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ORIGINAL ARTICLE

Influence of Dietary Supplemented Semi-refined Sunflower Oil with Vitamin E on Some of Serum Biochemical and Immunological Measures in Laying Hens

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Mohammad Narimani-Rad, Ali Nobakht, Alireza Lotfi; Influence of dietary supplemented semi-refined sunflower oil with vitamin E on some of serum biochemical and immunological measures in laying hens

ABSTRACT

The aim of this study was to investigation on effects of dietary supplemented semi-refined sunflower oil with vitamin E (as anti-oxidant agent) on some of serum biochemical (glucose, cholesterol, triglyceride, total protein and albumen) and immunological (heterophil, lymphocyte and H/R ratio) measures in laying hens. This study was conducted as 3×2 factorial experiment with three levels (2, 4 and 6 percents semi-refined sunflower oil) and two vitamin E levels (75 and 150 mg/kg) in six treatments (include three replicates and 12 bird in each replicate) in completely randomized design. Dietary supplementation of various levels of sunflower semi-refined oil with vitamin E caused significant changes in blood glucose level of layers ($P<0.05$). With increasing supplemented level, serum glucose level has decreased and with supplemented 6% oil, lowest blood glucose concentration was resulted. Submission of dietary semi-refined oil with vitamin E didn't have any significant effect on heterophile, lymphocyte count and H/L ratio in layers. It was concluded that dietary supplementation of 6 percent semi-refined oil caused lowers blood glucose and higher lymphocyte number and lower H/L ration in laying hens. Supplementation of other percent of semi-refined sunflower oil along with or without vitamin E didn't have significant effect on serum biochemical measures (with exception to glucose). Supplementing 150 mg Vitamin E alone or in combination with 6% semi-refined sunflower oil caused higher lymphocyte number in laying hens.

Key words: semi-refined oil, immunological measure, laying hen, vitamin E.

Introduction

Dietary fats and oils are suitable supplementation for energy obtaining in poultry feeds. Animal fats have structural long-chain and higher number of saturated fatty acids and commonly have lower digestibility and absorption rate and also lower ME in comparison with vegetable oils [1]. Regardless to vegetable oil benefits for broilers, unfortunately they are susceptible for oxidation reaction. Free radicals cuts "H" from "CH" group of fatty acids carbon chain and cause pro-oxidation reaction [2]. Anti-oxidant agents such as vitamin E [3] or selenium [4] must be presented in high oil included diets to prevention of occurrence oxidative damages arise from unsaturated fatty acids oxidation. Also vitamin E has immunological importance for bird and with naturalization of oxygen species (free radicals) via enzymatic pathways can protect cells and avoiding oxidative damages [5]. Zhao *et al*., [6] had reported significant interaction between Vitamin E and vaccination routine on the immune functions of experimental birds and suggested vitamin E's biological function appeared to be dose-dependent,

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especially with regard to its positive effect on the immune responses of young chickens.

Maximum dietary level of oil in laying hen diet with attention to cumulative egg production and performance may be is 10 percent that was suggested with Grobas *et al*., [7]. In other side, use of oxidized [8, 9] or semi-refined oils [10]is a new subject in poultry nutrition research works for possible economic efficiency with lowering feed cost and energy obtaining via cheaper sources. It is documented that oxidation of oil did not have negative effect on its ME levels for poultry [11], but in other hand oxidized oils may be hazardous for birds and can lowers feed efficiency and body weight [8]. Moraes *et al*., [10] had concluded that utilization of semi-refined rice oil didn't has any negative effect on oxidative stability and suggested that meat oxidative stability was not affected by oil type or inclusion levels, that similar results have been reported with supplementing soybean oil or canola oil [12].

With attention to high cost of refined vegetable oil and successful results with dietary supplementation of semi-refined rice oil for broilers [10], aim of this study was to investigation on effects of dietary supplemented semi-refined sunflower oil (as common dietary oils in poultry nutrition) with vitamin E (as anti-oxidant agent) on some of biochemical (total cholesterol, triglyceride, glucose, uric acid, albumen and total protein) and immunological measures (heterophile, lymphocyte count and H/L ratio) in laying hens.

Materials and Methods

-Experimental Design:

This study was conducted as 3×2 factorial experiment with three level of semi-refined sunflower oil (2, 4 and 6 percent) and two vitamin E level (150 and 750 mg/kg) in six treatments (include three replicates: 12 bird) and totally 212 Hy-line (W36) strain from 62 to 74 weeks in completely randomized design. Before onset, egg production records of hens were determined for identification of their egg production number. During 12 weeks of experiment, feed intake, egg production, egg weight, egg mass and FCR were recorded.

-Diet Formulation:

Diets were formulated according nutrient requirements of laying hens presented in NRC [13]; ME: 2750 Kcal/Kg, CP: 13.75% by UFFDA software. Experimental rations were including;
Group 1: supplementation 2% of semi-refined sunflower oil with 75 mg Vitamin E,
Group 2: supplementation 2% of semi-refined sunflower oil with 150 mg Vitamin E,
Group 3: supplementation 4% of semi-refined sunflower oil with 75 mg Vitamin E,
Group 4: supplementation 4% of semi-refined sunflower oil with 150 mg Vitamin E,
Group 5: supplementation 6% of semi-refined sunflower oil with 75 mg Vitamin E, and
Group 6: supplementation 2% of semi-refined sunflower oil with 150 mg Vitamin E [table1].

Environmental conditions such as lighting program (16 hours light: 8 hours darkness) were similar for all groups..

-Sample Analysis and Assays:

At end of experimental period, two birds were selected randomly and blood samples were taked from wings. Blood samples were divided to two tubes including EDTA contained tube and sample acid-washed tube respectively for leukocyte count and serum biochemical analysis. Blood cells were counted by staining and light microscope. Serum measures were determined by Alyson 300 auto analyzer (USA) and its commercial kits. Obtained data were analyzed by SAS software Ver. 9.1 and Duncan multiple range test were done for detection of significant differences at 0.05 %.

Results:

Dietary supplementation of various levels of sunflower semi-refined oil with vitamin E caused significant changes in blood glucose level of layers ($P<0.05$). With increasing supplemented level, serum glucose level has decreased and with supplemented 6% oil, lowest blood glucose concentration was resulted. Main resource of blood glucose is carbohydrate, and in present experiment, addition of oil caused lower carbohydrate intake and subsequent lower blood glucose rate for oil fed groups.

Submission of dietary semi-refined oil with vitamin E didn't have any significant effect on heterophile, lymphocyte count and H/L ratio in layers.

Discussion:

In broiler chickens, Rashidi *et al*., [14] had reported that dietary supplementation of tallow (as fat source) could elevate blood glucose levels but supplementation of fish oil with vitamin E could lower this measure. In present study in agreement with Rashidi *et al*., [14], supplementation of 2% semi-refined oil (without vitamin E) could increase blood glucose rate, but higher dosage (6%) had lowering effect. It may be because of less carbohydrate (as glucose source) intake with high fat contented diet, that couldn't supply more glucose for blood.

Table 1: Feed ingredients and ration composition of experimental layer hen diets.

Feed ingredients %	Treatments					
	1	2	3	4	5	6
Corn	47.67	47.67	40.23	40.23	34	34
Wheat	18.11	18.11	19.81	19.81	20.01	20.01
Soybean meal	15.73	15.73	16.83	16.83	18	18
Wheat bran	5	5	5	5	5	5
Semi-refined sunflower oil	2	2	4	4	6	6
Vitamin E	75	150	75	150	75	150
Inert (sand)	2	2	4	4	6	6
oyster shell	7.24	7.24	7.36	7.36	7.06	7.06
Bone meal	1.49	1.49	1.52	1.52	1.55	1.55
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix1	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
Calculated nutrients						
Meatbolizable energy (ME)(kcal/kg)	2750	2750	2750	2750	2750	2750
Crude protein (CP) %	13.75	13.75	13.75	13.75	13.75	13.75
Calcium %	3.27	3.27	3.22	3.22	3.22	3.22
Available phosphorus %	0.3	0.3	0.3	0.3	0.3	0.3
Sodium %	0.14	0.14	0.14	0.14	0.14	0.14
Lysine %	0.63	0.63	0.65	0.65	0.67	0.67
Metyonine + Systein %	0.52	0.52	0.52	0.52	0.52	0.52
Theronin %	0.55	0.55	0.55	0.55	0.55	0.55
Tryptophan %	0.18	0.18	0.18	0.18	0.18	0.18

1- per kg vitamin supplement include 8500000 IU vitamin A, 2500000 IU Vitamin D_3, 11000 IU Vitamin E, 2200 mg Vitamin K_3, 1477 mg Vitamin B_1, 4000 mg Vitamin B_2, 7840 mg Vitamin B_3, 34650 mg Vitamin B5, 2464 mg Vitamin B6, 110 mg Vitamin B9, 10 mg Vitamin B12, 400000 mg choline chloride.
2-per kg mineral supplement include 74400 mg Mg, 75000 mg Fe, 64.675 mg Zn, 6000 mg Cu, 876 mg iodine, 200 mg selenium.

Howell *et al*. [15] had investigated the relationship between diet and blood cholesterol levels and found that saturated fat in the diet, but not dietary cholesterol, influences blood cholesterol levels, but Mono and poly unsaturated fats may lower blood cholesterol levels when they replace saturated fat in the diet [16] Dietary supplementation of sunflower oil, fish oil, soybean oil and hazelnut oil couldn't decreases egg cholesterol content [16]. Results of present study following vitamin E supplementation, are according to [17] and in agreement with Küçükersan *et al*., [16], but because of utilization semi-refined oil couldn't has lowering effect on serum lipids, that reported by Celebi and Utlu [18]. Because of high lipid source in diet (present study) it seem that lipid metabolism were dominant on protein metabolism and total protein or albumen level didn't affected by semi- refined oil. Celebi and Utlu [18], reported that dietary flaxseed oil caused lower total cholesterol, LDL, VLDL and triglyceride level of serum in laying hens, but tallow oil caused higher serum lipids. It is interesting that supplemented sunflower oil had similar effect with flaxseed oil for serum lipid concentrations in layers. In present study, serum total cholesterol and triglyceride of layers didn't affected by dietary semi-refined oil or vitamin E. May cholesterol and triglyceride elevation effect of supplemented oxidized oil [9] didn't occurred with supplementation of semi-refined oil in present study. The ratio of n-6 to n-3 poly unsaturated fatty acids plays a major role in modulating cell-mediated and humoral immune responses of laying hens, and various n-3 fatty acids possess different potencies of immunomodulation [19]. In immunological approach, Moraes *et al*., [10] didn't find any considerable effect on tissue malondialdehyde value (TBARS) following dietary addition of semi-refined oil. Mohiti Asli *et al*., [17], concluded that dietary vitamin E can increase immune response in laying hens. In their study, serum cholesterol concentration didn't has any significant change following supplemented with dietary vitamin E, but vitamin E could increase antibody titer against Sheep red blood cells (as an immunological parameter)[17].

Zhao *et al*., [20] had an investigation on immune response of dietary vitamin E in layers vaccinated against Newcastle and avian influenza viruses. At end of their experiment, antibody titres against Newcastle disease virus or avian influenza virus and the plasma concentration of interleukin-1 were increased by the high level of Vitamin E supplementation. There was also significant interaction between VE and vaccination routine on the immune functions of experimental birds. They had suggested that vitamin E supplementation had immune response, dose-dependent. In present study, 6 percent semi-refined oil with 150 mg vitamin E caused increases lymphocyte number [table3], that this finding are in agreement with Moreas *et al*., [10], Zhao *et al*., [6] and Mohiti Asli *et al*., [17] and Wang *et al*., [19].

It was suggested that possible weakness of immune defense caused by semi-refined oil (possible oxidation damage) was moderated by vitamin E supplementation, and with supplementation of higher dosage (150 mg Vitamin E) immune response was occurred by higher lymphocyte count [table2].

Table 2: Effects of different levels of semi-refined sunflower oil and vitamin E on some of biochemical (total cholesterol, triglyceride, glucose, uric acid, albumen and total protein) in laying hens

Supplements (Mg/dl)	Glucose	Total cholesterol	Triglyceride	Uric acid	Total protein	Albumen
2% semi-refined sunflower oil	224.42 a	226.52	1819.7	6.94	7.88	3.07
4% semi-refined sunflower oil	196.67 ab	171	1760.3	5.52	8.02	3
6% semi-refined sunflower oil	178.32 b	130.5	1779.8	4.35	6.65	3.84
SEM	12.92	30.91	469.81	0.99	0.36	0.15
75 mg/kg Vitamin E	192.61	183.39	1868.6	5.24	7.66	2.96
150 mg/kg Vitamin E	207	168.63	1704.5	5.97	7.38	2.98
SEM	10.55	25.24	383.61	0.8	0.3	0.12
2% semi-refined sunflower oil × 75 mg/kg Vitamin E	208	240.34	2197.5	7.37	7.8	2.87
2% semi-refined sunflower oil × 150 mg/kg Vitamin E	240.84	212.74	1441.84	6.5	7.97	3.26
4% semi-refined sunflower oil × 75 mg/kg Vitamin E	193.67	171.84	1930	4.67	8.07	3
4% semi-refined sunflower oil × 150 mg/kg Vitamin E	199.67	170.17	1590.5	6.37	7.97	3
6% semi-refined sunflower oil × 75 mg/kg Vitamin E	176.17	138	1478.34	3.67	7.1	3
6% semi-refined sunflower oil × 150 mg/kg Vitamin E	180.5	123	2081.17	5.04	6.2	2
SEM	18.27	43.72	6641.43	1.4	0.51	0.21

*different letters (a and b) show significant difference, $p<0.05$.

Table 3: Effects of different levels of semi-refined sunflower oil and vitamin E on heterophile, lymphocyte count and H/L ratio in laying hens.

Supplements (number)	Heterophile (percent)	Lymphocyte (percent)	H/L
2% semi-refined sunflower oil	12.75	86.42	0.142
4% semi-refined sunflower oil	12.5	83.5	0.139
6% semi-refined sunflower oil	11.34	87.83	0.136
SEM	2.24	2.45	0.03
75 mg/kg Vitamin E	13.61	84.87	0.16
150 mg/kg Vitamin E	10.78	87.06	0.118
SEM	1.82	2	0.02
2% semi-refined sunflower oil × 75 mg/kg Vitamin E	13.84	85.5	0.148
2% semi-refined sunflower oil × 150 mg/kg Vitamin E	11.67	87.34	0.135
4% semi-refined sunflower oil × 75 mg/kg Vitamin E	14	84.67	0.166
4% semi-refined sunflower oil × 150 mg/kg Vitamin E	11	82.34	0.111
6% semi-refined sunflower oil × 75 mg/kg Vitamin E	13	84.17	0.165
6% semi-refined sunflower oil × 150 mg/kg Vitamin E	9.67	91.5	0.107
SEM	3.16	3.47	0.04

*different letters (a and b) show significant difference, $p<0.05$.

Increase H/L ratio is an indicator for stressful condition and infection [21]. Lower H/L ratio in present work mentioned that addition of semi-refined sunflower oil/ vitamin E didn't had any considerable stress for layers.

It was concluded that dietary supplementation of 6 percent semi-refined oil caused lowers blood glucose and higher lymphocyte number in laying hens. Supplementation of other percent of semi-refined sunflower oil along with or without vitamin E didn't have significant effect on serum biochemical measures (with exception to glucose). Supplementing 150 mg Vitamin E alone or in combination with 6% semi-refined sunflower oil caused higher lymphocyte number in laying hens.

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