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Effect of *Melissa officinalis* L., *Tanacetum balsamita* L. and *Ziziphora clinopodioides* L. on Performance, Blood Biochemical and Immunity parameters of Laying Hens

¹Ali Nobakht, ²Navid Hosseini Mansoub and ³Mohammad Ali Mohammad Nezhady

¹Islamic Azad University, Maragheh Branch, Maragheh, Iran

²Islamic Azad University, Mahabad Branch, Mahabad, Iran

³Faculty of Sciences, Islamic Azad University, Urmia Branch, Urmia, Iran

Corresponding Author: Ali Nobakht, Islamic Azad University, Maragheh Branch, Maragheh, Iran

ABSTRACT

Taking advantage of medicinal plants is developing in the poultry industry as an easy, economical and beneficial way in order to elevate the poultry's out come. This experiment was conducted to find out the effects of *Melissa officinalis* L., *Tanacetum balsamita* L. and *Ziziphora clinopodioides* L. on performance, egg quality, blood biochemical and immunity parameters of laying hens. This study was carried out with 288 of Hy-line (W36) hens in 8 treatment groups and 3 repetitions for each group. The groups were (1) Control group (2) (2% *Melissa officinalis* L., 3) 2% *Tanacetum balsamita* L. (4) 2% *Ziziphora clinopodioides* L. (5) 1% *Melissa officinalis* L. and 1% *Tanacetum balsamita* L. (6) 1% *Melissa officinalis* L. and 1% *Ziziphora clinopodioides* (7) 1% *Tanacetum balsamita* L. and 1% *Ziziphora clinopodioides* and (8) 0.67% of each three medicinal plants. According to the results, there were significant effects of these plants using on performance, egg quality and blood biochemical parameters of hens ($p < 0.05$). The highest percentage of egg production, the lowest feed conversion ratio, the highest weight and the highest index of yolk were seen in the 6th group. The highest egg weight was in the 5th group and the highest weight of white was in the 3rd group. Also these plants improved the immune system and blood biochemical parameters of laying hens. According to the results of this study, these three herbs can remarkably boost the performance and immunity of laying hens.

Key words: Biochemical parameters, egg production, immunity level, medicinal plants, poultry performance

INTRODUCTION

Antibiotics are growth promoters with high rate of usage in the poultry industry. They improve growth and the yield of feed but these chemical products have adverse effects like antibiotic resistance and the possibility of transferring some of these antibiotics to the human by using these poultry products. Since then the scientists are trying to find alternatives and one the best options is herbs and their derivatives. There are a lot of advantages in using medicinal plants such as easy usage, non side effects, no waste particulars in the target body and etc. (Abaza *et al.*, 2008).

Adding medicinal plants into the diets of broilers and laying hens have some benefits on their production performance and health status. Ghasemi *et al.* (2010) reported that inclusion 0.2% of

Thyme and Garlic powders in the diet of laying hens improves the yolk index and increases the lymphocytes rate of their blood. Eseceli and Kahraman (2004) found out that the oils of several plants could optimize the yolk index and the weight and thickness of eggshell. Mitsch *et al.* (2004) showed that usage of herbs in the diets of laying hens decrease the bacterial colony in their intestine. Cabuk *et al.* (2006) proved that Thymol and Carvacrol can improve the digestion of nutrients.

Adinee *et al.* (2008) found that the trans-carveol is the main effective substances of Melissa and it has been using for neuro-disorders, stomach, heart and intestine sicknesses. The antimicrobial effect of Tanacetum oil has been proved by many experiments; this oil has antibacterial effect on gram negative and gram positive, antiseptic, anti parasite and insecticides. Mohammadreza (2008) reported that the main components of ziziphora are carvacrol, menthol, neo-menthol and pulegone.

Most of the studies about the effects of these plants have been carried out by using their oils and extracts. There are few studies on the direct effects of powdered plants specially their combinations on performance, egg traits, blood biochemical and immunity parameters of laying hens. So, it is needed to run these kinds of experiments, therefore, in this experiment the effects of these three herbs and their combinations on performance, immune system and quality of eggs in laying hens have been investigated.

MATERIALS AND METHODS

This experiment was conducted with 288 laying hens Hy- line strain (w36) in 8 experimental groups with 3 repetitions and 12 hens were involved in each repetition. The investigation was carried out in early, 2011. The hens were 62 to 74 weeks old. The experimental groups are as follows: (1) Control group (2) 2% *Melissa officinalis* L. (3) 2% *Tanacetum balsamita* L. (4) 2% *Ziziphora clinopodioides* L. (5) 1% *Melissa officinalis* L. plus 1% of *Tanacetum balsamita* L. (6) 1% *Melissa officinalis* L. and 1% *Ziziphora clinopodioides* L. (7) 1% *Tanacetum balsamita* L. plus 1% *Ziziphora clinopodioides* L. and (8) 0.67% of each tree plants. All the plants were collected from Azarbaijan province and then identified and classified by experts. Basal diet were based on corn-soybean meal considering the necessary nutrients were recommended by NRC (1994) with energy level of 2800, kcal Kg⁻¹ and crude protein of 14% which has been organized by UFFDA software. Also, the plants were used in the powder form mixed with the basal diet.

The amounts of intake food, egg production, egg mass and feed conversion were measured weekly. At the end of experiment, 4 eggs from each repetition were randomly chosen and weighted and their mass was determined by sinking them into the water and salt soluble with different concentrations.

The height of internal soft white was measured by German made (model CE300) micrometer. The shells were cleaned then kept at the room temperature for 48 h. After that they were weighted by weighing machine which had 0.01 g accuracy. The thickness of the shells was measured by micrometer with the accuracy of 0.001 mm in the middle and 3 points of egg shell and the average was considered as the shell thickness. This process was taken on each 4 eggs and the average of them was considered as the final result for each group. The strength of the shell was determined by the mg weight of shell to every cm of the surface of it. The surface of the shell was calculated by the Coutts and Wilson (1990) formula:

$$\text{Surface of the shell} = 3.9782 \times (\text{egg's weight})^{0.7056}$$

Statistical analysis: Finally the data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (2005). Means were compared using the Duncan's multiple range test. Statements of statistical significance are based on $p < 0.05$.

RESULTS

The influence of different combinations of herbs were significant on performance of laying hens ($p < 0.05$) which is shown in the Table 1. The heaviest eggs (64.47 g) were resulted in group 5, whereas the highest percentage of egg production (62.74%) and best feed conversion ratio (2.66) were seen in the 6th group. On the other hand, the lowest weight of egg (61.96) was in the second group and the lowest percent of egg production (45.28) with the highest feed conversion ratio (3.05) were detected in the 5th group. Despite the insignificant differences in the intake food, numerically the highest intake food (107.14) belonged to the first and 8th groups and the lowest one (104.89) belonged to the 5th group.

The quality of eggs significantly affected by herbs and their mixture ($p < 0.05$) (Table 2). The highest egg especial gravity (1.079) and yolk index (4) were seen in the 6th group. There were no significant differences in the other parameters, they just differ numerically. The best haugh unit (87.94) was in the third group, yolk weight (19.82) was in 2nd group, the highest of yolk index (42.35), shell weight (6.87) and shell thickness (0.352) were in the 7th group.

Table 1: The effect of *Melissa officinalis*, *Tanacetum balsamita* and *Ziziphora clinopodioides* lam on performance of laying hens

Treatments ¹	Performance:				
	Egg weight (g)	Egg production (%)	Egg mass (g/bird/day)	Feed intake (g/bird/day)	Feed conversion ratio
1	62.66 ^b	60.96 ^a	38.20 ^a	107.14	2.80 ^b
2	61.96 ^b	54.65 ^{ab}	33.86 ^{ab}	106.21	3.14 ^b
3	62.93 ^a	50.36 ^a	37.99 ^a	105.14	2.77 ^b
4	63.09 ^{ab}	59.21 ^a	37.36 ^a	105.76	2.83 ^b
5	64.47 ^a	45.28 ^b	29.20 ^b	104.89	3.05 ^a
6	62.97 ^{ab}	62.74 ^a	39.51 ^a	105.25	2.66 ^b
7	63.38 ^{ab}	61.75 ^a	39.14 ^a	107.14	2.74 ^b
8	63.23 ^{ab}	61.83 ^a	39.16 ^a	106.66	2.72 ^b
SEM	00.51	00.25	02.03	000.33	0.45

Values in each column having different letters are significantly different ($p < 0.05$)

Table 2: The effect of *Melissa officinalis*, *Tanacetum balsamita* and *Ziziphora clinopodioides* lam on egg quality characters of laying hens

Treatments ¹	Special weight	Yolk index	Yolk color	Shell weight	White weight	Yolk weight	Shell	
	(mg cm ⁻³)	(%)	index	(g)	(g)	(%)	Haugh unit	thickness (mm)
1	1.070 ^{bc}	41.55	2.00 ^b	6.37	38.72 ^b	19.61	87.91	0.332
2	1.069 ^{bc}	40.86	3.17 ^{ab}	6.51	37.48 ^b	19.82	86.34	0.345
3	1.064 ^c	41.28	3.00 ^{ab}	6.70	44.19 ^a	19.61	87.94	0.351
4	1.072 ^{ab}	42.23	3.34 ^a	6.22	39.77 ^{ab}	18.18	78.16	0.335
5	1.069 ^{bc}	41.10	3.17 ^{ab}	6.18	39.02 ^b	18.64	77.55	0.339
6	1.079 ^a	41.28	4.00 ^a	6.50	40.35 ^{ab}	18.73	84.13	0.341
7	1.072 ^b	42.35	4.17 ^a	6.87	41.74 ^{ab}	19.28	83.98	0.352
8	1.066 ^{bc}	40.73	4.17 ^a	6.14	41.03 ^{ab}	19.35	81.39	0.332
SEM	0.002	0.96	0.38	0.29	01.51	00.49	04.14	0.017

Values in each column having different letters are significantly different ($p < 0.05$)

Table 3: The effect of *Melissa officinalis*, *Tanacetum balsamita* and *Ziziphora clinopodioides* lam on blood biochemical and immune parameters of laying hens

Treatments ¹	Glucose (mg dL ⁻¹)	Cholesterol (mg d L ⁻¹)	Triglyceride (mg dL ⁻¹)	Heterophile (%)	Lymphocyte (%)	Heterophile/ Lymphocyte
1	188.22	137.52 ^b	3087.2 ^a	16.50	83.00	0.213
2	208.85	233.45 ^a	1687.3 ^{ab}	12.17	85.50	0.150
3	194.16	129.38 ^b	1962.0 ^{ab}	11.83	85.84	0.141
4	219.35	207.28 ^{ab}	2049.5 ^{ab}	15.34	83.17	0.186
5	174.98	140.63 ^b	1559.7 ^{ab}	9.27	89.67	0.105
6	258.38	154.83 ^{ab}	1980.3 ^{ab}	12.00	87.50	0.141
7	171.95	152.87 ^{ab}	1768.3 ^{ab}	11.67	87.34	0.138
8	257.65	193.15 ^{ab}	1176.3 ^b	8.50	90.50	0.096
SEM	30.22	25.96	479.23	3.96	3.96	0.070

Values in each column having different letters are significantly different ($p < 0.05$)

Table 3 shows that, there are significant influence due to usage of herbs and their mixture on biochemical parameters of laying hens ($p < 0.05$). The lowest cholesterol level (129.38 mg dL⁻¹) was seen in the third group but unlike the effect of *Tanacetum* (3rd group), the highest level of cholesterol (207.28 mg dL⁻¹) was detected in the 4th group (*ziziphora*). The effect of three plants simultaneously (8th group) had the highest impact on lowering the triglyceride level (1176.3 mg dL⁻¹).

The plants and their combinations had no significant effect on the blood immune cells of the laying hens ($p > 0.05$). Meanwhile, the lowest percentage of heterophil (8.5), the highest percentage of lymphocytes (90.5) and the lowest rate of heterophil to lymphocyte (0.096) were seen in the 8th group.

DISCUSSION

Antibacterial and antifungal effects of these plants could increase the weight and percentage of egg production; also improve the feed conversion ratio in the treated groups. *Tanacetum* and *ziziphora* are special in this matter, because of their reducing effect in the amount of harmful microbes in the digestive system which can improve their immunity and performance. This is possible that it is result of synergetic influence of effective substances in increasing antimicrobial activity. Antimicrobial activity of essential oil of *tanacetum* has been demonstrated in both gram-negative and gram-positive bacteria. The antibacterial effect of *ziziphora* has been reported because of its Pulegone and carvacrol substances, specially pulegone on gram-negative and gram-positive bacteria particularly *Sallmonella typhimurium*.

Enhancement in the yolk index is related to stability of yellow pigments in the membrane of the yolk among the lipid molecules, the antioxidants can prevent these molecules from oxidative stress (Kirunda *et al.*, 2001). There can be another scenario too, some carotenoids in these plants like xanthophylls transferred to the yolk and increase its yellow color, as this item is related to the compounds of diet like corn and wheat. Farkhoy *et al.* (1994) indicated that by the inclusion of pigments with plant sources into the yolk, these make them more colorful.

Considering that the most part of white is water and this fact that most of herbs are bitter in taste, it can be concluded that this leads to more water consumption and increase in the eggs weight. Also, there is another possibility of getting heavier eggs and it can be because of increase in the amount of ovosin protein. The medicinal plants can stimulate the secretion of digestive

enzymes and by reducing the bacterial colony in digestive tracts and improve production and health status of laying hens and this can cause formation of more ovosin.

Despite the insignificant differences in the other egg's quality parameters, using tanacetum and ziziphora and their mixture numerically improved these parameters. For instance, the highest Haugh unit was seen in the group which has used tanacetum. The Flavonoid compounds in this plant can improve the digestive ability too, by their antibacterial effects.

The low level of blood biochemical parameters can be due to substances like carvacrol and thymol in Melissa and ziziphora which have reducing effects on cholesterol and triglyceride of blood. Since by using these plants in the diet and increasing the fibers level of it the bile secretion get stimulated, so in results body uses the cholesterol and triglyceride of blood to replace it and in consequence the blood level of these parameters decrease. Heydari *et al.* (2010) obtained the same results in 2010 as we did, they found that cholesterol and triglyceride level reduces by using thyme, ziziphora and nettle. Valchev *et al.* (2009) achieved results in agreement with our findings in pigs, they realized that Tanacetum can decrease the cholesterol level of blood in pigs.

The effect of combination of plants were more than their effect solely on the immune system, the combination of herbs increased the percentage of lymphocytes and decreased the heterophiles and its proportion (ratio) to the lymphocyte. Lymphocytes are the highest amount of white blood cells in the poultry which produce the antibodies and cell mediated immune response, indeed the decrease of heterophile and its proportion to the lymphocytes shows the enhance of poultry's immune system (Sturkie, 1995).

Finally, it can be concluded that the usage of combination of these herbs, specially mixture of tanacetum and ziziphora in the diet of laying hens can improve the performance, quality of eggs, blood biochemical parameters and their immune cells.

CONCLUSION

Based on the findings of this experiment, it can be concluded that these three herbs can significantly improve the egg quality of laying hens while they increase the performance and immunity of hens themselves. By the facts in this study, the use of these plants in the diet of hens can significantly be beneficial to farmers. But since there are few studies on these plants in poultry, so, more studies are needed to prove the fact and to achieve an optimum amount and mixture.

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