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Original Article

Effect of dietary supplementation with *Mentha pulegium* and *Rosmarinus officinalis* on carcass characteristics, oxidative stability and quality of Japanese quail breast meat

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ABSTRACT

Background and Objectives: The aim of this study was to investigate the effect of dietary supplementation with *Mentha pulegium* and *Rosmarinus officinalis* on the carcass characteristics, physicochemical properties, color, texture and oxidative stability of Japanese quail breast meat.

Materials and Methods: 120 Japanese quails were divided into 4 treatments and 3 replications including T1: basic diet (control), T2: basic diet + *M. pulegium* (1.5%), T3: basic diet + *R. officinalis* (1.5%) and T4: basic diet + *M. pulegium* (1.5%) + *R. officinalis* (1.5%).

Results: The results showed there was no significant difference in breast and thigh weight, pH, dry matter, ash, cooking loss and vitamin E content among the treatments. Live weight, protein and fat contents, redness (a*), yellowness (b*), phenol and carotenoid contents of all treatments increased significantly ($P<0.05$) in comparison to control. Supplementation of diet with *M. pulegium* and *R. officinalis* individually or in combination significantly reduced TBARS, lightness (L*) and hardness compared to control ($P<0.05$).

Conclusions: The results revealed that supplementation of diet with *M. pulegium* and *R. officinalis* improved the protein and fat contents, color and oxidative stability of Japanese quail breast meat.

Keywords: Breast meat quality, Dietary supplementation, *Mentha pulegium*, Oxidative stability, *Rosmarinus officinalis*

Introduction

In recent years, broilers have shown the fastest growing and the most efficient species among the animals used for meat production [1]. Poultry meat consumption has increased due to relatively low production cost, low fat content and high nutritional value compared to the meat of other animals such as pig, sheep and cow [2]. The world's population increased from 3 billion to 7 billion within 1960-2010 and poultry production increased 10-fold during the same period [3]. Japanese quail (*Coturnix japonica*) is among the poultry species produced for meat and eggs especially in Asia, Europe and the United States [4].

The quality and composition of meat are affected by various factors such as bird genotype, slaughter age and feeding method [5]. Lipid oxidation and microbial degradation are two important factors affecting the meat shelf life [6]. Poultry meat is among the most susceptible meats to lipid oxidation because of its unsaturated fatty acids with several double bonds [7]. Lipid oxidation is a highly destructive chemical reaction which exerts adverse effects on flavor, color and nutritional value and produces toxic compounds and myoglobin oxidation changes the color of meat [5,8].

The adverse effects of chemical preservatives on human health have led to their replacement with herbal additives [9]. Therefore, one of the most important purposes of using natural antioxidants is to diminish the oxidation of meat thereby improving its sensory properties and nutritional value. Several plants, their essential oils and their phenolic extracts have been used as effective antioxidants or antimicrobials [10, 11, 12, 13,14].

Mentha pulegium belonging to *Lamiaceae* family is often found in wetlands along streams and springs originated from the highlands of temperate regions of Europe and Asia. Its fresh and dried leaves as well as

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flowers are used in foods, beverages and medicines [15]. The flowers are used in traditional medicine for treating cold, bronchitis, nausea, cholera, tuberculosis and sinusitis [16]. Its essential oil has antimicrobial, antifungal and antioxidant properties [7]. The most invaluable compounds found in the essential oil of *M. pulegium* are thymol and carvacrol with antioxidant activity [17, 18].

Rosmarinus officinalis belonging to *Lamiaceae* family is native to the Mediterranean region and is of special importance in western countries. It is a valuable plant due to its antimicrobial and antimutagenic properties [19]. Its fresh leaves and flowers have antioxidant properties [20]. It contains a wide range of phenolic acids and phenolic diterpenes which are responsible for its high antioxidant activity [21], while monoterpenes develop its antimicrobial properties [22].

The aim of this study was to investigate the effect of dietary supplementation with *M. pulegium* and *R. officinalis* on carcass characteristics, quality and oxidative stability of Japanese quail breast meat.

Materials and Methods

Treatments & Diets

This experiment was laid out in completely randomized design with 120 birds, 4 treatments and 3 replications each with 10 birds. The treatments included T1: basic diet (control), T2: basic diet + *M. pulegium* (1.5%), T3: basic diet + *R. officinalis* (1.5%) and T4: basic diet + *M. pulegium* (1.5%) + *R. officinalis* (1.5%). The diet for each period was formulated based on NCR tables (Table 1). Japanese quails were reared in cages up to 42 days of age having free access to water and food. *M. pulegium* and *R. officinalis* leaves were dried and powdered. At the end of 42 days of age, 4 birds from each replication were slaughtered and live weight at the time of slaughter as well as weight of breast and thigh muscles were recorded. The breast muscles were refrigerated in polyethylene bags for further experiments. The test protocol was approved by the Animal Ethnic Committee of Amol University of Special Modern Technologies (Ir. Ausmt. Rec. 1400. 16).

Physical properties of breast meat

To measure the pH value, 5 g of the breast meat were homogenized with 25 mL of distilled water in stomacher and then the pH value of each sample was measured using a pH meter (Jenway 3, 505, Staffordshire, UK) at room temperature [23]. Drip loss was measured by weight loss (%) of a piece of meat calculated after 24 h at 4°C [24]. In order to measure cooking loss, a piece of breast meat was weighed and placed in a water bath at 75°C for 1 h. Then the cooked and dried sample was cooled at room temperature for 30 min, and then weighed. Cooking loss (%) was calculated using the weight difference [24].

Table 1. Chemical composition of basic diet

Ingredients	Kg
Corn	600
Soybean meal	334
Corn gluten germ	10
Vegetable oil	20
Oyster shell	11
Dicalcium phosphate	14.5
Common salt	3.2
L-Threonine	0. 4
DL-methionine	2
*Vitamin and mineral premix	5
Chemical composition (%)	
Crude protein	20.20
Calcium	0.86
Available phosphorus	0.41
sodium	0.18
Lysine	1.06
Methionin+Cystine	0.60
Metabolisable energy (Kcal/kg)	3020

*vitamin and mineral premix supplied per kilogram of diet: Vit A, 10000 IU; Vit D3,
9800 IU; Vit E, 121 IU; Vit B12, 20 µg; Riboflavin, 4.4 mg; Calcium pantothenate,
40 mg; Niacin, 22 mg; Choline, 840 mg; Biotin, 30 µg; Thiamin, 4 mg; Zinc sulfate,
60 mg; Manganese oxide, 60 mg.

Chemical composition of breast meat

The amounts of dry matter [25], fat [26], ash [27] and protein [28] of breast meat samples were analyzed by methods proposed by the International Society of Chemists.

Textural properties of breast meat

The hardness of the breast meat samples was measured by a texture analyzer (Brookfield Texture Pro CT V1.2 Build 9, England). The samples (diameter of 3 cm and thickness of 1 cm) were compressed to 50% of their original thickness at room temperature. The force-time deformation curves were plotted with 25 kg cell load at a speed of 2 mm/s and the hardness (N) was analyzed [29].

Color of breast meat

Color indexes (L*, a*, b*) of the breast meat samples were measured by Konica Minolta colorimeter (Model CR-400, Japan) and L* (lightness), a* (redness) and b* (yellowness) were assessed [29].

Oxidative stability of breast meat

The amount of phenolic compounds was determined on the basis of capric ion reduction [30,31]. This method is based on reducing Cu (II) ion to Cu (I). The samples were mixed with copper chloride solution ($CuCl_2$) and neocuproine reagent in acetate buffer, incubated at 50°C for 20 min and then the absorbance of the samples was read at 450 nm.

The meat was homogenized by a homogenizer (Omni International, Kennesaw, USA) to measure the carotenoid content based on standard beta-carotene curve at 470 nm using a spectrophotometer (T80 UV/VIS; PG Instruments Ltd, Leicestershire, UK). The standard curve was calculated with different concentrations of carotenoid pigments and a commercial carotene reagent [32, 33].

To measure vitamin E content, 10 mg of breast meat were homogenized by a homogenizer (Omni International, Kennesaw, USA). The samples then were exposed to Fe_3 solution, 2,4,6-tripyridyl–s-triazine reagent and acetate buffer (pH=4). The standard curve was prepared with different concentrations of vitamin E and the absorbance of samples was read at 595 nm [33, 34].

To measure Thiobarbituric Acid Reactive Substances (TBARS), 5 g of breast meat was homogenized with 15 mL of homogenizing solution in a centrifuge for 30 s. The resulting mixture was filtered through filter paper and 5 mL of the filter solution were mixed with 5 mL of 0.02 M aqueous thiobarbituric acid solution in a test tube and incubated in a water bath at 100°C for 40 min. The samples then were cooled using cold water. The resulting color was read at 532 nm by a spectrophotometer (T80+UV-VIS; PG Instruments Ltd, Leicestershire, UK). The results were calculated by standard 1,1,3,3-tetraethoxy propane curve and expressed in mg malondialdehyde /kg (MDA) meat. TBARS value was determined after 14 days of refrigerated storage [35].

Statistical analysis

The data normality and the equality of variance were tested by Shapiro-Wilk test and Levene's test, respectively. Data were analyzed by parametric one-way variance analysis (ANOVA) and the means were compared by post hoc Tukey test. The results were expressed as mean±SD. Data were analyzed by SPSS version 26 (SPSS Inc. Chicago II, USA). 5% was considered as the significance level.

Results

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the carcass characteristics of Japanese quail is shown in Table 2. Live weight of the treatments was higher than that of control with the difference being only significant between T4 and T1 (P<0.05). Breast and thigh weight of the treatments also was higher than that of control however the difference was not significant (P>0.05).

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the physical properties of Japanese quail breast meat is shown in Table 3. The birds fed *M. pulegium* showed lower drip loss than the birds fed *R. officinalis* (P<0.05), while cooking loss and pH were not affected.

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the chemical composition of Japanese quail breast meat is shown in Table 3. There was no significant difference in dry matter and ash contents among the treatments (P>0.05). Protein content of the treatments was higher than that of control as the highest (19.05%) and the lowest (18.20%) contents were observed for T4 and control, respectively. All treatments showed significant difference in protein content. Fat content of the treatments was higher than that of control and the highest amount of fat was found for T4 (9.5%). T3 and T4 showed a significant difference regarding fat content (P<0.05).

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the textural properties of Japanese quail breast meat is shown in Table 3. The hardness of the treatments was lower than that of control and there was a significant (P<0.05) difference among the treatments.

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the color indexes of breast meat is shown in Table 3. All color indexes of the treatments were significantly different from those of control (P<0.05). L* value of the treatments was lower than that of control, while a* and b* values of the treatments were higher than those of control.

Table 2. Effect of dietary supplementation with *M. pulegium* and *R. officinalis* on carcass characteristics of Japanese quail

Experimental groups	T1	T2	T3	T4	P-value
Final Body Weight (g)	199.88±1.50[a]	202.92±1.66[ab]	200.08±1.82[ab]	204.88±2.39[b]	0.002
Breast weight (g)	22.36±1.34[a]	23.56±1.56[a]	24.00±0.79[a]	23.40±0.83[a]	0.20
Thigh weight (g)	15.40±1.002[a]	16.56±0.48[a]	15.46±0.58[a]	15.40±0.96[a]	0.09

Data are reported as mean values±SD; Different letters [a,b,c,d] in each row indicate significant differences (P<0.05). T1: Control, T2: *M. pulegium*, T3: *R. officinalis*, T4: *M. pulegium* + *R. officinalis*.

Table 3. Effect of dietary supplementation with *M. pulegium* and *R. officinalis* on physical characteristics, chemical composition, texture and color of Japanese quail breast meat

Experimental groups	T1	T2	T3	T4	P-value
pH	6.30±0.36^{a}	6.35±0.23^{a}	6.54±0.08^{a}	6.54±0.16^{a}	0.26
Drip loss (%)	2.10±0.44^{ab}	1.90±0.26^{a}	2.64±0.41^{b}	2.24±0.23^{ab}	0.02
Cooking loss (%)	1.56±0.38^{a}	1.62±0.34^{a}	1.46±0.32^{a}	1.52±0.13^{a}	0.87
Dry matter (%)	71.60±8.26^{a}	75.00±3.46^{a}	79.80±2.16^{a}	78.60±4.82^{a}	0.09
Ash (%)	1.52±0.32^{a}	1.36±0.35^{a}	1.16±0.18^{a}	1.26±0.23^{a}	0.26
Crude protein (%)	18.20±0.05^{a}	18.48±0.04^{b}	18.70±0.11^{c}	19.05±0.07^{d}	0.001
Fat (%)	9.10±0.05^{a}	9.25±0.08^{ac}	9.37±0.11^{bc}	9.50±0.10^{b}	0.001
Hardness (N)	9.17±0.10^{a}	8.11±0.08^{b}	7.47±0.16^{c}	7.79±0.19^{d}	0.001
L*	64.73±0.17^{a}	61.23±0.58^{b}	62.01±0.69^{b}	61.33±0.73^{b}	0.001
a*	4.45±0.32^{a}	6.42±0.25^{b}	5.48±0.32^{c}	5.99±0.10^{b}	0.001
b*	12.40±0.16^{a}	14.89±0.13^{b}	16.83±0.11^{c}	18.60±0.26^{d}	0.001

Data are reported as mean values±SD; Different letters ^{a,b,c,d} in the same row indicate significant differences (P<0.05). T1: Control, T2: *M. pulegium*, T3: *R. officinalis*, T4: *M. pulegium* + *R. officinalis*.

The effect of diet supplementation with *M. pulegium* and *R. officinalis* on the oxidative stability is shown in Table 4. The total phenol and carotenoid contents of the treatments were higher than those of control with the difference being significant among all treatments (P<0.05). The highest amount of total phenol (1228.38 ppm) and carotenoid (111.46 ppm) was found for T3. There was no significant (P>0.05) difference regarding vitamin E content among the treatments. TBARS value of all treatments was lower than that of control as there was a significant difference between the treatments and control (P<0.05).

Discussion

Live weight of the treatments was higher than that of control with the difference being only significant between T4 and T1. Herbs and spices not only stimulate appetite and digestion but can contribute to the health and welfare of animals by affecting physiological functions like probiotics, prebiotics, organic acids and plant extracts, thereby improving their growth performance [12]. These positive effects can be attributed to the antioxidant compounds in *M. pulegium* (luteolin-7-rutinoside, diosmin, and apigenin) and *R. officinalis* (carnosol, carnosic acid, rosmanol, rosmarinic acid, oleanolic acid and ursolic acid) which prevent nutrients oxidation. The breakdown of amino acids will be prevented and their absorption will be improved due to the antimicrobial compounds in *M. pulegium* which decrease the harmful bacterial populations in the gastrointestinal tract. These antimicrobial effects are probably due to the alteration in the permeability of cell membranes [36,37,38]. Similarly, Sarmad et al. [39] reported that dietary supplementation with rosemary powder at 2 levels (5 and 10 gr/kg feed) significantly increased live weight of quails. Helal et al. [40] showed that dietary supplementation with mixture of *M. oleifera* and *R. officinalis* leaves increased rabbit live weight significantly. It has been previously reported that addition of *M. pulegium* to broilers diet had no effect on breast and thigh weight [41].

Table 4. Effect of dietary supplementation with *M. pulegium* and *R. officinalis* on oxidative stability of Japanese quail breast meat

Experimental treatments	1	2	3	4	P-value
Phenol (ppm)	77.85±1.52^{a}	826.78±6.71^{b}	1228.38±12.00^{c}	1112.32±47.24^{d}	0.001
Carotenoid (ppm)	88.84±0.62^{a}	95.74±0.70^{b}	111.46±1.04^{c}	107.78±1.61^{d}	0.001
Vit E (mg/100g)	0.58±0.02^{a}	0.60±0.01^{a}	0.58±0.02^{a}	0.57±0.01^{a}	0.20
TBARS (mg MDA/kg)	0.33±0.01^{a}	0.19±0.01^{b}	0.20±0.01^{b}	0.20±0.02^{b}	0.001

Data are reported as mean values±SD; Different letters ^{a,b,c,d} in each row indicate significant differences (P<0.05). T1: Control, T2: *M. pulegium*, T3: *R. officinalis*, T4: *M. pulegium* + *R. officinalis*.

The birds fed *M. pulegium* showed lower drip loss than the birds fed *R. officinalis* (P<0.05), while cooking loss and pH were not affected. The reason of this phenomenon may be attributed to the strong antioxidant activity of *M. pulegium*. Similarly, Abdel-Wareth et al. [42] reported that addition of jojoba seed oil in broiler diets decreased drip loss of leg and breast meat [42,43]. Dietary supplementation with lycopene improved the WHC of meat during storage and decreased drip loss [44]. These results have been proved in previous studies in pigs, lambs and broilers [45, 46, 47, 48]. It has been reported that diet supplementation with *M. oleifera* leaves had no effect on drip loss [49]. Mirshekar et al. [50] reported that the addition of different plant essential oils such as rosemary, green tea and echinacea EO to the broilers diet had no effect on pH [50]. Similarly, Young et al. added oregano to the broilers diet and found no effect on the pH value [51].

Dry matter and ash contents of Japanese quail breast meat were not significantly different among the treatments. However T2, T3 and T4 had significantly higher protein and fat contents than control treatment. Similarly, Pirmohamammadi et al. [16] showed that dietary supplementation with *T. vulgaris* and *M. pulegium* individually or in combination had no effect on dry matter of broilers thigh meat. Due to the synergistic effects of the active compounds of *M. pulegium* and *R. officinalis*, protein metabolism may improve. Rosemary is assumed to increase the secretion of enzymes that facilitate the digestion of nutrients in the gastrointestinal system. Also medicinal plants such as rosemary can increase the mRNA abundance especially amino acids and peptide transporters [39]. The addition of herbal plants to the broilers diet could improve the fatty acids profile of meat [52]. Similarly, Marcincakova et al. [52] reported that supplementation of diet with 1% *A. foliumin* and 1% *C. oxyacantha* increased the protein and fat contents of breast meat. Nasir and Grashorn [53] showed that the birds fed *E. purpurea* and *N. Sativa* significantly had higher crude protein content [53].

All experimental treatments had lower hardness as compared to control. Saracila et al [54] showed that dietary supplementation with chromium picolinate and creeping wood sorrel powder caused a decrease in the mechanical strength of muscle fiber and lower hardness of chicken meat in comparison to control [54]. Similarly, dietary supplementation with oregano, eucalyptus, thyme, garlic, lemon, rosemary, and sweet orange extracts in dried form decreased hardness of broiler chickens [55]. This result has been proved previously in meat of rabbits fed the whole *Tithonia tubaeformis* plant [56]. This phenomenon may be attributed to the limitation of fatty acids oxidation in tissues [57]. Breast meat of the broilers fed a mixture of vegetables and herbs (ginger, rosemary, chili) significantly showed lower shear force [58]. Wang et al. reported that diet supplementation with marigold extract reduced the shearforce of broiler meat compared to control [59].

a* and b* values of breast meat for all treatments fed *M. pulegium* and *R. officinalis* individually or in combination were increased significantly and they showed significantly lower L* value. An important factor affecting the consumer acceptance is the color of meat [60]. The reason for color changes in meat is the oxidation of red oxymyoglobin to metmyoglobin which develops a brown color in meat. It has been reported that natural antioxidants increase redness and delay the metmyoglobin formation thereby preventing meat discoloration [61]. Pirmohamammadi et al. [16] reported that addition of *T. vulgaris* and *M. pulegium* to broilers diet decreased L* value of thigh meat. Increase of a* value of ewe and broilers meat due to addition of *R. officinalis and M. Oleifera* leaves has been reported previously [49, 62]. Similar to the results of the current study, Aminzade et al. [63] showed that dietary supplementation with *M. piperita* increased a* and b* values of Japanese quail breast meat.

Based on the results of this study, all treatments had significantly higher total phenol and carotenoid content compared to control. The antioxidant activity of the herbal components is associated with the phenols attached to the conjugated groups which can inhibit oxygen free radicals [64]. Some researchers proved the positive effect of dietary supplementation with rosemary, nettle, chokeberry and *V. coignetiae* on total phenol content of breast and thigh meat [65, 66]. Accumulation of carotenoids in muscles as natural antioxidants increased the quality of poultry meat through developing yellow color and improving flavor [33]. In a study conducted by Young et al., dietary supplementation with oregano increased the yellowness of breast meat [51]. Similarly, Dietary supplementation with oregano EO increased the b* value of breast meat at 9 and 12 weeks of age [67].

The treatments showed no significant difference on vitamin E content. Vitamin E can extend the shelf life of meat products via delaying the lipid oxidation [33]. Similarly, Botsoglou et al. reported that the addition of 1% dehydrated rosemary leaves to the diet of turkeys had no effect on the vitamin E content of breast and leg meat [68].

TBARS value of all treatment was significantly lower than that of control. High concentration of unsaturated fatty acids with several double bonds in poultry meat makes it susceptible to oxidative degradation [7]. Many species of *Lamiaceae* family such as oregano, rosemary, thyme and marjoram have a significant amount of antioxidants [8]. Similarly, Olmeza and Yoruk [41] showed that dietary supplementation with *M. pulegium* reduced TBARS value of broilers meat. Rostami et al. [21] showed that addition of *R. officinalis* and vit E to broilers diet decreased TBARS value of breast meat at days 4, 7 and 17 at refrigerator temperature. Dietary supplementation with 1.5 and 2%

Squaw Mint Herb reduced TBARS value in Japanese quail meat after one month storage at freeze condition [69].

Conclusion

Dietary supplementation with *M. pulegium* and *R. officinalis* increased live weight and oxidative stability and improved quality properties (protein, fat and color) of Japanese quail breast meat.

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