International Journal of Animal and Veterinary Advances 6(1): 34-39, 2014
DOI:10.19026/ijava.6.5614
ISSN: 2041-2894; e-ISSN: 2041-2908

Submitted: November 22, 2013 Accepted: December 04, 2013 Published: February 20, 2014

Research Article

The Impact of Adding the Mixture of Medicinal Herbs to the Diet on the Qualitative Characteristics of Egg

Ali Karimi
Department of Animal Sciences, De Lasalle University, Manila, Philippine

Abstract: Like other creatures, the human life depends on the phenomena of surrounding environment and the medicinal herbs are always applied in the human and livestock and poultry's foods as well as the pharmaceutical industry in order to protect the health and treat the diseases. The impact of medicinal herbs on the livestock and poultry's performance, their characteristics and enhanced immunity can be measured and applied as well. In this regard, an experiment in the form of completely randomized design was conducted for investigating the impact of the mixture of medicinal herbs (Thyme, oregano, cumin, Alhagi, garlic and eucalyptus) in the diet on the performance, the quality of egg and the commercial laying hens' immune responses. The qualitative characteristics of egg including Haugh Unit, egg specific gravity, egg shell weight and shell thickness were measured and recorded at the end of each stage. The functional properties such as Feed Conversion Ratio (FCR), production percentage, egg mass weight and feed intake were recorded and measured weekly and the egg weight two times a week. The obtained data analysis was done through the statistical software SPSS 20 and Duncan test was utilized in order to compare the average traits. Different levels of medicinal herbs had no impact on the improved Feed Conversion Ratio (FCR), production percentage and egg mass weight in the whole period, but the medicinal herbs 2 and 3% significantly reduced the total feed intake and egg weight during the period compared to the control and probiotic treatment ($p<0.05$). The application of medicinal herbs at the level of 1% reduced the egg weight compared to the control treatment ($p<0.05$). There was no significant difference with the control treatment in any tested treatments in terms of qualitative characteristics of egg including the egg weight, shell weight, shell thickness, Haugh Unit, egg specific gravity, antibody titer against red blood cells, Lymphocytes, hematocrit, red blood cell volume, white blood cell count and serum cholesterol, but the experimental treatments reduced the red blood cells count, increased the yolk high cholesterol and decreased IgM and IgG.

Keywords: Cholesterol, egg laying hens, immune response, mixture of medicinal herbs, performance, probiotics (protexine), qualitative characteristics of egg

INTRODUCTION

The livestock and poultry sector is significantly important in the economy of our country. Providing the required protein for the country, the significant portion in making the value added in the agriculture, creating the employment opportunities in rural and urban communities and the wide participation of private sector for investment in the livestock production are among the characteristics of this sector. The egg is as a high quality and inexpensive source of protein compared to other sources of animal protein from the products of poultry farming in our country and the important steps are always taken in order to enhance its production. Among the animal proteins, the egg protein has higher critical value, thus it is considered as the first-grade proteins (Shen *et al*., 1992). The antibiotics were applied as the growth promoters and the feed conversion ratio improvers in the poultry industry. The frequent use of antibiotics leads to the survival and growth of resistant bacteria in addition to the adverse effects on the environment and creates a population of resistant bacteria. The various supplements are applied in order to improve the performance and balance of nutrients in the diet of livestock and poultry; however, the improved feed efficiency is only one of the important aspects in animal production (Amin, 2005). Not only the applied methods should lead to the produced high quality products, but also they should be compatible with the strict and growing laws of food safety. Based on this viewpoint, the foods with the natural origins especially the medicinal and aromatic herbs containing the active biological components can make the favorable prospects (Ghorbani, 2006).

The application of medicinal herbs as the additives is rapidly increasing because of their active ingredients, which have the beneficial effects on the host. In addition to the ingredients, there are other excipients which intensify and balance the therapeutic effects of plant as well as preventing the toxicity and adverse effects of active ingredients (Asadollahi *et al*., 2010). These herbal ingredients of plant sources are added to

the diet of livestock and poultry with the aim at improving the performance, immune system, gastrointestinal health and growth promoting. Medicinal herbs are applied in the powder, essence, juice, pulp, soaked and boiled forms (Craig, 1999). The most important compounds of medicinal herbs include the volatile or essential oils of eucalyptus such as flavonoids, carotenoids, saponins, plant steroids, phenolic compounds (including the simple phenols, polyphenols, phenolic acids, tannins, Chinones and Coumarins), Sulfides, Lectins, polypeptides, non-starch polysaccharides and some of oligosaccharides. Meanwhile, four first groups are consumed for the animals and the essential oils are particularly the largest and most important groups of these compounds (Mehri, 2001; Izat *et al*., 1990; Wagh *et al*., 2007).

In recent years, the new compounds called "probiotics" are applied as an additive in the livestock and poultry diets. In fact, most of the commercial products containing the live and beneficial microorganisms are called probiotics. In other words, the probiotics are the food supplements containing the live microorganisms and have the beneficial effects for the animals through creating the microbial balance in the gut. The probiotics are applied in order to prevent the growth and proliferation of harmful bacteria in the digestive tract, improve the feed efficiency and Feed Conversion Ratio (FCR) and finally increasing the growth and performance in addition to the reduced spread of disease. According to a report, the vast and sometimes inappropriate use of medicines had led to the drug resistance and also the drug residues in the meat of poultry have enhanced the consumers' concern, thus the use of plant extracts and essence for the treatment of this disease can be an appropriate treatment way in the future (Nobakht and Mehmannavaz, 2010).

The beneficial effects of medicinal herbs include stimulating the appetite and immune system, increasing the secretion of digestive enzymes and anti-bacterial, anti-viral, anti-parasitic and antioxidant activities. On this basis and according to the available hypotheses, this study investigates the effects of adding the mixture of medicinal herbs to the feed diet on the commercial laying hens' performance, the commercial laying hens' immune system responses and the qualitative characteristics of their egg.

In a report, the Feed Conversion Ratio (FCR) and the egg production in the laying hens were improved through adding 0.5 and 0.1% of thyme to the diet, but adding 1% of thyme to the diet increased the feed conversion ratio and reduced the feed intake and produced egg. The weight of egg was not affected according to Haugh Unit (Bolukbasi and Erhan, 2007). The albumen weight and yolk color were influenced by adding thyme extract and the hens received the diet with thyme had a small number of Escherichia coli (Arpasova *et al*., 2010). 2% of mixture of medicinal herbs (Malva, Alhagi and mint) numerically improved the increased daily weight and the feed conversion ratio because of the anti-bacterial and anti-fungal effects of compounds in plants due to the reduced harmful microbial population in the gastrointestinal tract; this improvement is obtained through promoting the chicks' health and immunity and preventing the decomposition of protein and amino acids by the harmful microbial population in the gastrointestinal tract (Nobakht and Aghdam-Shahriar, 2010). According to a research, the biosynthesis of bile acids in the liver is enhanced by increasing the cumin consumption and cumin can prevent the activity of enzyme 3-Hydroxy-3-methylglutaryl coenzyme A Reductase (HMG-CoA), which is the key regulatory enzyme in cholesterol synthesis (Srinvasan and Sambaiah, 1991). Adding 2.5% of cumin to the laying hens enhanced the weight of egg and yolk, but different amounts of cumin had no significant effect on the feed intake, production percentage and other parameters associated with the quality of egg including the specific gravity, Albumen and shell (Arshami *et al*., 2010a). In another study, the impact of replacing different amounts of Eucalyptus as a natural additive for growth promoting on the productive performance, quality of egg, carcass characteristics, blood parameters and immune response in the Japanese quail was investigated and it indicated the enhanced daily weight, number of egg, egg weight, egg mass weight, improved feed conversion ratio and quality of egg in the diets with Eucalyptus compared to the control diet (Hassan *et al*., 2011). Applying 3 g of eucalyptus per kilogram of diet improved the laying hens' productive performance and immunocompetence; moreover, the feed conversion ratio was improved in the hens given 3 g of eucalyptus as the diet because of their health and vitality (Abd El-Motaal *et al*., 2008). The gram-positive bacteria show greater sensitivity to the volatile oils of Eucalyptus than the gram-negative ones. The greater sensitivity of gram-positive bacteria to the Chemicals and vegetable essences is due to the differences in the wall construction (Soltaninejad *et al*., 2010). Different species of eucalyptus are different according to the percentage of ingredients and probably for this reason have different effects on the activity of various bacterial strains (Torabi Sogand *et al*., 2011). Another research was conducted on impact of adding the medicinal herbs, thyme, mint and oregano, to the laying hens' diet on the qualitative characteristics of egg, blood parameters and their immune responses; the highest productive percentage of egg and the best feed conversion ratio was seen in the treatment with two percentage of oregano. The lowest triglycerides of blood serum was obtained as the result of applying two percent of mint and the highest Haugh Unit and yolk color index through two percent of oregano. Furthermore, adding 2% of oregano to the laying hens' diets improved the qualitative characteristics of egg and the amount of blood parameters and percentage of

immune cells in the blood (Nobakht and Mehmannavaz, 2010). Different levels of oregano had significant effect on the qualitative characteristics of egg, but no significant effect was seen on the performance. The increased yolk weight was probably due to the existence of polyunsaturated fatty acids in this medicinal herb, thus the eggs with higher yolk weight were obtained. Moreover, the use of oregano transfers the yellow color to the yolk and makes its color richer because of various pigments (Nobakht and Mehmannavaz, 2010). As the result of increasing the amount of dietary fibers, the medicinal herbs enhance the rate of contents passing the digestive tract, thus they can increase the feed intake due to the palatability of diets, antimicrobial properties of medicinal plants and hens' improved overall health and digestive tract, increased secretion of pancreatic enzymes resulting in the appetite (Qureshi *et al*., 1983).

MATERIALS AND METHODS

This test was done in the salon 1 of Northern Poultry side in the Animal Science Research Institute situated in Karaj from October 15, 2012 to January 6, 2013 (for 12 weeks). The salon had dimensions of 10*30 m. The southern side of salon was applied in this test. In general, there were 1000 laying hen cages each which with three hens. There were (6) rows of laying hen cages in two floors with dimensions of 30*40*40 cm in this salon. There were seven 2* 2.5 m windows in the southern side of salon and three air conditioners in the northern side for air ventilation. Thirty 100-watt lamps with 3 m distance from each other and in 2.30 m height from the ground were considered for salon lighting. The applied drinking-system had the automatic nipple type and the handy feeder pan system was performed. The feed was distributed in the feeding systems two times a day at 7 am and 4 pm and several hours after distributing the grains, they were moved by the hand in order to be distributed equally.

Before transferring the hens, the salon and cages were disinfected using the antiseptic solution of formaldehyde gas. The chicken vaccination schedule was done according to the vaccination program of breed. The hens had Bovans breed and were transferred to the Research Institute of Animal Science at the age of 3 months (pullet) on February 4, 2012. These hens started laying eggs from the 21st week (marked) and the production peek was 70%. Ongoing egg laying began at the age of 25 weeks. The project started at the age of 49 weeks and ended at the age of 61 weeks. The catalogue of Bovans laying hens was applied in order to regulate the experimental diets and the standard table applied for measuring the chemical compounds and the energy of diet nutrients (NRC, 1994). Moreover, UFFDA software was applied in order to regulate the diets (Peste and Miller, 1994). A test in the form of completely randomized design was done on the total 300 laying hens at the age of 49 weeks for 84 days through 5 treatments, Treatment 1 = Base diet+ 0.1% of Probiotics (Protexine), Treatment 2 = Base diet+ 3% of medicinal herbs mixture powder, Treatment 3 = Base diet+ 2% of medicinal herbs mixture powder, Treatment 4 = Base diet+ 1% of medicinal herbs mixture powder, Treatment 5 = Base diet without any additives (control) and each treatment with 5 replications and each replication with 12 Bovans laying hens. Finally, two hens were randomly selected from each replication in the second phase of test (60 days) in order to investigate the antibody titer against the sheep red blood cells and they were injected with 1 cc (0.5%) of sheep red blood cells and the blood samples were taken after 10 days. Furthermore, two hens of each blood sampling replication were investigated in terms of blood parameters (Serum cholesterol, red blood cells, while blood cells, lymphocytes and hematocrit) in the day 70 of test.

During the main stage, some characteristics of laying egg were measured and determined daily and several ones in two time a week and the characteristics associated with the quality of egg were measured monthly. The studied characteristics are as follows: The performance characteristics, the daily feed intake (g), the feed conversion ratio (g/g), egg production (%), egg weight (g), egg mass weight, qualitative characteristics of egg, internal quality of egg, the egg yolk cholesterol concentration, external quality, egg shell (g), shell thickness, blood cells, red blood cells count and differential white blood cell count.

RESULTS

Orthogonal comparisons for performance of the entire course: The egg weight in the entire course and the feed intake were undergone several treatments ($p<0.05$), so that the maximum egg weight in the entire course belonged to the control treatment and the minimum was related to the diet containing mixture of medicinal herbs 3% and the maximum feed intake belonged to the control treatments, probiotics and mixture of medicinal herbs 1% and the minimum amount was related to the treatment 3% of mixture of medicinal herbs. The percentage of production and feed conversion ratio in the entire course were not undergone various treatments. The egg mass weight in the entire course was not undergone different treatments, so that its maximum value belonged to the control treatment and the minimum one to the treatment 3% of mixture of medicinal herbs. Impact of various treatments on the performance of entire course is shown in Table 1.

The use of different medicinal herbs had significant effects on the production percentage and the feed intake, but the egg weight, egg mass production and the

Table 1: Impact of various treatments on the performance of entire course

	Experimental group						
Evaluated parameters	Control	Probiotic	Mixture of medicinal herbs 1%	Mixture of medicinal herbs 2%	Mixture of medicinal herbs 3%	SEM	p.value
Egg weight of entire course (g)	63.74^{a}	63.24^{b}	63.20^{b}	63.22^{b}	63.09^{b}	0.0743	0.034
Feed intake of entire course (g)	110^{a}	109.71^{a}	109.61^{a}	108.88^{b}	108.22^{c}	0.1522	0.0
Production percentage of entire course	71.72	71.05	71.08	65.47	60.25	1.571	0.74
Egg mass weight of entire course (g)	45.72	44.94	44.95	41.39	38.01	1.016	0.067
Feed conversion ratio of entire course (g/g)	2.41	2.46	2.47	2.65	2.86	0.0611	0.111

Different letters in each column refer to the significant statistical differences ($p<0.05$)

Table 2: The impact of different treatments on the antibody titer against the SRBC, Immunoglobulin G (IgG) and Immunoglobulin M (IgM)

	Experimental group						
Evaluated parameters	Control	Probiotic	Mixture of medicinal herbs 1%	Mixture of medicinal herbs 2%	Mixture of medicinal herbs 3%	SEM	p.value
SRBC	8.20	9	7.25	7.60	7.60	0.281	0.383
IgG	6^{a}	2^{d}	4.50^{bc}	3.40^{c}	4.80^{ab}	0.332	0.001
IgM	3.20^{b}	7^{a}	2.75^{b}	4.20^{b}	2.80^{b}	0.402	0.001

Different letters in each column refer to the significant statistical differences ($p<0.05$)

Table 3: The impact of different treatments on the white and red blood cells, Hematocrit, lymphocyte and the volume of red blood cells

	Experimental group						
Evaluated parameters	Control	Probiotic	Mixture of medicinal herbs 1%	Mixture of medicinal herbs 2%	Mixture of medicinal herbs 3%	SEM	p.value
White blood cell ($\times 10^{3}$)	29.26	28.61	26.56	27.80	5.27	0.4839	0.069
Red blood cell ($\times 10^{6}$)	2.56^{a}	2.55^{a}	2.09^{b}	2.31^{ab}	2.28^{ab}	0.0522	0.036
Hematocrit (%)	30.67	29.60	27.83	30.25	28.50	0.605	0.24
Lymphocyte (%)	67	67.90	69.50	65.25	68.75	0.579	0.249
Volume of red blood cells	30.96	28.64	25.80	28.72	26.65	0.578	0.165

Different letters in each column refer to the significant statistical differences ($p<0.05$)

feed conversion ratio were not undergone different treatments and it was reported that the application of medicinal herbs led to higher feed intake than the control. The maximum percentage of egg production, the maximum egg mass production and the best feed conversion ratio belonged to the treatment containing 2% of oregano, while the produced eggs in the control group had the maximum weights (Nobakht and Mehmannavaz, 2010); the egg weight value was consistent with our results, but the results of egg mass production, feed conversion ratio, feed intake and production percentage were not consistent with our findings. According to a report based on the impact of different values of probiotics (Protexine) on the performance and qualitative characteristics of egg, no significant difference was seen among the treatments in the rate of egg production and the feed conversion ratio. The treatments had no significant differences in terms of mean egg weight and the egg weight in the control treatment was significantly higher than 25 g.t of probiotics (Shahsavari, 2006); it was consistent with the results of this study.

Orthogonal comparisons for humoral immunity: As shown in Table 2, the application of probiotics and the mixture of medicinal herbs at three levels had no impact on the Sheep Red Blood Cell (SRBC) ($p>0.05$). Adding the probiotics and mixture of medicinal herbs 1 and 2% to the laying hens' diet led to the lower Immunoglobulin G (IgG) than the control, but adding the mixture of medicinal herbs 3% to the laying hens' diet did not led to this reduction ($p<0.001$). The application of probiotics in the laying hens' diet led to the higher Immunoglobulin M (IgM) than the control and the mixture of medicinal herbs at three levels ($p<0.001$). The impact of different treatments on the antibody titer against the SRBC, Immunoglobulin G (IgG) and Immunoglobulin M (IgM) is shown in Table 2.

The impact of different treatments on the white and red blood cells, Hematocrit, lymphocyte and volume of red blood cells: The results associated with the impact of different treatments on the white and red blood cells, Hematocrit, lymphocyte and volume of red blood cells are presented in Table 3. As shown in this table, the application of mixture of medicinal herbs 3% in the laying hens' diet led to the lower amount of white blood cells than the mixture of medicinal herbs 1 and 2%, probiotics and control ($p>0.05$). Adding the mixture of medicinal herbs 1% to the laying hens' diet led to the lower amount of red blood cells than the

Table 4: Orthogonal comparisons for the qualitative characteristics of egg

	Experimental group				
Evaluated parameters	Control	Probiotic	Mixture of medicinal herbs	SEM	p.value
Egg weight (g)	66.20	64.47	67.89	0.631	0.093
Shell weight (g)	6.88	6.72	7.06	0.078	0.230
Shell thickness (µm)	425.3	414.8	428.3	0.379	0.3
Specific gravity (g/cm^3)	1.087	1.088	1.087	0.0006	0.708
Haugh unit	76.74	83.42	82.23	1.634	0.317

Different letters in each column refer to the significant statistical differences ($p<0.05$)

control, probiotics and the mixture of medicinal herbs 2 and 3% ($p<0.05$). The application of probiotics and the mixture of medicinal herbs at three levels had no impact on the Hematocrit, lymphocyte and the volume of red blood cells compared to the control ($p>0.05$). The impact of different treatments on the white and red blood cells, Hematocrit, lymphocyte and the volume of red blood cells is shown in Table 3.

This study investigated the impact of applying the medicinal herbs, Thyme, mint and oregano, on the performance of egg and the blood and immunity parameters in the laying hens. The use of different medicinal herbs had no significant impact on the Heterophile, lymphocyte and the ratio of Heterophile to lymphocyte (Nobakht and Mehmannavaz, 2010); it was consistent with our results for the lymphocyte.

According to a report based on the impact of Eucalyptus leaves powder on the laying hens' immune responses and performance at the age of 56 to 54 months, the experimental treatments consisted of 0, 1, 2 and 3 g of Eucalyptus leaf powder per kilogram of diet plus the basic diet. It was reported that the experimental treatments had no significant effect on the hematocrit (Abd El-Motaal *et al*., 2008); it was consistent with the results of this research.

Ademola *et al*. (2011) reported that the hens fed with 200 mg/kg garlic oil had significantly higher white blood cells count, but it had no effect on the red blood cells and it was consistent with the results of this research.

Al-Kassie (2009) reported that the broilers, fed with the thyme and cinnamon, had significantly higher levels of white blood cells, red blood cells, hematocrit and Hemoglobin than the control treatment; it was consistent with the results of this research.

Orthogonal comparisons for the qualitative characteristics of egg: The results of orthogonal comparisons for the qualitative characteristics of egg are presented in Table 4. According to the results of Table 4, adding probiotics and the mixture of medicinal herbs to the laying hens' diet had no impact on the egg weight, shell weight, shell thickness, specific gravity and Haugh Unit ($p>0.05$). Orthogonal comparisons for the qualitative characteristics of egg is shown in Table 4.

The impact of different levels of Cumin seeds on the production and the qualitative parameters of egg laid by hens at the age of 54 weeks was studied in a research and it was reported that different levels of Cumin had no significant effect on the parameters associated with the quality of egg such as the specific gravity, Albumen and its shell (Arshami *et al*., 2010b); it was consistent with the results of this study.

CONCLUSION AND SUGGESTIONS

Different rates of mixture of medicinal herbs had no impact on the improved feed conversion ratio, production percentage and egg mass weight in the entire course, but the mixture of medicinal herbs 2 and 3% led to the lower feed intake and the egg weight in the entire course than the probiotics and control treatments and the mixture of medicinal herbs 1% led to the lower egg weight than the control treatment.

There was no significant difference with the control treatment in any tested treatments in terms of qualitative characteristics of egg including the egg weight, shell weight, shell thickness, Haugh Unit, egg specific gravity, Lymphocytes, hematocrit, red blood cell volume and white blood cell count, but the experimental treatments affected the red blood cells count, yolk cholesterol, Immunoglobulin M (IgM) and Immunoglobulin G (IgG).

In this regard, the following suggestions can be offered:

- The impact of medicinal herbs mixture powder (Thyme, oregano, cumin, Alhagi, garlic and eucalyptus) should be comprehensively investigated on the immunity and blood parameters.
- The impact of medicinal herbs mixture powder (Thyme, oregano, cumin, Alhagi, garlic and eucalyptus) should be investigated on the yolk color.
- The impact of medicinal herbs mixture essence (Thyme, oregano, cumin, Alhagi, garlic and eucalyptus) should be investigated on the performance, the qualitative characteristics of egg, immunity and blood parameters.

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