

Effects of Supplementation of Different Source and Level of Oil to Diet on Lipid Peroxidation and Some Blood Parameters in the Layer Hens

¹I. Sadi Cetingul, ²Abdullah Eryavuz, ²Recep Aslan, ³Yilmaz Dunder and ⁴Fatma Inal
¹Department of Animal Nutrition and Nutritional Diseases, ²Department of Physiology,

³Department of Biochemistry University of Afyon Kocatepe,
Faculty of Veterinary Medicine, Afyonkarahisar, Turkey

⁴Animal Nutrition and Nutritional Diseases,
Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey

Abstract: This research has been performed to determine the effects of Hazelnut Oil (HO) and Sunflower Oil (SO) added in the layer ration at various levels as an energy source onto lipid peroxidation and blood parameters of laying hens. Eight hundred laying hens at 135 days of age were used as research material in this experiment. The hens were divided into 5 groups and then each group was divided into 10 subgroups containing 16 hens each. No oil was added in feeds of control group. The other groups were given 15 g kg⁻¹ HO; 15 g kg⁻¹ SO; 30 g kg⁻¹ HO and the last one was mixed oil given 30 g kg⁻¹ MO (15 g kg⁻¹ HO + 15 g kg⁻¹ SO). No difference has been observed in terms of Malondialdehyde (MDA), Glutathione (GSH), Total Cholesterol (TCH), mean corpuscular volume (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Cell Volume (MCV), glucose, White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HB), Packed Cell Volume (PCV) and Thrombocyte (TB) values among the groups. The source and level of the oil used in this study did not affect lipid peroxidation and hematological values.

Key words: Layer hens, different oil, blood parameter, RBC, WBC

INTRODUCTION

The main objective in oil supplementation to the poultry diets is to increase the energy value as well as to avoid a powdery structure (Gunstone *et al.*, 1986; Montgomery *et al.*, 1996; Anonymous, 2007). The advantage of oil usage in diets is its extra caloric effect (Gunstone *et al.*, 1986) occurred as the effect of unsaturated fatty acids on facilitating the resorption of saturated fatty acids (Montgomery *et al.*, 1996) keeping the supplemented oil to diets longer in the digestive tract thus, increase the absorption comparing the standard diets.

Since, they yield large amounts of energy upon oxidation in metabolism and are required for the absorption and transport of lipid-soluble vitamins through the bloodstream, lipids occur an essential part of the diet (Anonymous, 2007). In addition, as an important constituent of cell membranes, they also play, specific roles in membrane signaling events and thus, cell development. Therefore, the characteristics of fats have been a research issue for about 200 years for the

scientific world since many feed materials contain fat (Gunstone *et al.*, 1986).

However, it is suggested that fatty acid composition in poultry rations affects blood parameters (Alparslan and Ozdogan, 2006). Monounsaturated and polyunsaturated fats may lower blood cholesterol levels when they replace saturated fat in the diet.

Among a variety of fatty acids, n-3 and n-6 PUFA have been most extensively studied for their functions in health. However, research on the effects of monounsaturated fatty acids on health is incomplete.

In previous studies, it has been reported that the supplementation of different oils to poultry diets affected the blood cholesterol level (Mirghelenj *et al.*, 2004; Sultan, 2005), Glutathione (GSH) concentration (Ebeid *et al.*, 2008), PCV (Weng, 2002) and lipid peroxidation (Nemeth, 2004; Ergun *et al.*, 2005; Shahriar *et al.*, 2008).

Hazelnut without shell is consisting of 60% oil. The ratio of oleic acid, which is one of the monounsaturated fatty acids, to the total oil content of hazelnut is more than 80% and very similar to olive oil regarding the fatty acid composition (Cetingul and Inal, 2003). This increases

Table 1: Ration percent composition used in the study

Ingredients in diet	25-36 weeks of age*			36-44 weeks of age**		
	Control	15 g kg ⁻¹	30 g kg ⁻¹	Control	15 g kg ⁻¹	30 g kg ⁻¹
Corn	53.50	48.00	38.50	53.00	46.00	39.00
Wheat	12.00	15.00	21.00	17.00	21.00	24.00
Full Fat Soyb.meal	1.27	-	-	-	-	-
Soybean meal	19.50	19.00	16.43	16.00	14.50	12.00
Sunflower meal	3.00	5.78	10.50	3.04	6.04	11.20
Vegateble oil	-	1.50	3.00	-	1.50	3.00
Limestone	8.00	8.00	8.00	8.25	8.30	8.35
Dicalc phospate	2.00	2.00	1.85	2.00	1.95	1.75
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25	0.25	0.25
Mineral	0.10	0.10	0.10	0.10	0.10	0.10
Methionin	0.13	0.12	0.12	0.11	0.11	0.10

Guaranteed levels of vitamin per 2.5 kg and mineral supplements per 1 kg product: vit. A: 12 000.000 UI; vit. D₃: 2 000.000 UI; vit. E: 35.000 mg; vit. K₃: 4000 mg; vit. B₁: 3000 mg; vit. B₂: 7.000 mg; vit. B₆: 5.000 mg vit. B₁₂: 15 mg; niacin: 20.000 mg; D-Biotin: 45 mg Apo Karotenoik Asit Ester: 500 mg, Folik Asit: 1 000 mg, Kolin Klorid: 125 000 mg Vit C: 50 000 mg. Kal D-Pantothenate: 10.000 mg, Ksanthaxantine: 1 500 mg copper: 5.000 mg; cobalt: 200 mg; selenium: 150 mg; manganese: 80.000 mg; zinc: 60.000 mg; iodine: 1.000 mg; iron: 60.000 mg; DL-Methionine: 99 % pure, *: % 16.5 HP, 2750 kcal ME kg⁻¹, **: %15 HP, 2750 kcal ME kg⁻¹

the popularity of hazelnut oil (Garcia *et al.*, 1994; Kris-Etherton *et al.*, 1999a, b). The objective of this study was to determine the effects of Hazelnut Oil (HO) and Sunflower Oil (SO) added in the layer hens' ration at various levels as an energy source onto lipid peroxidation and blood parameters.

MATERIALS AND METHODS

The study of layer hens has been performed on 800 layer hens of Nick Brown race having 25 week age. There were 5 trial groups, which were namely control (without oil), 15 g kg⁻¹ HO; 15 g kg⁻¹ SO; 30 g kg⁻¹ HO and the last one mixed oil group 30 g kg⁻¹ MO (15 g kg⁻¹ HO + 15 g kg⁻¹ SO). Each of the groups, was formed by 160 layer hens and each group was further divided into 10 sub-groups. Each sub-group was formed by 4 cages where each cage contained 4 layer hens. Feed and water supplied ad libitum and artificial light was provided for 16 h a day. This study was performed through 135 days. Two kind of rations were used in the layer hens (Table 1): Diet 1 (16.5% CP, 2750 kcal ME kg⁻¹) was provided up to 36 weeks and diet 2 was provided (15% CP, 2750 kcal ME kg⁻¹) during the remaining duration, namely. Blood samples were collected by the cardiac puncture from the hens at the end of the trial. The blood samples were transferred into heparinised tubes. RBC and WBC were counted by microscope in a blood film diluted with Natt and Herrick's solution (Konuk, 1981). Hemoglobin HB was determined by acid hematin method (Konuk, 1981). PCV was measured by the microhematocrit method. The following indices were calculated; Mean corpuscular volume (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean cell volume MCV (Swenson and Reece, 1993). Malondialdehyde (MDA)

was estimated according to, method of Draper and Hardley (1990), which is based on the coupling MDA with thio-barbituric acid. All statistical analyses were made using SPSS program designed for Windows (SPSS, 2002). Evaluation of the data was performed by one-way analysis of variance while, significant differences among treatment means were tested using linear and quadratic contrasts at 5% probability level.

RESULTS AND DISCUSSION

Free radicals are generally very reactive and produced continuously in cell either as accidentally by-products of metabolism or deliberately during, for example phagocytosis. The most important reactant in free radical biochemistry in aerobic cells is oxygen and its radical derivatives (superoxide and hydroxyl radical), hydrogen peroxide and transition metals because oxygen radicals and other activated oxygen species are common products of cellular metabolism. As the membrane lipids in cells are prone to oxidation of unsaturated bonds, it is perhaps reasonable to advocate lipid peroxidation as a significant event in the development of membrane damage (Akkus, 1995). Lipid peroxidation is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and malondialdehyde (MDA) (Akkus, 1995). Since, lipid peroxidation presents free radicals damage in the cell, the presence of MDA is taken as an indicator of free-radical damage through membrane lipid peroxidation (Katz *et al.*, 1996). MDA concentration is affected by supplementing oils at level 50 g kg⁻¹ to poultry diets, thus increasing the lipid peroxidation (Nemeth, 2004; Ergun *et al.*, 2005). Our results, which showed that

Table 2: Some blood parameter

Group	MDA	GSH	TCH	MCH	MCHC	MCV	Glucose	WBC	RBC	HB	PCV	TB
Control	1.7±0.09	37.2±2.0	87±9.0	31.5±1.0	22.0±0.6	139±5.7	171±9.1	2.1±0.07	37.9±4.6	6.6±0.2	29.1±0.8	41.3±3.9
15 g kg ⁻¹ S.O	1.7±0.08	37.6±1.7	103±8.0	31.9±1.5	22.1±0.6	141±3.8	178±11	2.1±0.06	41.7±5.9	6.6±0.2	29.5±0.8	44.0±7.5
15 g kg ⁻¹ H.O	1.8±0.11	38.1±1.0	106±11	32.5±0.9	22.9±0.5	143±3.5	184±4.2	2.1±0.07	42.7±6.5	6.7±0.1	29.9±0.7	45.5±6.1
30 g kg ⁻¹ H.O	1.8±0.08	39.8±2.0	112±19	33.0±1.1	23.0±0.5	145±3.9	185±4.3	2.1±0.04	45.2±4.6	6.8±0.2	30.2±0.8	46.7±7.4
30 g kg ⁻¹ M.O	1.8±0.11	40.5±1.6	117±15	33.1±0.8	23.2±0.5	151±6.9	192±6.5	2.1±0.07	50.8±2.7	6.9±0.2	30.9±1.2	51.5±3.7
Combined	0.847	0.614	0.579	0.797	0.385	0.476	0.393	0.972	0.478	0.715	0.675	0.809
P Linear	0.291	0.209	0.237	0.255	0.975	0.221	0.220	0.736	0.249	0.224	0.266	0.489
Quadratic	0.988	0.419	0.687	0.912	0.549	0.699	0.170	0.864	0.258	0.836	0.723	0.576

SO = Sunflower Oil, HO = Hazelnut Oil, MO = Mixed Oil (15 g kg⁻¹ HO + 15 g kg⁻¹ SO), MDA = Malondialdehyde, GSH = Glutathion, TCH = Total Cholesterol, MCH = Mean Corpuscular volume, MCHC = Mean Corpuscular Hemoglobin Concentration, MCV = Mean Cell Volume, WBC = White Blood Cells, RBC = Red Blood Cells, HB = Hemoglobin, PCV = Packed Cell Volume, TB = Thrombocyte

Table 2 MDA was not affected by oil supplementation, suggest that lipid peroxidation was not increased by either HO or SO supplementations at level up to 30 g kg⁻¹ to laying hens diet.

Cells have developed comprehensive array of antioxidant defenses to prevent free radical formation or to limit their damaging effects. These include enzymes to decompose peroxides, proteins to sequester transition metals and range of compounds to 'scavenge' free radicals (Akkus, 1995).

Therefore, the antioxidant system involves both enzymatic and non-enzymatic antioxidants. The first step in the enzymatic system is Superoxide Dismutase (SOD), which catalyzes the dismutation of superoxide anion (O₂⁻) to H₂O₂.

The conversion from H₂O₂ to H₂O is by either Glutathione Peroxidase (GPx) or catalyze forms the second step of enzymatic system.

Superoxide dismutase and GPx enzyme activities and the balance between them are very crucial protection against oxidative stress (Franco, 1999; De Haan *et al.*, 1996; Gaeta *et al.*, 2002). GPx catalyses the reduction of hydrogen peroxide or lipid peroxides with reduced glutathione (GSH) (Chan and Decker, 1994). Nemeth (2004) found that added oil at level 50 g kg⁻¹ to diet of broiler decreased GSH concentration in liver. The supplementation of oils to laying hens diet did not change GSH concentration in the blood of laying hens in this study, which indicate that supplemented HO and SO at level 30 g kg⁻¹ to diet do not affect GSH concentration. In this study, the effect of addition of different oils to the ration on cholesterol levels was found to be insignificant (Table 2). This result shows similarity with the results of Mirghelenj *et al.* (2004) and Sultan (2005), who added different oils to the ration of layer hens.

In this study, supplementation of oil to the ration and different oils usage do not affect the glucose value in the blood (Table 2). This finding is supported by the study of Calyslar *et al.* (2004) who stated that addition of different levels of oil to the broiler diets does not affect the blood glucose level.

In a related study on quails, using different kinds of oils that were supplemented around 50 g kg⁻¹ level affected PCV. Accordingly, soybean oil and menhaden fish oil groups have higher value than the group fed with hydrogenated soybean oil (Weng, 2002). In this study supplementation of oil to the ration and different oils usage were not affect the PCV values. Odunsi *et al.* (2007) stated that analyzed the effects of hematological parameters, PCV, HB, RBC, WBC, MCV, MCH, MCHC values were not affected by 15 g kg⁻¹ oil supplementation in broiler diets. These findings are also supporting our findings (Table 2) regarding the PCV, HB, RBC, WBC, MCV, MCH, MCHC values.

CONCLUSION

In this study showed that lipid peroxidation and hematological values were not changed by either HO or SO supplementations at level 30 g kg⁻¹ to laying hens diet.

REFERENCES

- Anonymous, 2007. <http://www.holsteinfoundation.org/pdf/f2171DairyCattleNutrition.pdf>04-05-2007time 15:30 World dairy cattle nutrition.
- Akkus, I., 1995. Free radicals and their pathophysiological effects Mimoza Press, Konya, pp: 145.
- Alparslan, G. and M. Ozdogan, 2006. The effects of diet containing fish oil on some blood parameters and the performance values of broilers and cost efficiency. *Int. J. Poult. Sci.*, 5 (5): 415-419.
- Calyslar, S., O. Ozturkcan, K. Celik and T. Cicek, 2004. Effects of animal bone fat on performance some carcass characteristics and blood parameters of broiler chicks. II World's Poultry Congress Istanbul, Turkey.
- Cetingul, S. and F. Inal, 2003. The effects of hazelnut oil usage in poultry diets on performance and fatty acid composition of animal products. Selcuk University Health Science Institute, Ph.D thesis, Konya, Turkey.

- Chan, K.M. and E.A. Decker, 1994. Endogenous skeletal muscle antioxidants. *Crit. Rev. Food Sci. Nutr.*, 34: 403-426.
- DeHaan, J.B., F. Cristiano, R. Iannello, C. Bladier, M.J. Kelner and I. Kola, 1996. Elevation of ratio of Cu/Zn-superoxide dismutase to glutathione peroxidase activity induces features of cellular senescence and this effect is mediated by hydrogen peroxide. *Hum. Mol. Genet.*, 5: 283-292.
- Draper, H.H. and M. Hardley, 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.*, 186: 421-431.
- Ebeid, T., Y. Eid, A. Saleh and H.A. El-Hamid, 2008. Ovarian follicular development, lipid peroxidation, antioxidative status and immune response in laying hens fed fish oil-supplemented diets to produce n-3-enriched eggs. *Animal*, 2: 84-91.
- Ergun, S., M. Yontem, A. Yerlikaya, A. Ozata, K. Uysal and H. Kurt, 2005. Influence of dietary oils on liver and blood lipid peroxidation *Saudi Med. J.*, 26: 442-446.
- Franco, R.S., 1999. Regulation of antioxidant enzymes gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. *Free Radic. Biol. Med.*, 27: 1122-1132.
- Gaeta, L.M., G. Tozzi, A. Pastore, G. Federici and F. Piemonte, 2002. Determination of superoxide dismutase and glutathione peroxidase activities in blood of healthy pediatric subject. *Clinica Chimica Acta*, 322: 117-120.
- Garcia, J.M., I.T. Agar and J. Streif, 1994. Lipid characteristics of kernels from different hazelnut varieties. *Turk. J. Agric. For.*, 18: 199-202.
- Gunstone, F.D., J.L. Harwood and F.B. Padley, 1986. *The Lipid Handbook*, The University Press, Cambridge London. ISBN: 0412244802.
- Katz, D., D. Mazor, A. Dvilansky and N. Meyerstein, 1996. Effect of radiation on red cell membrane and intra cellular oxidative defense system. *Free Rad. Res.*, 24 (3): 199-204.
- Konuk, T., 1981. *Practice Physiology*. 2nd Edn. Ankara University Press, Ankara.
- Kris-Etherton, P.M., S. Yu-Poth, J. Sabate, H.E. Ratcliffe, G. Zhao and T.D. Etherton, 1999a. Nuts and their bioactive constituents: Effects on serum lipids and other factors that affect disease risk. *Am. J. Clin. Nutr.*, 70: 504-511.
- Kris-Etherton, P.M., T.A. Pearson, Y. Wan, R. Lhargrove, K. Moriarty, V. Fishell and T.D. Etherton, 1999b. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentration. *Am. J. Clin. Nutr.*, 70: 1009-1015.
- Mirghelenj, A., S. Rahimi and M.A. Kamali, 2004. Effects of omega-3 fatty acid sources in laying hen diets on blood plasma cholesterol. XXII World's Poultry Congress, Ystanbul-Turkey.
- Montgomery, R., T.W. Conway, A.A. Spector and D.M.D. Chappell, 1996. *Biochemistry a Case-oriented Approach*. 6th Edn. Mosby-Year Book Inc 11830 Westline Industrial Drive St. Louis, Missouri 63146 USA.
- Nemeth, K., 2004. Effect of nutrytyon and ambyent temperature on the lypyd peroxydadyon processes of broyler chyckens. Theses of Doctoral (Ph.D) Dyssertatyon Veszprem Unyversyty Georgykon Faculty of Agryculture Hungarian.
- Odunsi, A.A., T.O. Oladele, A.O. Olaiya and O.S. Onifade, 2007. Response of broiler chickens to wood charcoal and vegetable oil based diets world. *J. Agric. Sci.*, 3: 572-575.
- Shahriar, H.A., K.N. Adl, Y.E. Nezhad and R.S. Nobar, 2008. Meternal of dietary fat type and vitamin E supplementation affects the antioxidant and oxydatif status of hatching chicks 1st Mediterranean Summit of WPSA Advances and Challenges in Poultry Science Porto Carras, Chalkidiki-Greek.
- SPSS Inc, 2002. *SPSS for windows 11.00* Chicago, USA.
- Sultan, S.I.A., 2005. Effect of dietary fish oil on production traits and lipid composition of laying hens. *Int. J. Poult. Sci.*, 4 (8): 586-588.
- Swenson, M.J. and W.O. Reece, 1993. *Dukes Physiology of Domestic Animals*. 6th Edn. Cornell University Press, London, pp: 22-49.
- Weng, B.C.B., 2002. Immunomodulation by Dietary lipids: Soybean oil, Menhaden fish oil, Chicken fat and hydrogenated soybean oil in Japanese quail (*Coturnix coturnix japonica*) and Bobwhite quail (*Colinus virginianus*) Doctora These Blacksburg, Virginia USA.