Effects of *Thymus vulgaris* and *Mentha pulegium* on colour, nutrients and peroxidation of meat in heat-stressed broilers

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Abstract

This study was designed to investigate the effects of *Thymus vulgaris* (thyme) and *Mentha pulegium* (mentha) powders on meat colour, nutrient composition and malondialdehyde (MDA) where broiler chickens were under heat stress. Two hundred one-day-old male chicks were used in a completely randomized design with four treatments and five replicates each (10 birds per replication). Treatments were the control diet, 0.5% mentha, 0.5% thyme and 0.5% mixture of the two plants. The results showed no effect of dietary supplements on thigh meat redness and yellowness. Both plant products diminished the thigh lightness significantly compared with the control. No significant differences between treatments were observed for the ash, ether extract and crude protein content of the thigh muscle. Supplementation of thyme and mentha separately or together increased the moisture of the thigh muscle significantly, compared with the control. The combination of the plant products resulted in a higher thigh pH and significantly lower malondialdehyde (MDA) concentration in the thigh muscle compared with the control. In conclusion, dietary supplementation of thyme and mentha separately or in combination improved the meat quality of broiler chickens under heat stress through decreasing the MDA concentration and increasing the pH and moisture content of the thigh muscle.

Keywords: Lightness, malondialdehyde, moisture, pH, redness
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Introduction

High ambient temperature is a major problem in many parts of the world, especially in summer (Aksit *et al*., 2006). Exposure to high temperatures has been recorded as causing undesirable changes in the meat quality of broilers (Aksit *et al*., 2006). It has been reported that environmental stress and subsequent oxidative stress reduce the functional properties of muscle proteins and unbalance the antioxidant status of birds (Sacchetti *et al*., 2005). Heat stress during rearing is also one of the prominent ante mortem stressors, which results in a faster decline in pH and pale colour in breast meat in birds (Aksit *et al*., 2006). The higher lightness (L) during the summer was observed by Mc Curdy (1996) in turkey breast meat. Petracci *et al.* (2004) compared the breast muscle of broilers in summer and winter, and reported the higher lightness of meat for summer-reared broilers and lower redness (a) and yellowness (b) of meat for winter-reared ones. Lipid oxidation is a major cause of deterioration of muscle quality, which can directly affect the quality characteristics of flavour, colour, tissue, nutritional value and health in meat (Sharbati *et al*., 2015). Recently, the use of plant extracts as natural antioxidants has increased owing to restrictions on the use of synthetic materials. Natural antioxidants are biologically important protective cellular components (such as DNA, proteins, lipids and membranes) against reactive oxygen species, and have beneficial effects on human health (Tanabe *et al*., 2002). Plant extracts are capable of neutralizing free radicals and preventing oxidation of unsaturated fatty acids (Sacchetti *et al*., 2005). Garden thyme (*Thymus vulgaris* L.) belongs to the mint family (Lamiaceae). The origin of this plant is the Mediterranean region, the north of America and some parts of Asia (Stahl-Biskup & Saez, 2002). Thyme is a short wooden perennial grey coloured plant with a C3 metabolism system, which is 30 - 50 cm tall, depending the climate of the growth region (Stahl-Biskup & Saez, 2002). Thyme volatile phenolic oil has been reported to be among the top 10 essential oils, showing antibacterial, antimycotic, antioxidative, natural food preservative and anti-ageing properties (Naghdi Bhadi *et al*., 2004). Thymol and carvacrol are the major compounds of garden thyme (Chen *et al*., 2008).

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Antioxidant effects of various combinations of these plants have been demonstrated in several studies. Bolukbasi *et al.* (2006) reported that carvacrol and thymol could be responsible for the antioxidant effect of thyme. These researches indicated that the antioxidant capacity of thyme oil is more than that of vitamin E in thigh meat. Bolukbasi *et al.* (2006) reported that thyme oil (200 mg/kg) could reduce the thiobarbituric acid reactive substances (TBARS) of thigh and breast after slaughter and increase the weight gain in broilers. Adding powdered thyme to the meat of broilers causes lipid peroxidation stalling (Onibi, 2003).

Mentha is another medicinal plant, which belongs to the Labiatae family and includes 20 species that can be found all over the world. *Mentha pulegium* L. is one of the mentha species, commonly known as pennyroyal. It is native to Europe, North Africa, Asia Minor and the Near East (Mahdavi *et al*., 2013). The flowering aerial parts of *M. pulegium* were traditionally used for their antimicrobial properties in the treatment of cold, sinusitis, cholera, food poisonings, bronchitis and tuberculosis (Goordazi & Nanekarani, 2014). Three chemotypes of *M. pulegium* with major oil components are pulegone, piperitenone and piperitone and isomenthone/neoisomenthol (Cook *et al*., 2007). A mixture of *O. vulgare* (1%), *M. pulegium* (0.5%) and *Mentha piperita* L. (0.5%) has improved the carcass quality, weight gain, carcass efficiency and abdominal fat in broilers (Hernandez *et al*., 2004). Some research has been conducted on performance (above), but there is no report on the effects of these two plant products on the meat quality of broiler chickens. Hence the present research was conducted to reveal the effect of *T. vulgaris* and *M. pulegium* powders on quality, nutrient composition and meat colour of broiler chickens under heat stress.

Materials and Methods

Two hundred day-old male broiler chicks (Ross 308) were used in this experiment. All the birds were housed in 20 pens (1 m^2) of 10 each, and five replicate pens were allocated to each treatment. Continuous light was used in the house. All the birds were fed the same starter (from day 1 to day 10 of age) and grower (from day 11 to day 24) diets in mash form, but they received the different finisher diets (from days 25 to 42) (Table 1). All the experimental treatments were under heat stress and birds were fed the control diet (without plant supplements), 0.5% *T. vulgaris*, 0.5% *M. pulegium* and a blend of *T. vulgaris* and *M. pulegium* (0.25% of each). It has been stated that 0.5% of *M. pulegium* (Nobakht *et al*., 2011) and *T. vulgaris* (Rezaei *et al*., 2014) are enough to improve the performance in broiler chickens; consequently, such levels were used in the current experiment. The method of Yalcin *et al.* (2009) was used to induce the heat stress with some modifications (32 ºC as cyclic from 9:00 to 17:00). The total phenolic compounds of mentha and thyme were 12.6 mg/g and 28.5 mg/g dry weight, respectively. The colorimetric method of Folin-Ciocalteu reagent, as explained by Daneshyar *et al.* (2012; 2011), was used to determine phenolic compounds. First, 1 g dried plant tissue was extracted with 10 mL 80% methanol. For total phenol measurements, 0.5 mL of the extract, gallic acid (as the standard), Folin (diluted with water in the ratio 1 : 10) and 4 mL of sodium carbonate (1 M) solution were mixed and allowed to stand at room temperature for 15 minutes. Standards were prepared with concentrations of 0, 25, 50, 100, 250 and 500 mg/mL, and uptake of the samples was measured at a wavelength of 765 nm. Ultimately total phenols were expressed as mg/g dry weight of the plant sample. The experimental diets were used during the finisher period (from day 25 to 42 of age) and under the heat-stress condition. Average ambient relative humidity inside the house was 45%. At the end of the experiment (week 6), one chicken from each replicate pen (five per treatment) was selected randomly, marked and slaughtered. Two pieces of meat from each left thigh were removed to determine thigh meat indices. One piece was used for proximate analysis of dry matter (DM), crude protein (CP), ether extract (EE) and crude ash content. The other was transferred to the laboratory to check meat colour. The samples were collected in plastic trays, weighed and stored in airtight plastic bags in a freezer (−20 ºC) until they were required for analysis. They were then homogenized with a blender, and analysed.

The thiobarbituric acid reactive substances (TBARS) method was used to determine malondialdehyde (MDA) concentration in thigh meat. The pH of thigh meat was measured with a digital pH meter (TitroLine Easy, Schott Instruments, Mainz, Germany) after homogenization in distilled water. The DM contents of thigh samples were determined by oven-drying at 105 ºC for 18 h. The EE content of thigh samples was obtained by the Soxhlet extraction method, using anhydrous diethyl ether. The Kjeldahl method was used to analyse the total nitrogen content of thigh samples, and CP was expressed as nitrogen multiplied by 6.25. Meat moisture was determined with the vacuum oven method, according to the Association of Official Analytical Chemists (AOAC, 1999). The ground meat samples were dried for 48 hours in a vacuum-oven (23 kPa) at 98 ºC and cooled to room temperature in a desiccator prior to taking final weights. The crude ash content was determined after heating the samples in a muffle furnace at 550 ºC for 16 hours. Three parameters of meat colour, including lightness (L), redness (a) and yellowness (b) values were determined with a Minolta (CR-100) Chroma Meter (Norouzi *et al*., 2014; Sharbati *et al*., 2015). The data were analysed based on a completely randomized design using the general linear model procedure of SAS (2002). When treatment means were significant (P <0.05), the Duncan multiple range test was used to separate the means. The experimental protocols were reviewed and approved by the Animal Care Committee of Urmia University.

Table 1 Ingredient and nutrient **c**omposition of experimental diets

	Starter, 0 - 10 d	Grower, 11 - 24 d	Finisher, 25 - 42 d
Ingredients,%			
Maize	32.91	34.55	38.66
Wheat	20.00	25.00	25.00
Soybean meal	39.35	33.50	28.35
Soybean oil	3	2.80	3.18
Dicalcium phosphate	2.10	2.15	2.15
Lime stone	1.10	0.86	0.86
Lysine	0.29	0.22	0.20
DL-methionine	0.38	0.08	0.13
Vitamin-mineral mix*	0.5	0.5	0.5
Sodium chloride	0.37	0.34	0.34
Sand[#]	-	-	0.5
Total	100	100	100
Calculated analysis in g/kg			
Dry matter	859.9	861.9	858.3
Metabolizable energy, MJ/kg	11.97	12.23	12.48
Crude protein	219.9	200.0	179.9
Fat	49.3	48.3	53.3
Fibre	39.6	37.0	33.3
Calcium	10.0	9.00	8.90
Available phosphorus	4.50	4.50	4.40
Chloride	3.30	3.00	2.90
Sodium	1.60	1.50	1.50
Methionine	7.00	3.80	3.10
Lysine	14.3	12.40	10.90
Arginine	15.30	13.70	12.20
Methionine + cysteine	10.70	7.30	7.30
Tryptophan	2.90	2.60	2.30
Tyrosine	9.80	8.90	8.10
Threonine	8.50	7.70	6.90

* Supplied per kilogram of diet: Vitamin A, 9000 U; vitamin D_3, 2000 U; vitamin E, 18 U; vitamin B_{12}, 0.15 mg; riboflavin, 6.6 mg; calcium pantothenate, 10 mg; niacin, 30 mg; choline, 500 mg; biotin, 0.1 mg; thiamine, 1.8 mg; pyridoxine, 3 mg; folic acid, 1 mg; vitamin K_3, 2 mg; antioxidant (Ethoxyquin), 100 mg; zinc, 50 mg; manganese oxide, 100 mg; Cu, 10 mg; Fe, 50 mg; I, 1 mg; Se, 0.2 mg.
[#] Different levels of *T. vulgaris* L. and *M. pulegium* powder or a blend of the two were replaced with sand in the diets at finisher. The finisher diet was fed during the final study.

Results and Discussion

The effects of dietary supplementation of thyme and mentha powders on meat colour parameters (lightness, redness and yellowness) at 42 days of age are shown in Table 2. No effect of either supplement was observed on thigh meat redness and yellowness (P >0.05). Dietary supplementation of both plants diminished the thigh lightness compared with the control (P <0.05). Furthermore, the combination of thyme and mentha decreased thigh meat lightness numerically. The lower lightness (L) of thigh meat was observed in dietary supplementation of the two plants separately or in combination. It is accepted that heat stress stimulates the glycolysis process and consequently decreases the thigh pH quickly and hence causes the pale meat (Remignon *et al.*, 2006). But thyme and mentha powders prevented thigh paleness in the current experiment. Although no research has been done on the effects of thyme or mentha on meat quality in broilers under heat stress, the reduced meat lightness (L) of broiler chickens by thyme and mentha may have two reasons. The antioxidant properties of these plants may be the first reason (Hirasa & Takemasa, 1998; Tepe *et al*., 2006). Antioxidants retard or inhibit the oxidation of substances through initiation or propagation of oxidizing chain reactions. Consequently, natural antioxidants can protect the biologically important cellular components from oxidative processes caused by reactive oxygen species (ROS) (Velasco & Williams, 2011). These plants are sources of natural antioxidants (Nakatani, 1992; Gonçalves *et al*., 2009), which can improve meat shelf-life and quality mainly by retarding lipid oxidation and microbial growth (Velasco & Williams, 2011). Heat stress reduced the pH in the current experiment, which was followed by a pale colour of meat. The second possible reason for reduced meat lightness could be related to the ability of thyme and mentha powders to maintain acidity, prevent a loss in pH, and hence prevent meat colour changes. Yang & Chen (1993) reported increased redness and yellowness in ground chicken meat with low pH. Meat colour has been reported to be the most important factor of meat quality assessments by consumers and is related to meat freshness (Velasco & Williams, 2011). In the same way, Chen *et al.* (2008) indicated that dietary garlic increased pH of in pig meat.

Table 2 Effects of dietary supplementation of thyme and mentha powders on meat colour indices of broilers under heat stress at 42 days of age

Treatments	**Lightness (L)**	**Redness (a)**	**Yellowness (b)**
H	40.48[a]	5.46	6.67
T	38.22[b]	5.10	6.20
M	37.57[b]	5.62	5.58
TM	39.50[ab]	5.46	6.11
Pooled SEM	0.44	0.18	0.31
P-value	0.03	0.81	0.66

[a–b] Means within each column with different superscripts are significantly different (P <0.05).
H: heat stressed; M: heat stress + mentha; T: heat stress + thyme; TM: heat stress + mentha + thyme.

Changes in meat colour are due to oxidation of red oxymyoglobin to metmyoglobin (MMG), which gives meat an unattractive brown colour. Some reports demonstrated that natural antioxidants could postpone meat colour loss by extending the red colour (a*) and delaying MMG formation (Velasco & Williams, 2011). Although the authors observed lowered lightness by the phytogenic supplements in the recent study, inconsistent results have been reported by different phytogenic plants. For example, Cornforth (1994) and Carpenter *et al.* (2007) observed no changes of L*, b* and a* indices of raw pork patties from grape seed and bearberry extracts. Similarly, Lopez-Bote *et al.* (1998) detected no effects of oregano oil (1%) on fresh chicken breast meat. The use of oral garlic in the diet of pigs increased the pH, and reduced the L, a*, b* of meat (Onibi, 2003). Using extracts of rosemary and sage reduced the lipid oxidation and cholesterol concentration of broiler meat during storage for nine days (Debut *et al.,* 2003). In a study to evaluate the quality of broiler meat with green tea extract (0.1 and 0.2 g per kg), a* and b* were both increased by rosemary and sage extract supplementation. These discrepancies are because the researches and the current study used different plants.

The effects of dietary treatments on ash, fat, protein, moisture and pH of thigh meat of broilers under heat stress at 42 days of age are indicated in Table 3. There were no significant differences between the treatments in the levels of the ash, EE and CP of the thigh meat (P >0.05). Dietary supplementation of thyme and mentha separately or in combination increased thigh moisture compared with the control (P <0.05). Supplementation of thyme or mentha numerically increased the thigh pH, but only the combination of the two caused significantly higher thigh pH (P <0.05). Furthermore, all the dietary supplements resulted in a lower thigh MDA concentration (P <0.05) (Figure 1). It has been reported that heat stress reduces meat pH (Cornforth, 1994). Cornforth (2002) stated that meat with a high pH has a higher water-binding capacity.

Oxidative free radicals can cause oxidation of cellular lipid, DNA, and carbohydrates, and consequently damage the function of normal cells (Bianchi & Antunes, 1999). The combined use of antioxidants could reduce the effects of stress due to oxi-reduction properties, which may have an important role in free radical quenching and neutralization, in addition to improving meat organoleptic characteristics (taste, sight, smell and touch) and quality (Jem *et al*., 2008).

Table 3 Nutrient composition of thigh meat in broilers fed thyme and mentha powder under heat stress at 42 days old

Treatment[1]	Ash (%)	Ether extract (%)	Crude protein (%)	Moisture (%)	pH
H	2.11	1.59	18.91	74.75^{b}	6.56^{b}
T	2.01	1.78	18.56	76.18^{a}	6.71^{ab}
M	2.10	1.75	18.22	76.01^{a}	6.59^{ab}
TM	2.09	1.70	18.63	76.49^{a}	6.74^{a}
Pooled SEM	0.02	0.03	0.10	0.23	0.93
P-value	0.13	0.21	0.13	0.04	0.04

$^{a–b}$ Means within each column with different superscripts are significantly different (*P* <0.05).
H: heat stressed; M: heat stress + mentha; T: heat stress + thyme; TM: heat stress + mentha + thyme.

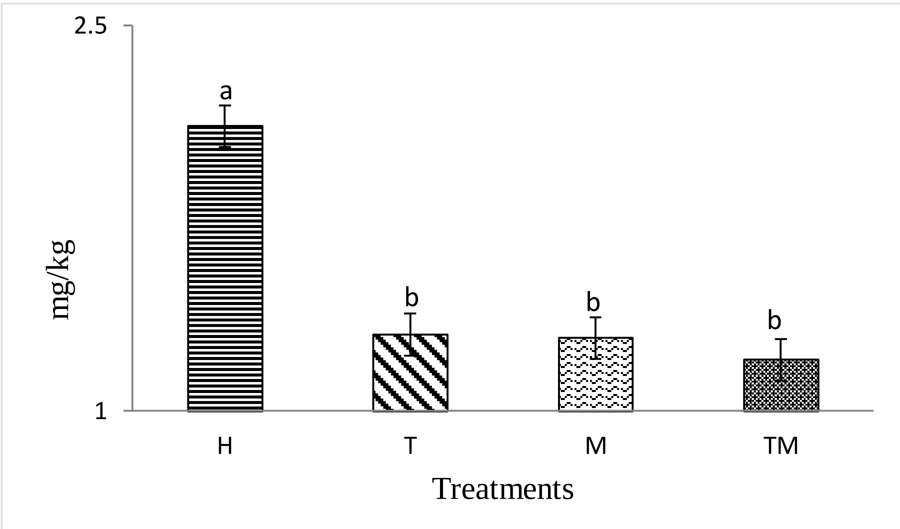

Figure 1 Thigh malondialdehyde (MDA) concentrations of broilers under heat stress (H) and fed mentha (M), thyme (T) of the combination of the two (TM) at day 42 of age (pooled SEM = 0.08, *P* <0.05).

Thyme and mentha powders reduced the thigh meat MDA in the current experiment. This could be the consequence of antioxidant components of these plants. The positive effects of some plant extracts have been detected on lipid oxidation and MDA formation in different types of meats. Dietary supplementation with *Forsythia suspensa* extract has reduced the oxidative stress of broiler chickens under high ambient temperatures (Wang *et al*., 2008). Tanabe *et al.* (2002) added the methanolic extracts of certain herbs and spices and observed the positive effect of *Zanthoxylum piperitum*, *Salvia officinalis* and *Zingiber officinale* Rosc. extracts on pork lipid oxidation. In addition, dietary incorporation of oregano, rosemary, and sage essential oils can retard lipid oxidation (MDA formation) in meat during refrigerated and frozen storage (Bianchi & Antunes, 1999).

Conclusions

According to the results of this study, dietary supplementation of thyme and mentha separately or together decreases the MDA and lightness and increases the pH and moisture of meat in heat-stressed chickens and hence can improve the meat quality during heat stress.

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Authors' contributions

AP and MD designed, conducted the growth trial and collected the samples from the birds. JA and FH did the MDA determinations. PF was the co-principal investigator and MD was principal investigator of the research and approved the article before publication.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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