

PRESERVATIVE EFFECT OF DIETARY OREGANO AND GERMANDER ON QUALITY AND STORAGE STABILITY OF RAW BROILER MEAT

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ABSTRACT

The effects of dietary *Origanum syriacum* L. and *Teucrium polium* L., alone and in combination, on the quality and stability of raw broiler meat were evaluated. Broilers (N=140) were reared for 21 days, divided into five groups under completely randomized design receiving one of five dietary treatments (w/w): 1) Control, 2) 1.5% germander (GER), 3) 2.5% oregano (ORE), 4) 1.5% ORE combined with 2.5% GER (CM), 5) 0.02% butylated hydroxyanisole (BHA). Meat was stored under refrigeration at 4 °C for up to 7 days, and analyzed for thiobarbituric acid-reactive substances (TBARS), protein oxidation, and CIE color values at 0, 4, and 7 days. A similar procedure was used for sensory evaluation and other quality measurements. Both CM and ORE treatments had the significant effect ($P \leq 0.05$) on decreasing TBARS values. In addition, CM treatment had the greatest anti-carbonyl effect and exhibited the greatest stabilizing effect on L* values at day 7. CM and ORE treatments were also best able to maintain a* values. The preservative effect of ORE and CM was significantly higher ($P \leq 0.05$), retarding the development of off-odor and rancidity. In conclusion, the current CM level still needs further investigation to be recommended as a commercial additive.

Keywords: Oregano, Germander, Dietary antioxidant, Lipid oxidation, Sensory attributes.

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INTRODUCTION

Poultry meat is a widely available and high-quality protein food sector in low-income countries (FAO, 2013). This is due to its lowest price and production cost compared to other meat sources such as beef, sheep, and goat (Chouliara *et al.*, 2007; Marangoni *et al.*, 2015; Kralik *et al.*, 2017). Since a large population relies on poultry meat, it is necessary to maintain and improve its quality and safety. Most popular applications used to enhance meat quality and stability (Avila-Ramos *et al.*, 2013) are usually done by dietary commercial additives (Wood and Enser, 1997; De Zawadzki *et al.*, 2017). These additives also enhance the growth and health status of animals, prevent dietary oxidation, and raise their immunity (Gopi *et al.*, 2014; Ahmad *et al.*, 2017). Several antibiotics and synthetic additives (e.g. butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin) are used in animal commercial feed to achieve these benefits (Lee *et al.*, 2001; Avila-Ramos *et al.*, 2013; Ri *et al.*, 2017). For instance, butylated hydroxyanisole (BHA) is currently considered safe as a technological additive in feedstuffs for most animal species, with a maximum level of 150 mg/kg of feedstuffs alone or in combination with butylated hydroxytoluene (BHT) and/or ethoxyquin (E 324) (Rychen *et al.*, 2018). However, minimizing these additives in poultry feed is also another important target (Gopi *et al.*, 2014; Mehdi *et al.*, 2018). In addition, the

possibility of their residual contamination (Vondruskova *et al.*, 2010) and antibiotic resistance to several bacterial strains (Van der Fels-Klerx *et al.*, 2011) encourages researchers to find natural alternatives.

Dietary supplements such as herbal plants and their extracts are an excellent approach and offer safer alternatives (William and Losa, 2001; Ri *et al.*, 2017; Amaral *et al.*, 2018; Zhai *et al.*, 2018). These plant additives are well studied and their positive role in improving animal growth performance, health, feed efficiency, immune system, and meat quality has been documented (Gill, 2000; Manzanilla *et al.*, 2001; Gopi *et al.*, 2014; Salahaen *et al.*, 2015; De Zawadzki *et al.*, 2017). Dietary medicinal plants and their extracts such as oregano, rosemary, and sage generally show positive effects on the quality and storage stability of poultry meat (Lopez-Bote *et al.*, 1998; Botsoglou *et al.*, 2003a, b; Al-Hijazeen *et al.*, 2019; Al-Hijazeen and Al-Rawashdeh, 2019). These plant materials have antimicrobial, anticancer, and antioxidant properties due to their content of polyphenolic compounds (Capecka *et al.*, 2005; Al-Bandak, 2007). Their extracts and essential oils have been extensively studied and analyzed using different methods (Okamura *et al.*, 1994; Afifi *et al.*, 2009; Ibrahim *et al.*, 2012a, b). For example, oregano plant (commonly known as Za'tar) extract contains several phenolic compounds (e.g. terpenoids) such as carvacrol and thymol, which represent its main antioxidant components (Kosar *et al.*, 2003; Al-Bandak, 2007;

Ibrahim *et al.*, 2012a; Krishan and Narang, 2014). The direct and indirect (dietary) antioxidant effect of oregano on meat quality and storage stability had been documented (Simitzis *et al.*, 2008; Rotolo *et al.*, 2013; Al-Hijazeen, 2018, 2019). For example, Botsoglou *et al.* (2003a,b) studied the antioxidant effect of dietary oregano oil supplemented with α -tocopherol acetate on raw, cooked, and long-term frozen turkey meat and found a positive effect on quality and storage stability. Another local medical plant that has not been evaluated as an animal feed supplement is germander (*Teucrium polium* L.). This plant is widely available in semi-arid areas and deserts of the Mediterranean region such as Jordan and is locally used for different medical purposes (Mahmoudi and Nosratpour, 2013; Jaradat, 2015). Germander plant extracts have been analyzed using different methods and found to contain variable compounds depending on the collection method, geographical origin, genetics, species, storage conditions, and several other factors (Aburjai *et al.*, 2006; Afifi *et al.*, 2009; Al Bahtiti, 2012). In addition, germander extract has antimicrobial activity against different bacterial species such as *Escherichia coli*, *Staphylococcus*, *Bacillus*, and *Streptococcus* (Al Bahtiti, 2012; Al-Kufaishi and Al-Mashhedy, 2012). Al-Kufaishi and Al-Mashhedy (2012) reported that germander has strong antioxidant properties due to the presence of several compounds such as tannins, saponins, flavonoids, terpenoids, glycosides, and primary and secondary amines. Its extracts have antidiabetic, anti-inflammatory, and anti-spasmodic properties and are used for different medical aspects (Khleifat *et al.*, 2002; Rasekh *et al.*, 2005). The essential oil of germander grown in the wild in Jordan contains 8-cedren-13-ol (24.8%), β -caryophyllene (8.7%), sabinene (5.2%), and germacrene D (6.8%) as reported by Aburjai *et al.* (2006). In addition, flavonoids in Jordanian *T. polium* (Ja'adeh) extracts have been shown to have effective antioxidant and DPPH radical scavenging activity (Al Bahtiti, 2012), the highest antioxidant activity being caused by luteolin-7-O-glucoside (Djilas *et al.*, 2006). Furthermore, Jaradat (2015) reviewed the phytochemical compounds isolated from different parts of *T. polium* and classified them into: a) volatile oils (different regions); b) compounds from the aerial parts of *T. polium* (isoprenoids, teuvincentins, and neo-clerodanediterpenoids); c) compounds identified by gaschromatographic and spectroscopic techniques; d) compounds in the aqueous phase (alcoholic extract) such as glucose and raffinose; e) major flavonoids; f) four sesquiterpenoid compounds identified by NMR technique. However, the effect of these plant additives on the meat's flavor, odor, and some sensory attributes poses a challenge to finding the appropriate level that can be used. A strong flavor (odor and taste), especially that formed by adding germander plant, may not be acceptable to consumers. The mechanism of how these

plant supplements works has many explanations but is still not yet clear. For example, their effect on the internal digestive system and their physiological effect through enhancing different blood parameters are still under investigation. In addition, their indirect effect on the antioxidant capacity in the muscle fiber before slaughtering might be another clarification (Avila-Ramos *et al.*, 2013; Park *et al.*, 2015).

The dietary combination of oregano and germander in this study is a new investigation, and no research conducted before has evaluated the effect of this on broiler meat quality and freshness. The objectives of the current study were to 1) investigate the effects of adding oregano (*Origanum syriacum* L.) and germander (*Teucrium polium* L.) herbal plants, alone and in combination, on the quality and storage stability of raw chicken meat and 2) compare these effects with that of a synthetic antioxidant (BHA) considering a basic diet formulation.

MATERIALS AND METHODS

Birds and diet formulation: In the current study, all procedures involved were approved by the animal ethics committee at the Department of Animal Production, Mutah University (Ref: 123/14/120). One hundred and forty broilers were raised for 21 days (21 to 42 days) according to general commercial husbandry practices. The dietary treatments were: 1) control (without supplement), 2) 1.5% germander (GER), 3) 2.5% oregano (ORE), 4) 1.5% ORE combined with 2.5% GER, and 5) 0.02% synthetic BHA. All dietary treatments were randomly assigned to one of five groups having seven replicates of four birds each according to a completely randomized design (CRD). Dried supplements of oregano (*Origanum syriacum* L., locally known as Za'tar) and germander (*Teucrium polium* L., locally known as Ja'adeh) were obtained (Identified by botanist and agronomist at the National Agriculture Research Center/ Amman; Jordan) from the wild in the Al-Karak region of south Jordan. The plants were harvested, dried, and ground into a fine powder, then vacuum packaged and stored at -18°C till further use. BHA additive was dissolved in soybean oil (carrier) to give a homogeneous distribution in the feed according to Al-Hijazeen (2019). Throughout the raising period, all birds were grown in floor cages and were offered one of the five dietary supplements and water *ad libitum* using a regular feeder and drinker. All dietary treatments were prepared to be isocaloric and isonitrogenous (Table 1).

Meat preparation: All birds (140), 6-week-old broilers raised on a corn–soybean meal diet, were harvested at the research facility of Mutah University (Agriculture College: Department of Animal Production) according to the regulations for poultry slaughtering (Jordanian

Ministry of Agriculture Guidelines). In addition, this study was evaluated and approved by the Department of Animal Production, and all birds were checked by veterinary officials and certified for health and welfare. The chicken carcasses were chilled in an ice–water mixture for 2 h and drained in a closed cold laboratory area, and the meat (breast and thigh) was deboned 24h after harvesting. Boneless (breast and thigh) muscles were washed, the skin and external fat removed, packaged under vacuum in oxygen-impermeable bags, and frozen at -18°C until used as in the protocol described by Al-Hijazeen *et al.* (2018).

The frozen meat was thawed in a laboratory cooler (4°C) and double ground through 15mm and 3mm plates (Moulinex, Type DKA1, France) before use.

Patties of both meat types were prepared using meat from the five different dietary treatments (batches):1) control (without additives), 2) 1.5% GRE, 3) 2.5% ORE, 4) combination of 1.5% GRE and 2.5% ORE, 5) 0.02% BHA. Meat patties (50g each) were separately packaged using oxygen-permeable envelopes (polyethylene, size: 11×25 cm; Future for Plastic Industry, Al-Mountaz Bags Co. Ltd, Jordan), stored at 4°C in a cooler for up to 7 days, and analyzed for lipid and protein oxidation, and color values at 0, 4, and 7 days. The same processing procedure was used for sensory analysis and other quality measurements. However, thigh meat patties were stored at 4°C for up to 4 days before doing each sensory estimation test.

Table 1. Ingredients and chemical composition (%) of the treatment diets.

Item Ingredients (%)	Control	GER	ORE	CM	BHA
Corn	49.97	50.85	51.2	51.72	49.97
Soybean meal	30.56	33.25	33.9	34.87	30.53
DL-Methionine	0.05	0.07	0.07	0.07	0.05
Limestone	1.54	1.69	1.69	1.69	1.53
Soybean oil	6	6	6	6	6
Salts	0.27	0.33	0.34	0.34	0.27
Minerals and Vitpremix ^a	0.1	0.1	0.1	0.1	0.1
Antifungal ^b	0	0	0	0	0.0
Monocalcium phosphate	0.66	0.84	0.84	0.86	0.66
Broconconcentrate ^c	2.06	0	0	0	2.12
GER	0	1.5	0	1.5	0
ORE	0	0	2.5	2.5	0
BHA	0	0	0	0	0.02
Wheat bran	8.49	5.08	3.07	0.07	8.46
Threonine	0.3	0.28	0.28	0.28	0.3
Chemical composition (%)^d					
DM (%)	90.0	90.2	90.3	90.4	90.0
ME (Mcal/kg)	3050	3050	3050	3050	3050
Crude protein	20	20	20	20	20
Crude fiber	4.27	4.0	3.84	3.59	4.26
Methionine	0.38	0.38	0.38	0.38	0.38
Lysine	1.054	1.05	1.064	1.073	1.054
Calcium	0.9	0.9	0.9	0.9	0.9
Non-phytate phosphorus	0.35	0.35	0.35	0.35	0.35

^aVitamin premix provides (per kilogram of premix): vitamin A, 700,000 IU; vitamin D3, 150,000 IU; vitamin E, 75mg; vitamin B1, 100 mg; vitamin K, 175 mg; vitamin B5, 600 mg; manganese oxide, 4000 mg; ferrous sulfate, 9000 mg; zinc oxide, 6000 mg; magnesium oxide, 2500 mg; potassium iodide, 70 mg; sodium selenite, 125 mg; copper sulfate, 100 mg; cobalt sulfate, 50 mg; dicalcium phosphate, 7000 mg; sodium chloride, 10000 mg.

^bMold inhibitor for animal feed (Kemin Industries, USA).

^cBrocon Concentrate® (Wafa, B V, Alblaserdam, Holland) provides (on as-fed basis): metabolizable energy, 2200 kcal/kg; crude protein, 35%; crude fiber, 4.8%; non-phytate phosphorus, 2.2%; methionine, 1.6%; lysine, 2.4%; cysteine, 0.3%.

^dFormulated diets were calculated on the basis of analyzed values of feed ingredients (feed composition tables) from poultry NRC (1994).

Thiobarbituric acid-reactive substances (TBARS)

measurement: The oxidation of meat lipids was evaluated by a TBARS protocol (Ahn *et al.*, 1998) with minor changes. The TBARS number was reported as mg of malondialdehyde (MDA) per kg of meat.

Protein oxidation (DNPH): Protein oxidation (DNPH: 2,4-dinitrophenylhydrazine) was measured by the popular total carbonyl method as described by Lund *et al.* (2008). In addition, the carbonyl content was calculated as

nmol/mg protein along with an absorption coefficient of 22,000/M/cm as proposed by Levine *et al.* (1994).

Color measurement: Meat color was estimated by a Konica Minolta Color Meter (CR-400, Konica Minolta, Osaka, Japan). The colorimeter was calibrated using an illuminant source C on a standard white ceramic plate. The color was expressed as CIE L* (lightness), a* (redness), and b* (yellowness) values (AMSA, 2012). Obvious defects in the meat surface were excluded from the target examination. Mean values of colorimeter measurements on both sides of the sample surface were used for statistical analysis.

Sensory evaluation: A hedonic line-scale was used to estimate selected sensorial characteristics of the meat samples, following the procedure proposed by Al-Hijazeen (2014). The panelists (ten specialized academic faculty from Mutah University Agriculture College) evaluated the raw meat color, oxidation odor, spice odor, and general acceptability. In addition, all treatments were prepared using the same method described in the previous quality analysis section.

Water separation: Samples of 20 g (raw meat/water mixture) were placed into 50 mL centrifuge tubes to measure their water separation as described by Sebranek *et al.* (2001) with minor modification. The samples were placed in tubes then centrifuged at 10,000 rpm for 20 min (High Speed Centrifuge, TG16G, Hunan Kaida, China). After centrifugation, the excess water was decanted and

tubes were re-weighed. Water separation percentage (an expression of water-holding ability) was calculated as the ratio of centrifuged water to the original sample weight. This method evaluates how strongly water is bound or retained by meat proteins.

pH values of raw meat: The pH value of the ground (breast) raw meat (ultimate pH) samples was determined using a pH meter (PL-600, pH/mV/Temp Meter, Taiwan) before cooking as described by Sebranek *et al.* (2001).

Statistical analysis: The experiment was organized under completely randomized design (CRD). The collected data were statistically analyzed by the procedures of a generalized linear model (Proc. GLM, SAS program, version 9.3, 2012) and Tukey's multiple range test was employed for comparing treatment means. The treatment means were considered significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

Lipid oxidation: Initial TBARS values depend strongly on the free radicals formed at the beginning of auto-oxidation reactions (Ahn *et al.*, 2009; Al-Hijazeen *et al.*, 2016). These TBARS values are linked with the reducing capacity, especially in fresh or raw meat which undergoes primary and pre-blinding preparation. In the current study, all supplements showed a small antioxidant effect in raw chicken meat at day 0 (Table 2).

Table 2. TBARS values of raw ground chicken meat (breast and thigh) treated with different dietary herbal supplements and refrigerated at 4°C.

Time	Control	GRE	ORE	CM	BHA	SEM
Breast						
	----- TBARS (mgmalondialdehyde/kg meat) -----					
Day 0	0.131 ^{az}	0.122 ^{abz}	0.109 ^{bz}	0.104 ^{bx}	0.18 ^{aby}	0.004
Day 4	0.182 ^{ay}	0.151 ^{by}	0.121 ^{ey}	0.114 ^{ex}	0.127 ^{ey}	0.005
Day 7	0.244 ^{ax}	0.185 ^{bx}	0.139 ^{ex}	0.126 ^{ex}	0.143 ^{ex}	0.005
SEM	0.005	0.006	0.0016	0.0057	0.004	
Thigh						
Day 0	0.227 ^{az}	0.215 ^{az}	0.198 ^{az}	0.184 ^{ay}	0.213 ^{az}	0.0132
Day 4	1.108 ^{ay}	0.795 ^{by}	0.443 ^{ey}	0.346 ^{exy}	0.537 ^{ey}	0.0522
Day 7	2.113 ^{ax}	1.227 ^{bx}	0.864 ^{bex}	0.626 ^{ex}	0.882 ^{bex}	0.0876
SEM	0.045	0.073	0.058	0.073	0.0381	

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA. n = 4.

^{a-c}Values with different letters within a row are significantly different ($P \leq 0.05$).

^{x-z}Values with different letters within a column are significantly different ($P \leq 0.05$).

However, both CM and ORE dietary additives exhibited a significant ($P \leq 0.05$) antioxidant effect compared to the control treatment (day 0) using breast meat because of the lower MDA formation in raw compared to cooked meat (Al-Hijazeen, 2014; Al-Hijazeen and Al-Rawashdeh, 2019) or other meat sources which differ in their fat content. The antioxidant mechanism of action for all supplements could be due to

the absorption of plant phenolic compounds by muscle (Botsoglou *et al.*, 2003a, b). These phenolic exogenous antioxidants will consistently raise a bird's internal reducing capacity with a similar effect to that of dietary vitamin E. (Li and Liu, 2012; Avila-Ramos *et al.*, 2013; Park *et al.*, 2015). In addition, fresh meat usually maintains some of its reducing capacity directly after slaughtering. However, the reducing capacity is highly

dependent on its muscle reducing enzyme activity (Ahn *et al.*, 2009). This explanation agrees with that of Park *et al.* (2015) who concluded that dietary oregano powder enhances superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activity, lowering the TBARS values of duck meat, and therefore they reported that phenolic antioxidant compounds are absorbed by the muscle and support its antioxidant defense system. The results show that all feed supplements significantly ($P \leq 0.05$) decreased TBARS values compared with the control after day 4, for both meat sample types. This is also related to the greater progress of lipid oxidation and its primary products, and so increases the variation between these treatments and the control samples. For instance, adding ORE with chestnut (*Castanea sativa* Mill) wood extract as a feed supplement in pork diet raises the antioxidant capacity of the blood by increasing the levels of glutathione peroxidase and glutathione reductase, which delays lipid oxidation (Ranucci *et al.*, 2015). In addition, it has been found that supplementation of animal diet with oregano decreases lipid oxidation in meat samples (Botsoglou *et al.*, 2003 a, b; Chouliara *et al.*, 2007; Janz *et al.*, 2007). However, no research studies have investigated the effect of dietary germander plants

or their extract on poultry meat quality. Both CM and ORE supplements exhibited the greatest effect on decreasing lipid oxidation (TBARS) during storage. On the other hand, both GER and BHA had a smaller effect on MDA formation. However, there was no significant difference ($P > 0.05$) among ORE, CM, and BHA at day 7 for either breast or thigh lean meat. In addition, the effect of adding germander dried plant supplement alone was smaller than that for its combination with oregano. Based on previous studies, lower TBARS values using raw meat cause less significant variation, and this should be clearer if the meat is cooked and produces more free radicals (Al-Hijazeen, 2014; Al-Hijazeen *et al.*, 2016, 2019).

Protein Oxidation (DNPH): Protein oxidation in meat systems is highly correlated with oxidized lipids and their secondary products (Ahn *et al.*, 2009; Hes, 2017). In addition, it also affects fresh meat quality characteristics such as color, flavor, texture, odor, protein solubility, and functionality (Howell *et al.*, 2001; Lund *et al.*, 2011; Amaral *et al.*, 2018). So, the results showed no significant differences ($P > 0.05$) between all treatments at day 0 of storage (Table 3).

Table 3. Effect of dietary ORE and GER on protein oxidation during storage.

Time	Control	GER	ORE	CM	BHA	SEM
Breast meat						
Day 0	0.718 ^{az}	0.689 ^{ay}	0.646 ^{ax}	0.584 ^{ax}	0.661 ^{ay}	0.0512
Day 4	0.921 ^{ay}	0.784 ^{abxy}	0.688 ^{bx}	0.637 ^{bx}	0.706 ^{bxy}	0.044
Day 7	1.191 ^{ax}	0.908 ^{bx}	0.762 ^{bx}	0.731 ^{bx}	0.821 ^{bx}	0.0458
SEM	0.026	0.042	0.045	0.0731	0.036	
Thigh meat						
Day 0	0.838 ^{az}	0.815 ^{ay}	0.713 ^{ay}	0.626 ^{ay}	0.734 ^{ay}	0.0535
Day 4	1.375 ^{ay}	0.964 ^{by}	0.846 ^{bexy}	0.715 ^{exy}	0.875 ^{bey}	0.0485
Day 7	1.703 ^{ax}	1.308 ^{bx}	0.995 ^{ex}	0.966 ^{ex}	1.167 ^{bex}	0.067
SEM	0.0375	0.0681	0.0532	0.0754	0.0389	

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA. n = 4.

^{a-e}Values with different letters within a row are significantly different ($P \leq 0.05$).

^{x-z}Values with different letters within a column are significantly different ($P \leq 0.05$).

The variation at day 0 was not enough to show a significant antioxidant effect of the supplements. Generally, this effect is caused by the improvement in reducing capacity (antioxidant phenolic compounds) of fresh raw meat and changes in muscle fiber metabolism. This is due to a lower total carbonyl content when using raw chicken meat (Al-Hijazeen and Al-Rawashdeh, 2019) compared to cooked (Al-Hijazeen *et al.*, 2016). For example, Xiao *et al.* (2011) obtained similar results of a low total carbonyl content (0.46 to 0.81 nmol/mg protein) when testing raw ground chicken meat. However, this variation was clearer after day 4 of storage, especially when testing ORE and CM samples. The effect of ORE and CM dietary supplements was significant ($P \leq 0.05$)

compared to the control treatments. Furthermore, GER and BHA had a significant ($P \leq 0.05$) effect, delaying total carbonyl formation compared to the control at day 7 using both meat types. The results show that the CM treatment had the greatest effect on carbonyl formation compared to the other dietary supplements. On the other hand, GER was the weakest anti-carbonyl formation agent during storage. These sequences in the development of protein oxidation in the meat samples are highly correlated with the initial total carbonyl content (day 0) and the formation of free radicals (Al-Hijazeen *et al.*, 2016). There was little numerical difference between these treatments at the beginning of the current study. The variation could be clearer and more significant due to

an increase in the progress of lipid-protein oxidation. This shows the improvement of enzymes' reducing capacity at the beginning of storage (24h after slaughtering), and also the effect of meat composition (Al-Hijazeen and Al-Rabadi, 2017) among all treatments (Avila-Ramos *et al.*, 2013; Park *et al.*, 2015). The control samples had a higher fat content compared to the other treatments as in the proximate composition analysis. This may be due to the higher feed intake which reflects the effect of plant (GER and ORE) flavor on the birds' feed consumption.

Meat color: Both visual and instrumental evaluation of meat color depends on the chemical status of myoglobin (heme group) pigment (AMSA, 2012; Suman and Joseph, 2013). Fresh meat discoloration also depends strongly on the reducing capacity, especially in the early postmortem phase (Mancini and Hunt, 2005; Keokammerd *et al.*, 2008). The effect of natural dietary additives on an animal's muscle reducing capacity is also expected and documented (Kumar *et al.*, 2015; Park *et al.*, 2015; Ranucci *et al.*, 2015). There was a significant ($P \leq 0.05$) decrease in L^* values during storage (days 0–7). For instance, the L^* values of control treatment samples (breast and thigh) decreased significantly ($P \leq 0.05$) during storage (Tables 4 and 5). These results are in agreement with previous research studies done on chicken meat (Chouliara *et al.*, 2007; Keokammerd *et al.*, 2008; Al-Hijazeen *et al.*, 2016; Al-Hijazeen and Al-Rawashdeh, 2019). However, CM supplementation had the greatest effect on maintaining L^* values which were highest on day 7 among all treatments using both meat types. All supplements had a significant effect ($P \leq 0.05$) on maintaining L^* values compared to the control at day 7 using thigh meat. The different myoglobin concentration in the two meat types (breast and thigh) is

another reason why variation becomes clearer during storage. CM and ORE treatments had the greatest significant ($P \leq 0.05$) effect compared to the control treatment at day 0 using breast meat. CM and ORE also had the greatest effect on maintaining a^* values compared to treatments with the other supplements. The preservation effect of directly adding herbal plant extracts to meat has also been documented (Keokammerd *et al.*, 2008; Velasco and Williams, 2011; Avila-Ramos *et al.*, 2013; Al-Hijazeen *et al.*, 2016). In addition, it is well known that natural dietary supplements can improve meat lipid stability (Li and Liu, 2012; Cimmino *et al.*, 2018) and quality characteristics such as color (Kumar *et al.*, 2015; Rossi *et al.*, 2017; Zahid *et al.*, 2018). Meat color is also affected by and correlated with lipid and protein oxidation during storage (Xiong, 2000; Suman and Joseph, 2013). On the other hand, GRE had the smallest effect on maintaining a^* values. Meat discoloration also depends on many endogenous factors such as enzyme reducing activity, oxidation of lipids and free radicals, the ultimate pH of meat, pro-oxidants, and others (Mancini and Hunt, 2005; Neethling *et al.*, 2017). This ability of CM to maintain a^* values also reflects the improvement in the total muscle reducing capacity (Keokammerd *et al.*, 2008; Avila-Ramos *et al.*, 2013; Park *et al.*, 2015) of these polyphenols in the early postmortem stage. The ability of these plant supplements to stabilize fresh meat color provides more evidence of muscle tissue absorption and bioavailability of polyphenolic compounds (Botsoglou *et al.*, 2003a, b; Pandey and Rizvi, 2009). Finally, the changes in b^* values were inconsistent and affected by the interaction with both a^* and L^* values. In addition, there were no significant differences ($P > 0.05$) in b^* values among all treatments at day 7 using thigh meat.

Table 4. CIE L^* , a^* , and b^* color values of ground chicken (breast) patties during storage at 4 °C.

Time	Control	GER	ORE	CM	BHA	SEM
L^*						
Day 0	64.25 ^{ax}	64.52 ^{ax}	64.64 ^{ax}	64.91 ^{ax}	64.67 ^{ax}	0.167
Day 4	63.62 ^{by}	63.85 ^{aby}	64.05 ^{aby}	64.2 ^{ay}	63.92 ^{abxy}	0.106
Day 7	62.58 ^{cz}	63.09 ^{bcz}	63.72 ^{aby}	63.86 ^{az}	63.56 ^{aby}	0.148
SEM	0.1	0.167	0.128	0.082	0.202	
a^*						
Day 0	8.02 ^{cx}	8.22 ^{bex}	8.42 ^{abx}	8.65 ^{ax}	8.46 ^{abx}	0.06
Day 4	6.41 ^{cy}	6.87 ^{by}	7.05 ^{aby}	7.36 ^{ay}	6.82 ^{by}	0.075
Day 7	6.18 ^{cz}	6.42 ^{bcz}	6.84 ^{ay}	6.95 ^{az}	6.72 ^{aby}	0.075
SEM	0.04	0.073	0.072	0.073	0.085	
b^*						
Day 0	20.88 ^{bx}	20.98 ^{bx}	21.21 ^{bx}	21.73 ^{ax}	21.73 ^{ax}	0.094
Day 4	19.05 ^{by}	19.27 ^{aby}	19.33 ^{aby}	19.2 ^{aby}	19.68 ^{ay}	0.137
Day 7	18.77 ^{ay}	19.03 ^{ay}	18.73 ^{az}	19.03 ^{ay}	18.85 ^{az}	0.115
SEM	0.154	0.101	0.1	0.114	0.104	

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA. n=4.

^{a-c}Values with different letters within a row are significantly different ($P \leq 0.05$).

^{x-z}Values with different letters within a column are significantly different ($P \leq 0.05$).

Table 5. CIE L*, a*, and b* color values of ground chicken meat (thigh) during storage at 4 °C.

Time	Control	GER	ORE	CM	BHA	SEM
L*						
Day 0	55.77 ^{ax}	56.29 ^{ax}	56.22 ^{ax}	56.37 ^{ax}	56.14 ^{ax}	0.046
Day 4	54.97 ^{ax}	55.31 ^{ay}	55.6 ^{ax}	55.25 ^{axy}	55.77 ^{ax}	0.212
Day 7	52.14 ^{by}	53.66 ^{az}	54.16 ^{ay}	54.39 ^{ay}	54.03 ^{ay}	0.212
SEM	0.305	0.187	0.196	0.286	0.13	
a*						
Day 0	9.77 ^{ax}	9.97 ^{ax}	9.96 ^{ax}	9.98 ^{ax}	9.95 ^{ax}	0.0919
Day 4	8.48 ^{by}	8.99 ^{ay}	9.17 ^{ay}	9.3 ^{ay}	9.13 ^{ay}	0.0778
Day 7	7.19 ^{cz}	8.23 ^{bz}	8.87 ^{ay}	8.92 ^{ay}	8.72 ^{az}	0.0929
SEM	0.0597	0.0879	0.0804	0.1013	0.1037	
b*						
Day 0	16.51 ^{ay}	16.96 ^{axy}	16.84 ^{ax}	16.79 ^{ay}	16.95 ^{ax}	0.166
Day 4	16.90 ^{axy}	16.74 ^{ay}	16.51 ^{ax}	16.66 ^{ay}	16.87 ^{ax}	0.18
Day 7	17.31 ^{ax}	17.55 ^{ax}	17.24 ^{ax}	17.35 ^{ax}	17.37 ^{ax}	0.127
SEM	0.137	0.198	0.193	0.115	0.135	

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA. n = 4.

^{a-c}Values with different letters within a row are significantly different ($P \leq 0.05$).

^{x-z}Values with different letters within a column are significantly different ($P \leq 0.05$).

Sensory evaluation: Several research studies have estimated the effects of dietary plant supplements on the quality and sensory characteristics of animal meat (Rotolo *et al.*, 2013; Ri *et al.*, 2017; Rossi *et al.*, 2017). Most of these found positive effects of supplementation on sensory attributes (Rossi *et al.*, 2013; Kirkpinar *et al.*, 2014; Zahid *et al.*, 2018). Generally, CM, ORE, and BHA had the greatest effect on maintaining meat color stability. However, GER had the smallest effect compared to treatment with the other supplements. These results are in agreement with and linked to previous instrumental (Minolta) data. Oregano and germander dried plant supplements were clearly detected and recognized by the panelists in all meat samples (odor attributes). The CM treatment samples had the highest spice odor values among the treatments. The extensive spice odor reflects the ability of muscle tissues to absorb these plant polyphenols. Similar results were obtained in several studies evaluating the effect of dietary oregano

plants and their essential oil on meat sensory evaluation (Kirkpinar *et al.*, 2014; Janacua-Vidales *et al.*, 2019). Results for the attribute oxidation odor agreed with the previous findings for lipid and protein oxidation (Tables 4 and 5). However, the CM treatment had the greatest effect, giving the lowest oxidation odor values among the other treatments. ORE and CM were evaluated as having the most significant effect ($P \leq 0.05$) on retarding the development of off-odor and rancidity in the raw chicken meat. Oxidized meat odor is affected by the number of total volatile compounds (aldehydes, hydrocarbons, sulfuric compounds) formed by lipid peroxidation and secondary compounds (Ahn *et al.*, 2009; Al-Hijazeen, 2014), so any significant effect existing should be due to the antioxidant action of these dietary plants. Regarding overall acceptability, the CM and ORE treatment samples had significantly higher ($P \leq 0.05$) values than control and BHA treatment samples.

Table 6. Sensory attribute mean values of raw thigh meat patties.

Treatment	Sensory attributes*			
	Redness	Spice odor	Oxidation odor	Overall acceptability
Control	5.81 ^c	0.74 ^b	6.95 ^a	3.37 ^c
GER	6.85 ^{bc}	7.42 ^a	3.034 ^{bc}	5.94 ^b
ORE	7.97 ^a	7.37 ^a	2.037 ^c	7.48 ^a
CM	7.94 ^a	8.2 ^a	1.918 ^c	7.69 ^a
BHA	7.82 ^{ab}	0.93 ^b	3.219 ^b	7.15 ^{ab}
SEM	0.26	0.318	0.286	0.366

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA. n=10.

*Samples were evaluated on day 3.

SEM: standard error of the means.

^{a-c}Means within the same column with different superscripts are significantly different ($P \leq 0.05$).

Water separation percentage and ultimate pH:

Generally, there were no significant differences ($P \leq 0.05$) in water separation percentage (WS) among all supplements using both breast and thigh meat. However, the CM dietary treatment showed the lowest WS in both breast and thigh meat samples. On the other hand, the control treatment resulted in the highest WS values ($P \leq 0.05$). This is due both to meat composition (fat and protein profile) and early postmortem events such as the rate of pH decline, protein degradation, and protein oxidation (Huff-Lonergan and Lonergan, 2005). In addition, these changes will affect the isoelectric point of meat protein, and so indirectly its water-holding ability. Furthermore, there were no significant differences ($P > 0.05$) in ultimate pH values between treatments (supplements) at day 7. However, the control treatment showed the significantly lowest values ($P \leq 0.05$). This low ultimate pH value could be the reason behind the higher WS in control samples. A greater decline in pH in the postmortem period usually means a lower water-binding ability (more drip loss and purge expected) of meat protein as in the case of pale, soft, exudative meat (Huff-Lonergan and Lonergan, 2005; Bee *et al.*, 2007; Garcia *et al.*, 2010). Finally, it is well known that healthy birds with a good immune system, higher reducing capacity, and a normal pH decline usually produce better-quality meat.

Table 7. Effect of different dietary supplementation on water separation percentage and ultimate pH (breast and thigh) of raw chicken meat.

Treatment	WS breast	WS thigh	Ultimate pH*
Control	23.70 ^a	21.03 ^a	5.34 ^b
GER	21.99 ^b	20.04 ^{ab}	6.12 ^a
ORE	21.31 ^b	19.75 ^b	6.18 ^a
CM	21.28 ^b	19.57 ^b	5.91 ^{ab}
BHA	21.40 ^b	19.73 ^b	6.29 ^a
SEM	0.31	0.271	0.155

Treatments: control; GER: germander; ORE: oregano; CM: combination; BHA.

WS: water separation percentage of raw fresh chicken meat.

*Ultimate pH (24h after slaughtering) of raw breast meat.

Conclusion: Dietary CM had the greatest significant effects ($P \leq 0.05$) on preventing meat deterioration and stabilizing meat quality. The lowest TBARS and total carbonyl values were detected when testing meat samples from the CM treatment. However, the antioxidant effect of GER and ORE alone was lower than when adding them in combination (CM). In addition, the CM treatment also had the greatest effect on stabilizing color values (a^* and L^* values). No significant difference ($P > 0.05$) was found among additives regarding their ultimate pH values or WS. The CM meat samples had better sensorial attributes and overall acceptability. Overall, the positive

effect on chicken meat quality of adding both GER and ORE was superior to that for the individual additives. This combination could be a prospective natural replacement which enhances meat quality and freshness especially during the postmortem stage.

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