

# Independent and combined effects of *Satureja khuzistanica* essential oils and dietary acetic acid on fatty acid profile in thigh meat in male broiler chicken

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**ABSTRACT** A 2 × 6 factorial experiment was conducted to evaluate the effect of *Satureja khuzistanica* essential oils (SkEO; 0, 200, 300, 400, 500, and 600 mg/bird/day) administered via oral gavage and dietary acetic acid (AA; 0 and 20 g/1 kg) on fatty acids (FA) composition in thigh meat of Ross 308 broiler chickens at days 34, 38, and 42 of age. Dietary AA reduced DWG, DFI, and European economic efficiency index, and increased FCR compared with the nonacidified diet. In day 34 of age, saturated FA (SFA) percentage reduced and polyunsaturated FA (PUFA), n-3, and n-6 percentages increased in the birds that received 400 mg SkEO. Mean monounsaturated FA (MUFA) percentage was greater, whereas PUFA, n-3, n-6, and total FA (TFA) percentages were lesser in the birds fed on the acidified diet. In day 38 of age, mean PUFA, TFA, n-3, and n-6 percentages were greater while MUFA and cis FA (CFA) concentrations were lesser in the thigh muscle of the birds

that received 400 mg SkEO. Mean MUFA, PUFA, n-3, n-6, CFA, and TFA percentages were lower in the birds maintained on the acidified diet. In day 42 of age, mean SFA percentage reduced in the birds given 300 mg SkEO, while TFA percentage lowered in the birds that received 200 and 600 mg SkEO. The acidified diet decreased MUFA, TFA, and CFA percentage and increased SFA and the n-6 to n-3 fatty acids ratio of thigh meat in chicken. The results led to the conclusion that the daily enteral administration of SkEO through oral gavage may feasibly modify the fatty acids profile of thigh meat in favor of increased PUFA. Dietary AA and its interaction with SkEO inconsistently modified concentration of certain classes of fatty acids in broiler thigh meat, particularly in advanced ages. Almost all alterations induced by AA-involving treatments in fatty acids composition of thigh meat were on the contrary to the SkEO influences as they were in favor of an increased SFA proportion.

**Key words:** fatty acid composition, meat quality, n6 to n3 ratio, organic acid, savory

**Abbreviations:** AA: acetic acid, CFA: *cis* fatty acid, DWG: daily weight gain, DFI: daily feed intake, EEEI: European economic efficiency index, FA: Fatty acid, FAME: Fatty acid methyl esters, FCR: feed conversion ratio, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, SkEO: *Satureja khuzistanica* essential oil, TFA: *trans* fatty acid

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## INTRODUCTION

Most commercial chicken meat is always criticized for insalubrious composition of fatty acids notwithstanding several desired nutritional features, such as high protein and low lipid content (Tan et al., 2018). Chicken meat

should ensure greater content of n-3 fatty acids (FA), a balanced n-3 polyunsaturated fatty acids (PUFA)/n-6 PUFA ratio, and a proper PUFA/saturated FA ratio (PUFA/SFA) because solid evidence exists to support their roles in reducing the risk of certain metabolic diseases such as coronary artery disease, hypertension, diabetes, inflammatory, and autoimmune disorders (Briggs et al., 2017). Dietary manipulation has been recognized as a practically adoptable method to modify PUFA proportions in chicken meat (Rymer and Givens, 2005; Cui et al., 2019) because fatty acid profile of chicken meat is highly correlated with the fatty acid composition of the food (Ramay and Yalçın, 2019).

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Therefore, it is widely accepted that n3/n6 ratio in chicken meat may forthrightly increase through supplementing diets with n3-enriched fat sources such as fish oil/meal, marine algae, and flax seed oil/seed.

However, inclusion of such fat sources reportedly brings about certain adverse effects leading to quality deterioration of meat (Zhao et al., 2019). At the outset, the oxidative stability of meat decreases with high levels of dietary long chain n-3 FA, a phenomenon which adversely affects meat flavor and consumer acceptability (Forte et al., 2018; Abd El-Samee et al., 2019). In addition, use of n3-enriched fat sources may be accompanied by a lack of cost-effective accessibility and probable risk of rancidity of commercial preparation before or during inclusion in diet. Therefore, the current grains-derived starch-enriched diets for broiler chicken have to be modified using certain ingredients or adding appropriate additives other than n-3 enriched feed ingredients to realize increased n3/n6 ratio with no off-flavor consequences of meat. Such hypothesis may be tested through feeding certain easily absorbable active moieties which impose direct effects on cell metabolism. We selected carvacrol and acetic acid for the same purpose while the hypothesis was already considered by Forte et al., (2018), Shirani et al., (2019), and Zhao et al., (2019) by inclusion of oregano (*Origanum vulgare* L.) aqueous extract, *Pulicaria gnaphalodes* powder, and chlorogenic acid-enriched extract from *Eucommia ulmoides* leaf into broiler diets, respectively.

Carvacrol is validated for antioxidant (Lahmara et al., 2018), anti-inflammatory (Liu et al., 2019), and fat metabolism influencing (Khosravinia, 2015; Reis et al., 2018) properties based on results of the several in vitro and in vivo studies. This small fat-soluble molecule is found in medicinal plants belonging to Lamiaceae family which attracted great concern by poultry nutritionists owing to their high contents of a essential oils rich in many convinced phenolic monoterpene metabolites including carvacrol, menthol, and thymol. *Satureja khuzistanica*, as a member of the same family, contains up to 4.5% essential oils comprising up to 94% of carvacrol (Khosravinia, 2016). It was shown that carvacrol encounters fat metabolism in definite biochemical pathways (Shahbazi et al., 2014; Alagawany et al., 2015) in animals but its effect on fatty acid composition in chicken meat has been investigated in few studies (Forte et al., 2018); thus, it needs to be characterized in detail.

Traditionally, vinegar (a solution of 4 to 5 percent acetic acid by weight) is recommended as a lipid metabolism influencing medication in humans (Petsiou et al., 2014) as well as chickens. Commercially, acetic acid (AA) is included in acidifier preparations contributing profitability in poultry diets as a nonantibiotic additive. Acetic acid may favor lipolysis in the adipose tissue, a phenomenon that may led to a plasma-modified fatty acid profile and plasma-decreased cholesterol content in broiler chicken (Pucci et al., 2000). Moreover, it was demonstrated that AA activates AMPK $\alpha$ , which in turn upregulates the expression of lipid oxidation genes

in the liver to reduce fat accumulation (Li et al., 2018). Based on these observations, AA may potentially be used to modify the fatty acid profile of chicken meat independently of fatty acid composition in diet.

We hypothesized that daily administration of SkEO by oral gavage and concomitant inclusion of AA into diet may be used as a nutritional tool to impose modifications in fatty acid profile in broiler meat independent of the supplementary lipid constituents in diet. Therefore, this study intended to evaluate the effects of SkEO and dietary AA supplementation as well as their interactions on fatty acid composition of thigh meat in male broiler chickens.

## MATERIALS AND METHODS

### *Birds and Diets*

Two hundred fifty-two male Ross 308 10-day-old broiler chicks with an average weight of  $300 \pm 5$  g were used in 84 wire cages up to day 42 of age. The birds were chosen from a male pre-experimental flock raised in a wood shaving furnished floor pen placed in a power-ventilated grow-out house up to day 10 of age, following the protocols of Animal Care Committee of the Lorestan University, Iran. The environmental temperature and relative humidity were kept at  $32 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$ , respectively, during the early raising period. The birds were also received a 23:1 h lighting to darkness lightening regimen, except for the first 3 D when illumination provided clock round. Throughout the same period, the birds ensured free access to water and a crumble prestarter diet and vaccinated against Newcastle and Bronchitis viruses on the day 4 of age. At the commencement of the day 10, the experimental birds were transferred to the battery cages ( $45 \times 50 \times 40$  cm for length, width, and attitude, respectively) arranging in 7 rows (blocks) perpendicular to the air flow direction, where the birds underwent a 3-day acclimation period before starting the experiment.

At day 14, experimental period initiated by feeding a basal starter (14 to 21 D), grower (22 to 35 D), and finisher (36 to 42 D) pelleted diet (Table 1) with or without 20 g/1 kg AA inclusion. Then, birds in each group received 0, 200, 300, 400, 500, 600 mg/bird/day SkEO via oral gavage daily. Lightening schedule was set identical to the pre-experimentation period. Ambient temperature during the first week of the experimentation period was kept at  $29^\circ\text{C}$  and then gradually reduced by  $2^\circ\text{C}$  to  $3^\circ\text{C}$  weekly to reach about  $24^\circ\text{C}$  at the end of the fourth wk when it was kept constant.

### *Preparation of Agents*

The SkEO preparation was provided 0from Khorraman Medicinal Plants Laboratory, Khorramabad, Lorestan. Based on the analysis conducted using GC-Mass, it contained carvacrol (94.50%), p-Cymene (0.96%), and  $\gamma$ -Terpenene (0.51%) and many other ingredients in

**Table 1.** Ingredient, nutrient, and fatty acid composition of the basal diets (%).

| Ingredients (%)                   | Prestarter (1-10 day) | Starter (11-21 day) | Grower (22-35 day) | Finisher (36-42 day) |
|-----------------------------------|-----------------------|---------------------|--------------------|----------------------|
| Yellow maize                      | 61.32                 | 63.08               | 66.32              | 68.72                |
| Soybean meal                      | 33.94                 | 31.54               | 28.80              | 26.50                |
| Soybean oil                       | 1.00                  | 1.50                | 1.50               | 1.50                 |
| Calcium phosphate                 | 1.30                  | 1.40                | 1.10               | 1.10                 |
| CaCo <sub>3</sub>                 | 1.33                  | 1.34                | 1.20               | 1.20                 |
| DL-Methionine                     | 0.27                  | 0.28                | 0.20               | 0.10                 |
| L-Lysine HCl                      | 0.02                  | 0.04                | 0.05               | 0.05                 |
| Salt                              | 0.14                  | 0.14                | 0.14               | 0.14                 |
| Vitamin premix <sup>1</sup>       | 0.25                  | 0.25                | 0.25               | 0.25                 |
| Mineral premix <sup>2</sup>       | 0.25                  | 0.25                | 0.25               | 0.25                 |
| L-Threonine                       | 0.04                  | 0.04                | 0.05               | 0.05                 |
| Sodium bicarbonate                | 0.14                  | 0.14                | 0.14               | 0.14                 |
| Nutrient composition (calculated) |                       |                     |                    |                      |
| ME (kcal/kg)                      | 2942                  | 3014                | 3055               | 3084                 |
| Crude protein (%)                 | 20.43                 | 19.57               | 18.62              | 17.72                |
| Crude fiber (%)                   | 2.55                  | 2.52                | 2.52               | 2.51                 |
| Crude fat (%)                     | 3.49                  | 4.04                | 4.15               | 4.23                 |
| Lysine (%)                        | 1.09                  | 1.04                | 0.97               | 0.92                 |
| Threonine (%)                     | 0.79                  | 0.76                | 0.72               | 0.68                 |
| Methionine + cystine (%)          | 0.59                  | 0.57                | 0.54               | 0.52                 |
| Tryptophan (%)                    | 0.28                  | 0.27                | 0.25               | 0.24                 |
| Calcium (%)                       | 0.85                  | 0.87                | 0.75               | 0.72                 |
| Available P (%)                   | 0.42                  | 0.43                | 0.37               | 0.36                 |
| Na (%)                            | 0.14                  | 0.14                | 0.14               | 0.14                 |
| Fatty acid <sup>3</sup>           |                       |                     |                    |                      |
| SFA                               | 20.62                 | 20.61               | 20.41              | 20.64                |
| MUFA                              | 29.10                 | 29.15               | 29.48              | 29.55                |
| PUFA                              | 49.58                 | 49.59               | 49.96              | 49.79                |
| n-3                               | 3.97                  | 3.88                | 3.82               | 3.85                 |
| n-6                               | 45.61                 | 45.71               | 46.14              | 45.94                |
| n-6/n-3                           | 11.49                 | 11.78               | 12.08              | 11.93                |
| TFA                               | 0.38                  | 0.36                | 0.28               | 0.31                 |
| CFA                               | 78.30                 | 78.38               | 79.16              | 79.03                |

<sup>1</sup>Provides per kg of feed: vitamin A, 9,000,000 international units; vitamin D<sub>3</sub>, 2,000,000 international units; vitamin E, 18,000 units; vitamin K<sub>3</sub>, 2,000 mg; vitamin B<sub>1</sub>, 1,800 mg; vitamin B<sub>2</sub>, 6,600 mg; vitamin B<sub>3</sub>, 10,000 mg; vitamin B<sub>5</sub>, 30,000 mg; vitamin B<sub>6</sub>, 3,000 mg; vitamin B<sub>9</sub>, 1,000 mg; vitamin B<sub>12</sub>, 15 mg; vitamin H<sub>2</sub>, 100 mg; choline chloride, 250,000 mg; antioxidant, 1,000 mg.

<sup>2</sup>Provides per kg of feed: manganese, 100,000 mg; zinc, 85,000 mg; copper, 10,000 mg; selenium, 200 mg; iodine, 1,000 mg; iron, 50,000 mg.

<sup>3</sup>SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n<sub>6</sub>/n<sub>3</sub>: the ratio of n-6 to n-3 PUFA; TFA: *trans* fatty acid; CFA: *cis* fatty acid.

trace. Acetic acid obtained from Kimia Company, Tehran, Iran, with 99.9% purity.

## Performance Records

Individual body weight and pen-wise feed intake were recorded in days 14 and 42 of age and used to calculate daily weight gain (**DWG**), daily feed intake (**DFI**), and feed conversion ratio (**FCR**) for entire experimental period. European economic efficiency index (**EEEI**) at day 42 of age was calculated as  $EEEI = [(LW \times S) / (FCR \times AS)]$ , where LW is live weight (kg), S is survival rate (%), FCR is feed conversion ratio and AS is the slaughter age in terms of day (Euribrid, 1994).

## Slaughter and Measurements

At days 34, 38, and 42 of age, one bird from each replicate (7 birds per treatment) was randomly taken, weighed, and then killed. The thigh meat was dissected from each half-carcass for the determination the FA composition.

## Fatty Acid Identification

**Lipid Extraction** Primarily, about 40 g of the finely ground meat collected from each sample bird was weighed, and then 80 mL of methanol was added and homogenized for 2 min then again 80 mL of methanol was added and homogenized in a Waring blender for 2 min. After that, 80 mL of chloroform was added and then blended for 2 min. Next, 80 mL of chloroform was added and blended for 2 min. Then the water was added according to the sample water content. The mixture was filtered through Whatman paper n<sup>o</sup> 1 with vacuum, and the filtrate was transferred to a separatory funnel. After phase separation, the chloroform phase was collected, and the solvent was evaporated by using a rotary evaporator.

**Saponification** One gram of extract was subjected to saponification adding 6 ml of methanol and gently shaking until becoming uniform. A 4 ml solution of KOH in methanol at 0.5 N was added and subjected to reflux for 20 min at 90°C.

**Methylation** Three ml of BF<sub>3</sub> was added to 50 ml of saponified oil and submitted to reflux for 30 min

with constant agitation using a magnetic stirrer. Agitation was continuously maintained during cooling when 3 ml of distilled water was added. The organic phase of the sample was extracted 3 times with 2 ml of hexane using a separatory funnel. The organic phase was mixed and 0.5 g of anhydrous  $MgSO_4$  was added to eliminate excess humidity and then passed through Whatman #1 paper to filter out the  $MgSO_4$ .

**Gas Chromatography–Mass Spectrometry** The methyl esters were analyzed using a gas chromatograph coupled with a TurboMass-Autosystem XL mass spectrometer connected to flame ionization detector (FID) and a computer with TotalChrom Navigator Software (Aligent, 7890B). The samples were injected in a 20% splitless mode in an Elite WAX column (polyethylene glycol of 105 m, 0.25 mm, RESTEK, RTX-2330). Injector and FID temperatures were maintained at 230°C. The oven temperature programming was as follows: 180°C for 3 min followed by an increase until 250°C. This temperature was maintained for 15 min.

### Statistical Analysis

The experiment was conducted in a  $2 \times 6$  factorial fashion with 12 treatments in 7 replicates (cage) of 3 birds each. A complete randomized block design was used to evaluate the response of broiler chickens to SkEO (0, 200, 300, 400, 500, or 600 mg/bird/day)

administration by oral gavage combined with 2 levels of dietary AA. All data were analyzed using PROC Mixed in Statistical Analysis System, version 9.1 (SAS Institute, 2003). The Tukey test was used for multiple treatment comparisons (Kramer, 1956). For all tests, the maximum likelihood for type III error was set at 5 percent ( $P < 0.05$ ). Specific orthogonal contrasts (linear and quadratic) were applied to determine the effects of varying levels (0, 200, 300, 400, 500, and 600 mg/bird/day) of SkEO administered through oral gavage.

## RESULTS

Dietary AA reduced DWG and DFI of the birds by 6.22 g (10.27%) and 7.34 g (6.60%), respectively, compared with the control birds ( $P < 0.05$ ). Feed conversion ratio of broiler birds fed diets containing AA increased (1.93 vs. 1.84) compared with the birds maintaining on the nonacidified diet ( $P < 0.05$ ). Supplementation of AA in diet, therefore, decreased EEEI by 40 units compared with the control birds during days 14 to 42 of the birds' age (192 vs. 232) ( $P < 0.05$ ; Table 2).

In day 34 of age, saturated fatty acids (SFA) percentage reduced and polyunsaturated fatty acids (PUFA), n-3, and n-6 percentages increased in the birds that received 400 mg SkEO by oral gavage compared with the birds receiving other treatments. Mean *cis* fatty acid (CFA) proportion in the birds that received

**Table 2.** Mean daily weight gain (DWG), daily feed intake (DFI), feed conversation ratio (FCR), and European economic efficiency index (EEEI) in broiler chickens that received *Satureja khuzistanica* essential oils (SkEO) by gavage method and acetic acid (AA) in days 14 to 42 of age.

| SkEO (mg/bird/day) | AA (g/kg) | DWG                | DFI                 | FCR               | EEEI             |
|--------------------|-----------|--------------------|---------------------|-------------------|------------------|
| 0                  |           | 53.71              | 105.40              | 2.00              | 185              |
| 200                |           | 60.77              | 111.84              | 1.85              | 230              |
| 300                |           | 59.28              | 110.13              | 1.88              | 224              |
| 400                |           | 57.10              | 106.79              | 1.89              | 211              |
| 500                |           | 56.95              | 106.16              | 1.87              | 209              |
| 600                |           | 56.75              | 104.44              | 1.84              | 212              |
| SEM                |           | 2.42               | 3.17                | 0.04              | 16.00            |
|                    | 0         | 60.54 <sup>a</sup> | 111.13 <sup>a</sup> | 1.84 <sup>b</sup> | 232 <sup>a</sup> |
|                    | 20        | 54.32 <sup>b</sup> | 103.79 <sup>b</sup> | 1.93 <sup>a</sup> | 192 <sup>b</sup> |
| SEM                |           | 1.39               | 1.88                | 0.02              | 9.23             |
| 0                  | 0         | 56.81              | 108.35              | 1.90              | 211              |
| 0                  | 2         | 57.68              | 104.50              | 1.84              | 189              |
| 200                | 0         | 60.67              | 111.10              | 1.86              | 230              |
| 200                | 2         | 58.23              | 107.50              | 1.90              | 218              |
| 300                | 0         | 58.95              | 110.10              | 1.87              | 226              |
| 300                | 2         | 58.71              | 107.40              | 1.79              | 217              |
| 400                | 0         | 58.70              | 108.50              | 1.85              | 225              |
| 400                | 2         | 56.50              | 104.50              | 1.85              | 210              |
| 500                | 0         | 58.28              | 108.10              | 1.86              | 223              |
| 500                | 2         | 55.30              | 104.70              | 1.91              | 201              |
| 600                | 0         | 58.65              | 108.25              | 1.84              | 222              |
| 600                | 2         | 55.15              | 104.65              | 1.87              | 203              |
| SEM                |           | 2.10               | 2.10                | 0.04              | 14.50            |
|                    |           | <i>P</i> -values   |                     |                   |                  |
| SkEO               |           | 0.426              | 0.515               | 0.264             | 0.459            |
| AA                 |           | 0.002              | 0.005               | 0.025             | 0.004            |
| SkEO $\times$ AA   |           | 0.621              | 0.583               | 0.675             | 0.681            |
| Linear             |           | 0.938              | 0.339               | 0.090             | 0.674            |
| Quadratic          |           | 0.168              | 0.207               | 0.342             | 0.183            |

<sup>a,b</sup>Means in the same column without the same superscript differ significantly ( $P < 0.05$ ).

Abbreviation: SEM, standard error of the mean.



**Table 3.** Mean saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3, n-6, n-6/n-3 ratio, *trans* fatty acids (TFA), and *cis* fatty acids (CFA) concentrations (%)<sup>1</sup>/ratios in thigh meat in broiler chickens received *Satureja khuzistanica* essential oils (SkEO) by gavage method and dietary acetic acid (AA) in day 34 of age.

| SkEO (mg/bird/day) | AA (g/kg) | SFA                      | MUFA                 | PUFA                   | n-3                   | n-6                      | n-6/n-3 | TFA                    | CFA                      |
|--------------------|-----------|--------------------------|----------------------|------------------------|-----------------------|--------------------------|---------|------------------------|--------------------------|
| 0                  |           | 30.00 <sup>a,b</sup>     | 44.35 <sup>c</sup>   | 23.99 <sup>b</sup>     | 1.618 <sup>b</sup>    | 22.38 <sup>a</sup>       | 13.84   | 0.385 <sup>b,c,d</sup> | 67.96 <sup>c</sup>       |
| 200                |           | 29.93 <sup>a,b</sup>     | 46.45 <sup>a,b</sup> | 21.89 <sup>d,e</sup>   | 1.512 <sup>b,c</sup>  | 20.38 <sup>c,d</sup>     | 13.54   | 0.532 <sup>a</sup>     | 67.81 <sup>c</sup>       |
| 300                |           | 29.24 <sup>b,c</sup>     | 46.37 <sup>a,b</sup> | 22.99 <sup>c</sup>     | 1.583 <sup>b,c</sup>  | 21.40 <sup>b</sup>       | 13.55   | 0.348 <sup>d</sup>     | 69.00 <sup>a</sup>       |
| 400                |           | 28.42 <sup>c</sup>       | 44.60 <sup>c</sup>   | 24.74 <sup>a</sup>     | 1.786 <sup>a</sup>    | 22.96 <sup>a</sup>       | 12.84   | 0.412 <sup>b,c</sup>   | 68.93 <sup>a,b</sup>     |
| 500                |           | 29.96 <sup>a,b</sup>     | 45.88 <sup>b</sup>   | 22.27 <sup>d</sup>     | 1.530 <sup>b,c</sup>  | 20.74 <sup>c</sup>       | 13.57   | 0.365 <sup>c,d</sup>   | 67.79 <sup>c</sup>       |
| 600                |           | 30.15 <sup>a</sup>       | 47.28 <sup>a</sup>   | 21.28 <sup>e</sup>     | 1.469 <sup>c</sup>    | 19.81 <sup>d</sup>       | 13.52   | 0.423 <sup>b</sup>     | 68.14 <sup>b,c</sup>     |
| SEM                |           | 0.199                    | 0.218                | 0.152                  | 0.032                 | 0.144                    | 0.281   | 0.012                  | 0.202                    |
|                    | 0         | 29.63                    | 45.41 <sup>b</sup>   | 23.30 <sup>a</sup>     | 1.629 <sup>a</sup>    | 21.67 <sup>a</sup>       | 13.33   | 0.444 <sup>a</sup>     | 68.27                    |
|                    | 20        | 29.61                    | 46.23 <sup>a</sup>   | 22.42 <sup>b</sup>     | 1.536 <sup>b</sup>    | 20.88 <sup>b</sup>       | 13.63   | 0.378 <sup>b</sup>     | 68.27                    |
| SEM                |           | 0.119                    | 0.127                | 0.088                  | 0.019                 | 0.083                    | 0.162   | 0.007                  | 0.116                    |
| 0                  | 0         | 30.91 <sup>a</sup>       | 43.56 <sup>d</sup>   | 24.05 <sup>b</sup>     | 1.63 <sup>b</sup>     | 22.41 <sup>b</sup>       | 13.54   | 0.410 <sup>b,c</sup>   | 67.20 <sup>d</sup>       |
| 0                  | 2         | 29.09 <sup>c,d</sup>     | 45.14 <sup>c</sup>   | 23.94 <sup>b</sup>     | 1.60 <sup>b,c</sup>   | 22.34 <sup>b,c</sup>     | 13.62   | 0.360 <sup>b,c</sup>   | 68.72 <sup>a,b,c</sup>   |
| 200                | 0         | 29.36 <sup>b,c,d</sup>   | 45.30 <sup>c</sup>   | 23.37 <sup>b,c</sup>   | 1.65 <sup>b</sup>     | 21.72 <sup>b,c,d</sup>   | 13.45   | 0.664 <sup>a</sup>     | 68.00 <sup>b,c,d</sup>   |
| 200                | 2         | 30.51 <sup>a,b</sup>     | 47.61 <sup>b</sup>   | 20.42 <sup>f</sup>     | 1.38 <sup>c,d</sup>   | 19.04 <sup>f</sup>       | 12.50   | 0.400 <sup>b,c</sup>   | 67.62 <sup>c,d</sup>     |
| 300                | 0         | 29.67 <sup>a,b,c,d</sup> | 46.47 <sup>b,c</sup> | 22.98 <sup>b,c,d</sup> | 1.62 <sup>b</sup>     | 21.36 <sup>c,d,e</sup>   | 13.47   | 0.360 <sup>b,c</sup>   | 69.09 <sup>a,b</sup>     |
| 300                | 2         | 28.81 <sup>d,e</sup>     | 46.26 <sup>b,c</sup> | 22.99 <sup>b,c,d</sup> | 1.54 <sup>b,c,d</sup> | 21.45 <sup>b,c,d,e</sup> | 13.49   | 0.335 <sup>c</sup>     | 68.92 <sup>a,b,c</sup>   |
| 400                | 0         | 27.54 <sup>e</sup>       | 41.43 <sup>e</sup>   | 28.52 <sup>a</sup>     | 2.01 <sup>a</sup>     | 26.52 <sup>a</sup>       | 12.95   | 0.423 <sup>b</sup>     | 69.53 <sup>a</sup>       |
| 400                | 2         | 29.31 <sup>b,c,d</sup>   | 47.76 <sup>a,b</sup> | 20.96 <sup>e,f</sup>   | 1.57 <sup>b,c,d</sup> | 19.40 <sup>f</sup>       | 13.50   | 0.400 <sup>b,c</sup>   | 68.32 <sup>a,b,c,d</sup> |
| 500                | 0         | 29.65 <sup>a,b,c,d</sup> | 46.40 <sup>b,c</sup> | 22.00 <sup>d,e</sup>   | 1.52 <sup>b,c,d</sup> | 20.48 <sup>e</sup>       | 13.45   | 0.363 <sup>b,c</sup>   | 68.03 <sup>b,c,d</sup>   |
| 500                | 2         | 30.28 <sup>a,b,c</sup>   | 45.36 <sup>c</sup>   | 22.55 <sup>c,d</sup>   | 1.54 <sup>b,c,d</sup> | 21.01 <sup>d,e</sup>     | 13.53   | 0.368 <sup>b,c</sup>   | 67.54 <sup>d</sup>       |
| 600                | 0         | 30.62 <sup>a,b</sup>     | 49.29 <sup>a</sup>   | 18.91 <sup>g</sup>     | 1.35 <sup>d</sup>     | 17.56 <sup>g</sup>       | 13.47   | 0.443 <sup>b</sup>     | 67.75 <sup>b,c,d</sup>   |
| 600                | 2         | 29.69 <sup>a,b,c,d</sup> | 45.28 <sup>c</sup>   | 23.64 <sup>b</sup>     | 1.59 <sup>b,c</sup>   | 22.05 <sup>b,c</sup>     | 13.52   | 0.403 <sup>b,c</sup>   | 68.52 <sup>a,b,c,d</sup> |
| SEM                |           | 0.278                    | 0.308                | 0.216                  | 0.045                 | 0.204                    | 0.253   | 0.117                  | 0.285                    |
|                    |           | <i>P</i> -value          |                      |                        |                       |                          |         |                        |                          |
| SkEO               |           | <0.001                   | <0.001               | <0.001                 | <0.001                | <0.001                   | 0.246   | <0.001                 | <0.001                   |
| AA                 |           | <0.001                   | <0.001               | <0.001                 | 0.001                 | <0.001                   | 0.205   | <0.001                 | 0.970                    |
| SkEO × AA          |           | <0.001                   | <0.001               | <0.001                 | <0.001                | <0.001                   | 0.362   | <0.001                 | <0.001                   |
| Linear             |           | <0.001                   | <0.001               | <0.001                 | 0.080                 | <0.001                   | 0.387   | 0.018                  | 0.659                    |
| Quadratic          |           | 0.328                    | 0.001                | 0.001                  | 0.001                 | <0.001                   | 0.108   | 0.335                  | <0.001                   |

<sup>a-f</sup>Means in the same column without the same superscript differ significantly ( $P < 0.05$ ).

Abbreviation: SEM, standard error of the mean.

<sup>1</sup>As the percent of fatty acids.

200 mg SkEO ( $P < 0.05$ ) and monounsaturated fatty acids (MUFA) percentage in the birds that received 600 mg SkEO ( $P < 0.05$ ) were greater than those in the birds subjected to the other treatments. The ratio of n-6 to n-3 fatty acids was affected by administration of neither SkEO nor the acidified diet ( $P > 0.05$ ). Mean MUFA was greater while PUFA, n-3, n-6, and *trans* fatty acid (TFA) percentages were lesser in the birds grown on the acidified diet ( $P < 0.05$ ; Table 3). A significant SkEO × AA interaction was observed on SFA, MUFA, PUFA, n-3, n-6, TFA, and CFA concentration in thigh meat of broiler chickens. Interestingly, SFA and MUFA percentage decreased in thigh meat of the birds administered with 400 mg SkEO and fed on the nonacidified diet. Mean PUFA, n-3, n-6, and CFA percentages were increased in the birds that received 400 mg SkEO and offered the nonacidified diet, while TFA percentage was lower in the birds that received 300 mg SkEO and the acidified diet than those that received 200, 400, and 600 mg SkEO by oral gavage and grown on the diet with no AA inclusion ( $P < 0.05$ ; Table 3).

In day 38 of age, PUFA, TFA, n-3, and n-6 percentages were greater in thigh meat of the birds that received 400 mg SkEO, while the MUFA and CFA concentrations were lesser in the same birds than birds treated with other SkEO and AA combinations ( $P < 0.05$ ; Table 5). Mean SFA percentage was affected by orally gavaging SkEO in a quadratic trend ( $P < 0.05$ ). Mean

MUFA, PUFA, n-3, n-6, CFA, and TFA percentages were lesser in the birds maintained on the acidified diet, while SFA percentage increased in the same birds compared with the control birds ( $P < 0.05$ ; Table 4). A significant SkEO × AA interaction was observed on SFA, MUFA, PUFA, n-3, n-6, n-6/n-3, TFA, CFA concentration in thigh meat of broiler chickens. Mean SFA increased in the birds that received 200 mg SkEO and fed on the acidified diet ( $P < 0.05$ ). However, MUFA percentage reduced in the birds that received 400 mg SkEO through oral gavage and grown on the acidified diet, but PUFA, TFA, and n-6 fatty acids percentages were greater in the birds administering 400 mg SkEO and grown on nonacidified diets compared with the control birds. Mean n-3 fatty acids percentage increased in the thigh meat of the birds receiving 500 mg SkEO and fed on the nonacidified diet than control birds. Moreover, the ratio of n-6 to n-3 fatty acids reduced in the birds that received 300 mg SkEO and were fed by the acidified diet ( $P < 0.05$ ; Table 4).

In day 42 of age, oral administration of SkEO by gavage increased PUFA percentage in thigh meat compared with the control birds ( $P < 0.05$ ). Mean SFA percentage reduced in the birds that received 300 mg SkEO, while TFA percentage lowered in the birds that received 200 and 600 mg SkEO than those experiencing other treatments ( $P < 0.05$ ). Mean n-3 and n-6 fatty acids percentage was affected by oral gavaging of SkEO in a quadratic and linear fashion,

**Table 4.** Mean saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3, n-6, n-6/n-3 ratio, *trans* fatty acids (TFA), and *cis* fatty acids (CFA) concentrations (%)<sup>1</sup>/ratios in thigh meat in broiler chickens that received *Satureja khuzistanica* essential oils (SkEO) by oral gavaging method and dietary acetic acid (AA) in day 38 of age.

| SkEO (mg/bird/day) | AA (g/kg) | SFA                    | MUFA                 | PUFA                   | n-3                   | n-6                    | n-6/n-3                | TFA                | CFA                      |
|--------------------|-----------|------------------------|----------------------|------------------------|-----------------------|------------------------|------------------------|--------------------|--------------------------|
| 0                  |           | 29.33 <sup>c</sup>     | 47.05 <sup>a</sup>   | 22.120 <sup>b</sup>    | 1.435 <sup>b,c</sup>  | 20.69 <sup>b</sup>     | 14.42                  | 0.364 <sup>c</sup> | 68.81 <sup>a</sup>       |
| 200                |           | 30.64 <sup>a</sup>     | 46.16 <sup>b</sup>   | 21.73 <sup>b</sup>     | 1.386 <sup>c</sup>    | 20.34 <sup>b</sup>     | 14.73                  | 0.359 <sup>c</sup> | 67.53 <sup>b,c</sup>     |
| 300                |           | 30.65 <sup>a</sup>     | 45.55 <sup>c</sup>   | 22.146 <sup>b</sup>    | 1.473 <sup>b</sup>    | 20.67 <sup>b</sup>     | 14.08                  | 0.355 <sup>c</sup> | 67.34 <sup>b,c</sup>     |
| 400                |           | 30.17 <sup>a,b</sup>   | 43.95 <sup>e</sup>   | 24.187 <sup>a</sup>    | 1.587 <sup>a</sup>    | 22.60 <sup>a</sup>     | 14.25                  | 1.036 <sup>a</sup> | 67.10 <sup>c</sup>       |
| 500                |           | 30.07 <sup>a,b</sup>   | 44.86 <sup>d</sup>   | 23.65 <sup>a</sup>     | 1.541 <sup>a</sup>    | 22.10 <sup>a</sup>     | 14.38                  | 0.290 <sup>c</sup> | 68.22 <sup>a,b</sup>     |
| 600                |           | 29.69 <sup>b,c</sup>   | 46.32 <sup>b</sup>   | 22.157 <sup>b</sup>    | 1.447 <sup>b,c</sup>  | 20.71 <sup>b</sup>     | 14.34                  | 0.523 <sup>b</sup> | 67.95 <sup>a,b,c</sup>   |
| SEM                |           | 0.187                  | 0.158                | 0.158                  | 0.017                 | 0.159                  | 0.214                  | 0.019              | 0.225                    |
|                    | 0         | 29.63 <sup>b</sup>     | 45.81 <sup>a</sup>   | 23.02 <sup>a</sup>     | 1.500 <sup>a</sup>    | 21.52 <sup>a</sup>     | 14.37                  | 0.596 <sup>a</sup> | 68.23 <sup>a</sup>       |
|                    | 20        | 30.55 <sup>a</sup>     | 45.50 <sup>b</sup>   | 22.31 <sup>b</sup>     | 1.457 <sup>b</sup>    | 20.85 <sup>b</sup>     | 14.37                  | 0.380 <sup>b</sup> | 67.42 <sup>b</sup>       |
| SEM                |           | 0.137                  | 0.110                | 0.091                  | 0.013                 | 0.092                  | 0.154                  | 0.011              | 0.134                    |
| 0                  | 0         | 28.38 <sup>e</sup>     | 48.09 <sup>a</sup>   | 21.93 <sup>e</sup>     | 1.44 <sup>d</sup>     | 20.49 <sup>e</sup>     | 14.23 <sup>a,b,c</sup> | 0.370 <sup>c</sup> | 69.65 <sup>a</sup>       |
| 0                  | 2         | 30.28 <sup>b,c</sup>   | 46.02 <sup>c,d</sup> | 22.31 <sup>e</sup>     | 1.43 <sup>d</sup>     | 20.88 <sup>d,e</sup>   | 14.62 <sup>a,b,c</sup> | 0.359 <sup>c</sup> | 67.97 <sup>b,c,d</sup>   |
| 200                | 0         | 29.03 <sup>d,e</sup>   | 44.91 <sup>e</sup>   | 24.25 <sup>a,b</sup>   | 1.58 <sup>a,b,c</sup> | 22.67 <sup>a,b</sup>   | 14.36 <sup>a,b,c</sup> | 0.364 <sup>c</sup> | 68.79 <sup>a,b</sup>     |
| 200                | 2         | 32.24 <sup>a</sup>     | 47.42 <sup>a,b</sup> | 19.21 <sup>f</sup>     | 1.19 <sup>e</sup>     | 18.01 <sup>f</sup>     | 15.10 <sup>a</sup>     | 0.354 <sup>c</sup> | 66.27 <sup>e</sup>       |
| 300                | 0         | 30.43 <sup>b,c</sup>   | 46.44 <sup>c</sup>   | 21.62 <sup>e</sup>     | 1.39 <sup>d</sup>     | 20.23 <sup>e</sup>     | 14.60 <sup>a,b,c</sup> | 0.380 <sup>c</sup> | 67.68 <sup>b,c,d,e</sup> |
| 300                | 2         | 30.87 <sup>b</sup>     | 44.66 <sup>e</sup>   | 22.68 <sup>c,d,e</sup> | 1.56 <sup>a,b,c</sup> | 21.12 <sup>c,d,e</sup> | 13.56 <sup>c</sup>     | 0.330 <sup>c</sup> | 67.01 <sup>c,d,e</sup>   |
| 400                | 0         | 29.44 <sup>c,d,e</sup> | 44.27 <sup>e,f</sup> | 24.91 <sup>a</sup>     | 1.59 <sup>a,b</sup>   | 23.32 <sup>a</sup>     | 14.70 <sup>a,b,c</sup> | 1.810 <sup>a</sup> | 67.37 <sup>b,c,d,e</sup> |
| 400                | 2         | 30.91 <sup>b</sup>     | 43.64 <sup>f</sup>   | 23.46 <sup>b,c,d</sup> | 1.59 <sup>a,b</sup>   | 21.88 <sup>b,c,d</sup> | 13.80 <sup>b,c</sup>   | 0.263 <sup>c</sup> | 66.84 <sup>e</sup>       |
| 500                | 0         | 30.08 <sup>b,c,d</sup> | 44.50 <sup>e,f</sup> | 23.76 <sup>b,c</sup>   | 1.60 <sup>a</sup>     | 22.16 <sup>b,c</sup>   | 13.86 <sup>a,b,c</sup> | 0.310 <sup>c</sup> | 67.94 <sup>b,c,d</sup>   |
| 500                | 2         | 30.06 <sup>b,c,d</sup> | 45.23 <sup>d,e</sup> | 23.55 <sup>b,c,d</sup> | 1.48 <sup>c,d</sup>   | 22.06 <sup>b,c</sup>   | 14.90 <sup>a,b</sup>   | 0.270 <sup>c</sup> | 68.50 <sup>a,b,c</sup>   |
| 600                | 0         | 30.43 <sup>b,c</sup>   | 46.62 <sup>b,c</sup> | 21.68 <sup>e</sup>     | 1.41 <sup>d</sup>     | 20.27 <sup>e</sup>     | 14.44 <sup>a,b,c</sup> | 0.343 <sup>c</sup> | 67.95 <sup>b,c,d</sup>   |
| 600                | 2         | 28.96 <sup>e</sup>     | 46.02 <sup>c,d</sup> | 22.64 <sup>d,e</sup>   | 1.49 <sup>b,c,d</sup> | 21.15 <sup>c,d,e</sup> | 14.24 <sup>a,b,c</sup> | 0.703 <sup>b</sup> | 67.96 <sup>b,c,d</sup>   |
| SEM                |           | 0.243                  | 0.210                | 0.224                  | 0.023                 | 0.225                  | 0.281                  | 0.026              | 0.316                    |
|                    |           | <i>P</i> -value        |                      |                        |                       |                        |                        |                    |                          |
| SkEO               |           | <0.001                 | <0.001               | <0.001                 | <0.001                | <0.001                 | 0.249                  | <0.001             | <0.001                   |
| AA                 |           | <0.001                 | 0.010                | <0.001                 | 0.001                 | <0.001                 | 0.976                  | <0.001             | <0.001                   |
| SkEO × AA          |           | <0.001                 | <0.001               | <0.001                 | <0.001                | <0.001                 | 0.001                  | <0.001             | <0.001                   |
| Linear             |           | 0.799                  | <0.001               | <0.001                 | <0.001                | <0.001                 | 0.340                  | <0.001             | 0.197                    |
| Quadratic          |           | <0.001                 | <0.001               | <0.001                 | <0.001                | <0.001                 | 0.415                  | <0.001             | <0.001                   |

<sup>a-f</sup>Means in the same column without the same superscript differ significantly ( $P < 0.05$ ).

Abbreviation: SEM, standard error of the mean.

<sup>1</sup>As the percent of fatty acids.

respectively ( $P < 0.05$ ). The n-6/n-3 ratio decreased in the birds that received 400 and 500 mg SkEO compared with those orally gavaging 600 mg SkEO. Mean PUFA and n-3 fatty acids percentage increased in the thigh meat of the birds treated with 600 mg SkEO. The acidified diet significantly decreased MUFA, TFA, and CFA percentage and increased SFA and the n-6 to n-3 fatty acids ratio in thigh meat of the birds ( $P < 0.05$ ; Table 3). A significant combined effect of SkEO × AA was exhibited on SFA, MUFA, PUFA, n-3, n-6, n-6/n-3, TFA, CFA, in thigh meat at day 42 of age. Mean MUFA concentration increased in the birds that received 200 mg SkEO by oral gavage synchronized with feeding by the acidified diet compared with other birds ( $P < 0.05$ ). Oral gavage of 600 mg SkEO concomitant feeding of the acidified diet increased SFA concentration and n-6/n-3 ratio in thigh meat of the birds compared to the birds receiving other treatment regimens ( $P < 0.05$ ; Table 5).

## DISCUSSION

Many studies are available concerning SkEO or its major constituent (carvacrol) effects on broiler chicken performance when administered through food and water or by gavage method (Mikaili et al., 2010; Abdel-Wareth et al., 2012; Parvar et al., 2013; Khosravinia, 2015; Khosravinia et al., 2015; Khosravinia, 2016; Shad et al., 2016; Mirderikvandi et al., 2019)). Results from

the same reports collectively reveal that a diet containing 400 mg of carvacrol/kg may improve FCR at a constant WG but it usually decreases feed intake. Water supplementation of 400 mg/L SkEO also could improve breast weight of broilers under a tropical climate (Parvar et al., 2013), results which confirmed by Khosravinia et al. in a series of studies (Khosravinia et al., 2013, Khosravinia, 2015, Khosravinia et al., 2015, Khosravinia, 2016). Such beneficial effects are mainly attributed to the carvacrol antioxidant as well as anti-inflammatory effects (Alagawany et al., 2015; Lahmara et al., 2018; Forte et al., 2018). Moreover, essential oils, such as SkEO, have shown to assist in improved digestive processes (Lee et al., 2004), increased absorption of some nutrients (Dehghani et al., 2018), and consequently enhanced growth and productive performance via modification and activation of gastrointestinal tract structure and function and to inhibit/prevent gut disorders (Alagawany et al., 2015).

Despite the considerable body of literature on effects of phytochemical additives on productive performance in birds, scarce information on influence of the same additives on meat composition has been published and then conveyed to the public. It was shown that addition of thymol and carvacrol to diet reduced SFA and increased total PUFA and n-6 in serum and thigh meat and increased total MUFA in thigh meat in broiler chicken (Hashemipour et al., 2013). Dietary oregano, a carvacrol enriched medicinal plant, influenced broiler's meat

**Table 5.** Mean saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3, n-6, n-6/n-3 ratio, *trans* fatty acids (TFA), and *cis* fatty acids (CFA) concentrations (%)<sup>1</sup> ratios in thigh meat in broiler chickens that received *Satureja khuzistanica* essential oils (SkEO) by gavage method and dietary acetic acid (AA) in day 42 of age.

| SkEO (mg/bird/day) | AA (g/kg) | SFA                    | MUFA                     | PUFA                   | n-3                     | n-6                    | n-6/n-3              | TFA                  | CFA                    |
|--------------------|-----------|------------------------|--------------------------|------------------------|-------------------------|------------------------|----------------------|----------------------|------------------------|
| 0                  |           | 29.95 <sup>a,b</sup>   | 45.61                    | 23.16 <sup>b</sup>     | 1.492 <sup>b</sup>      | 21.67 <sup>b</sup>     | 14.56 <sup>a,b</sup> | 0.300 <sup>b</sup>   | 68.47                  |
| 200                |           | 30.51 <sup>a</sup>     | 44.82                    | 23.84 <sup>a,b</sup>   | 1.502 <sup>b</sup>      | 22.34 <sup>a,b</sup>   | 14.91 <sup>a,b</sup> | 0.000 <sup>d</sup>   | 68.66                  |
| 300                |           | 28.38 <sup>c</sup>     | 45.11                    | 24.23 <sup>a</sup>     | 1.563 <sup>a,b</sup>    | 22.67 <sup>a</sup>     | 14.56 <sup>a,b</sup> | 0.168 <sup>c</sup>   | 69.17                  |
| 400                |           | 30.04 <sup>a,b</sup>   | 44.92                    | 24.05 <sup>a</sup>     | 1.575 <sup>a</sup>      | 22.48 <sup>a</sup>     | 14.31 <sup>b</sup>   | 0.337 <sup>b</sup>   | 68.63                  |
| 500                |           | 29.31 <sup>b</sup>     | 44.71                    | 24.48 <sup>a</sup>     | 1.633 <sup>a</sup>      | 22.85 <sup>a</sup>     | 14.04 <sup>b</sup>   | 0.407 <sup>a</sup>   | 68.78                  |
| 600                |           | 29.53 <sup>b</sup>     | 44.62                    | 24.18 <sup>a</sup>     | 1.472 <sup>b</sup>      | 22.71 <sup>a</sup>     | 15.55 <sup>a</sup>   | 0.060 <sup>d</sup>   | 68.74                  |
| SEM                |           | 0.216                  | 0.240                    | 0.168                  | 0.030                   | 0.159                  | 0.282                | 0.015                | 0.355                  |
|                    | 0         | 29.23 <sup>b</sup>     | 45.32 <sup>a</sup>       | 24.00                  | 1.564                   | 22.43                  | 14.39 <sup>b</sup>   | 0.262 <sup>a</sup>   | 69.05 <sup>a</sup>     |
|                    | 20        | 30.01 <sup>a</sup>     | 44.61 <sup>b</sup>       | 23.98                  | 1.514                   | 22.47                  | 14.92 <sup>a</sup>   | 0.162 <sup>b</sup>   | 68.43 <sup>b</sup>     |
| SEM                |           | 0.154                  | 0.139                    | 0.097                  | 0.017                   | 0.092                  | 0.163                | 0.008                | 0.205                  |
| 0                  | 0         | 30.42 <sup>b,c</sup>   | 44.94 <sup>b,c,d</sup>   | 23.19 <sup>d,e</sup>   | 1.50 <sup>a,b,c,d</sup> | 21.69 <sup>c,d,e</sup> | 14.45 <sup>b</sup>   | 0.258 <sup>c</sup>   | 67.88 <sup>b,c</sup>   |
| 0                  | 2         | 29.48 <sup>c,d</sup>   | 46.27 <sup>a,b</sup>     | 23.13 <sup>d,e</sup>   | 1.48 <sup>b,c,d</sup>   | 21.65 <sup>d,e</sup>   | 14.67 <sup>a,b</sup> | 0.343 <sup>b,c</sup> | 69.05 <sup>a,b,c</sup> |
| 200                | 0         | 29.84 <sup>c</sup>     | 46.85 <sup>a</sup>       | 22.22 <sup>e</sup>     | 1.42 <sup>c,d</sup>     | 20.80 <sup>e</sup>     | 14.73 <sup>a,b</sup> | 0.000 <sup>e</sup>   | 69.07 <sup>a,b,c</sup> |
| 200                | 2         | 31.18 <sup>a,b</sup>   | 42.79 <sup>e</sup>       | 25.45 <sup>a</sup>     | 1.58 <sup>a,b,c</sup>   | 23.87 <sup>a</sup>     | 15.09 <sup>a,b</sup> | 0.000 <sup>e</sup>   | 68.25 <sup>a,b,c</sup> |
| 300                | 0         | 28.23 <sup>d,e</sup>   | 45.18 <sup>a,b,c,d</sup> | 24.91 <sup>a,b</sup>   | 1.66 <sup>a,b</sup>     | 23.24 <sup>a,b</sup>   | 14.00 <sup>b</sup>   | 0.337 <sup>b,c</sup> | 69.75 <sup>a,b</sup>   |
| 300                | 2         | 28.52 <sup>d</sup>     | 45.04 <sup>b,c,d</sup>   | 23.56 <sup>c,d</sup>   | 1.46 <sup>b,c,d</sup>   | 22.10 <sup>c,d</sup>   | 15.12 <sup>a,b</sup> | 0.000 <sup>e</sup>   | 68.60 <sup>a,b,c</sup> |
| 400                | 0         | 29.39 <sup>c,d</sup>   | 45.09 <sup>b,c,d</sup>   | 23.70 <sup>a,b,c</sup> | 1.62 <sup>a,b,c</sup>   | 22.79 <sup>a,b,c</sup> | 14.14 <sup>b</sup>   | 0.370 <sup>b</sup>   | 69.13 <sup>a,b,c</sup> |
| 400                | 2         | 30.69 <sup>a,b,c</sup> | 44.74 <sup>b,c,d</sup>   | 23.70 <sup>c,d</sup>   | 1.53 <sup>a,b,c</sup>   | 22.17 <sup>b,c,d</sup> | 14.49 <sup>b</sup>   | 0.303 <sup>b,c</sup> | 68.14 <sup>b,c</sup>   |
| 500                | 0         | 30.38 <sup>b,c</sup>   | 44.24 <sup>c,d,e</sup>   | 24.00 <sup>b,c,d</sup> | 1.55 <sup>a,b,c</sup>   | 22.45 <sup>b,c,d</sup> | 14.49 <sup>b</sup>   | 0.487 <sup>a</sup>   | 67.75 <sup>b,c</sup>   |
| 500                | 2         | 28.25 <sup>d,e</sup>   | 45.19 <sup>a,b,c,d</sup> | 24.95 <sup>a,b</sup>   | 1.71 <sup>a</sup>       | 23.24 <sup>a,b</sup>   | 13.60 <sup>b</sup>   | 0.327 <sup>b,c</sup> | 69.82 <sup>a,b</sup>   |
| 600                | 0         | 27.13 <sup>e</sup>     | 45.60 <sup>a,b,c</sup>   | 25.26 <sup>a</sup>     | 1.63 <sup>a,b,c</sup>   | 23.63 <sup>a</sup>     | 14.52 <sup>b</sup>   | 0.120 <sup>d</sup>   | 70.74 <sup>a</sup>     |
| 600                | 2         | 31.94 <sup>a</sup>     | 43.65 <sup>d,e</sup>     | 23.09 <sup>d,e</sup>   | 1.31 <sup>d</sup>       | 21.78 <sup>c,d,e</sup> | 16.59 <sup>a</sup>   | 0.000 <sup>e</sup>   | 66.74 <sup>c</sup>     |
| SEM                |           | 0.285                  | 0.340                    | 0.237                  | 0.043                   | 0.225                  | 0.398                | 0.021                | 0.502                  |
|                    |           | <i>P</i> -value        |                          |                        |                         |                        |                      |                      |                        |
| SkEO               |           | <0.001                 | 0.077                    | <0.001                 | 0.005                   | <0.001                 | 0.012                | <0.001               | 0.811                  |
| AA                 |           | <0.001                 | 0.001                    | 0.903                  | 0.051                   | 0.799                  | 0.026                | <0.001               | 0.040                  |
| SkEO × AA          |           | <0.001                 | <0.001                   | <0.001                 | <0.001                  | <0.001                 | 0.022                | <0.001               | <0.001                 |
| Linear             |           | 0.015                  | 0.011                    | <0.001                 | 0.237                   | <0.001                 | 0.371                | 0.141                | 0.686                  |
| Quadratic          |           | 0.027                  | 0.493                    | 0.004                  | 0.004                   | 0.011                  | 0.024                | <0.001               | 0.424                  |

<sup>a-f</sup>Means in the same column without the same superscript differ significantly ( $P < 0.05$ ).

Abbreviation: SEM, standard error of the mean.

<sup>1</sup>As the percent of fatty acids.

composition in terms of total phenolic content, antioxidant capacity, and thiobarbituric acid-reactive substances and improving meat resistance to oxidation (Forte et al., 2018). Animals that received thymol, as an isomer of carvacrol, exhibited greater antioxidant enzyme activities and greater concentration of PUFA in phospholipids of the brain than the untreated control birds (Youdim and Deans, 2000). Enhancement of UFA in thigh lipids may result from attenuation of fatty acid oxidation in thigh meat. It was thought that antioxidant activity of thymol and carvacrol inhibited lipid peroxidation of thigh lipids, especially PUFAs. For this reason, PUFA in the thigh and serum in birds fed diets supplemented with thymol and carvacrol enhanced linearly compared with the control birds. These results in line with our findings confirm the results by Hashemipour et al. (2014) who found a reduction in the total content of SFA and PUFA in the serum and thigh muscle of the broilers fed with a diet containing a phytochemical preparation. These results may also receive greater practical application so as Avila Ramos et al. (2017) which reported that breast meat of the broilers fed with oregano oil and acidulated soybean oil accumulated thymol, but more carvacrol: 552% and 648%, respectively, compared with the control birds. They concluded oregano oil supplementation in diet increased deposition of thymol and carvacrol in broiler meat, a phenomenon that may protect PUFA from peroxidation and enhance their amassing in meat. Also, it was shown that SkEO is able to

induce an appreciated alteration in the proportion of anabolic to catabolic steroids in mevalonate pathway in favor of the anabolic moieties (Khosravinia, 2015).

On the other hand, AA as an ingredient of many commercial acidifier preparations in poultry industry is mostly considered for promising effects on performance and health of birds when included in diets by a range of 0.5 to 3 percent. The results of the present study did not confirm the same idea where almost all productive performance parameters depreciated in the birds feeding by the AA-added diet. We may suggest reasons such as difference in dose of acid, purity of the product, and application of AA alone in combination with other acids to interpret out finding compared with the outcomes from other research studies. However, our findings agree with those of Kopecky et al. (2012) who reported no change or slight decrease in performance of the broilers grown on AA-supplemented diets compared with the control birds during days 21 and 28 of age. The same results with AA-included diets have been reported by Afsharmanesh and Pourreza (2005), Abdel-Fattah et al. (2008), and Attia et al. (2013). In most of these studies, the adverse effects of AA are modestly attributed to the reduced feed intake due to the bitter taste of the acid (El-Hakim et al., 2009) and great change in hemostasis through altered pH and exchange of ions through biological membranes resulting in fail to establish internal balance causing deteriorated performance and gut mucosal health (Abdelrazek et al., 2016). A

recent study has demonstrated that AA treatment increased AMPK $\alpha$  phosphorylation, which subsequently increased expression and transcriptional activity of peroxisome proliferator-activated receptor  $\alpha$  and upregulated the expression of lipid oxidation genes in a cell culture. These changes ultimately led to increased levels of lipid oxidation in BRL-3A cells. Furthermore, elevated AMPK $\alpha$  phosphorylation reduced the expression and transcriptional activity of the sterol regulatory element-binding protein 1c, which reduced the expression of lipogenic genes, thereby decreasing lipid biosynthesis in BRL-3A cells. Consequently, triglyceride content in acetate-treated BRL-3A cells was significantly decreased (Li et al., 2018). Although dietary AA *per se* exerted no appreciable effect on fatty composition in broiler thigh meat at 3 ages concerned, SkEO  $\times$  AA interactions inconsistently modified ratio of certain fatty acid classes in broiler thigh meat in particular, in advanced ages as indicated by the declined MUFA content in the birds provided with 200 mg SkEO by oral gavage when maintained on the acidified diet compared with other birds. Further evidences were provided when administration of 600 mg SkEO accompanied by feeding the acidified diet increased SFA concentration and n-6/n-3 ratio in thigh meat of the birds compared to the birds receiving other treatment regimens.

The results of our experiment led to the conclusion that the daily enteral administration of SkEO via oral gavage in broiler chicken may feasibly modify the fatty acids composition of thigh meat in favor of increased PUFA and n3 percentages. However, this approach may impose slight modification in fatty acids composition of chicken meat compared with fortification of diets with fat sources enriched in PUFA, but it has the speculated advantage of acting as a supportive partner in lipids stability and delaying meat off-flavor, an inference which recently pointed out by Forte et al., (2018) but needs to be characterized in detail. Dietary AA *per se* as well as its interaction with SkEO inconsistently modified ratio of certain fatty acid classes in broiler thigh meat, particularly in advanced ages. Almost all modifications induced by AA-involving regimens were on the contrary to the SkEO influences as they act mainly in favor of an increased SFA proportion in thigh meat of broiler chicken.

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