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Effects of Varying Levels of Nettle *(Urtica dioica L.)*, Pennyroyal *(Mentha pulegium L.)* Medicinal Plants and Enzyme on Performance and Egg Traits of Laying Hens

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Abstract: A study was conducted to investigate the effects of varying levels of nettle *(Urtica dioica L.)*, pennyroyal *(Mentha pulegium L.)* medicinal plants and enzyme on performance and egg traits of laying hens. In this study 288 Hy Line (W36) laying hens from 24-36 weeks of egg were used as 2×3 factorial experiment with 2 levels of nettle (0, 0.5%) and pennyroyal (0, 0.5%) and enzyme (0 and 0.05%) in a completely randomized design with 8 treatments, 3 replicates and 12 birds in each replicate. Using 0.5% of pennyroyal significantly decreased the egg production percent, egg mass and increased feed conversion, 0.5% of nettle significantly decreased the amount of daily feed intake. Interaction between enzyme and pennyroyal significantly decreased the amounts of egg production percent and egg mass, interaction between pennyroyal and nettle significantly decreased the amounts of egg production percent, egg mass and feed intake. Interaction between enzyme, pennyroyal and nettle significantly decreased the egg production percent and egg mass. Using pennyroyal, nettle and interaction between pennyroyal, nettle and between pennyroyal, nettle and enzyme significantly increased the eggshell thickness. The overall results showed that dietary supplementation 0.5% of pennyroyal, 0.05% of nettle itself or by combination together had adverse effects on performance of laying hens.

Key words: Egg traits · Enzyme · Laying hens · Medicinal plants · Performance

INTRODUCTION

Newly, some medicinal herbs and their associated essential oils or extracts are being concerned as potentially growth promoters [1]. At present the scientists are working to improve feed efficiency and growth rate of livestock using useful herbs [2]. Research on the use of herbal mixtures in poultry diets have produced inconsistent results. In an experiment, broilers fed with 0.5% of peppermint performed better than those fed with 1.5% peppermint concerning weekly body weight gain [3]. Adding 2% of nettle *(Urtica dioica L.)* dried areal parts powder into laying hens diets significantly increased egg yield, egg mass and eggshell weight, whereas the highest haugh unit was recorded by using 2% of savory *(Satureja hortensis L.)* [4]. In another study by using different levels of Thymyus *valgaris, Lamiaceae menthapiperita* and *Oreganum valgare* the highest egg production percent, egg mass and the best feed conversion, the highest haugh unit and egg yolk color index were resulted by using 2% of *Oreganum valgare*, whereas the highest amount of feed intake was observed by adding 2% of *Lamiaceae menthapiperita* [5].

Mentha, the genus in Labiatae family, 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 a native species in Europe, North Africa, Minor Asia and the near East. The flowering aerial parts of *Mentha pulegium L.* have been traditionally used antimicrobial properties in the treatment of cold, sinusitis, cholera, food poisonings, bronchitis and tuberculosis [6].

The Nettle *Urtica dioica L.* (Urticaceae) is widely grown in different parts of the world and has been used to improve human health. Numerous analysis of nettle have revealed the presence of more than fifty different chemical constituents. It has been extensively studied and found to contain starch, gum, albumen, sugar and two resins. Histamine, acetylcholine, choline and serotonin are also present. In a study an anti-coagulant was isolated from nettle leaves [7]. In an experiment the addition of 2% nettle to broiler diet led to increase their body weight [8].

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Recent studies in broilers showed that using nettle in blend with other medicinal plants had positive effects on performance, carcass traits and blood biochemical and immunity parameters. Using 1.5% of different mixtures of nettle, pennyroyal and Zizaphora medicinal plants in broiler diets improved their performance and carcass quality [9]. An application of 0.75% of mixtures of nettle, pennyroyal and Zizaphora in the grower period had positive effects on the performance and carcass traits of broilers [10]. Recently reported that the addition 2% of pennyroyal aerial parts powder to broiler diets significantly decrease their performance [11]

Non starch polysaccharides (NSP) have adverse effects on digestion of nutrients present in considerable amount in cell walls and internal cell components of herbs and other feed ingredients. Several studies showed the positive effects of NSP degrading enzymes on laying hens performance [12].

This experiment was conducted to investigate the effects of varying levels of nettle *(Urtica dioica L.)*, pennyroyal *(Mentha pulegium L.)* medicinal plants and enzyme on performance and egg traits of laying hens.

MATERIALS AND METHODS

Animals and Dietary Treatments: In this study 288 Hy Line (W36) laying hens from 24-36 weeks of egg were used as 2×3 factorial experiment with 2 levels of nettle and pennyroyal (0, 0.5%) and enzyme (Safizyme) (0 and 0.05%) in a completely randomized design with 8 treatments, 3 replicates and 12 birds in each replicate. The diets were formulated (Table 1) to meet the requirements of laying hens as established by the NRC [13].

Dried aerial parts of pennyroyal and nettle were supplied from local market and their compositions were determined according to AOAC [14]. After fine milling, mixed with other ingredients. Enzyme supplied from veterinary pharmacy. The lighting program during the experimental period consisted of a period of 16 hours light and 8 hour of darkness. Average of environmental temperature was 15°C.

Performance Parameters and Egg Traits: Birds were individually weighted at the beginning and at the end of the study and body weight gain was calculated. Feed intake, feed conversion, egg production percent, egg mass and egg weight were determined weekly on bird bases. Mortality was recorded if it occurred. The collected eggs were classified as normal or damaged; the latter included the following: fully cracks eggs (an egg with broken shell and destroyed membrane), hair cracks eggs (an egg broken shell but intact membrane), the eggs without shell (an egg without shell but with intact membrane). For measuring of egg traits at the end of experiment period 3 egg sampled collected from each replicate. Determination of eggs specific gravity was done

Table 1: The ingredients and nutrients composition of starter diets of broilers (1-21 days)

Diets¯ / ingredients¯	Control Group	0.05% Enzyme	0. 5% pennyroyal	0. 5%Pennyroyal + 0.05%Enzyme	0. 5% Nettle	0. 5% Nettle+ 0.05% Enzyme	0. 5% Pennyroyal + 0.05%Nettle	0. 5% Pennyroyal + 0.05% Nettle + 0.05%Enzyme
Yellow corn	55	55	55	55	55	55	55	55
Soybean meal	16.46	16.46	16.49	16.49	16.74	16.74	16.49	16.49
Wheat	18.8	18.8	17.49	17.49	17.49	17.49	17.49	17.49
Vegetable oil	0.05	0.05	0.33	0.33	0.33	0.33	0.33	0.33
Pennyroyal	0	0	0.5	0.5	0	0	0.5	0.5
Nettle	0	0	0	0	0.5	0.5	0.5	0.5
Enzyme	0		0.05	0.05	0	0	0.05	0.05
Oyster shell	7.85	7.85	7.85	7.85	7.85	7.85	7.85	7.85
Dicalcium phosphate	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08
Salt	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated composition								
Metabolisable energy (Kcal/Kg)	2800	2800	2800	2800	2800	2800	2800	2800
Crude protein (%)	14	14	14	14	14	14	14	14
Calcium (%)	3.28	3.28	3.28	3.28	3.28	3.28	3.28	3.28
Available phosphorous (%)	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Laysin (%)	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Methionine+Cysteine (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Threonine (%)	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
Thryptophan (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17

[1]Vitamin premix per kg of diet: vitamin A (retinol), 8500000 IU; vitamin D3 (Cholecalciferol), 2500000 IU; vitamin E (tocopheryl acetate), 11000 IU; vitamin k_3, 2200 mg; thiamine, 1477 mg; riboflavin, 4000 mg; panthothenic acid, 7840 mg; pyridoxine, 7840 mg; cyanocobalamin, 10 mg; folic acid, 110 mg; choline chloride, 400000 mg.

[2]Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe), 75000 mg; Mn (MnSO4.H2O, 32.49% Mn), 74.4 mg; Zn (ZnO, 80.35% Zn), 64.675 mg; Cu (CuSO4.5H2O), 6000 mg; I (KI, 58% I), 867 mg; Se (NaSeO3, 45.56% Se), 200 mg.

by floated eggs in salty water. Content of egg shells were cleaned and shells were maintained in environmental temperature for 48 h until dried, then weighed with a digital scale in an accuracy of 0.01 (g). The thickness of egg shell was measured by micrometer with accuracy of 0.001(mm) in the middle of egg and in three spots on four eggs. Then their average was considered as final thickness of egg shell for each experimental unit. Colour index of the yolk (Roche colour index), haugh units were determined [15].

Statistical Analysis: The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute [16]. Means were compared using the Duncan multiple range test. Statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

The effects different levels of pennyroyal, nettle and enzyme and their interaction are presented in Table 2.

The addition of enzyme to laying hens diets at 0.05% did not have any significant difference on their performance. Using 0.5% of pennyroyal in laying hens diets had negative effects on their performance and significantly decreased the egg production percent and egg mass and increased feed conversion (P<0.05).

Table 2: The effects of different levels of pennyroyal, nettle and enzyme on laying hens performance

Performance → Supplements ↓	Egg Weight (g)	Egg production (%)	EggMass (g)	FeedIntake (g)	Feed conversion
Enzyme levels					
0	61.53	73.87	45.50	105.68	2.31
0.05%	61.27	73.33	45.81	105.23	2.32
SEM	0.29	1.05	0.72	0.75	0.04
Pennyroyal levels					
0	61.20	77.24^{a}	47.37^{a}	106.42	2.26^{b}
0.5%	61.61	70.16^{b}	43.94^{b}	104.49	2.36^{a}
SEM	0.29	1.05	0.72	0.75	0.04
Nettle levels					
0	61.49	74.78	46.64	107.20^{a}	2.31
0.5%	61.31	72.62	44.67	103.71^{b}	2.32
SEM	0.29	1.05	0.72	0.75	0.04
Pennyroyal × Enzyme					
Pennyroyal (0) × Enzyme (0)	61.18	78.07^{a}	47.98^{a}	106.80	2.24
Pennyroyal (0.5%) × Enzyme (0)	61.87	69.48^{c}	43.02^{b}	106.54	2.38
Pennyroyal (0) × Enzyme (0.05%)	61.21	76.41^{ab}	46.75^{ab}	106.04	2.30
Pennyroyal (0.5%) × Enzyme (0.05%)	61.34	70.84^{bc}	44.86^{ab}	104.42	2.34
SEM	0.41	1.49	1.02	1.06	0.05
Nettle × Enzyme					
Nettle (0) × Enzyme (0)	61.74	75.90	46.82	107.27	2.28
Nettle (0.5%) × Enzyme (0)	61.32	71.65	44.18	104.08	2.34
Nettle (0) × Enzyme (0.05%)	61.25	73.66	46.46	107.12	2.34
Nettle (0.5%) × Enzyme (0.05%)	61.30	73.58	45.16	103.33	2.30
SEM	0.41	1.49	1.02	1.06	0.05
Nettle × Pennyroyal					
Nettle (0) × Pennyroyal (0)	60.87	79.25^{a}	48.18^{a}	107.73^{a}	2.26
Nettle (0.5%) × Pennyroyal (0)	61.61	75.23^{ab}	46.55^{ab}	105.10^{ab}	2.27
Nettle (0) × Pennyroyal (0.05%)	62.61	70.32^{b}	45.09^{ab}	106.66^{a}	2.36
Nettle (0.5%) × Pennyroyal (0.05%)	61	70^{b}	42.79^{b}	102.31^{b}	2.37
SEM	0.41	1.49	1.02	1.06	0.05
Nettle × Pennyroyal × Enzyme					
Nettle (0) × Pennyroyal (0) × Enzyme (0)	60.98	79.81^{a}	48.67^{a}	107.99	2.24
Nettle (0.5%) × Pennyroyal (0) × Enzyme (0)	61.38	76.32^{abc}	47.28^{ab}	105.59	2.24
Nettle (0) × Pennyroyal (0.5%) × Enzyme (0)	62.49	71.99^{abc}	44.96^{ab}	106.54	2.33
Nettle (0.5%) × Pennyroyal (0.5%) × Enzyme (0)	61.25	66.98^{c}	41.08^{b}	102.58	2.44
Nettle (0) × Pennyroyal (0) × Enzyme (0.05%)	60.58	78.69^{ab}	47.69^{ab}	107.47	2.29
Nettle (0.5%) × Pennyroyal (0) × Enzyme (0.05%)	61.84	74.13^{abc}	45.81^{ab}	104.62	2.30
Nettle (0) × Pennyroyal (0.5%) × Enzyme (0.05%)	61.92	68.65^{bc}	45.22^{ab}	106.78	2.38
Nettle (0.5%) × Pennyroyal (0.5%) × Enzyme (0.05%)	60.75	73.03^{abc}	44.51^{ab}	102.05	2.30
SEM	0.57	2.10	1.44	1.50	0.07

Values in the same row not sharing a common superscript differ significantly (p<0.05).
SEM = Standard error of mean

Table 3: The effects of different levels of pennyroyal, nettle and enzyme on egg traits of laying hens

Performance → Supplements ↓	Egg specific gravity (mg/cm^3)	Egg yolk Color index	Eggshell Weight (g)	Eggshell Thickness (mm)	Haughunit
Enzyme levels					
0	1.07	4.39	5.93	42.61	90.77
0.05%	1.075	4.28	5.85	42.33	90.69
SEM	0.003	0.09	0.078	0.43	1.20
Pennyroyal levels					
0	1.077	4.45	5.93	41.83b	91.74
0.5%	1.075	4.22	5.86	43.10a	89.72
SEM	0.003	0.09	0.078	0.43	1.20
Nettle levels					
0	1.078	4.34	5.99	41.81b	90.93
0.5%	1.074	4.33	5.79	43.12a	90.52
SEM	0.003	0.09	0.078	0.43	1.20
Pennyroyal × Enzyme					
Pennyroyal (0) × Enzyme (0)	1.079	4.61	5.97	42.09	91.67
Pennyroyal (0.5%) × Enzyme (0)	1.076	4.17	5.89	43.13	89.86
Pennyroyal (0) × Enzyme (0.05%)	1.075	4.28	5.89	41.58	91.80
Pennyroyal (0.5%) × Enzyme (0.05%)	1.074	4.28	5.82	43.07	89.58
SEM	0.004	0.13	0.11	0.60	1.69
Nettle × Enzyme					
Nettle (0) × Enzyme (0)	1.082	4.50	6.05	42.16	90.41
Nettle (0.5%) × Enzyme (0)	1.073	4.28	5.81	43.06	91.12
Nettle (0) × Enzyme (0.05%)	1.074	4.17	5.93	41.47	91.45
Nettle (0.5%) × Enzyme (0.05%)	1.076	4.39	5.77	43.19	89.93
SEM	0.004	0.13	0.11	0.60	1.69
Nettle × Pennyroyal					
Nettle (0) × Pennyroyal (0)	1.077	4.45	5.95	40.13b	94.07
Nettle (0.5%) × Pennyroyal (0)	1.077	4.40	5.90	43.54a	89.40
Nettle (0) × Pennyroyal (0.05%)	1.078	4.22	6.03	43.50a	87.80
Nettle (0.5%) × Pennyroyal (0.05%)	1.072	4.22	5.68	42.70a	91.64
SEM	0.004	0.13	0.11	0.60	1.69
Nettle × Pennyroyal × Enzyme					
Nettle (0) × Pennyroyal (0) × Enzyme (0)	1.083	4.78	6.04	41.06ab	92.31
Nettle (0.5%) × Pennyroyal (0) × Enzyme (0)	1.075	4.44	5.89	43.11ab	91.04
Nettle (0) × Pennyroyal (0.5%) × Enzyme (0)	1.081	4.22	6.06	43.26ab	88.52
Nettle (0.5%) × Pennyroyal (0.5%) × Enzyme (0)	1.071	4.11	5.72	43ab	91.21
Nettle (0) × Pennyroyal (0) × Enzyme (0.05%)	1.072	4.11	5.86	39.19b	95.83
Nettle (0.5%) × Pennyroyal (0) × Enzyme (0.05%)	1.079	4.44	5.91	43.96a	87.77
Nettle (0) × Pennyroyal (0.5%) × Enzyme (0.05%)	1.076	4.22	6.01	43.74a	87.07
Nettle (0.5%) × Pennyroyal (0.5%) × Enzyme (0.05%)	1.072	4.33	5.63	41.41ab	92.08
SEM	0.006	0.18	0.16	0.85	2.39

Values in the same row not sharing a common superscript differ significantly ($p<0.05$).
SEM = Standard error of mean

The addition 0.5% of nettle to laying hens diets only significantly decreased the amount of daily feed intake ($P<0.05$). In interaction between pennyroyal and enzyme the amounts of egg production percent and egg mass significantly decreased, whereas interaction between nettle and enzyme could not significantly affect the performance of laying hens. In interaction between pennyroyal and nettle the amounts of egg production percent, egg mass and feed conversion significantly decreased ($P<0.05$). The interaction between pennyroyal, nettle and enzyme significantly decreased the egg production percent and egg mass of laying hens ($P<0.05$). Enzyme and nettle alone or together did not have any significant adverse effects on performance of laying hens, but when their accompanied with pennyroyal, the adverse effects were happened. These results about pennyroyal is in agreement with results of Arjomandi *et al* (2011), who reported that addition pennyroyal to laying hens diets negatively affected their performance [11]. It is known from the literature that pennyroyal herb contains 1-2% essential oil of which pulegone is the principal component (60-90%) [17]. Later studies the liver toxicity of pulegone has been confirmed and a mechanism of action has been proposed based on its metabolism to menthofuran and other reactive metabolites, which are the ultimate hepatotoxins (substance which is poisonous to the liver)

[18]. Therefore, because of toxicity of this herbal medicinal plants or products containing pennyroyal, peppermint and mint (plant/oil), probability of increasing liver damage and in severe cases (use of the above) can resulted in an pulmonary edema and internal hemorrhage which subsequently it can influence performance of body especially in laying hens that are sensitive to toxic herbals. The results observed in this experiment not in agreement with findings of Nobakht and Mehmannavaz who reported that the addition 2% of *Oreganum valgare* significantly increase the egg production percent and egg mass of laying hens [5]. In another study it was shown that in broilers using 0.5% of pennyroyal significantly improve the performance of broilers [1]. The addition different levels of nettle alone or together with enzyme could not improve the performance of laying hens. The observation about nettle and pennyroyal in present experiment not supported by Modiry *et al*. results who reported that using 1.5% of different mixtures of nettle, pennyroyal and Zizaphora medicinal plants in broiler diets improved their performance and carcass quality [9]. The observed differences in results of current experiment with previous experiment may be related to experiment status, medicinal plants laying hens used in these experiment. Using pennyroyal and nettle and their interaction significantly increased the eggshell thickness. Reduction of egg production causes high amounts of calcium supply for per egg and with precipitation high amounts of calcium supply for eggshell formation, so the thickness of eggshell increases.

CONCLUSION

From the results of the present study, it was concluded that using 0.5% of pennyroyal or 0.5% of nettle and interaction between them, significantly decrease the performance of laying hens.

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