

INVESTIGATING THE EFFECT OF FENUGREEK SEED POWDER AND GARLIC POWDER IN THE DIET ON IMMUNE RESPONSE OF COMMERCIAL LAYING HENS' EGG**SEYED MOHAMMAD MOTAMED^{a1} AND SEYED MOZAFAR MEHDIZADE TAKLIMI^b**^aDepartment of Animal Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran^bFaculty Member of Animal Science Research Institute (ASRI) Karaj, Iran**ABSTRACT**

In order to investigate comparative impacts of fenugreek powder and garlic powder levels in the diet on efficiency and qualitative traits of commercial laying hens, an experiment was performed as completely randomized layout with six treatments and four replications including 12 laying hens; totally, 288 laying hens for 84 days. The treatments included treatment (1): control (without additives), treatment (2): control diet+1% garlic powder+0.5% fenugreek powder, treatment (3): control diet+0.5% fenugreek powder, treatment (4): control diet+1% garlic powder+1% fenugreek powder, treatment (5): control diet +1% garlic powder, treatment (6): control diet+1% fenugreek powder. The performance of poultries immune system was investigated which had been chosen randomly through injecting sheep red blood cells (SRBC); also, produced antibody was studied in two hens of each replication. Also, the amount of serum cholesterol and other blood parameters were measured. Data analysis was conducted using SAS software, and Duncan test was used to compare the traits means. Results showed that, there is a significant difference in the experimental groups in terms of the traits associated with immune system in antibody titer against (SRBC) and immunoglobulin G with the control group. The best treatment was the treatment containing 1% garlic powder. Also, there was no significant difference in antibody titer against Newcastle disease, immunoglobulin M, heterophile ratio to lymphocytes H/L and white blood cells. There was a significant reduction in the amount of LDL compared to the control group, and the best treatment contained 1% garlic powder; also, in blood parameters there was no significant difference in serum cholesterol and calcium, phosphorus, total protein, globulin and hematocrit but, a significant difference was generated in the amount of blood albumin and red blood cells compared to the control group; the best treatment was the treatment containing 1% garlic powder. Totally, experimental group containing 1% garlic powder + 1% fenugreek powder and the treatment containing 1% garlic powder showed more appropriate response about blood parameters and immune system.

KEYWORDS: Fenugreek Powder, Garlic Powder, Blood parameters, Serum Cholesterol, Immune Response, Laying Hens

Egg is an important animal protein resource which is greater than other protein resources in terms of amino acid score, and is a rich source of minerals, vitamins and other nutrients. Moreover, lower price of egg has caused that, all segments of society to be able to access this high quality protein resource. Anyways, quality and quantity of produced egg highly depend on nutritional and health management of laying hens farms. Garlic and fenugreek are medicinal plants of which useful effects have been known for long time, and their effective materials and the mechanism of their effect have been studied through experimental works. Although, numerous researches have been conducted on the effect of garlic and fenugreek on the health and growth yield of broiler chickens and other laboratory animals, less information is available about their effect on laying hens yield and egg quality. Accordingly, the present research has been done to investigate the effect of various levels of garlic and fenugreek powder on the cholesterol serum, other blood parameters and immune response of laying hens. Fenugreek is an annual herbaceous plant with single flowers which are light yellow or violet white with Strong smell and bitter taste and are aromatic. This plant is one-year, herbaceous, standing with 15 to 50 cm height that is planted in most of areas in Iran and of which flowering is in May, June and July (Qhraman, 1999). Fenugreek seed has 45 to 60% carbohydrate (mostly Galactomannan mucilage), 20 to 30% protein enriched by Lysine tryptophan, 5 to 10% lipid, considerable amounts of pyridine-like alkaloid (including 0.2 to 0.36% Trigonelline, 0.5%), flavonoids, free amino acids (arginine, histidine and lysine, and 0.09% hydroxyisoleucine), calcium and iron, 0.6 to 1.7%

saponins, glycosides, cholesterol and citostrol, vitamins (A, B1, C) and 0.015% volatile oils (American Botanical Council, 2000). Garlic is a biennial plant, grass, and a stem with a height of 20 to 30 cm and even more. The bulk of garlic is water (61%). Moreover, organic acids, amino acids, fatty acids, carbohydrates, minerals, enzymes, vitamins, prostaglandins, terpenoids, flavonoids, glycosides and sulfur derivatives are found in garlic structure (Pourabdollah & Pourabdollah, 2001). There is a wide range of organic acids including citric acid, malic acid, succinic acid, oxalic acid, Malonic acid, lactic acid, Gluconic acid, alpha acid in the garlic composition. Amino acids including phenylalanine, lysine, arginine, asparagine, histidine, leucine, alanine, isoleucine, glycine and alpha-aminobutyric acid form amino acid profile of garlic. Two fatty acids of inoleic acid and palmitic acid have been also identified in garlic composition. About carbohydrate content of garlic also, there are free single sugars such as fructose, glucose and arabinose and free oligosaccharides including sucrose and maltose in garlic extract. Garlic is full vitamin A, B1, B2, niacin, ascorbic acid, folacin, biotin, vitamin D and vitamin E. Enzymes of garlic also include polyfructosidase, oxidase, proteolytic, pectin-esterase, peroxidase and alliinase which causes to convert alliin to allicin that is an effective material of garlic. Alliin is one of the most important material of garlic which is among sulfur derivatives which is converted to allicin affected by alliinase when garlic is chopped. Allicin is the most effective recognized material existing in garlic composition (Kasuga and Kansawa, 1980). Lotfollahian et al., (2007) reported that, using 0.5% green herbal supplement of growth in the diet of broiler chickens is useful and appropriate. The cost of consumptive feed per

kg of live weight increase in the group that used diet containing 0.5% green herbal supplement of growth was less than control group and production index also in the same group was higher than the control group and other experimental diets. Masjedi et al. (2010) studied the effect of garlic extract on the amount of feed consumption and glucose level, cholesterol serum triglycerides of diabetic rats' blood by streptozotocin. Results showed that, garlic extract decreased glucose level, cholesterol and triglycerides of diabetic rats' blood significantly; so that, glucose level of diabetic rats' blood that had received garlic extract, was same as the healthy rats. Also, the results showed that, garlic extract can be effective in adjustment of glucose, cholesterol and triglyceride level in diabetic patients. MottaqiTalab and Jamshidzahi (2008) in an experiment on 360 broiler chickens for four days with one of nine experimental diets without additives, basal diet plus 20 ppm virginiamycin antibiotic, basal diet plus 4, 2, 6 kg/ton of olive leaf powder and basal diet plus two types of olive leaf extract with levels of 200 and 400 gr/ton in a completely randomized layout reported that, virginiamycin antibiotic can be replaced effectively by olive leaf powder as an appropriate option. Ullah Khan et al. (2009) studied the effect of fenugreek essence on the internal organs of broiler chickens. In this study, treatments of 0.00 (control), 10, 20 and 30 ml/l of fenugreek essence were applied. In the study on internal organs it was found that, liver weight was insignificantly higher in treatment of 20 and 30 ml/l compared to the other treatments. For gizzard weight also there was no significant difference between fenugreek and control treatments. Intestinal weight was significantly higher in control treatment than fenugreek treatments. Totally, the results indicated that, fenugreek in broiler chickens' diet relatively causes to increase the size of

some internal organs (except the intestines). Al-Troudi and Hussein. (2009) investigated the effect of fenugreek seed powder and blood parameters of broiler chickens. In this study, four treatments were used including: 1. control treatment (basal diet without additives), 2. Basal diet plus gentamicin antibiotic, 3, 4. Basal diet plus 1% and 2% of fenugreek seed powder respectively. Results showed that, adding fenugreek seed powder results in increase of body weight, growth speed, feed consumption improvement and FCR improvement. Red blood cells content and hemoglobin concentration also was increased as a result of using fenugreek while, cholesterol and blood sugar concentration decreased, and these effects showed an increasing trend by increasing the amount of fenugreek from 0.00 to 2%.Hernandes et al., (2004) used 200 ppm of oregano, cinnamon, pepper extract and 5000 ppm of artemisia, thyme and rosemary in diet of broiler chickens. Results showed that, the amount of growth in these treatments in the growth period of 14-21 days was higher than the control group.

MATERIALS AND METHODS

This study was conducted in the research farm at the Animal Science Research Institute (ASRI) of Iran. In this study, two hundred eighty eight piece 49-wk-old laying hens (Bovans strain) were randomly assigned into 6 groups with 4 replicates of 12 birds each (48 laying hens per group) and fed diets supplemented with; Treatment 1= control (without additives), treatment 2= control diet+1% garlic powder+0.5% fenugreek powder, treatment 3= control diet+0.5% fenugreek powder, treatment 4= control diet+1% garlic powder+1% fenugreek powder, treatment 5= control diet +1% garlic powder, treatment 6= control diet+1% fenugreek powder, respectively for 12 wk.

Table 1: Composition of the Diets and Their Nutrient

Ingredients	Control(%)	G1+F0.5(%)	F0.5(%)	G1+F1(%)	G1(%)	F1(%)
Corn	34	33.300	33.750	33.150	33.450	33.450
Wheat	30	29.700	29.900	29.500	29.850	29.850
Soybean meal	22.535	22.035	22.385	21.885	22.235	22.235
NaCl	0.1	0.1	0.1	0.1	0.1	0.1
Dicalcium phosphate	2.26	2.26	2.26	2.26	2.26	2.26
Vegetable oil	1.2	1.2	1.2	1.2	1.2	1.2
Oyster shell	9.252	9.252	9.252	9.252	9.252	9.252
Baking soda	0.15	0.15	0.15	0.15	0.15	0.15
Phytase enzyme	0.003	0.003	0.003	0.003	0.003	0.003
Vit and minerals	0.5	0.5	0.5	0.5	0.5	0.5
Fenugreek powder	0.00	0.5	0.5	1.00	0.00	1.00
Garlic powder	0.00	1.00	0.00	1.00	1.00	0.00
Energy (kcal/kg)	2773	2773	2773	2773	2773	2773
Calcium (%)	4.15	4.15	4.15	4.15	4.15	4.15
Crud protein(%)	15.73	15.73	15.73	15.73	15.73	15.73
Phosphorus(%)	0.41	0.41	0.41	0.41	0.41	0.41

Na(%)	0.18	0.18	0.18	0.18	0.18	0.18
Methionine (%)	0.63	0.63	0.63	0.63	0.63	0.63
Methionine+cystine (%)	0.90	0.90	0.90	0.90	0.90	0.90
Lysine(%)	0.87	0.87	0.87	0.87	0.87	0.87
(%)Tryptophan	0.2	0.2	0.2	0.2	0.2	0.2

F_{0.5}: the diet containing 0.5% fenugreek powder, F₁: the diet containing 1% fenugreek powder, G₁: the diet containing 1% garlic powder, G₁+F_{0.5}: the diet containing 0.5% fenugreek + 1% garlic powder, G₁+F₁: the diet containing 1% fenugreek powder+1% garlic powder

Table 1. Composition of the diets and their nutrients. In order to adjust experimental diets, standard tables of feed requirements for laying hens were used and to compute chemical compounds and energy of the diets' feed also standard tables were used (NRC, 1994). In order to adjust the diets, UFFDA software was used (Peste et al., 1994). After adjusting the diets, required foods were provided for the diets. In this stage, the amounts of experimental medicinal plant (garlic powder and fenugreek powder) required in each diet were weighted and were mixed by soybean, corn and wheat meal and then, other components of the diet were added to it. For measuring sheep red blood bell (SRBC) antibody titration, 0.2 ml sterile sheep red blood cell (0.8%) suspension injected through limb vein of two hens per replicate. After 10 days blood samples collected and total response titration (IgM+IgG) measured by hemoagglutination as described by , IgG and IgM measured by separating mercapto ethanol resistant (MER) antibody (IgG) and this amount is deducted from the total antibody response sensitive to mercapto ethanol (IgM). Blood cells counted at day 70 of study after blood collecting (two hens per replicate) in venojects having EDTA. Counting of red blood cells (RBC) and differentially white blood cells (WBC) was using Hemocytometer. Packed Cell Volume (hematocrit) measured by centrifuging heparin coated capillary tubs for 15 min at 12000 rpm. For measuring total cholesterol, at day 70 of study, after blood collecting (two hens per replicate), serum separated and total cholesterol measured by spectrophotometer using commercial kits (Parsazmoon, Iran). Experimental design of this study was completely random design (CRD). In this regard, in 70th day of the experiment, two hens of each replication were bled and the sample was sent to the laboratory. The following model was assumed in the analysis of all traits. $y_{ij} = \mu + a_i + b_j + ab_{ij} + e_{ij}$ Where y_{ij} is the observed value, μ is mean population, a_i is the effect of fenugreek powder, b_j is the effect of garlic powder, ab_{ij} is the interaction of garlic and fenugreek, e_{ij} is test error. Data were analyzed by GLM method and SAS(2002) software for Windows. The differences between means were determined by ANOVA. Means were compared by Duncan's multiple range tests with 95% confidence interval. In addition, Means were compared by orthogonal contrasts.

RESULTS

Immunity System Indices in Blood

Sheep Red Blood Cells (SRBC)

According to the variance analysis, the effect of various treatments have been significant on SRBC ($P < 0.05$) so that, the mean SRBC in the group of 1% garlic and 1% garlic+1% fenugreek and 0.5% fenugreek has a significant difference with the control group. But, there is no significant difference between the treatments of 1% fenugreek and 1% garlic+0.5% fenugreek; but in terms numerical has had an increase compared to the control group. Treatment of 1% garlic powder has the maximum value (6.12) and the control treatment has the minimum value (4.000). The obtained results has no consistency with Hashemi Attar et al, (2008).

Immunoglobulin G (IgG)

According to the variance analysis, the effect of various treatments have been significant on immunoglobulin G ($P < 0.05$) so that, the mean of training group containing 1% garlic and 1% garlic+1% fenugreek and 1% fenugreek have a significant difference. Also, there is no significant difference among treatments containing 1% fenugreek+0.5% garlic, 1% fenugreek and control group. In terms of numerical, the treatment of 1% garlic has the maximum value (4.30) and the control treatment has the minimum value (2.45) which causes to increase cellular immunity. The obtained results has no consistency with Ao et al,(2010).

Immunoglobulin M (IgM)

According to the variance analysis, the effect of various treatments did not become significant on the data of immunoglobulin M(IgM) ($P > 0.05$). In terms of numerical, the treatment containing 1% garlic+ 1% fenugreek had the maximum value (1.72) and control treatment and 0.5% fenugreek had the minimum value (1.48). The obtained results has no consistency with Ao et al, (2010).

Newcastle Titer

According to the variance analysis, the effect of various treatments did not become significant on the data of Newcastle titer ($P > 0.05$). Means comparison of various traits in different treatments showed that, in terms of numerical, the treatment containing 1% fenugreek had the maximum value (7.75) which has had an increase compared to the control treatment, and control treatment and 0.5% fenugreek had the minimum value (6.00). The obtained results are consistent with Hashemi Attar et al, (2008).

White Blood Cells (WBC)

According to the variance analysis, the effect of various treatments did not become significant on the data

of WBC ($P>0.05$). Means comparison of various traits in different treatments showed that, in terms of numerical, the treatment containing 0.5% fenugreek had the maximum value (30.07), and control treatment had the minimum value (26.30). The obtained results are consistent with Hashemi Attar et al, (2008).

Lymphocytes

According to the variance analysis, the effect of various treatments did not become significant on the data of lymphocytes ($P>0.05$). Means comparison of various traits in different treatments showed that, in terms of numerical, the treatment containing 0.5% fenugreek had the maximum value (69.00) and 1% garlic powder which has had an increase compared to the control treatment, and control treatment had the minimum value (67.75).

Heterophile

According to the variance analysis, the effect of various treatments did not become significant on the data of heterophile ($P>0.05$). Means comparison of various traits in different treatments showed that, in terms of numerical, the treatment containing 1% garlic powder had the maximum value (29.60); all experimental groups have been increased compared to the control treatment, and control treatment had the minimum value (28.00).

Ratio of Heterophile to Lymphocytes (H/L)

According to the variance analysis, the effect of various treatments did not become significant on the data of H/L Ratio ($P>0.05$). Means comparison of various traits in different treatments showed that, in terms of numerical, the treatment containing 1% garlic powder+ 0.5% fenugreek, 0.5% fenugreek, 1% garlic+1% fenugreek, and 1% fenugreek had the maximum value (0.43), and the treatment containing 1% garlic powder had the minimum value (0.4).

Table 2: Effects of Dietary Supplementation of Feed Additives on Immune Response of Laying Hens

Immune response	Control	F0.5	F1	G1	G1+F0.5	G1+F1	SEM	Significance
Newcastle titer	7.50	6.00	7.75	6.25	6.12	7.25	0.29	0.17
LYP (%)	67.75	69.00	68.50	69.00	68.25	68.60	0.50	0.98
Heterophile (%)	28.00	28.75	29.50	29.60	29.50	29.50	0.54	0.97
WBC ($\times 10^3$)	26.30	30.07	29.20	26.40	28.02	28.96	0.52	0.22
HET/LYP(%)	0.41	0.43	0.43	0.40	0.43	0.43	0.01	0.98
SRBC	4.00 ^c	5.31 ^{ab}	4.61 ^{bc}	6.12 ^a	4.40 ^{bc}	5.85 ^a	0.41	0.001
IgG	2.45 ^c	3.00 ^{bc}	3.96 ^{ab}	4.30 ^a	2.59 ^c	4.11 ^a	0.45	0.003
IgM	1.48	1.48	1.48	1.71	1.60	1.72	0.38	0.96

The numbers which have been marked by the letters have significant difference ($p<0.05$)

HEMATOLOGY

Blood Serum Cholesterol

According to the variance analysis, the effect of various treatments did not become significant on the data of cholesterol ($P>0.05$). In terms of numerical, the treatment containing 1% fenugreek had the minimum value (151.75); while, control treatment had the maximum amount of cholesterol (242.00). The results are consistent with Al Troudi and Hussein. (2009), Ao et al. (1995), Augusiti.(1997), Orellana et al. (1992), saffa. (2007) and are in conflict to Masjedi et al. (2010) and Petit et al. (1995).

LDL

According to the variance analysis, the effect of different treatments became significant on LDL ($P<0.05$); so that, the mean of treatments containing 1% garlic powder, 1% fenugreek, 1% garlic powder+0.5% fenugreek decreased compared to the control group and there is a significant difference between them. The treatment containing 1% garlic powder+1% fenugreek powder and control group had no significant difference. The concentration of blood serum LDL was significantly decreased in response to the

use of garlic and fenugreek. By the way, the difference of the means of various treatments was statistically significant; so that, control treatment had the highest LDL (39.00) and the treatment of 1% garlic had the minimum LDL (27.33). Results of the present study showed that, the use of garlic and fenugreek in the diet of laying hens increases the concentration of triglycerides in the blood serum of laying hens through affecting triglyceride metabolism. The achieved results are consistent with Masjedi et al. (2010), Al Troudi and Hussein (2009), Ao et al. (1995), Augusiti (1997), Orellana et al. (1992), saffa (2007), Skan et al. (1993) and are in conflict to Petit et al. (1995).

Blood Hematocrit

According to the results of variance analysis, effect of various treatments was not significant on the blood hematocrit data ($P>0.05$). Also, means comparison of blood hematocrit data states that, there is no significant difference among various treatments. In terms of numerical, the experimental group containing 1% garlic powder+1% fenugreek had the maximum value (30.42) while, the control group had the minimum amount of hematocrit.

Red Blood Cells (RBC)

According to the results of variance analysis, effect of various treatments was significant on RBC data ($P < 0.05$). In terms of numerical, the maximum value

(2.77) was for the experimental group containing 1% garlic powder+1% fenugreek powder; also, the group containing 1% garlic powder+0.5% fenugreek powder had the minimum value (2.15) of RBC. The obtained results are consistent with Al_Troudi and Hussein (2009).

Table 3: Effect of Feed Additives on Hematology of Laying Hen

Hematology	Control	F _{0.5}	F ₁	G ₁	G ₁ +F _{0.5}	G ₁ +F ₁	SEM	Significance
Serum cholesterol (mg/dl)	242.00	159.00	151.75	191.75	161.13	175.00	11.90	0.25
(LDL(mg/dl)	39.00 ^a	32.50 ^{bc}	30.50 ^{cd}	27.33 ^d	28.00 ^{cd}	35.66 ^{ab}	1.11	0.0005
(%)Blood hematocrit	26.50	27.67	28.37	28.25	27.40	30.42	0.43	0.17
(R.B.C ($\times 10^6$	2.29 ^{bc}	2.22 ^c	2.53 ^{abc}	2.67 ^{ab}	2.15 ^c	2.77 ^a	0.06	0.02

The numbers which have been marked by the letters have significant difference ($p < 0.05$)

BLOOD PARAMETERS

Blood Globulin

According to the results of variance analysis, effect of various treatments was not significant on blood globulin data ($P > 0.05$). In terms of numerical, the experimental group had enhancement compared to the control group; so that, the group containing 1% garlic powder had the maximum value (4.76) and the control group had the minimum value (3.45).

Blood Albumin

According to the results of variance analysis, effect of various treatments was significant on blood albumin data ($P < 0.05$). In terms of numerical, the experimental group containing 1% garlic powder + 1% fenugreek had the maximum value (2.76) and the treatment containing 1% fenugreek had the minimum value (2.25). The obtained results are consistent with Chehrei et al. (2011) and are in conflict to Ao et al. (2010).

Total Protein

According to the results of variance analysis, the effect of various treatments was not significant on total

protein data ($P > 0.05$). There was a significant difference between the treatment containing 1% garlic powder and 1% fenugreek powder. In terms of numerical, the maximum amount of mean total protein (7.36) was for the group containing 1% garlic powder and the maximum value (5.70) was for the group with 1% fenugreek powder. The obtained results are in conflict with Ao et al. (2010).

Blood Calcium

According to the results of variance analysis, the effect of various treatments on blood calcium was not significant ($P > 0.05$). But, in terms of numerical, the amount of blood calcium has increased in all experimental groups compared to the control group. Also, the treatment containing 1% garlic powder had the maximum value (38.53) and control group had the minimum value (34.80).

Blood Phosphorus

According to the results of variance analysis, the effect of various treatments on blood phosphorus was not significant ($P > 0.05$). But, in terms of numerical, the treatment containing 1% garlic powder + 1% fenugreek had the maximum value (7.53) and the group containing 1% fenugreek powder had the minimum value (6.00).

Table 4: Effect of Different Levels of Dietary Feed Additives on Blood Biochemistry of Laying Hen

Blood biochem.	Control	F _{0.5}	F ₁	G ₁	G ₁ +F _{0.5}	G ₁ +F ₁	SEM	Significance
Calcium(mg/dl)	34.80	37.20	36.50	38.53	37.06	36.00	1.04	0.96
Phosphorus(mg/dl)	7.35	7.05	6.00	6.63	7.50	7.53	0.21	0.24
Protein(mg/dl)	6.35	6.70	5.70	7.36	7.06	6.69	0.19	0.15
Albumin(mg/dl)	2.50 ^{abc}	2.40 ^{bc}	2.25 ^c	2.60 ^{ab}	2.70 ^a	2.76 ^a	0.05	0.01
Globulin(mg/dl)	3.45	4.50	3.85	4.76	4.36	4.20	0.16	0.27

The numbers which have been marked by the letters have significant difference ($p < 0.05$)

DISCUSSION

Improvement of immunity response in the chickens' body can be stated that, in the treatments receiving garlic powder and fenugreek powder, considering that both plants have been recognized as stimulating of the immunity system, boost humeral immunity and enhance cytokinins particularly IFN and lymphocytes T which lead to increase of cellular

immunity. About the effects of fenugreek powder and garlic powder on cholesterol concentration and blood serum LDL, considering the compounds existing in these two plants as modifiers, the blood lipids could have a considerable decrease in all the experimental treatments; so that, all experimental groups have significant differences compared to the control group the maximum of which was for the group containing 1% garlic powder.

Similar to the present study, Saffa (2007) reported a reduction in total concentration of plasma cholesterol as well as LDL as a result of adding garlic and fenugreek to the diet of laying hens. The results showed that, 2% garlic and fenugreek had beneficial effects on the cholesterol metabolism without having any adverse effect on the laying hens' performance. In broilers also, Al Troudi and Hussein (2009) reported the reduction of serum cholesterol concentration as a result of using fenugreek. In this study, plasma cholesterol concentration has been decreased parallel with increasing fenugreek from 0.00 to 2%. Ao et al. (1995) also investigated the effect of using garlic powder on plasma cholesterol concentration of laying hens and reported that, by increasing the amount of garlic powder in the diet from 0.00 to 3%, blood serum cholesterol concentration has been decreased significantly while, concentration of saturated fatty acids was decreased and concentration of unsaturated fatty acids was increased. Generally, the conducted experiments showed that, adding garlic powder and fenugreek powder to the diet of laying hens reduce cholesterol concentration and saturated fatty acids by bile excretion resulted from excessive secretion induced by excessive excitatory actions of the material existing in both plants without having any adverse effect on the laying hens' performance. Skan et al. (1993) created fatty acids to increase the ratio of unsaturated fatty acids with double links which has reduced blood serum LDL and will have beneficial effects on the health of laying hens and consumers. But, about cholesterol, although all the treatments had a reduction cholesterol concentration numerically, the reductions were not significant. About red blood cells (RBC), the experimental treatments had a significant difference to the control group which represents erythropoietin hormones stimulation in the adrenal to proliferate red blood cells in the chickens' body. Garlic has anti-platelet aggregation. This effect of garlic has been recognized absolutely. Actually, adenosine as one of organosulfate compounds obtained from butanol extract of garlic bulb has the strongest anti-platelet aggregation effect among the effective material of garlic (Makheja and Bailey, 1990). Ahoiin has an anti-clotting power at least similar to aspirin (Pourabdollah & Pourabdollah, 2001). Conducted investigations show that, using 800 mg/day of garlic powder reduces platelets aggregation by 56% after four weeks in the patents with platelets aggregation and high risk of ischemic attack (localized anemia) (Azad et al., 1991). Al-Troudi and Hussein (2009) studied the effect of fenugreek powder on blood parameters of broilers. The results showed that, adding fenugreek powder increases red blood cells and blood hemoglobin concentration and these effects showed an increasing trend by increasing the amount of fenugreek from 0 to 2%. In this study also, about albumin, there was significant difference among the experimental treatments and control group when using fenugreek powder and garlic powder. But in the other items (calcium, globulin, proteins, phosphorus) there was not significant an improvement compared to the control group. Higher level of blood albumin (which is a protein product) in the groups that have received garlic and fenugreek powder can be related to the increase of nutrient

absorption including protein and its presence in the blood serum.

CONCLUSION

Fenugreek and garlic powder affected significantly the immunity system of laying hens and caused a relative improvement. Also fenugreek and garlic powder had a considerable effect on the blood lipids and particularly LDL and had the highest impact on the other parameters of blood in particular albumin. The treatments containing 1% garlic powder + 1% fenugreek powder were the best treatments. Therefore, it can be concluded that, the use of garlic powder and fenugreek powder causes health, immunity, increase of herd efficiency and consequently economic benefit enhancement. By increasing the use percentage of these plants in the future studies, we may observe considerable improvement in the other experiments.

ACKNOWLEDGMENT

We gratefully acknowledge the efforts of the staff of the department of Animal Science, Azad Islamic University, Varamin, Iran and Animal Science Research Institute (ASRI) in Karaj, Iran

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