	1581.3 ± 24.7 ^b	1604.3 ± 28 ^b	1607.4 ± 26.2 ^b	1701.7 ± 26.2 ^a	1691.5 ± 26.2 ^a
CCWG (g)	1672.5 ± 26.8 ^{ab}	1615.5 ± 25.3 ^b	1642.2 ± 28.6 ^b	1642.1 ± 26.8 ^b	1748.2 ± 26.8 ^a	1741.9 ± 26.8 ^a
Fat Pad (%)	2.01 ± 0.05 ^{ab}	2.11 ± 0.05 ^a	2.09 ± 0.05 ^a	1.91 ± 0.05 ^{bc}	1.8 ± 0.05 ^c	1.91 ± .05 ^{bc}
Breast (g)	422.9 ± 9.6 ^{bc}	409.4 ± 9.1 ^c	419.5 ± 10.3 ^{bc}	422.2 ± 9.6 ^{bc}	451.7 ± 9.6 ^a	445.2 ± 9.6 ^{ab}
Breast (%)	25.21 ± 0.22	25.27 ± 0.21	25.4 ± 0.24	25.62 ± 0.23	25.73 ± .23	25.43 ± 0.23

Values shown are means ± SEM ($n = 80$).

1CCWG = chilled carcass without giblets; HCW = hot carcass weight; SW = slaughter weight.

²AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold. Different letters within a row indicate a significant difference at $P < 0.05$.

Table 4. Effects of phyto-genic supplementation on meat color and water holding capacity

Parameters ¹	Experimental groups ²					
	C	AVHGP	SCP	BHGP	AVSSL	SG
pH	5.73 ± 0.01	5.73 ± 0.01	5.73 ± 0.01	5.71 ± 0.01	5.75 ± 0.01	5.73 ± 0.01
L^*	53.34 ± 0.30 ^a	52.71 ± 0.25 ^a	51.69 ± 0.2 ^b	51.88 ± 0.2 ^b	51.23 ± 0.2 ^b	52.20 ± 0.3 ^b
a^*	4.14 ± 0.08	4.13 ± 0.09	4.04 ± 0.06	4.16 ± 0.07	4.27 ± 0.08	3.96 ± 0.07
b^*	1.26 ± 0.10 ^a	1.40 ± 0.10 ^a	1.05 ± 0.10 ^a	1.1 ± 0.10 ^a	1.11 ± 0.10 ^a	1.62 ± 0.10 ^b
Drip loss	1.43 ± 0.14 ^a	1.41 ± 0.10 ^a	1.45 ± 0.10 ^a	0.97 ± 0.08 ^b	2.14 ± 0.15 ^c	0.86 ± 0.08 ^{bd}

Values shown are means ± SEM ($n = 80$).

¹ a^* = redness; b^* = yellowness; L^* = lightness.

²AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold. Different letters within a row indicate a significant difference at $P < 0.05$.

SCP, respectively, whereas diets supplemented with AVHGP had 62.0% greater TBARS values, compared with CTRL-fed broilers (results not presented in graphic or tabular form).

Meat Aroma, Flavor, Taste, and Texture

Descriptive panelists did not detect ($P > 0.05$) any difference in aromas (cooking

Figure 1. Antioxidant properties of phytogetic additives determined by stable free radical DPPH assay. C = control; AVHGP = AV/HGP/16; AVSSL/12 = AVSSL; SCP = Superliv Concentrate Premix; BHGP = AGP premix: bacteriostatic herbal growth promoter with essential oils; SG = Superliv Gold.

chicken, brothy, blood serum metallic, green herb, wet feather, or barnyard; Table 5) and basic tastes (salt, sweet, sour, and bitter; Table 6) of breast fillets among treatment groups. Panelists noted greater ($P < 0.05$) green herb flavor intensity in fillets from birds supplemented with AVHGP than those consuming CTRL- and SCP-diets, as well as birds drinking SG-supplemented water; otherwise, phytogetic supplementation had no effects ($P > 0.05$) on the intensity of cooked chicken, brothy, chicken fat, organy, blood serum, metallic, cardboard, wet feather, or barnyard flavors (Table 7). Although panelists failed to detect differences in metallic- ($P > 0.05$) and astringency- ($P > 0.05$) feeling factors among samples, panelists indicated that moisture release intensity was greater ($P < 0.05$) in fillets from birds fed CTRL, AVHGP, and BHGP than those drinking SG-supplemented water (Table 8).

Gene Expression

Feeding diets supplemented with AVHGP and BHGP upregulated ($P < 0.05$) the muscle expression of SOD2, ERK1, ERK2, and JNK compared with the CTRL-fed birds (Figure 2b and e-g, respectively). However, the GPX1 mRNA abundance was increased ($P < 0.05$) in breast muscle of BHGP-fed birds compared with the CTRL-fed group (Figure 2c), but the expression of

Table 5. Effect of phytogetic supplementation on aromas of broiler breast fillets

AROMA	Experimental groups ¹					
	C	AVHGP	SCP	BHGP	AVSSL	SG
Cooked chicken	4.9 ± 0.19	4.9 ± 0.19	4.9 ± 0.19	4.8 ± 0.19	4.9 ± 0.19	4.9 ± 0.19
Brothy	2.0 ± 0.71	1.8 ± 0.71	2.0 ± 0.71	1.8 ± 0.71	2.0 ± 0.71	1.8 ± 0.71
Blood serum metallic	3.0 ± 0.25	2.9 ± 0.25	3.0 ± 0.25	3.0 ± 0.25	3.0 ± 0.25	3.1 ± 0.25
Green herb	0.0 ± 0.49	0.2 ± 0.49	0.0 ± 0.49	0.2 ± 0.49	0.4 ± 0.49	0.3 ± 0.49
Wet feather	1.5 ± 0.77	1.5 ± 0.77	0.9 ± 0.77	1.6 ± 0.77	1.2 ± 0.77	1.1 ± 0.77
Barnyard	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06

Values shown are means ± SEM ($n = 94$).

¹AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold.

Table 6. Effects of phytogetic supplementation on basic taste of broiler breast fillets

Basic taste	Experimental groups ¹					
	C	AVHGP	SCP	BHGP	AVSSL	SG
Salt	2.6 ± 0.24	2.7 ± 0.24	2.7 ± 0.24	2.6 ± 0.24	2.7 ± 0.24	2.7 ± 0.24
Sweet	0.2 ± 0.07	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.07
Sour	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
Bitter	0.5 ± 0.21	0.5 ± 0.21	0.5 ± 0.21	0.6 ± 0.21	0.6 ± 0.21	0.4 ± 0.21

Values shown are means ± SEM ($n = 94$).

¹AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold.

Table 7. Effects of phytogenic supplementation on flavor of broiler breast fillets

Flavor	Experimental groups ¹					
	C	AVHGP	SCP	BHGP	AVSSL	SG
Cooked chicken	6.1 ± 0.21	6.0 ± 0.21	6.0 ± 0.21	5.9 ± 0.21	6.1 ± 0.21	6.1 ± 0.21
Brothy	1.7 ± 0.56	1.8 ± 0.56	1.4 ± 0.56	1.4 ± 0.56	1.7 ± 0.56	1.7 ± 0.56
Chicken fat	0.0 ± 0.33	0.2 ± 0.33	0.0 ± 0.33	0.0 ± 0.33	0.2 ± 0.33	0.2 ± 0.33
Organy	0.8 ± 0.57	0.6 ± 0.57	0.7 ± 0.57	0.9 ± 0.57	1.0 ± 0.57	1.1 ± 0.57
Blood serum	2.8 ± 0.42	2.7 ± 0.42	2.7 ± 0.42	2.6 ± 0.42	2.7 ± 0.42	2.8 ± 0.42
Metallic	1.5 ± 0.36	1.3 ± 0.36	1.7 ± 0.36	1.4 ± 0.36	1.6 ± 0.36	1.2 ± 0.36
Green herb	0.0 ± 0.54 ^b	0.7 ± 0.54 ^a	0.0 ± 0.54 ^b	0.5 ± 0.54 ^{ab}	0.5 ± 0.54 ^{ab}	0.2 ± 0.54 ^b
Cardboard	1.2 ± 0.43	1.2 ± 0.43	1.1 ± 0.43	1.0 ± 0.43	1.2 ± 0.43	1.1 ± 0.43
Wet feather	0.9 ± 0.5	0.7 ± 0.5	0.6 ± 0.5	0.4 ± 0.5	0.9 ± 0.5	0.9 ± 0.5
Barnyard	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03

Values shown are means ± SEM (n=94).

¹AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold. Different letters within a row indicate a significant difference at $P < 0.05$.

Table 8. Effects of phytogenic supplementation on texture of broiler breast fillets

Texture	Experimental groups ¹					
	C	AVHGP	SCP	BHGP	AVSSL	SG
Metallic FF	2.9 ± 0.45	2.9 ± 0.45	3.1 ± 0.45	2.9 ± 0.45	3.0 ± 0.45	3.0 ± 0.45
Astringency FF	6.7 ± 0.29	6.6 ± 0.29	6.7 ± 0.29	6.7 ± 0.29	6.7 ± 0.29	6.8 ± 0.29
Moisture release	1.1 ± 0.36 ^a	1.2 ± 0.36 ^a	0.9 ± 0.36 ^{ab}	1.2 ± 0.36 ^a	0.9 ± 0.36 ^{ab}	0.7 ± 0.36 ^b

Values shown are means ± SEM (n = 94).

¹AVHGP = AV/HGP/16; AVSSL = AV/SSL/12; BHGP = bacteriostatic herbal growth promoter with essential oils; C = control; SCP = Superliv Concentrate Premix; SG = Superliv Gold. Different letters within a row indicate a significant difference at $P < 0.05$.

SOD1 (Figure 2a), HSP70 (Figure 2d), and NRF2 (Figure 2h) did not differ ($P > 0.05$) among the treatment groups.

DISCUSSION

This study examined the effects of 5 phytogenic additives (3 were supplemented in feed and 2 in water) on carcass characteristics and meat quality in modern broiler chickens. Interestingly, supplementing AVSSL and SG in the drinking water, but not feeding phytogenic-supplemented diets, improved BW as well as HCW and CCOG. Beside a high probable difference in active ingredient content between the 2 categories of additives that was not divulged during, or since the experiment, and represents a limitation in this study, it is plausible that this observed difference in growth performance might be due to higher water intake and consequently higher phytogenic additive intake. In fact, water intake of broilers is approximately 2 to 3 times as much as feed intake (Williams, 1996; Feddes et al., 2002), which may result in higher levels of phytogenic additive intake and, consequently, a higher amount of active ingredients reaching the intestinal tract of the birds when compared with feed-phytogenic additives. Furthermore, it is possible that

water-phytogenic additive had a higher absorption rate, which is supported by the modulation of gut segment morphology (villi high, crypt depth, and muscle thickness; results not shown).

In addition, both water (AVSSL and SG) phytogenic additives improved breast muscle mass and reduced fat pad size which suggests an enhancement of muscle protein synthesis and reduction of fat deposition through inhibition of lipogenesis and/or increase of lipolysis process.

Herbs and spices are traditionally defined as any part of a plant that is used in the diet for their aromatic properties with no or low nutritional value (Smith and Winder, 1996). However, recent studies have demonstrated that natural herbal additives contain various phytochemicals and can possess powerful antioxidant activity (Park and Yoo, 1999; Dragland et al., 2003; Liu et al., 2006). Several culinary and medicinal herbs are important sources of dietary antioxidants (Dragland et al., 2003). In agreement with these studies, the DPPH assay showed that both water- and feed-phytogenic additives exhibited greater radical scavenging effects and total antioxidant activities when compared with the CTRL-fed diet. Antioxidant-rich herbs have the potential to protect against oxidative

Figure 2. Effect of phytogetic additives on the expression of stress- and antioxidant-related genes in broiler breast muscle. SOD1 (a), SOD2 (b), GPX1 (c), HSP70 (d), ERK1 (e), ERK2 (f), JNK (g), and NRF2 (h). mRNA abundances were measured by qPCR. Data are presented as mean $\pm$ SEM ($n = 6$ /group). Different letters indicate significant difference at $P < 0.05$. C = control; AVHGP = AV/HGP/16; AVSSL/12 = AVSSL; SCP = Superliv Concentrate Premix; BHGP = AGP premix: bacteriostatic herbal growth promoter with essential oils; SG = Superliv Gold.

damage by reducing lipid peroxidation and improve the quality of a product (Wood and Enser, 1997). This is evidenced in the present study by the reduction in TBARS, a byproduct of lipid peroxidation, at day 1 postmortem in breasts from phytogetic-fed birds compared with the untreated group.

At molecular levels, the first line antioxidant defense comprises 3 key enzymes, namely, SOD,

catalase (CAT), and GPX. These enzymes play an indispensable role in catalyzing and detoxifying of superoxide and hydroxyl radicals and, thereby, in the antioxidant protective capacity of any biological system (Lü et al., 2010). In this study, although all the studied phytogetic additives exhibited a high total antioxidant activity, only AVHGP and BHGP upregulated the expression of SOD2, but

not SOD1, gene. According to Rosen et al. (1993), the metalloenzyme SOD1 requires Cu and Zn as metal cofactors, whereas SOD2 needs Mn. This suggested that these 2 additives might contain high levels of Mn or active ingredient(s) that specifically and directly trigger SOD2 transcription and/or its metal cofactor. Research has revealed that the induction of detoxifying enzyme activities occurs at the transcriptional levels and is regulated by a *cis*-acting element that is present in the promoter region defined as an antioxidant response element (ARE; Rushmore and Pickett, 1990). In agreement with previous studies (Yu et al., 2000), the upregulation of the expression of mitogen-activated protein kinase (MAPK)-related genes (ERK1/2 and JNK1) in the present study indicates that AVHGP and BHGP phyto-genic additives might activate SOD2 transcription via induction of MAPK and ARE. Although MAPK are well-known to be associated with stress conditions (Harrison et al., 2004; Darling and Cook, 2014), emerging studies have shown that MAPK cascades also regulate a diverse class of “normal” biological processes from gene expression to cell proliferation and differentiation (Robinson and Cobb, 1997; Schaeffer and Weber, 1999). The unchanged levels of HSP70 mRNA—the gold standard of stress marker—between all the groups indicated that the induction of MAPK expression was unlikely due to stress in the present experimental conditions; however, exactly how MAPK were activated in this study and what are the upstream mediators remain to be identified.

As lipid oxidation has been reported to cause rancidity and deterioration in flavor and color of meat (Suryanti et al., 2014), it is imperative to determine the effect of phyto-genic supplementation on meat organoleptic quality (pH, color, and sensory parameters). The magnitude of the range of pH, L*, a*, and b* values was similar to that reported in previous studies (Van Laack et al., 2000; Woelfel et al., 2002; Janisch et al., 2011), indicating that these values were in the normal range. Interestingly, all the phyto-genic additives except AVHGP reduced the breast lightness (L* values). Although the underlying mechanisms are not known, it is possible that these additives affected the pigment and myoglobin content in broiler breast muscles. The relative amounts of myoglobin in the breast muscle depend on the oxygen availability, myoglobin autoxidation rate, and reducing capacity (Ledward, 1992). On the other hand, the oxygen availability depends on the oxygen partial pressure, penetration depth, and oxygen consumption rate of the muscle (Ledward, 1992). It was hypothesized that the

phyto-genic additives might modulate the thickness of different myoglobin layers in the breast muscle, which, in turn, affect the depth of light penetration and L* values. It is also conceivable that during postmortem glycolysis, the supplemented phyto-genic additives modulate the sarcoplasmic protein denaturation and precipitation on the myofibrils, thereby affecting light scattering and penetration, which merits further in-depth investigations.

Many herbs and their extracts have been added to a variety of food and diets to improve their sensory characteristics. Contrary to expectation, professional descriptive panelists did not discern any difference in aroma and basic tastes among treatment groups. However, they identified a more intense green herb flavor in breast fillets from AVHGP-fed than CTRL-fed birds. This could be of particular interest for consumers preferring a “natural” meat flavor.

Taken together, these results demonstrated that water-phyto-genic additives are more efficient in improving growth performance than the feed-additives. Although further in-depth studies are warranted, phyto-genic supplementation appears to enhance meat quality probably through modulation of MAPK- and antioxidant-related genes.

AUTHOR CONTRIBUTIONS

J.F., E.S.G., and S.D. performed the live bird trial; S.D. purchased the reagents; S.O., J.F., E.S.G., F.L.Y., C.M.O., M.K., N.A., and S.D. assisted in the processing of the trial and collection of meat quality data; J.F. and S.D. conducted gene expression analysis and statistical analysis; D.A. and S.L. measured antioxidant activity; and S.D. wrote the manuscript.

Conflict of interest statement. None declared.

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