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Chemical Composition and Dietary Effects of Pennyroyal and Dill on Biochemical, Hematological, and Oxidative Stress Biomarkers in Broiler Chickens

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ABSTRACT

The current study was conducted to determine chemical constituents present in *M. pulegium* L and *A. graveolens* L. essential oils and investigate dietary effects of the herbal powders on some biochemical, hematological and oxidative stress parameters. A 42-day fully randomized trial was conducted using 240 broilers (Ross 308) divided into 4 main groups with three replicates, supplemented with the aerial parts of the plant materials as follows: (I) Control (corn-soybean meal only); (II) 1% pennyroyal; (III) 1% dill; (Ⅳ) the combination (0.5% pennyroyal+0.5% dill). Numerous active compounds were detected in the essential oils of both plants. 1,8-cineole was found to be the major constituent in pennyroyal's essential oil and Carvone in dill. The serum cholesterol, triglyceride as well as High and Low Density Lipoprotein (HDL and LDL) levels were significantly different among treatments (P< 0.05). However, the combination of both plants had more pronounced effects on the aforementioned parameters. A significant increase in total protein content also was observed in the groups supplemented with herbal powders. Nevertheless, dill powder had no significant effect on glucose levels. Heterophil and lymphocyte counts also were different between groups (P< 0.05). Moreover, activities of glutathione peroxidase, superoxide dismutase and catalase were significantly changed following the herbal supplementation. A significant decrease in malondialdehyde content and increase in total antioxidant capacity were recorded in all supplemented groups. Conclusively, supplementation with the dried powders can improve serum biochemistry and enhance the antioxidant status. However, it seems like the combinatorial supplementation is more effective.

Keywords: *A. graveolens*, Combinatorial supplementation, Herbal supplementation, *M. pulegium*.

INTRODUCTION

Since many years ago, vast varieties of feed additives such as antibiotics have been broadly utilized in the poultry industry. In fact, it is a well-known concept that manipulation of gut function and microbial habitat of domestic animals with feed additives is an effective way to enhance growth performance and feed efficiency (Rahimi *et al.*, 2011). Accordingly, almost 80% of domestic animals are supplemented with synthetic additives because of medication, growth promotion or other purposes (Lee *et al.*, 2001). However, in the recent years, numerous reports about antimicrobial resistances and antibiotic residues in humans' food have raised the fear that some of the bacterial species might no longer be susceptible to the available antibiotics. As a result, the resistance to antibiotics will become a worldwide crisis, endangering whole life on the earth, soon (Ventola, 2015).

Production of the primary poultry products, including meat and eggs, progressively has

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grown all over the world. From 1995 to 2005, consumption and thus production of poultry products have increased globally (percentage increase) for chicken meat (53%), turkey meat (13%), duck meat (67%), goose meat (53%), chicken eggs (39%), and other eggs (27%) (Scanes, 2007), resulting in an intensive competition among poultry products producers. Various amounts of synthetic additives are used to promote growth performance, increase carcass quality, and decrease susceptibility of animals to pathogens. However, there are some strict rules that limit utilization of these feed additives. Therefore, seeking for novel, safe, cost effective, readily available and natural agents, particularly in the poultry industry, is absolutely crucial.

In the past few years, several researchers have focused on various herbal supplements, because of their safety, accessibility, naturalness and cheapness (Hadj Ayed *et al.*, 2018; Hajati *et al.*, 2018; Helal *et al.*, 2018; Mokhtari *et al.*, 2018; Qorbanpour *et al.*, 2018). Various herbal derivatives including dried powder, extract, essential oil and even green algae itself have been recently tested in poultry for different reasons including, growth promotion, fighting against oxidative stress, aflatoxins and microbes (Yakhkeshi *et al.*, 2012; Ciurescu *et al.*, 2016; Calislar and Demirtas, 2017; Rahimian *et al.*, 2017; Bagherzadeh Kasmani *et al.*, 2018; Rooy *et al.*, 2018). Most of herbal supplements are versatile, meaning that they can play several roles simultaneously when administered to chickens. For example, Ciurescu and collaborators (2016) showed that Camelia (*Camelina sativa* L. Crantz variety) had the ability to enhance immune system and growth performance as well as improve meat quality in broiler chickens.

Oxidative stress caused by excessive levels of reactive oxygen species including superoxide, hydroxyl and hydrogen peroxide radicals that are induced under stressful conditions, such as heat exposure or coccidiosis, can retard birds' performance (Dalloul *et al.*, 2006). In normal conditions, both enzymatic and non-enzymatic antioxidant systems eliminate excessive oxidative radicals. Oxidative status are mainly assessed by measuring Total Antioxidant Capacity (TAC), and activities of three key antioxidant enzymes including Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT). Moreover, lipid peroxidation level can be assessed by measuring Malondialdehyde (MDA) in biological samples (Wang *et al.*, 2008). Supplementation with synthetic antioxidants (e.g., α-tocopheryl acetate or butylated hydroxytoluene) has been proposed as an effective way to alleviate oxidative damages in the poultry industry. However, recently, the global trend has been changed and researchers have concentrated on herbal materials.

Mentha pulegium L is one of the 20 species in Mentha genus from Labiatae family with worldwide spread. The plant is commonly known as pennyroyal of which the aerial parts have been traditionally used for its antimicrobial properties in the treatment of cold, sinusitis, cholera, food poisonings, bronchitis and tuberculosis (Mahdavi *et al.*, 2013). Moreover, pennyroyal is a powerful antioxidant (El-Ghorab, 2006). *Anethum graveolens* L (dill), an important member of the Apiaceae, has been extensively used as a traditional herbal medicine throughout Europe, Asia, and America (Sharopov *et al.*, 2013). Some important pharmacological effects of dill include antibacterial (Lopez *et al.*, 2005), antioxidant (Singh *et al.*, 2005) and cancer chemopreventive (Zheng *et al.*, 1992).

Considering the urgent demand for natural feed additives and numerous advantages of herbal supplements, this study was designed to evaluate possible beneficial effects of dried powders of aerial parts of two common plants alone and in combination with each other in broiler chickens. With this aspect, several biological and hematological factors, as well as oxidative stress biomarkers were measured. Chemical composition of these plants was also studied to better understand which of the chemical constituents are responsible for the biological effects.

MATERIALS AND METHODS

Animals and Experimental Protocols

Two hundred and forty (240) uniform 1-day old male broiler chicks (Ross 308) with average weight of 41.65 g were purchased from a local commercial hatchery. The animals were randomly divided into 4 major groups with three replicates, each containing 20 birds. Birds were housed in floor pens of identical size (1×2 m) using wood shaving as litter. Animal care was performed exactly according the recommendations in the Guide for the Care and Use Committee of Islamic Azad University, Kermanshah Branch, Iran, and that reported by Yakhkeshi *et al*. (2012). Briefly, lighting schedule was 23 L/1 D and temperature was gradually reduced from 32°C by lowering 3°C weekly (Yakhkeshi *et al.*, 2012). All of the animals had free access to food and water during the experimental course (42 days). Nutritional requirements of the chickens were met using two different corn-soybean meal diets for the starter (day1- 21) and grower (day 22- 42) based on the NRC guidelines (NRC, 1994). Trace mineral and vitamin premixes (0·25%) were added to the basal diet, but no commercial antioxidant was included in the basal diet. More details about the diets are available in Table 1. The dietary treatments were: (I) Control (basal diet); (II) Basal diet supplemented with 1% dried powder of aerial parts of pennyroyal; (III) Basal diet supplemented with 1% dried powder of aerial parts of dill; and (Ⅳ) Basal diet supplemented with the combination of 0.5% pennyroyal and 0.5% dill. The dried powder of the plant materials was thoroughly mixed with basal diet.

Plant Material

M. *pulegium* L and A. *graveolens* L were purchased freshly from the local market at the middle of summer 2017. Pharmacognostic verification of the plants was done with the aid of Department of Botany and Herbal Medicine, Faculty of Agriculture and Technology, Kermanshah University, Kermanshah, Iran.

Extraction of Essential Oils

Aerial parts of both plants were air-dried and grinded into fine powder (250 g), then subjected to hydro-distillation for ~ 3 hours using a Clevenger-type apparatus. The obtained oils were dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4°C until further analysis.

Table 1. Ingredient and nutrient composition of the basal diets.

Item (%)	Starter (1- 21)	Grower (22-42)
Corn	58.74	54.20
Soybean meal	32.66	23.82
Wheat	0	15
Fish meal	3	3
Soy oil	2.45	1.17
Bone meal	1.60	1.51
Oyster shell	0.65	0.5
Common salt	0.25	0.23
Mineral mixture [a]	0.25	0.25
Vitamin mixture [b]	0.25	0.25
DL-Methionine	0.15	0.07

[a] BTrace mineral mix provided (mg kg^{-1} of diet): $MnSO_4$= 248 mg; $FeSO_4 \cdot 7H_2O$= 125 mg; ZnO= 211 mg; $CuSO_4$= 25 mg; CaI_2= 25 mg, Se= 0.5 mg, choline= 625 mg. [b] Supplied per kilogram of diet: Vitamin A= 22500 IU; Vitamin D3= 5000 IU; Vitamin E= 45 IU; Vitamin B1= 4.3 mg; Vitamin B2= 16.5 mg; Vitamin B12= 0.04 mg; Pantothenic acid= 24.5 mg; Folic acid= 2.5 mg; Niacin= 74 mg; Pyridoxine= 7.3 mg, Biotin= 0.0f mg.

Gas Chromatography/Mass Spectroscopy (GC/MS) Analysis of the Essential Oils

GC/MS analysis was carried on a Varian Gas Chromatograph (Thermofinigan, USA) series 3800 fitted with a VF-5 ms fused silica capillary column (60 m×0.25 mm, film thickness 0.25 µm) coupled with a 4000 series mass detector under the following conditions: Injection volume 0.5 µL with split ratio 1:60, helium as the carrier gas at 1.0 mL min^{-1} constant flow mode, injector temperature 260°C, oven temperature was programmed from 70 to 180°C at 3°C min^{-1}. Mass spectra: Electron Impact (EI+) mode, 70 eV and ion source temperature 290°C. Mass spectra were recorded over 50–500 amu range.

Identification of Components

Identification of the essential oil constituents was performed on the basis of Retention Index [RI, determined with respect to homologous series of n-alkanes (C8–C40, Polyscience Corp., Niles IL) under the same experimental conditions], co-injection with standards and MS Library search (NIST 05 and Wiley) and by comparing with the MS literature data (Adams and Sparkman, 2007).

Sampling

On day 42, all of the birds in each group were humanely euthanized with carbon dioxide and blood specimens were directly punctured from the heart (approximately 6 mL), then transferred to heparinized tubes and tubes filled with separator gel. Serum samples were separated from the none-heparinized tubes following centrifugation at 1,400 g at 6°C for 30 minutes and placed in the Eppendorf tubes, then stored at -20°C until analysis onset. The heparinized samples were used for counting of heterophil and lymphocyte. Two drops of blood was smeared on two glass slides, then, stained with Wright stain for 15 minutes.

Biochemical Analysis

Biochemical analysis was performed using automated biochemistry analyzer (BT1500) in Central Laboratory of Veterinary Faculty. All of the parameters including glucose, total protein, Triglyceride (TG), cholesterol, high and low density lipoprotein were measured using standard assay kit (PARSAZMUN, Tehran, Iran) based on the instructions and recommendations of the manufacturer in serum samples.

Oxidative Stress Parameters

Various oxidative stress biomarkers including the activities of antioxidant enzymes, Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD) and Catalase (CAT) as well as Malondialdehyde (MDA) content as a marker of lipid peroxidation and total antioxidant capacity (TAC) were measured in the serum samples according to the previously described procedures (Rezaei and Dalir-Naghadeh, 2006; Nazarizadeh and Asri-Rezaie, 2016).

The enzymatic activities were measured spectrophotometrically. Very briefly, SOD activity was determined based on xanthine-xanthine oxidase assay (McCord and Fridovich, 1969) using commercially available standard kit (RanSod, RanDox Co., UK). Catalase activity was detected by monitoring the disappearance of hydrogen peroxide. The absorbance alteration at 240 nm was monitored for 30 s against a blank containing phosphate buffer instead of substrate (Kotze and McClure, 2001). The activity of GSH-Px was evaluated spectrophotometrically with GSH-Px detection kit (Ransel, RanDox Co., UK) according to the manufacturer's instructions and previously described guidelines (Nazarizadeh and Asri-Rezaie, 2016). The

activities of the aforementioned enzymes were expressed as U L^{-1}.

Malondialdehyde content as a biomarker of lipid peroxidation was measured as Thiobarbituric Acid Reactive Substances (TBARS) based on the previously described procedure with trifling alteration (Nair and Turner, 1984). The level of malondialdehyde was expressed as nmol per liter. Total antioxidant capacity was determined using a standard kit (Randox Laboratories Ltd., UK) and expressed as milli-mole per liter.

Statistical Analysis

All of the hematological and biochemical data was expressed as mean±Standard Deviation (SD). Differences between the means were compared by one-way Analysis Of Variance (ANOVA-1) followed by post-hoc Bonferroni test. Statistical analysis was performed using SPSS software, version 22 (IBM Corp., Chicago, IL, USA). A "P" value less than .05 was considered significant.

RESULTS

Chemical Composition of the Essential Oils

Several chemical constituents were identified in the essential oils of M. *pulegium* L and A. *graveolens* L (Tables 2 and 3). Table 2 shows that 28 different components exist in the essential oil of M. *pulegium* L, which constitute 95.76% of total composition. The predominant

Table2. Chemical composition of essential oil from aerial parts of *Mentha pulegium* L.[a]

Components	Retention time (s)	(%)
α-Myrcene	24.01	1.02
α-Terpineol	23.25	7.79
Pulegone	**25.37**	**14.82**
Cadinene	26.56	0.01
Menthol	33.82	3.28
Limonene	9.56	3.02
Sabinene	20.22	0.82
Camphene	22.51	0.61
Piperitenone	28.50	9.17
Neomenthol	29.50	2.82
Piperitone	**30.43**	**12.12**
Menthyl acetate	25.00	0.72
β–Caryophyllene	31.01	0.42
trans-Ocimene	31.31	0.14
Isomenthone	32.02	1.56
menthofuran	32.50	3.21
1-Octen-3-ol	34.50	0.09
Germacrene D	35.90	1.89
Geranyl acetate	36.30	2.06
Terpinolene	37.20	1.54
Piperitone oxide	39.03	3.06
α-Terpinene	40.01	0.05
Carvone	45.00	1.13
1,8-Cineole	**46.50**	**18.46**
Linalool	51.09	3.03
Linalyl acetate	40.03	0.02
Menthone	42.56	2.89
Terpinen-4-ol	44.12	0.02

[a] The dominant compounds are indicated in bold.

Table3. Chemical composition of essential oil from aerial parts of *Anethum graveolens* L.[a]

Components	Retention time (s)	(%)
α-Pinene	6.32	1.02
Myrcene	8.53	0.14
α-Phellandrene	**9.39**	**22.36**
p-Cymene	10.38	1.1
Dill ether	**18.63**	**15.41**
Limonene	12.64	1.38
cis-Dihydrocarvone	15.41	0.35
Camphor	15.83	0.31
trans-Dihydrocarvone	17.92	1.58
neoiso-Dihydrocarveol	8.42	0.14
Carvone	**23.59**	**54.37**
(E)-Anethole	19.1	0.17
Dillapiole	8.24	0.32
Cadinol	31.17	0.09
γ-Muurolene	22.51	0.22
α-Tujene	7.36	0.14

[a] The dominant compounds are indicated in bold.

elements are 1,8-cineole (18.46%), Pulegone (14.82%) and Piperitone (12.12%). As can be seen in Table 3, Carvone is considerably high (54.37%) in the essential oil of A. *graveolens* L. It is followed by α-Phellandrene (22.36%) and dill ether (15.41%).

Biochemical and Hematological Factors

The effects of herbal supplementation with 1% dried powders of pennyroyal and dill on various biochemical factors as well as heterophil and lymphocyte counts are depicted in Table 4. As presented, both of the plants could significantly decrease cholesterol, LDL and triglyceride levels compared to the control values, however, the combination imposed more pronounced effects on decreasing the aforementioned parameters. Athough pennyroyal powder could decrease glucose levels significantly, dill powder had no significant effects on glucose contents. Dietary treatment of the chickens with the plant materials could increase HDL contents significantly when compared with the control group. However, the combination was found to be more effective in increment of HDL content. The herbal powders also exerted exactly similar effects on total protein concentrations.

Table 4. Effect of dietary *Mentha pulegium* (Pennyroyal) and *Anethum graveolens L* (Dill) supplement on serum cholesterol, glucose, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglyceride (TG) and Total Protein (TP) as well as Heterophil (Hetro) and Lymphocyte (Lympho) numbers in broilers (n= 60). [a]

Parameter	Control	Pennyroyal	Dill	Pen+Dill	P value
Glucose	195.59±1.94^{a}	188.10±3.56^{b}	194.26±2.16^{a}	190.68±2.12^{d}	<0.027
Cholesterol	131.76±0.84^{a}	108.88±9.94^{b}	119.49±12.91^{c}	98.68±0.78^{d}	<0.001
HDL	51.06±0.33^{a}	56.66±0.51^{b}	52.92±1.03^{c}	58.87±0.51^{d}	<0.001
LDL	57.03±0.43^{a}	41.82±0.33^{b}	52.59±0.39^{c}	34.83±0.42^{d}	<0.001
TG	101.07±0.81^{a}	73.91±1.50^{b}	81.31±0.63^{c}	70.59±0.87^{d}	<0.001
TP	3.47±0.07^{a}	3.64±0.03^{b}	3.52±0.01^{c}	3.69±0.02^{d}	<0.001
Hetro	24.62±0.44^{a}	18.65±0.42^{b}	21.63±0.34^{c}	18.86±0.46^{d}	<0.047
Lympho	68.63±0.51^{a}	72.57±0.58^{b}	70.27±0.50^{c}	70.54±0.03^{c}	<0.001

[a] Means within a row with different superscript letters (a–d) denote significant differences ($P< 0.05$) with the control. Unit of all biochemical factors is mg dL^{-1}.

Moreover, heterophil and lymphocyte counts were changed following treatment with the plant materials. The plant powders could significantly decrease the number of heterophil, but increased lymphocyte count.

Oxidative Stress Biomarkers

Various oxidative stress biomarkers were assessed and the obtained results are presented in Table 5. Generally, both of the herbal powders could alter the biomarkers significantly compared to the control group, except for GSH-Px activity. Moreover, the combination of both powders was more effective when compared to each plant alone. As indicated in Table 5, SOD activity was significantly increased following treatment with various types of supplements. On the other hand, the activity of CAT was decreased following the supplementation ($P< 0.05$). In addition, the activity of GSH-Px was constant for both of the plants and no alteration was recorded, but the combination could increase the enzymatic activity, significantly. Malondialdehyde was measured to access the status of lipid peroxidation in serum samples of the broilers. The results showed that herbal powders could decrease MDA content. As presented in Table 5, MDA level decreased almost 2 folds in the combination group when compared to the untreated group. On the other hand, the herbal materials could enhance total antioxidant capacity, significantly.

DISCUSSION

This comprehensive study was conducted to evaluated possible beneficial effects of two well-known plant materials, namely, M. *pulegium* L and A. *graveolens* L, in the broiler chickens. Numerous biochemical and hematological factors were examined to confirm these effects. More importantly, several oxidative stress biomarkers were measured to have a broad overview about the antioxidant potential of these plants. Moreover, chemical composition of the herbal supplements was studied to find the predominant components in them. The efficacy of the herbal powders was evaluated alone and in combination with each other to investigate the possibility of synergistic effects.

The obtained data showed that 28 different components were present in the essential oil of M. *pulegium* L, of which the most predominant ones were 1,8-cineole (18.46%), Pulegone (14.82%) and Piperitone (12.12%). Furthermore, 16 chemical constituents could be detected in the essential oil of dill, of which more than 50% of the oil was comprised of Carvone (54.37%). The findings about chemical composition of pennyroyal was moderately different compared to previous reports claiming that Pulegone is the major components of pennyroyal (Brahmi *et al.*,

Table 5. Effect of dietary *Mentha pulegium* L (Pennyroyal) and *Anethum graveolens* L (Dill) supplement on Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT) activities, as well as Malondialdehyde level (MDA) and Total Antioxidant Capacity (TAC) in serum samples of broilers (n= 60). [a]

Parameter	Control	Pennyroyal	Dill	Pen+Dill	P value
SOD	98.71 ± 3.85^{a}	112.28 ± 2.94^{b}	119.49 ± 6.91^{b}	134.61 ± 9.48^{c}	<0.001
GSH-Px	146.26 ± 10.94^{a}	150.49 ± 8.59^{a}	153.46 ± 9.86^{a}	173.68 ± 5.49^{b}	<0.041
CAT	56.81 ± 2.33^{a}	52.16 ± 1.41^{b}	50.32 ± 1.19^{b}	44.27 ± 2.15^{c}	<0.021
MDA	8.13 ± 0.41^{a}	6.39 ± 0.12^{b}	5.97 ± 0.69^{b}	4.17 ± 0.22^{c}	<0.001
TAC	18.37 ± 0.31^{a}	22.58 ± 0.53^{b}	20.91 ± 0.68^{c}	26.59 ± 0.37^{d}	<0.001

[a] Means within a row with different superscript letters (a–d) denote significant differences ($P< 0.05$) with the control. Enzymatic activity (SOD.CAT, GSH-Px)= U L^{-1}, MDA= nmol L^{-1}, TAC= Milli mol L^{-1}.

2016; Bouyahya *et al.*, 2017). However, the data obtained from dill analysis matched those reported previously from Tajikistan and Romania (Radulescu *et al.*, 2010; Sharopov *et al.*, 2013). A possible explanation for the observed differences in the chemical composition is that several factors including climate, region, cultivation system, soil nutrients and extraction method can affect chemical composition of plant's essential oil (Mehdizadeh *et al.*, 2016).

Pulegone, a naturally occurring monoterpene, is responsible for pleasant smell of pennyroyal. Carvone is also a monoterpene hydrocarbon found in excessive quantities in the seeds of caraway, dill, and spearmint, belonging to the family of terpenoid (Gon *et al.*, 2008). Carvone possess several biological effects and usages. According to the recent report, a phytogenic feed additive based on thymol, carvacrol, and cinnamic aldehyde could improve live body weight, reduce total bacterial count in the environment, lymphocyte counts, and exert hepatoprotective effects in broiler chickens (Reis *et al.*, 2018). Another feed additive based on thymol and carvacrol could alleviate the intestinal injury by improving intestinal integrity and modulating immune responses in the C. perfringens-challenged broiler chickens (Du *et al.*, 2016). In a separate study, supplementation with the same combination enhanced performance, increased antioxidant enzyme activities, retarded lipid oxidation, enhanced digestive enzyme activities, and improved immune response of broilers (Hashemipour *et al.*, 2013).

In the current research, dried powder of the plants was used because it is the most common and easiest way of utilization. Apparently, plant supplements when used in the form of herb or essential oil have different effects in live birds (Cross *et al.*, 2007). However, only a few studies have been conducted on utilization of dried solid plants in broiler chickens. Therefore, in designing the current research, the dried powder was used so that the results would be practical.

According to our experiments, supplementation with both of the plants could decrease cholesterol, TG and LDL levels, but pennyroyal was found to be more effective than dill. The probable explanation for this finding is that pennyroyal essential oil contains biologic compounds that are more diverse. Besides, pennyroyal could reduce glucose contents, but dill had no effects. Our data also clearly showed that these plants had synergistic effects and, when used in combination, their effects were potentiated. Consistently, Torki *et al.* (2018) showed that adding dill essential oil to the diet of laying hens had similar effects. Possible mechanism beyond the effects of dill on lipid profile is that it can bind to bile acids and thereby reduce lipid absorption in intestine, leading to increased lipid excretion and reduced blood lipid concentration (Torki *et al.*, 2018). Furthermore, Qureshi *et al.* (1983) suggested that the activity of key enzymes including 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) reductase and cholesterol-7 hydroxylase fatty acid synthase, which play pivotal role in lipid metabolism, as well as pentose phosphate pathway were reduced by consumption of herbal supplements, possibly leading to decreasing lipid concentration in blood. This study showed that herbal supplements had the ability to increase total protein contents of serum. The exact mechanisms beyond the observed beneficial effects in broilers have not yet been completely elucidated. However, a possible theory is that harmful bacteria of the gastrointestinal tract are able to break down amino acids, resulting in the decreased level of absorption (Nobakht *et al.*, 2011). Therefore, the antimicrobial properties of M. *pulegium* L (Goodarzi and Nanekarani, 2014) can reduce the harmful bacterial populations throughout the intestine and its antioxidant effects can protect amino acids against oxidation and thus improve the levels of absorbed amino acids. In agreement with this hypothesis, Erhan *et al.* (2012) demonstrated that the

addition of pennyroyal reduced E. coli count and increased the lactic acid bacteria count of the jejunum in broilers.

We observed a slight alteration in heterophil and lymphocyte counts probably due to antimicrobial and antioxidant effects of the herbal supplements. Since the herbal materials are cable of reducing harmful pathogens in the broilers, as a result, the number of heterophil has been decreased. This finding is consistent with the previous report (Hashemipour *et al.*, 2013). Tissues and cells are protected against detrimental effects of active oxygen species and oxidant molecules by cooperative activity of antioxidant enzymes, SOD, GSH-Px, and CAT. The physiological concentrations of these enzymes are tightly controlled. A minor alteration in the concentrations may result in malfunction of the whole system and vulnerability of biomolecules to oxidative damages (Nazarizadeh and Asri-Rezaie, 2016). The results obtained from this study showed that herbal powders could increase SOD activity in serum of broilers. Considering that SOD has the largest catalytic efficiency of any known enzyme (Nazarizadeh and Asri-Rezaie, 2016), therefore, the increased activity is probably to cope with free radicals. In agreement with this result, supplementation with *Curcuma longa* L dried powder in the diet of broilers containing aflatoxin B_1 could restore SOD activity in liver (Gowda *et al.*, 2009). It also was observed that GSH-Px activity did not change following receiving the plant materials, but the combination of both could elevate the enzyme activity. A possible explanation is that the herbal supplements can either increase the biosynthesis of Glutathione (GSH), substrate of the enzyme or reduce the oxidative stress leading to less degradation of GSH, or having both effects (Ukperoro *et al.*, 2010). Consistent with our data, Wang *et al*. (2008) demonstrated that dietary administration of broiler chickens under high ambient temperature with *Forsythia suspensa* extract could restore GSH-Px activity. Following the dietary treatment with herbal powders, we observed that CAT activity was reduced significantly, and a further reduction was noticed with the combination. This finding can be attributed to direct antioxidant nature of the herbal supplements that could eliminate the enzyme substrate (hydrogen peroxide) or protect against peroxidation of lipids and thus reduce the enzyme activity.

Measurement of lipid peroxidation is a gold marker of oxidative damage caused by ROS, and the assessment of MDA is a reliable method to gain such determination (Nazarizadeh and Asri-Rezaie, 2016). Moreover, the potential to oxidative stress in the body can be attained by measuring the antioxidant capacity of tissues and organs (Tiwari *et al.*, 2014). In fact, the capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants (Ghiselli *et al.*, 2001). The results of the current research showed that both of the herbal powders were able to enhance total antioxidant capacity of the birds and protect against lipid peroxidation. These findings clearly propose that both of the plant materials can boost up anti-oxidative defense and simultaneously prohibit free radical formation matching to those reported previously (Wang *et al.*, 2008). The improvement of serum antioxidant capacity by dietary phytogenics could be due to the active components and their phenolic group constituents, which exhibit a strong antioxidant effect or induction of the antioxidant enzyme activities (Mehmet *et al.*, 2005; Polat *et al.*, 2011).

Our data clearly demonstrates that essential oils obtained from M. *pulegium* L and A. *graveolens* L contain various amounts of several biologically active constituents. Moreover, supplementation with the dried powders of these plants can improve biochemical and hematological parameters probably because of antimicrobial and antioxidative effects. Furthermore, the combination of these plants has synergistic effects. The herbal

supplements are also able to protect against oxidative stress and eliminate free radicals as evidenced by measuring several biomarkers of oxidative stress. A major limitation of the current study is its design based on single time point evaluation. However, multiple time-point studies can extend our knowledge about the effects of herbal supplements on oxidative stress biomarkers during various life stages of broilers. Furthermore, growth performance and carcass quality should be evaluated. Such determinations should be covered in further studies.

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شناسایی ترکیبات شیمیایی و بررسی اثرات خوراکی پونه و شوید بر پارامترهای بیوشیمیایی، خونی و نشانگرهای زیستی استرس اکسیداتیو در جوجه های گوشتی

ف. محمدی

چکیده

مطالعه کنونی با هدف ۱) شناسایی ترکیبات شیمیایی موجود در روغن اسانسی *M. pulegium* L. و *A. graveolens* L و ۲) بررسی اثرات خوراکی پودر گیاهان مذکور بر برخی از پارامترهای بیوشیمیایی، خونی و استرس اکسیداتیو طراحی و اجرا گردید. کارآزمایی کاملا تصادفی با استفاده از ۲۴۰ قطعه جوجه گوشتی (راس ۳۰۸)، تقسیم شده به ۴ گروه اصلی با ۳ تکرار و تحت تیمار قسمت های هوایی مواد گیاهی به ترتیب ذیل اجرا گردید: ۱) کنترل (فقط جیره مبتنی بر سویا و ذرت)، ۲) ۱٪ پونه، ۳) ۱٪ شوید، ۴) ترکیبی از هر دو گیاه (۰/۵٪ + ۰/۵٪). ترکیبات شیمیایی فعال متعددی در اسانس روغنی هر دو گیاه شناسایی شد. 1,8-cineole بیشترین ترکیب شمیایی موجود در اسانس روغنی پونه و Carvone در شوید بود. مقادیر کلسترول سرمی، تریگلیسرید و همچنین لیپوپروتئین با چگالی زیاد و اندک به طور معنی داری بین گروه های تحت تیمار متفاوت بود($P< 0.05$). با این وجود، ترکیبی از هر دو گیاه اثرات چشمگیرتری بر فاکتورهای فوق الذکر داشت. همچنین یک افزایش معنی دار در مقادیر پروتئین تام در گروه های تحت تیمار با مکمل گیاهی مشاهده شد. با این وجود، پودر شوید هیچ اثر معنی داری بر میزان گلوکز نداشت. تعداد هتروفیل و لنفوسیت در بین گروه ها متفاوت بود ($P< 0.05$). بعلاوه، فعالیت آنزیم های گلوتاتیون پراکسیداز، سوپراکسید دیسموتاز و کاتالاز به دنبال مصرف مکمل های گیاهی به طور معنی داری تغییر یافت. یک افزایش معنی دار در مقادیر مالون دی آلدئید و کاهش در ظرفیت تام آنتی اکسیدانی در تمامی گروه های تیمار ثبت شد. مطمعنا تیمار با پودرهای گیاهی می تواند باعث بهبود بیوشیمی سرم و ارتقاء وضعیت آنتی اکسیدانی شود. اما، به نظر می رسد که ترکیبی از هردو گیاه موثرتر است.