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Effect of Botanical Extracts on Amino Acid and Fatty Acid Profile of Broiler Meat

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ABSTRACT

The objectives of current study were to evaluate the effect of botanical extracts as antioxidants on amino – and fatty acids profile of broiler meat. In total, 200 one-day-old broiler chicks were divided according to a completely randomized design into eight dietary treatments with five replicates of five chicks each. Dietary treatments were: A - positive control (PC) with antibiotics and synthetic antioxidants, B - negative control (NC), C - NC+*Trigonellafoenum-graecum*, D -NC+*Nigella sativa* seed, E -NC+*Violaodorata*, F -NC+*Trachyspermumammi*, G -PC+*Trachy spermumammi*+*Trigonellafoenum-graecum*+*Violaodorata,* and H -NC+ *Trachyspermumammi*, *Trigonellafoenum-graecum* and *Viola odorata*. Meat amino – and fatty acid contents were analyzed after slaughter at 35 days of age. Data were submitted to one-way analysis of variance and means were compared by Duncan's Multiple Range test. The levels of histidine, isoleucine, leucine, lysine and threonine were significantly higher ($p \leq 0.05$) in the H, F, E, H and F groups respectively. Arginine, tryptophan, valine, methionine, and phenylalanine were significantly higher ($p \leq 0.05$) in the C, F, E, G and H groups. Total essential amino acid levels were highest (40.36%) in C group ($p \leq 0.05$). The meat of H broilers presented significantly higher ($p \leq 0.05$) unsaturated fatty acids (54.8%) and omega-3 contents (2.77%) and the lowest saturated fatty acid content (44.8%). Group F present the highest linoleic acid and omega-6 levels and group H the highest ($p \leq 0.05$) arachidonic and eicosapentaenoic acid (EPA) contents. It was concluded that botanical extracts aid health-beneficial meat production and the simultaneous inclusion of *Trachyspermumammi*, *Trigonellafoenum-graecum,* and *Viola odorata* in broiler diets is a more suitable option than the other dietary regimes.

INTRODUCTION

The poultry industry plays a vital role in supply of healthy meat products to the public. Broiler meat production from broiler is one of the major food-producing enterprises globally. Although broiler exhibits relatively fast growth rate and heavy body size, the nutritional profile of its meat has been criticized under conventional human dietary regimes (Fanatico *et al.*, 2007).

The breast and thigh are the preferred parts of consumers of various regions. However, the chemical composition of meat fat, including its amino acid and fatty acids profile, have been currently intensively researched due to their direct association with cardiovascular diseases in humans. The accretion of amino acids like lysine in skeletal satellite cells in the muscle is largely influenced by the activation of insulin-like growth factor (*IGF-1*) (Tesseraud *et al.,* 2003).Plant extracts, like that of *Aloe vera*, can enhance muscle integrity by elevating *IGF-1*

(Muhammad *et al.,* 2013). It was demonstrated that curcumin extracts enhance the growth performance of pigs by regulating the gene expression of *IGF-1* and up regulating the activity of antioxidant enzymes (Upadhaya *et al.,* 2016). Muscular health and growth are well regulated by curcumin feeding (Alamdari *et al.,* 2009). Botanical extracts can aid muscle regeneration and differentiation, reduce muscle atrophy and enhance anti-inflammatory responses (Rondanelli *et al.,* 2016).

Fatty acid composition of dietary fats can affect saturated fatty acids (SFA) as well as unsaturated fatty acids (UFA) levels in broiler breast and thigh muscles (Zhang *et al.,* 2013). Fat sources rich in UFA can be included in broiler diets to produce leaner meat with much better ω-3 profiles. The desired ω-3 fatty acids, such as linolenic acid (LNA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), in the breast and thighs of chicks can be achieved by using omega 3 rich oils (Sadeghi *et al.,* 2012). Unfortunately, UFA-rich oils are subjected to faster oxidative stress and the inclusion of rancid fats with high peroxide value can impair broiler performance and severely affect immunity (McGill *et al.,* 2011).

The dietary addition of antioxidants can improve broiler performance and immunity by reducing thiobarbituric acid levels in liver and increasing serum and liver vitamin A and E concentrations (Taverez *et al.,* 2011). The supplementation of broiler feeds with plant-derived rich in antioxidant compounds enhance oxygen scavenging responses to safeguard fat sources, both outside and inside body. This effect of natural antioxidants reflects results in a decline in cholesterol and triglyceride levels (Ahmed *et al.,* 2015). Phenolic compounds like quercetin and vitamin E decrease fatty acid generation, particularly of SFA (Sohaib *et al.,* 2015). Antioxidant-rich diets potentially inhibit saturated fatty acid levels is by modulating the activity of 9-desaturase enzyme complex, which converts SFA into UFA (Gnoni *et al.,* 2009). A researcher group documented a declining trend in SFA production in the breast muscle of broilers fed gallic acid and linoleic acid (Jung *et al.,* 2010). Similarly, the inclusion genistein and hesperidin in broiler diets reduced SFA level in the breast meat (Kamboh & Zhu, 2013). Black cumin seeds can be used up to 1.5% in layer feed to reduce egg cholesterol and enhance UFA content in egg yolk (Yalc *et al.,* 2009). Chia seed in diet also effective in controlling fat content in meat and significantly lowering SFA content eventually having better SFA: UFA and ω-6:ω-3 ratio in muscles (Ayerza *et al.,* 2002). Feeding layers with black cumin seeds decreased yolk triglyceride content and cholesterol level in up to 40% (Nabiela *et al.,* 2006).

Recent studies have shown that nutrition has a major influence on tissue gene expression. Dietary antioxidant supplements from plant sources can enhance the quality of meat by minimizing oxidative stress on lipids and protein molecules and enhancing the activities of glutathione peroxidase, catalase, and superoxide dismutase, ultimately improving the amino acid and fatty acid profile of meat (Upton *et al.,* 2009). Due to their potential health benefits, consumers are willing to buy meat from broilers fed botanical extracts (Rizzo *et al.,* 2008). After reviewing the potentials of natural extracts, this study aimed at evaluating the effects of the dietary inclusion of botanical extracts on the amino acid and fatty acid profiles of broiler meat.

MATERIALS AND METHODS

Preparation of Trachyspermumammi, Trigonellafoenum-graecum, Nigella sativa and Viola odorata extracts

Dried flowers of *Viola odorata* (sweet violet) and seeds of *Trigonellafoenum-graecum* (fenugreek), *Nigella sativa* (black cumin) and *Trachyspermumammi* (Ajwain) were purchased from local sources. Seeds were ground to 30-mm particle size. The processes of sonication and extraction were performed according to the methodology proposed by Kim & Lee (2002). Briefly, 200 g of each sample were soaked in 2-L flask with 1 L of 80% methanol. After the sonication for 24 hours, samples were filtered, and again submitted to sonication for 24 hours. Filtrates were transferred to round bottom flasks on a rotary evaporator at 40°C and allowed to evaporate until the 300 mL of the contents remained, which then were standardized to 400 mL by adding distilled water.

Experimental design and bird management

Birds were reared at Research and Development Farm of Sindh Feed, Pvt. Limited, Pakistan. The laboratory analysis of the meat and eggs were performed at the Department of Food Science & Technology, University of Karachi and Pakistan Council for Scientific & Industrial Research (PCSIR) Laboratories Complex, Karachi, Pakistan.

A total of 200 one-d-oldRoss-308 broilers were divided into eight treatments with five replicates of five birds each according to a completely randomized design. Bird were reared on litter at a stocking density

0.6ft²/bird for a period of 35 days. A photoperiod of 22 hours of light and 2 hours of darkness was applied.

Isocaloric (23, 21.12 and 20% crude protein) and isonitrogenous (3000, 3300 and 3200 kcal/kg) diets (starter, grower and finisher respectively) were formulated according to recommendations of the Ross-308 manual using ingredients available in the market. The software FeedLive was used for diet formulation (Table 1).

Table 1 – Ingredients and nutritional composition of the starter, grower, and finisher broiler diets (Ross 308 standards)

Ingredients	Starter	Grower	Finisher	Nutrient composition	Starter	Grower	Finisher
Yellow corn (90% DM)	58.15	61.8	59.7	Metabolizable energy (kcal/kg)	3000	3300	3200
Soybean meal Argentina	31.25	28.25	21.25	Crude protein %	23	21.12	20.0
Fish meal 50% C.P	4.25	2.75	2.0	Calcium %	0.92	0.87	0.81
Canola meal	0	0	6.2	Available phosphorus %	0.48	0.44	0.42
Sunflower meal 33% C.P	2.0	1.75	3.2				
Limestone/ Marble	1.0	1.075	0.93	Digestible lysine %	1.28	1.153	1.03
Sodium bicarbonate.	0.2	0.225	0.2	Digestible Met+Cys %	0.95	0.872	0.8
Salt (NaCl)	0.175	0.20	0.2	Digestible arginine %	1.37	1.234	1.136
Choline chloride 70%	0.2	0.20	0.2	Digestible Tryptophan %	0.238	0.214	0.195
Sunflower oil	1.5	2.5	5	Digestible Threonine %	0.858	0.775	0.711
Lysine sulphate	0.33	0.33	0.28	Digestible isoleucine %	0.86	0.787	0.708
D. L methionine	0.3	0.28	0.23	Digestible valine %	0.96	0.872	0.813
L-Threonine	0.135	0.12	0.1	Linoleic acid %	2.1	2.7	4.061
L- Isoleucine	0	0.01	0	Sodium %	0.20	0.20	0.19
*Phyzyme 10000 TPT	0.010	0.010	0.01	Chloride %	0.22	0.21	0.20
** Vitamin/ mineral premix	0.50	0.50	0.5	Potassium	0.99	0.99	0.92
TOTAL	100	100	100				

*Phyzyme 10000 TPT contained phytase 10000 FTU/g

** Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12.000 IU; cholecalciferol, 3.000 IU; vitamin E, 80 mg; vitamin K3, 3.2 mg; vitamin B1, 3.2 mg; vitamin B2, 8.6 mg; niacin 65 mg; vitamin B6, 4.3 mg; vitamin B12, 20 µg; Ca-D- pantothenate, 20 mg; Folic acid, 2 mg; biotin, 0.2 mg; Mn, 120 mg; Zn,110 mg; Fe, 60 mg; Cu, 16 mg; Se, 0.3 mg, Iodine 1.25mg

Treatment groups were:

A: Positive control (PC) diet containing an antibiotic (lincomycin (4.4%) at 100 mg/kg); vitamin E at 40 mg/kg and a commercial synthetic antioxidant product at 150 mg/kg (SELDOX®, Trow Nutrition, Netherlands), contain ingethoxyquin, BHA, BHT and citric acid.

B: Negative control (NC), diet with no inclusion of antioxidants or antibiotics.

C: NC + supplemented with fenugreek crude extract at 1ml/kg diet.

D: NC + supplemented with black cumin seed crude extract 1 mL/kg diet

E: NC + supplemented with sweet violet crude extract 1 mL/kg diet.

F: NC + supplemented with ajwain extracts 1mL/ kg feed

G: PC + supplemented with ajwain, fenugreek and sweet violet extracts, each at 0.5 mL/kg feed.

H: NC + supplemented with ajwain, fenugreek and sweet violet extracts, each at 0.5 mL/kg feed.

Parameters evaluated

At 35 days of age, birds were slaughtered, and eviscerated. Carcass yield (%) was calculated by the following equation.

$$Carcass\% = \frac{Carcass\ weight}{Live\ weight} \times 100$$

Breast and thighs were cut up from the carcass using sharp knives and their yields (%)were calculated on whole carcass basis using following equations.

$$Breast\% = \frac{Breast\ weight}{Carcass\ weight} \times 100$$

$$Thigh\% = \frac{Thigh\ weight}{Carcass\ weight} \times 100$$

Amino Acid Analysis

Breast and thigh meat samples were collected to determine their amino acid profile. Briefly, 50-mg samples were hydrolyzed for 18 h in 6N HCl. The sample container was sealed to prevent oxidation and stored at 110°C for hydrolysis. The amino acid composition of

the meat samples all birds fed the A, B, C, D, E, F, G and H diets was determined according to the method of Schuster (1988) using an amino acid analyzer. Samples were extracted in citrate buffer (2.2 pH) solution followed by filtration to obtain a clear solution of the free amino acids. An aliquot of this clear filtrate was injected into the column (Shim-pack ISC-07/S1504 Na) of the HPLC-based amino acid analyzer (RF-10AXL, Shimadzu Corporation, Tokyo, Japan) equipped with fluorescence detector (FLD-6A). Sodium hypochlorite and o-phthalaldehyde solutions were used as reaction solutions for post-column derivation of amino acids.

Fatty Acid Analysis

For fatty acid analysis, fats were hydrolyzed and fatty acids were derived from total fat content according to the procedure of O'Fallon *et al.* (2007). Briefly, 50 µL of fat/oil was placed in a Pyrex screw cap tube, and 1 mL of internal standard, 0.7 mL KOH solution and almost 5.3 mL of methanol were added and mixed. Tubes were capped and placed on a preheated water bath at 55°C for 90 minutes. The tubes were then cooled in water followed by H_2SO_4addition in the samples. The tubes were then again kept in water bath at 55°C for 1.5 hours, followed by cooling and addition of 3 mL of hexane. Tubes were then placed into a vortex mixer for 5 minutes. The fatty acid methyl ester (FAME) hexane layer (upper layer) was separated, filtered and then analyzed. Gas chromatography was performed in GC-2010 apparatus (Shimadzu Corp., Japan) 07947 equipped with FID detector, split injector and SP-2560 silica fused capillary column (100m x 0.25mm x 0.2µm, Supelco) under the following operating program: injection volume 1 µL at 250°C, detector temperature of 260°C, column temperature of 140°C for 5 minutes and then ramped to 240°C at 4°C per minute, remained stable for 15 minutes. Helium was used as carrier gas at flow rate of 1.12mL/ min and linear velocity of 20 cm/s; split ratio 1:100. Results were expressed as percentages relative to FID response area.

Statistical Analysis

The collected data were subjected to one-way analysis of variance (Steel *et al.,* 1997)using the SPSS Statistics 20.0 software (IBM Deutschland GmbH, Ehningen, Germany) and means were compared using Duncan's Multiple Range (DMR) test (Duncan, 1955) following the statistical model:

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

Where,

Y_{ij}= Observation of dependent variable recorded on i^{th} treatment

µ = Population mean

τ_i= Effect of i^{th} treatment (i = 8 i.eA, B, C, D, E, F, G and H)

ε_{ij} = Residual effect of j^{th} observation on i^{th} treatment NID ~ 0, σ^2

RESULTS AND DISCUSSION

Carcass traits

Average carcass yield parameters are shown in Table 2. Thigh yields were significantly different ($p \leq 0.05$) among the dietary treatments. Maximum thigh yield (20.6±0.115) was determined in the birds fed the positive control diet. The lower carcass yield of the birds fed botanical extracts may be due to the fat content in their carcasses, as fat contribute to total carcass weight (Table 2). Tavarez *et al.* (2011) also observed that feeding antioxidant to broilers had no effect on carcass weight, carcass or breast meat yield, but in general increased serum vitamin E and vitamin A levels. The highest breast meat yield was obtained in group G, which was fed the positive control diet supplemented with antioxidants from botanical sources. Botanically-derived compounds like curcumin

Table 2 – Carcass yield of broilers fed botanical extracts

Treatments	Carcass	Thigh	Breast	Fats
	----------(%)----------			
A (Positive control; PC)	66.5 ± 0.173^{d}	20.6 ± 0.115^{c}	21.0 ± 0.115^{d}	7.20 ± 0.056^{d}
B (Negative control; NC)	64.5 ± 0.173^{ab}	18.0± 0.577^{a}	19.0 ± 0.577^{a}	7.53 ± 0.066^{e}
C (NC+ fenugreek)	64.1 ± 0.0^{a}	19.6 ± 0.23^{bc}	20.2± 0.115^{bc}	6.60 ± 0.00^{bc}
D (NC+ black cumin seed)	64.4 ± 0.23^{ab}	18 ± 0.773^{a}	18.87 ± 0.881^{a}	6.50 ± 0.115^{bc}
E(NC+ sweet violet)	64.0 ± 0.115^{a}	18 ± 0.577a	18.8 ± 0.057^{a}	5.00 ± 0.115^{a}
F (NC+ajwain)	64.8 ± 0.23 ^{b}	17.8± 0.115^{b}	19.87 ± 0.666^{b}	6.73 ± 0.666^{c}
G (PC+ajwain+ fenugreek+sweet violet)	65.4 ± 0.115^{c}	19.0 ± 0.058^{ab}	23.6 ± 0.346^{e}	6.40± 0.057^{b}
H (NC+ajwain+fenugreek+sweet violet)	64.0 ± 0.208^{a}	19.8 ± 0.115^{bc}	20.73± 0.105^{cd}	6.43± 0.12^{b}
p-value	0.000	0.000	0.000	0.000

Means ± SE within a column with different lowercase letters are significantly different at ($p \leq 0.05$)

have been used to promote muscle health, strength and growth (Nkukwana *et al.,* 2014). Similarly, scientific reviews on the effects of botanical extracts on muscle health (Rondanelli *et al.,* 2016) describe their positive role in muscle growth and integrity.

Essential amino acid profile of broiler meat

The meat contents of essential amino acids, including histidine, isoleucine, leucine, lysine and threonine were significantly ($p \leq 0.05$) lower in the negative control group (diet B) compared to all other treatments (Table 3). The breast meat of group H birds presented the highest ($p \leq 0.05$) histidine (4%) levels (Table 3). Isoleucine level (4.5%) was significantly ($p \leq 0.05$) in the meat of birds fed ajwain extracts (diet F) and leucine (7%; $p \leq 0.05$) in the meat of those fed sweet violet extract (diet E). The highest lysine level (9.1%) was achieved with diet F and the lowest with the negative-control diet (B) (Table 3). Threonine (4.2%) levels were higher better ($p \leq 0.05$) in the meat of broilers fed ajwain extracts. The breast meat of birds fed fenugreek extract (diet C) presented higher arginine (5.9%; $p \leq 0.05$) compared with the other treatments (Table 3). Meat tryptophan values (Table 3) were significantly higher ($p \leq 0.05$) in the F-group broilers (1.2%) and valine (4.1%; $p \leq 0.05$) in E-group broilers compared with all other treatments. Table 3 shows higher meat methionine content in group G and phenylalanine in group H, both significantly higher ($p \leq 0.05$) than in the other groups. The meat of broilers fed sweet violet extract (diets contained the low esttryptophan, methionine and phenylalanine levels (Table 3). The breast meat of group C broilers presented the highest sum of all essential amino acids (40.36%) followed by group H (40.19), both of which were significantly higher ($p \leq 0.05$) than other groups. The essential amino acid analysis of the broilers fed negative control diet showed that it contained lower values of many essential amino acids compared with those fed the other tested diets. The feeding of botanical extracts to broilers in general increased the individual levels of essential amino acids, and consequently the sum of all essential amino acid.

The overall improvement of essential amino acid contents in the meat of broilers fed the plant extracts suggest the potential benefits of their inclusion in broiler diets. These results may be due to the presence of bioactive compounds in the evaluated herbs. The findings of the current study are supported by reports of the presence of sulfur and non-sulfur compounds in *Allium sativum* (Amagase *et al.,* 2001). Some of

Table 3 – Essential Amino acid profile of meat from broilers fed botanical extracts

Treatments	Histidine	Isoleucine	Leucine	Lysine	Threonine	Tryptophan	Valine	Methionine	p-Alanine	Arginine	Total Essential AA
A (Positive control; PC)	2.95±0.12^{b}	3.5±0.17^{b}	5.6±0.26^{ab}	6.2±0.18^{b}	3.35±0.25^{b}	0.85±0.64^{b}	3.6±0.10^{b}	1.5±0.05^{ab}	2.6±0.13^{bc}	5.00±0.17^{c}	35.15±0.03^{b}
B (Negative control; NC)	2.52±0.09^{a}	2.7±0.09^{a}	5.4±0.12^{a}	5.3±0.91^{a}	3.0±0.01^{a}	0.9±0.58^{bc}	3.2±0.18^{ab}	1.4±0.17^{ab}	2.7±0.056^{cd}	6.20±0.18^{d}	33.33±0.17^{a}
C (NC+ fenugreek)	3.07±0.05^{b}	4.5±0.06^{c}	6.2±0.16^{c}	7.5±0.08^{c}	3.8±0.01^{c}	0.9±0.00^{bc}	3.55±0.23^{b}	1.4±0.91^{ab}	3.54±0.125^{e}	5.90±0.09^{d}	40.36±0.35^{e}
D (NC+ black cumin seed)	3.92±0.00^{c}	3.7±0.17^{b}	5.9±0.09^{bc}	6.00±0.12^{b}	4.2±0.12^{d}	0.75±0.29^{ab}	3.2±0.0^{ab}	1.7±0.091^{bc}	2.35±0.1^{ab}	4.90±0.11^{c}	36.62±0.42^{c}
E(NC+ sweet violet)	2.32±0.09^{a}	3.0±0.15^{a}	7.0±0.12 ^{d}	7.1±0.17^{c}	3.5±0.12^{bc}	0.6±0.02^{a}	4.1±0.13^{c}	1.3±0.012^{a}	2.3±0.08^{a}	3.63±0.06^{b}	34.48±0.35^{b}
F (NC+ajwain)	2.9±0.09^{b}	4.5±0.08^{c}	7.2±0.12^{d}	5.5±0.23^{a}	4.2±0.057^{d}	1.2±0.01^{d}	4.1±0.00^{c}	1.9±0.09^{cd}	2.9±0.04^{de}	3.01±0.09^{a}	37.41±0.17^{d}
G (PC+ajwain+ fenugreek+sweet violet)	2.44±0.05^{a}	2.9±0.87^{a}	6.2±0.09^{c}	8.1±0.09^{d}	3.8±0.09^{c}	1.1±0.06^{cd}	2.9±0.12^{a}	2.0±0.12^{d}	3.1±0.58^{e}	4.7±0.90^{c}	37.29±0.23^{d}
H (NC+ajwain+fenugreek+sweet violet)	4.00±0.07^{c}	3.8±0.12^{b}	6.2±0.12^{c}	9.1±0.09^{e}	2.7±0.00^{a}	1.1±0.06^{cd}	2.9±0.12^{a}	1.6±0.40^{ab}	4.1±0.40^{f}	4.69±0.01^{c}	40.19±0.12^{e}
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Means ± SE within a column with different lowercase letters are significantly different at ($p \leq 0.05$).

these compounds are reported as alliin (Puvača *et al.,* 2015) composed of the sulfur-containing amino acid γ-L-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (Stoll & Seebeck, 1948), methiin, (+)-S-(trans-1-propenyl)-L-cysteine sulfoxide, and cycloalliin (Fujiwara *et al.,* 1958).

Non-essential amino acid profile of broiler meat

There was a significant ($p \leq 0.05$) increase in non-essential amino acid levels (46%) in the breast meat of birds fed sweet violet extract (diet E), as shown in Table 4.The meat of broilers fed the E diet meat presented higher proline (4.5%), glycine (6.8%) and alanine (8.2%) contents compared with the other treatments ($p \leq 0.05$). The meat from birds fed ajwain extract had significantly ($p \leq 0.05$) higher tyrosine (3.6%), cysteine (1.4%) and total amino acid values (80.81%) than that of the other treatments (Table 4). Diet D promoted significantly higher ($p \leq 0.05$) aspartic acid values (14.5%) as compared with the other diets (Table 4). The lowest values ($p \leq 0.05$) of tyrosine, aspartic acid and, consequently, of total non-essential amino acids were obtained in the meat of broiler fed the G diet (Table 5).

Higher non-essential amino acids levels were obtained in the meat of broilers feds weet violet extract, and the combination of fenugreek, ajwain and sweet violet extracts resulted in a decline in meat non-essential amino acid content. Total amino acids contents were better in all groups supplemented with antioxidants from plant-derived sources. The process of muscle growth and protein accretion in broilers was best described by Tesseraud *et al.* (2003), who explained that the accretion of amino acids like lysine in skeletal muscle satellite cells is greatly influenced by the activation of insulin-like growth factor (IGF-1). Antioxidant-rich botanical extracts from plants like *Aloe vera* boost the biological role of *IGF-1* to modulate energy kinetics, myoglobin accretion and muscular integrity (Muhammad *et al.,* 2013). Muscle health and growth may be regulated by feeding curcumin, which was shown to improve broiler growth performance by regulating IGF-1 gene expression and enhancing the activity of antioxidant enzymes (Upadhaya *et al.,* 2016; Alamdari *et al.,* 2009). Recently, Rondanelli *et al.,*(2016) described that botanical extracts may aid muscle regeneration and differentiation, reduce muscle atrophy, and demonstrated it has anti-inflammatory and anti-fatigue properties, preventing muscle damage.

Table 4 – Non-Essential Amino acid profile of meat from broilers fed botanical extracts

Treatments	Tyrosine	Aspartic acid	Glutamic acid	Serine	Proline	Glycine	Alanine	Cysteine	Total Non-Essential AA	Total AA
A (Positive control; PC)	2.4±0.23^{bc}	8.0±0.16^{a}	12.0±0.46^{cd}	3.2±0.1^{d}	3.1±0.91^{c}	5.0±0.06^{b}	6.0±0.29^{cd}	0.7±0.11^{ab}	40.4±0.23^{ab}	75.55±0.58^{b}
B (Negative control; NC)	2.1±0.56^{b}	10.3±0.15^{d}	10.0±0.11^{ab}	2.7±0.09^{b}	2.6±0.91^{b}	6.1±0.13^{c}	5.8±0.11^{bc}	1.0±0.15^{bc}	40.6±0.11^{b}	73.93±0.16^{a}
C (NC+ fenugreek)	2.9±0.09^{d}	9.8±0.92^{d}	8.6±0.06^{a}	2.9±0.92^{c}	3.6±0.91^{d}	5.2±0.91^{b}	5.3±0.06^{b}	0.65±0.05^{a}	38.95±0.00^{ab}	79.31±0.00^{cd}
D (NC+ black cumin seed)	2.1±0.09^{b}	14.5±0.22^{e}	10.5±0.45^{bc}	3.1±0.06^{d}	2.5±0.9^{ab}	4.5±0.06^{a}	6.5±0.06^{d}	0.6±0.58^{a}	43.3±0.11^{c}	80.0±0.91^{d}
E (NC+ sweet violet)	2.5±0.06^{c}	7.6±0.00^{a}	13.0±0.92^{d}	2.2±0.06^{a}	4.5±0.28^{e}	6.8±0.17^{d}	8.2±0.34^{f}	1.2±0.81^{cd}	46.0±0.35^{d}	80.48±0.23^{e}
F (NC+ajwain)	3.6±0.12^{e}	9.3±0.17^{bc}	8.6±0.91^{a}	2.9±0.18^{bc}	4.1±0.11^{e}	6.1±0.13^{c}	7.35±0.03^{e}	1.4±0.18^{d}	43.4±0.12^{c}	80.81±0.18^{e}
G (PC+ajwain+ fenugreek+sweet violet)	1.8±0.09^{a}	7.5±0.29^{a}	10.0±0.46^{ab}	3.2±081^{d}	2.8±0.15^{bc}	6.2±0.18^{c}	5.5±0.29^{bc}	0.8±0.9^{ab}	37.8±0.92^{a}	75.09±0.29^{b}
H (NC+ajwain+fenugreek+sweet violet)	2.3±0.09^{bc}	9.1±0.18^{b}	11.5±0.64^{bcd}	3.13±0.10^{d}	2.1±0.23^{a}	4.5±0.23^{a}	4.9±0.23^{a}	0.7±0.01^{b}	38.3±0.82^{a}	78.48±0.20^{c}
p-value	0.003	0.018	0.008	0.000	0.000	0.007	0.007	0.009	0.025	0.000

Means ± SE within a column with different lowercase letters are significantly different at ($p \leq 0.05$).

Table 5 – Fatty acid profile (C15-C22) of meat from broilers fed botanical extracts

Fatty acids \ Treatments	A	B	C	D	E	F	G	H	*p*-value
C 15-1	18.28±0.96^{a}	18.68±0.55^{ab}	17.09±0.65^{a}	17.24±1.06^{a}	16.51±0.22^{a}	17.98±1.14^{a}	17.23±0.35^{a}	20.94±1.20^{b}	0.00
C 16-0	15.250±3.37^{cd}	18.21±0.37^{f}	16.44±0.78^{de}	17.51±0.22^{ef}	15.67±0.64^{d}	13.94±0.42^{c}	10.93±0.98^{b}	9.09±0.25^{a}	0.000
C 18-0	30.61±1.10^{a}	33.12±0.44^{ab}	32.6±1.63^{ab}	31.95±1.25^{ab}	30.84±0.21^{ab}	30.06±1.15^{a}	33.8±0.20^{b}	32.05±0.50^{ab}	0.000
C18-1n9c	17.66±1.75^{b}	13.93±0.26^{a}	16.17±0.40^{ab}	16.41±0.24^{ab}	17.83±0.21^{b}	20.73±1.38^{cd}	22.0±0.84^{d}	18.44±0.48^{bc}	0.000
C18-2n6t	0.32±0.13^{bc}	0.21±0.00^{a}	0.28±0.00^{ab}	0.27±0.01^{ab}	0.31±0.01^{bc}	0.37±0.03^{c}	0.31±0.00^{bc}	0.29±0.00^{bc}	0.000
C 20	0.29±0.00^{d}	0.19±0.01^{cd}	0.21±0.01^{c}	0.19±0.01^{cd}	0.00±0.00^{a}	0.32±0.03^{d}	0.15±0.00^{b}	0.44±0.01^{c}	0.000
C 20-1n9	8.76±0.34^{a}	8.97±0.29^{a}	11.78±0.35^{b}	11.93±0.48^{b}	9.8±0.37^{a}	9.71±0.72^{a}	9.32±0.29^{a}	11.91±0.15^{b}	0.000
C 20-3n6	0.11±0.00^{b}	0.00±0.00^{a}	0.13±0.01^{b}	0.108±0.01^{b}	0.00±0.0^{a}	0.22±0.00^{c}	0.26 ± 0.02^{d}	0.00 ± 0.0^{a}	0.000
C 20-5n3	0.91±0.018^{a}	1.11±0.097^{ab}	1.04±0.04^{ab}	0.87±0.03^{a}	1.22±0.09^{b}	1.30±0.12^{b}	1.22±0.14^{b}	2.63±0.03^{c}	0.000
C 22	0.17±0.00^{ab}	0.12±0.01^{a}	0.17±0.01^{ab}	0.18±0.01^{ab}	0.23±0.012^{b}	0.32±0.08^{c}	0.19±0.01^{ab}	0.17±0.01^{ab}	0.004
C 22-2	2.42±0.20^{d}	0.88±0.02^{a}	2.28±0.02^{d}	1.62±0.03^{bc}	2.40±0.10^{d}	2.68±0.28^{d}	1.84±0.04^{c}	1.29±0.01^{b}	0.000
SFA	51.16±1.16^{bc}	55.76±1.08^{d}	50.94±1.01^{bc}	51.13±1.76^{bc}	51.62±2.17^{cd}	46.73±0.52^{ab}	47.33±1.94^{abc}	44.8±1.43^{a}	0.000
UFA	48.84±1.75^{b}	44.24±0.19^{a}	49.06±1.22^{bc}	47.61±1.12^{ab}	48.38±0.76^{ab}	53.27±2.08^{cd}	51.67±1.39^{bcd}	54.8±1.74^{d}	0.000
MUFA	45.03±0.03^{abc}	41.97±0.03^{a}	45.39±0.39^{c}	45.91±0.91^{abc}	44.4±1.16^{ab}	48.38±2.33^{bcd}	48.92±1.86^{cd}	50.71±1.10^{d}	0.000
PUFA	3.82±0.21^{c}	2.27±0.03^{a}	3.66± 0.10^{c}	2.96±0.23^{b}	3.95±0.27^{cd}	4.59±0.41^{d}	3.74±0.15^{c}	4.50±0.14^{d}	0.000
OMG-3	0.91±0.01^{a}	1.24±0.04^{bc}	1.04±0.02^{a}	1.00±0.00^{a}	1.22±0.12^{b}	1.38±0.02^{cd}	1.43±0.03^{d}	2.77±0.06^{e}	0.000
OMG-6	0.56±0.01^{c}	0.21±0.01^{a}	0.41±0.01^{b}	0.38±0.01^{b}	0.40±0.00^{b}	0.68±0.04^{d}	0.57±0.02^{c}	0.36±0.024^{b}	0.000

Means ± SE within a row with different lowercase letters are significantly different at ($p \leq 0.05$).

C 15-1 = pentadecanoic acid; C 16-0 = palmitic acid; C 18-0 = stearic acid; C18-1n9c = oleic acid; C18-2n6t = linoleic acid; C 20= arachidonic acid; C 20-1n9 = eicosenoic acid; C 20-3n6 = eicosatrienoic acid; C 20-5n3 = eicosapentaenoic acid; C 22 = behenic acid; C 22-2 = docosadienoic acid; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; OMG-3 = omega-3 fatty acids; OMG-6 = omega-6 fatty acids.

Meat Fatty Acid Composition

The results of the meat fatty acid analysis are shown in Table 5. Pentadecanoic acid (C15-1) levels of 18.68% and 20.94% were determined in the B and H groups, respectively, and both were significantly ($p \leq 0.05$) higher than all other groups. Palmitic acid (C16) level was highest (18.21%) in the broilers fed the negative-control diet (group B) and lowest in those group H (9.09%), both at $p \leq 0.05$. The highest ($p \leq 0.05$) stearic acid values were obtained in group H (33.8%) and the lowest ($p \leq 0.05$) in Groups A (30.61%) and F (30.06%). Oleic acid levels were significantly ($p \leq 0.05$) higher in group G (22%) and lower in group B (13.93%), respectively, than in the other groups. Linoleic acid (C18-2n6) values obtained were significantly ($p \leq 0.05$) higher in group F and lower in group B (0.21%) than in the other groups. Arachidonic acid (C20) was significantly higher ($p \leq 0.05$) in group H chicken fats (0.44%) compared with other groups. Eicosenoic acid (C20-1n9) values in groups C (11.78%) and D (11.93%) were higher ($p \leq 0.05$) compared with the other groups. The meat of broilers in group G presented higher eicosatrienoic acid (C20-3n6) levels compared with the other treatments ($p \leq 0.05$). The highest eicosapentaenoic acid (EPA: C20-5n) was determined in group H (2.63%; $p \leq 0.05$). The highest ($p \leq 0.05$) behenic (C22) and docosadienoic (C22-2) acids levels were obtained in broilers fed ajwain extracts. The meat of the broilers fed the negative control diet presented the highest saturated fatty acid (SFA; 55.76%) levels ($p \leq 0.05$), and the lowest unsaturated fatty acid (UFA; 41.47%) and polyunsaturated fatty acid (PUFA; 2.24%) levels compared with other groups. Group H presented had significantly higher ($p \leq 0.05$) UFA (54.8%) and mono-unsaturated fatty acid (MUFA; 50.71%) levels compared with other all groups (Table 5). Maximum PUFA was observed in group F samples (4.59%) followed by group H (4.5%) and both were significantly better than other groups. Higher omega-3 fatty acid levels were obtained in groups F, G and H. Group H presented the highest omega-3 fatty acid level (2.77%) compared with the other groups ($p \leq 0.05$), which was almost three-fold higher than that obtained with the positive control diet (0.91%). The meat of broilers in groups C, D, E, F and G presented 1.04%, 1.0%, 1.22%,1.38%, and 1.43% omega-3 levels, respectively, which were significantly higher than obtained with the positive control diet ($p \leq 0.05$).The highest and the lowest omega-6 levels were obtained in groups F (0.68%) and B (0.21%), respectively, both were significantly different from the other groups ($p \leq 0.05$).

The inclusion of botanical extracts in broiler diets in the present study resulted in a remarkable increase in the meat levels of unsaturated fatty acids and a clear decline in saturated fats. The meat of the broilers

fed the negative-control diet (B group) presented the highest SFA and lowest UFA levels among all groups. On the other hand, the meat of broilers fed the negative control diet with the combination of the evaluated botanical extract (group H) contained 10% more UFA due to the reduction of almost 10% SFA, almost the double of PUFA and three times higher omega-3 and omega-6 fatty acids compared with the B group (negative control). It was demonstrated that UFA levels and UFA/SFA ratios in the breast and drumstick meat increased with the dietary supplementation of 2 or 5% olive oil (Zhang *et al.,* 2013). The inclusion of black cumin seeds in layer diets resulted in better fatty acid profiles and lower cholesterol and triglyceride levels in the egg (Yalc *et al.,* 2009; Nabiela *et al.,* 2006). Similarly, the botanical compound quercetin reduced SFA levels in broiler fats, especially of myristic and stearic acids (Sohaib *et al.,* 2015). Basil seed and chamomile in broiler diet reduced muscle lipid and triglyceride levels (Ahmed *et al.,* 2015). It was demonstrated that chia seeds reduced SFA and SFA:PUFA and omega-6:omega-3 ratios in chicken meat (Ayerza *et al.,* 2002). The meat fatty acid profiles obtained in the present study when botanical extracts were included in the diet are consistent with the results of Nkukwana *et al.* (2014), who reported higher C18,C 18-3n6,C 20, C20-2,C 20-3n6 and C 15 contents in the breast meat of broilers fed *Moringa olifera* leaf meal. It is very clear that botanical extracts with high levels of antioxidant compounds can protect oils and proteins in living tissues (Zhang *et al.,* 2010).

CONCLUSION

The extracts of spices and sweet violet flowers evaluated positively influenced broiler physiology. Overall, they improved meat quality as shown by the higher meat content of amino acids (especially of essential AA), as well as by the reduced meat levels of saturated fatty acids and the increased levels of UFA, MUFA, PUFA, and omega-3 and optimal fatty acid ratios. In conclusion, the evaluated natural botanical antioxidants are good modulators of amino acid and fatty acid contents in broiler meat, and the inclusion of a combination of ajwain, fenugreek and sweet violet extracts in the diet may efficiently replace the conventional antibiotic growth promoters.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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