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ORIGINAL ARTICLE

Effects of Graded Fat/oil on Egg Production and Quality, Some Biochemical Parameters of Blood and Immunity in Laying Hens

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ABSTRACT

This study was conducted to investigate the effects of using fat and oil and their replacement in diet on egg production and traits, biochemical parameters of blood and immunity of laying hens. A total of 288 High Line (W-36, 50 weeks of age) laying hens were used in a CRD with 6 treatments and 4 replicate (12 hens per each) for a trial period of 12 weeks. The experimental treatments included: T1: control (without fat/oil), T2: 4% fat (beef tallow, BT), T3: 4% oil (soybean oil, SO), T4: 2% BT + 2% SO, T5: 4% BT for first six weeks + 4% SO for second six weeks of trial period and T6: 4% SO + 4% SO by method similar to T5. There were significant differences (P>0.05) between treatments only in egg weight and some biochemical parameters of blood that the highest egg weight (65.12 g) and the lowest albumin (2.59 mg/dl) were observed by 4% SO (T3) and when dietary fat graded to 2% SO for a period of 6 weeks and followed by 2% BT for subsequent 6 weeks of trial, blood glucose significantly (P<0.05) decreased (149.88 mg/dl). By exerting changes in dietary fat/oil type, selected immunity factors (Heterophil, H; Lymphocyte, L; and H/L ratio) not significantly changed. From the results of presented study it is concluded that SO in 4% levels seems to be a good oil source for laying hen and adding BT to dietary fat not suitable.

Key words: Egg quality; laying hen; fat; oil; performance.

Introduction

Generally, fats and oils are used in poultry diet as resources of energy. Content energy and productivity of lipids are linked to their saturation level and the length of molecular chain [1-2]. Other advantages of utilizing fats and oils in poultry diet include decrease of nourishment dust, increase in absorption and digestion of lipoproteins, significance amount of necessary fatty acids and their lower heat toward carbohydrates and proteins. Also they assist vitamin A and Ca absorption. Chemical structure of fats and oils are different in the case that the oils with vegetable base such as soya have more unsaturated fatty acids and absorption productivity than animal fats [3-5]. Productivity of energy utilization is high in dietary containing fat which can be assigned to lower heat of dietary [6-7]. Oils and fats contain Linoleic acid. ($^{15, 9, 12}$ D, LA) , omega-3 , Omega-6 and arachidonic acid (^{5,8,11,14,} D, AA), are vital fats for human health which can be decrease the heart disease. In laying hens utilizing fats in diet will decrease feed intake and as a result improves the conversion coefficient of dietary in eggs [8]. But Harms and Russell [9] reported that the kind of fat has no efficacy on laying hens performance.

It is proven that high utilization of fat in laying hen's diet in comparison to oil can be decrease linoleic acid and size of egg [10]. Cholesterol entrance into egg is a method of natural blood

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cholesterol control in laying poultry. It has been previously documented by Hirata *et al*. [11] that there is no difference between egg yolk of hens fed soaben oil, coconut oil, pig fat and beef tallow. Brisson [1] stated that, utilization of oil in comparison with beef tallow declined circulation of serum cholesterol in broiler chickens as well as increase of unsaturated fats than saturated fats. Harris [12] and Grundy [13] reported that blood cholesterol level of poultry have direct connection with fatty acids type as the unsaturated fats can active and increase high density lipoproteins cholesterol (HDLs-CH, good) of blood which transferred cholesterol from cells to liver, while low DLs-CH (bad) reversely act. On the one side, Simopoulos and Robinson [14] reported that a high ratio of omega-6 to omega-3 fatty acids has been linked with an increased risk of cancer, cardiovascular disease, allergies, depression, obesity, and auto-immune disorders. Hence using unsaturated oils instead of fats and also omega-3 FA series instead of omega-3 is preferred in fowl's diet supplementing [15-16].

Therefore, this study was conducted to investigate the effects of using fat and oil and their replacement in diet on egg production and traits, biochemical parameters of blood and immunity of laying hens.

Materials and Methods

Birds and Husbandry:

This experiment was conducted with two hundred and eighty eight Hy Line (W36) laying hens from 50 to 62 weeks of age in a completely randomized design with 6 treatments and 4 replicate with 12 hens in each replicate. Experimental groups were included: control group (without using fat or oil), group 2: contain 4% BT, group 3: contain 4% SO, group 4: contain 2% BT + 2% SO, group 5: contain 4% BT in the first six weeks of experiment period and 4% SO in the second six weeks of experiment period and finally group 6: contain 4% SO in the first six weeks of experiment period and 4% BT in the second six weeks of experiment period. Experimental diets were adjusted on the bases of corn grain, soybean meal. The rations were isocaloric (2750 Kcal/Kg) and isonitrogenous (13.75 %) with different levels of fat and oil and were administered according to laying hens nutrient recommendations [17].

Along the experiment, environmental conditions were equal for all the groups. The hens were photo stimulated at 22 weeks of age and received 16 hours of light started from week 26 of age. This photo schedule was continued to 60 weeks of age. Light schedule included an environmentally controlled lighting, a reverse 16 h: 8 h light: dark cycle as well as temperature controlled with an *ad-libitum* feeding. Egg production were recorded daily and summarized on a weekly basis throughout the experimental period. Abnormal eggs having multiple yolks, shell-less and those with defective shells were recorded. Shell-less eggs were, however, not included in the weight data.

At the end of trial, four eggs were randomly selected and collected from each replicate for determine egg production and quality parameters. Individual egg weights were recorded for all the eggs of each hen, daily throughout trial period.

Table 1: List of Nourishment & Mixture of Dietaries Used in Experiment.

Ingredients	Control	Experimental diet
Corn	69.70	40.23
Wheat	0	19.81
Soybean Meal	15.21	5
Wheat Bran	5. 7	16.83
Fat/Oil	0	4
Inert	0	4
Oyster Shell	7.28	7.36
Bone Powder	1.3	1.52
Salt	0.26	0.25
Vitamin Supplementation	0.25	0.25
Mineral Supplementation ANALIZE	0.25	0.25
Metabolic Energy (kcal/kg)	2750	2750
Protein (%)	13.75	13.75
Ca	3. 22	3.32
Phosphorus	0.3	0.3
Sodium	0.14	0.14
Lysine	0.64	0.65
DL- methionine + L- cysteine	0.52	0.52
L-Threonine	0.57	0.55
Tryptophan	0.17	0.18

Experimental groups were prepared on 4% graded fat/oil and treatments included: T1: control (without fat/oil), T2: 4% fat (beef tallow, BT), T3: 4% oil (soybean oil, SO), T4: 2% BT + 2% SO, T5: 4% BT for first six weeks + 4% SO for second six weeks of trial period and T6: 4% SO + 4% SO by method similar to T5. 3For each kg of the diets; vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 IU; vitamin B1, 1,800 mg; vitamin B2, 6,600 mg; vitamin B3, 10,000 mg; vitamin B6, 3,000 mg; vitamin B12,15 mg; vitamin E, 18,000 mg; vitamin K3, 2,000 mg; vitamin B9, 1,000 mg; vitamin B5, 30,000 mg; folic acid, 21 mg; nicotinic acid, 65 mg; biotin, 14 mg; choline chloride, 500,000 mg; Mn, 100,000 mg; Zn, 85,000 mg; Fe, 50,000 mg; Cu, 10,000 mg; I, 1,000 mg; Se, 200 mg.

Experimental Parameters Measured:

The eggs were floated in salty water and then infracted for measuring thick albumen. Following formula is for this measuring on the bases of haugh unit: $(1.7\ w^{0.73} - 7.57 + H) = \text{Haugh unit} - 100\text{Log}$, so that W = weight of egg (g) and H = height of albumen (ml). In order to measurement of the yolk height, standard model of altimeter was used according to method of Carter [18].

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. In order to estimate the strength of shell (mg) criterion was used for each (cm): Shell surface = $3.9782 \times (\text{egg weight})^{7056.0}$ that shell surface is on the basis of (cm^2), egg weight (g), shell weight (mg/cm^2).

Blood biochemical samples were measured by Pars Azmoon diagnosis kits and Auto-analyzer system (American designed). Yolk cholesterol and triglyceride of egg samples (from last week) were extracted and measured by Folch *et al*. [19].

Statistical Analyses:

All data were analyzed through SAS software for windows [20] in order to compare the differences among averages according to below statistical model: $Y_{ij} = \mu + T_i + E_{ij}$ so that Yij is the error of experiment, Eij the effect of dietary, Ti population average and μ the quantity of experiment observation.

Result:

The performance results of the current study are shown in Table 2. Significant differences ($P<0.05$) are shown over the weights of eggs produced from group of 4% SO. In this group the heaviest eggs were 65.12 (g) and the lightest one was 63.73 (g). Although in the other groups no significant difference was observed, the utilization of oil and fat had efficacy on the performance. The most productive percentage of egg was 77.68, mass productive 50.25 and the best dietary conversion coefficient was 2.22 in control treatment.

Effects of different levels of fat and oil of hen diet on egg quality are shown in Table 3. Egg quality parameters included shell weight and thickness, haugh unit, shell thickness yolk cholesterol and triglycerides not significantly changed by diets containing different levels of fat or oil.

Results of measured blood biochemical parameters are shown in Table 4.

The utilization of equal mixture of fat and oil caused a significant decrease in serum glucose of blood (149.88 mg/dl). The least amount of albumin was significantly determined in group 3 (4% soybean oil, SO). Although utilizing a mixture of fat and oil in hen diets decreased the cholesterol of blood serum.

The percentage and ratio of blood cells are shown in Table 5. No significance difference were determined for immunity parameters such as Heterophil, H; Lymphocyte, L; and H/L ratio.

Table 2: The Efficacy of Fat and Oil on the Performance of Laying Hens.

Experimental Groups	Egg Production (%)	Egg Weight (g)	Egg Mass Production (g)	Feed intake (g)	Feed Conversion ratio
1	77.68	64.68 [ab]	50.25	111.23	22.2
2	75.45	64.12 [bc]	48.29	113.46	38.2
3	75.12	65.12 [a]	48.97	111.38	31.2
4	76.28	63.73 [c]	48.67	111.48	30.2
5	75.12	63.95 [bc]	48.15	111.52	33.2
6	73.9	63.95 [bc]	46.75	111.17	44.2
SEM	2.33	0.27	1.39	0.92	0.08

[a,b,c] Means within a rows with no common superscripts differ significantly ($P<0.05$).

Table 3: The Efficacy of Fat and Oil on Egg Quality.

Experimental Groups	Weight (g/cm^3)	Shell Weight (g)	Haugh Unit	Shell Thickness (ml)	Unit Weight of Shell (mg/cm^2)	Yolk Cholesterol (mg/g)	Yolk Triglyceride (mg/g)
1	1.082	5.27	91.28	0.313	70.68	189.5	1666
2	1.081	5.40	93.54	0.335	73.72	210.25	1845
3	1.089	5.64	88.38	0.340	76.56	197	1871.8
4	1.083	5.82	85.64	0.335	75.91	182.25	1606.3
5	1.086	5.73	88.12	0.318	77.55	170.75	1665
6	1.084	5.43	88.58	0.328	75.51	197	1865
SEM	0.004	0.23	2.58	0.010	2.71	12.82	97.47

Table 4: The Efficacy of Experimental Dietaries on Blood Biochemical Parameters of Laying Hens.

Treatment	Glucose (mg/dl)	Protein (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Albumin (mg/dl)	Uric Acid (mg/dl)
1	174.39 [ab]	4.39	1616.7	117.11	2.48 [ab]	2.84
2	162.51 [ab]	4.48	1975	175.41	2.63 [a]	2.75
3	210.52 [a]	3.96	1702.9	127.11	2.29 [b]	2.44
4	149.88 [b]	4.77	2162.5	119.19	2.59 [ab]	3.05
5	185.57 [ab]	3.78	1188	90.69	2.71 [a]	2.22
6	186.36 [ab]	4.82	2626.5	95.36	2.56 [ab]	3.03
SEM	13.77	0.45	685.4	29.64	0.09	0.54

[a,b,c] Means within a rows with no common superscripts differ significantly (P<0.05).

Table 5: The Efficacy of Experimental Dietaries on the Percentage and Ratio of Laying Hens' Blood Cells.

Treatment	Heterophil	Lymphocyte	Heterophil/Lymphocyte
1	85	90.75	0.095
2	10.75	88.38	0.123
3	1275	86	0.152
4	9.38	90.38	0.104
5	6.63	92.75	0.074
6	8.88	89.88	0.103
SEM	2.04	2.19	0.026

Discussion:

Egg Production and Quality Parameters:

Although significant difference was not observed among experimental groups in egg production, the highest value (77.67%) was related to control group. The heaviest eggs were observed in group 3 which contained 4% SO. This difference was not numerically significant in comparison to control group but it was significant in comparison to other groups contain fat/oil and mixture of fat and oil. Aydin and cook [21] reported that linoleic acid of soybean oil can affects the size of egg and replacement of fat with oil in hen diet can lead to smaller eggs. This can be attributed to diets containing fat without sufficient linoleic acid. Harms and Russell [9] reported that the kind of fat has no efficacy on laying hen's performance. Considering high percentage of egg production and weight in control group along less dietary utilization, the high egg mass production and lowest feed conversion ratio was observed in control group.

There was no significant difference among experimental groups according to egg traits. But utilizing BT, SO and both in combination together and to be replacement was led to non-significant increase of weight, shell weight, yolk weight and thickness of shell [22]. Diet supplementing with BT caused to increase of yolk cholesterol, while diet supplementation with oil or mixture of oil and fat decreased the yolk cholesterol in comparison to control. The increase of yolk cholesterol in eggs of hens fed in BT diets is due to fat structure contain more saturated fatty acids than oils that can increase blood cholesterol and transported to yolk [23-24]. The amounts of egg yolk triglycerides were gained by diet contain equal mixture of fat and oil. Hirata *et al.* [11] reported that the amount of yolk cholesterol does not differ among hens fed by soya oil, coconut oil, pig fat and beef tallow.

Serum Biochemical Values:

Diet supplementing with a mixture of SO and BT leads to significant decrease in blood glucose because of absorbing and metabolism of suitable fatty acids [25] and the highest amount of serum glucose was observed in groups 5 and 6. Celebi and Utlu [26] reported that diet supplementation with fat decreases blood glucose. Brisson [1] previously declared that when hen diet incorporated with more oil instead of fat, the amount of blood cholesterol will decrease according to increase of unsaturated fat acids ratio to saturated fat acids. While in the recent studies researchers has been reported that diet rich in unsaturated fatty acid increase the amount of serum glucose because of decline insulin secretion [25,27].

However, there are several reports that increasing fatty acids of poultry diet and at last blood serum cholesterol is decreased [3,12-13]. Celebi and Utlu [26] reported that blood and egg yolk triglycerides of hens fed diets supplemented with fat, were increased. The least amount of blood serum albumin was observed in group 3 containing 4% of soya oil. From the viewpoint of medical science, serum albumin level is tested for investigation of liver performance and its sudden increase indicates a problem and double pressure on liver and vice versa. [28-29]. Olomu and Baracos [30] reported that decrease the protein density can be due to increase lipid/protein ratio. Because, fats for transmission in blood must were mixed with proteins in the form of complex compositions of hydrophile lipoprotein and with consider to density of pure lipids is less from watter. Therefore, decrease the protein density can be due to increase lipid/protein ratio [29-30].

Immune System Parameters:

There was no significant difference between the values of Heterophil (H) and Lymphocyte (L) and H: L ratio.

However, the high percentage of Lymphocyte, least percentage of Heterophil and lessen ratio of H: L were observed in group 5 via dietary replacement of fat with oil for first 6 weeks of experiment period. Heterophils are phagocyte cells that specified avoiding against infectious factors like viruses, bacteria and other external factors by trapping and exterminating of them. Lymphocytes are leukocytes in lymphoid tissues such as thymus, spleen and lymphatic glands. In normal condition, lymphocytes contain the most white blood cells of poultry are responsible for producing antibody and immune representation. Heterophils to lymphocytes ratio are a kind of important index in analyzing immune level of body. The more the ratio is, the more the immune level will be.

Conclusion:

From the results of presented study it is concluded that diet supplementation by oil and mixture of fat and oil could improved ($P<0.05$) the performance, egg traits and some blood biochemical and also immunity parameters ($P>0.05$) in comparison to diet containing fat.

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