Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1616-1619

Received: 01-05-2018
Accepted: 02-06-2018

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Ameliorative role of Pudina (*Mentha arvensis*) leaf powder against oxidative stress on laying hens

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Abstract
The present study was conducted to evaluate the effect of Pudina (*Mentha arvensis*) leaf powder on antioxidant parameters in the diet of laying hens. A total of one hundred twenty White Leghorn laying hens of 36 weeks of age were randomly assigned into four treatment groups (T1, T2, T3 and T4) with six replicates of five birds each. The birds were fed with different levels of pudina leaf powder at 0, 5.0, 7.5 and 10.0 g / kg layer feedupto 12th weeks of age. At the end of fourth week or 28th day of experimentation, about 2 ml of blood was collected randomly from two birds of each replicate to determine antioxidant parameters. The results revealed that there was a significant (P<0.05) decrease in the level of LPO (T2, 1.91±0.04; T3, 1.77±0.03 and T4,1.68±0.05 nM MDA/ ml) and significant (P<0.05) increase in GSH (T2,1.69±0.05; T3, 2.52±0.06and T4, 2.74±0.04 mM/ ml) and CAT (T2, 538.55 ±4.42; T3, 597. 84±4.12 and T4, 648.02 ±6.40 mM H_2 O_2 utilized/min/mg Hb) levels in the pudina supplemented groups compared to the control groups (2.08±0.06 nM MDA/ ml, 1.03±0.04 mM/ ml and 350.14±2.58 mM H_2 O_2 utilized/min/mg Hb) respectively. The overall best performance was observed in birds of group T4 fed pudina leaves @ 10.0 g/kg. It can be concluded that pudina leaf powder can effectively be used as feed additive in laying hens as a strong antioxidant to protect against oxidative damage.

Keywords: Pudina, White leghorn, laying hens, antioxidant, proximate composition

Introduction
The importance of livestock in India goes not only beyond the function of food production but also considered as an important source of draught power, means of transportation, manure for crop production, fuel for indigenous use and also signifies a social dignity in certain societies in many parts of the country [1]. Among the livestock sector, poultry industry represents one of the important sub-sectors of livestock production in India. Presently, India stands the third largest in egg production in the world with per capita consumption of eggs over 68 eggs per annum [2]. The concern about poultry production is increasingly directed not only to the quantity and quality of the final product but also its implication for food security, environment and animal welfare. Phyto additives have gained the attention of researchers because they help in phytoadditives help in preventing common diseases including the oxidative stress in human and animals and also in maintaining their health [3].

Oxidative stress is a condition in which oxidation exceeds the anti-oxidant reactions, creating an imbalance between oxidative and anti-oxidant systems, with prevalence of reactive oxygen species (ROS) [4]. Peroxidation in the erythrocytes membrane may change the membrane function in stability causing reduction of the survival of RBCs [5]. It is one of the main issues for the present generation of various chronic and degenerative diseases, ageing, diabetes, atherosclerosis, ischemic heart disease, cancer, immunosuppression, neurodegenerative diseases etc [6]. Many *in vitro* and epidemiological studies on various edible and medicinal plants strongly supported the use of phytogenic substances having antioxidant property and are able to exhibit protective effects against the oxidative damage in biological systems.

Mentha arvensis (Pudina, Field Mint, Ginger Mint or Corn Mint) belongs to the Genus *Mentha* and Family Lamiaceae, is known to have strong antioxidant property [7, 8]. Ethanol extract *of M. arvensis* showed the presence of phytochemicals such as alkaloids, carbohydrates, glycosides, tannins, triterpenoids, flavonoids etc. Chemically, the essential oils of *Mentha arvensis* are composed mainly of terpenoids and phenylpropanoids [9] and also have predominance of oxygeneated monoterpenes [10].

The literature survey revealed that since there is scanty of scientific studies carried out on antioxidant properties of pudina (*M. arvensis*) leaf powder in poultry, the purpose of this research was to evaluate the potential of the plant as a feed additive for laying hens by measuring its effect on anti-oxidant parameters. Since, there are few literatures available on *Mentha arvensis* on laying hens. Hence comparative study was made to evaluate the effect of different level of Pudina (*Mentha arvensis*) leaf powder on antioxidant parameters in laying hens.

Materials and Methods

A feeding trial of 12 weeks duration was conducted in the month of August to November, 2015, to evaluate the anti-oxidant properties of pudina (*Mentha arvensis*) leaf powder in laying hens. A total of one hundred twenty white leghorn laying hens (36 weeks of age) randomly divided into four treatment groups (T1, T2, T3 and T4) with six replicates of five birds each. The groups were and supplemented with 0, 5.0, 7.5 and 10.0 g pudina leaf powder/kg of basal feed. The feeds and additive were analyzed for proximate principles, Ca and P contents [11]. The experiment was carried out in Californian cages in a completely randomized design. Each hen was housed in a single cage. The additive, pudina leaf powder was procured from R.K.Dehydration, Gujarat. Estimation of some oxidative stress related biochemical parameters viz. lipid peroxidase (LPO), reduced glutathione (GSH) and catalase (CAT) in erythrocytes were carried out.

Collection of samples

At the end of fourth week or 28th day of experimentation, about 2 ml of blood was collected randomly from two birds of each replicate through wing vein in sterile polypropylene vials containing anti-coagulant heparin under sterile conditions. After collection of blood, the vials were tightly capped and shaken gently to facilitate proper mixing of blood with heparin. The vials were carried in icebox containing ice and transported to the laboratory immediately. The heparinized blood samples were centrifuged at 2000 rpm for 15 min. Plasma and buffy coat were removed carefully and the resulting erythrocyte pellet was washed thrice with 0.15 M NaCl solution. The 33 per cent dilution of the packed RBC was prepared in phosphate buffer saline (PBS) at pH 7.4 [12]. The washed erythrocyte pellets were suspended in PBS at equal amount and kept at 4 ^{0}C till further analysis. This 33 per cent packed RBC was used for the estimation of lipid peroxidation and reduced glutathione. The 1:10 dilution of packed erythrocytes in PBS (pH 7.4) was used for the estimation of catalase and superoxide dismutase.

Lipid peroxidase (LPO)

Membrane lipid peroxidative damage in erythrocytes was examined in terms of malondialdehyde (MDA) production [13]. The peroxidative activity was determined at 535 nm wavelength. Calculation was done by using the molar extinction coefficient (EC) of MDA-TBA complex, 1.56X10^8 M^{-1} cm^{-1} and the amount of LPO was expressed as nM MDA formed per ml of packed cell.

Reduced glutathione (GSH)

Reduced Glutathione was assayed by the 5, 5 Dithiobis 2 nitrobenzoic acid (DTNB) method [14]. Calculation was done by using the molar extinction co-efficient (EC=13100 M^{-1} cm^{-1}) and results were expressed in mM GSH per ml of sample.

Catalase (CAT)

Catalase in erythrocytes was estimated using spectrophotometric [15]. The activity of enzyme catalase was expressed as mM H_2O_2 utilized Min^{-1} mg^{-1} H band and calculated was done using the specific formula.

Statistical analysis

The data obtained were analyzed statistically (SPSS, 17th Version) [16] and comparison of means was done using Duncan's multiple range tests [17].

Results and Discussion

Results on anti-oxidant parameters revealed that the LPO concentration showed a significant (P<0.05) decreasing trend with the increase level in pudina leaf powder (Table 3). Maximum (2.08 nM MDA/ml) and minimum (1.68 nM MDA/ml) lipid peroxidase concentrations were observed in T_1 and T_4 groups, respectively. The reduced glutathione (GSH) levels in erythrocytes was significantly (P<0.05) increased with the increasing levels of pudina leaf powder. The maximum (2.74mM/ ml) and minimum (1.03 mM/ ml) GSH values were observed in laying hens of T4 and T1 groups respectively while, the CAT enzyme concentration showed a significant (P<0.05) increasing trend with the subsequent increase in the level of pudina. The maximum CAT enzyme level was obtained in T_4 (648.02mM H_2O_2 utilized/ min/ mg Hb) and minimum in T_1 (350.14±2.58mM H_2O_2 utilized/ min/ mg Hb) group.

Free radicals are generated either under certain environmental condition and/or during normal cellular functions inside the body. Antioxidants function against the molecules that form free radicals, destroying them before they can activate the oxidative damage to the cell. The length of this oxidative damage depends upon chain breaking antioxidants, such as the enzymes superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (reduced GSH). Earlier researchers reported that the improved LPO production was also caused by heat stress [18]. Lipid peroxidation induced by H_2O_2 in RBC and its membrane was normalized by the supplementation of dietary herbal treatment [19]. The levels of LPO in pudina treated groups were significantly decreased due to either antioxidant activity of pudina because of its components *viz.* flavonoid and polyphenol or probably due to its hypocholesterolemic effect [20]. The GSH dependent enzymes also help to avert dissemination of free radicals inside the body [21]. In agreement to our study, researchers reported that administration of peppermint extract caused significant reduction of lipid peroxidation by ROS (Reactive Oxygen Species) scavenging ability and enhanced the concentration of endogenous antioxidants through which it exhibits membrane protection and protects the cells [22]. In support of the present study, it was reported that feeding of peppermint and parsley leaves and their mixture oils against hepatotoxicity rats improved GSH and decreased in MDA (lipid peroxidation malondialdehyde) concentrations [23]. The concentration of antioxidant enzymes such as catalase (CAT) and glutathione (GSH) were significantly increased while the concentration of MDA (Lipid peroxidase) were significantly decreased in the rats treated with *Mentha arvensis* extract [24]. The simultaneous incorporation of *Mentha arvensis* with Al expressed reduced LPO levels and increased the enzymatic level of CAT in albino mice compared to Al alone treated group [25]. However, it was suggested that supplementation of leaf powder of *Mentha spicata* @ 5 g / day for a period of 60

days in type 2 diabetic patients significantly decreased lipid peroxidation and catalase levels while GSH levels were significantly ($P<0.05$) in erythrocytes [26]. There exists a high correlation between the quantity of phenols in plant extracts and its antioxidant property [27]. Therefore, it was demonstrated that presence of certain active ingredients such as flavonoid, polyphenol etc. in pudina (*Mentha arvensis*) protects the excess formation of free radical and thereby reduces further oxidative damage [28].

Conclusion

From the present study, it can be concluded that pudina leaf powder at the rate of 10.0 g/ kg basal diet can effectively be used as feed additive in laying hens as a strong antioxidant to protect against oxidative damage. Furthermore, dose-response relationship experiments are suggested in future in order to determine a threshold dose below which there would be no toxic effect.

Acknowledgement

The authors are thankful to Dean and Department of Animal Nutrition, College of Veterinary and Animal Sciences, Pantnagar for providing the necessary facilities and help to carry out the investigation.

Table 1: Ingredient (%) and chemical composition (% DM basis) of experimental diets

Ingredient	%
Maize	53.00
Soyabean meal	27.00
Deoiled rice bran	8.80
Limestone powder	9.00
Dicalcium phosphate	1.40
Common salt	0.30
DL-methionine	0.15
TM-premix	0.10
Choline chloride	0.15
Toxin binder	0.05
Total	100.00
Chemical composition (%)	**Percent**
Dry matter	90.79
Crude protein	18.03
Ether extract	2.52
Crude fibre	5.78
Calcium	3.87
Total ash	12.00
Acid insoluble ash	1.53
Total phosphorus	0.73
Available phosphorus *	0.40
Metabolisable energy (kcal/kg)*	2601.38
Lysine *	1.10
Methionine *	0.48
Linoleic acid	1.39

*Calculated value

[1]Trace mineral premixsupplied (per kg diet): Magnesium- 300 mg, Manganese- 55 mg, Iodine-0.4 mg, Iron- 56 kg diet): vitamin A- 8250 IU, vitamin D_3- 1200 ICU; vitamin E- 40 IU, mg; Zinc- 30 mg and Copper 4 mg. [2] Vitamin premix supplied (per vitamin K- 1 mg; vitamin B_1- 2 mg, vitamin B_2 - 4 mg; niacin- 60 mg, pantothenic acid-10 mg, cyanocobalamin-10 microgram and choline- 500 mg

Table 2: Chemical composition of pudina leaf powder

Chemical composition		Analyzed
Dry matter	:	98.10
Crude protein	:	14.53
Crude fibre	:	21.03
Crude fat	:	2.95
Total ash	:	9.38
Nitrogen free extract	:	52.11
Calcium	:	1.90
Phosphorus	:	0.32

Table 3: Effect of anti-oxidant parameters of pudina leaf powder on laying hens

Parameters	T1	T2	T3	T4
LPO (nM MDA/ ml)	2.08^{a}±0.06	1.91^{b}±0.04	1.77^{bc}±0.03	1.68^{c}±0.05
Reduced GSH (mM/ ml)	1.03^{d}±0.04	1.69^{c}±0.05	2.52^{b}±0.06	2.74^{a}±0.04
CAT (mM H_2O_2 utilized /min/mg Hb)	350.14^{d}±2.58	538.55^{c} ±4.42	597. 84^{b}±4.12	648.02^{a} ±6.40

[ab] Values with different superscripts column wise differ significantly ($P<0.05$)

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