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Impact of poultry oil supplementation on liver health and fatty liver syndrome in caged layers

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Poultry oil is commonly used as a cost-effective energy source in poultry feed. However, when used at higher levels, raw poultry oil has been found to have adverse effects on the liver of birds. Unfortunately, many poultry farmers remain unaware of these harmful effects. The objective of this experiment was to investigate how poultry oil impacts liver health and contributes to fatty liver syndrome in layers. Using a completely randomized design, 120 Lohmann Single Comb (LSL-Lite) layers (25-week-old) were randomly assigned to 4 experimental groups, each of which had 30 birds. Increasing amounts of poultry oil (0, 1.5, 3.0 and 4.5%) were used in experimental diets. Hens were fed these experimental diets for 20 weeks. The results showed maximum liver fat percentage in 4.5% poultry oil in diet, severe liver congestion, degeneration and cell necrosis in caged layers. Plasma lipid profile (cholesterol, HDL, LDL, triglycerides) and liver enzymes (AST, ALT, ALP and LDH) were significantly ($p<0.05$) affected by poultry oil addition. Higher liver scores indicative of fatty liver syndrome was observed in layers receiving 4.5% poultry oil, while the control group showed the lowest incidence. This study indicated that poultry oil dietary addition at a higher level (4.5%) lead to an increased serum lipid profile and has a negative impact on liver health, ultimately causing fatty liver syndrome in caged layers.
Keywords: Poultry oil, poultry feed, liver histopathology, fatty liver syndrome, laying hens.

INTRODUCTION

Poultry farming is a major income source for small farmers in developing countries, fulfilling crucial protein needs through meat and egg production (Farooq *et al*., 2022). Poultry sector is the fastest-growing agricultural sub-sector, particularly in developing nations and is projected to expand in future due to increasing population, higher income and urbanization, driving greater demand for meat and eggs (Pichet *et al*., 2022). However, poultry industry in Pakistan faces various disease challenges, including infectious diseases such as Newcastle disease, fowl typhoid, and salmonella (Reynolds and Barry. 2022; Mahmood and Rabia 2021) as well as non-infectious disorders like nutritional deficiencies, nematode infestation and fatty liver syndrome in laying hens (Retno *et al*., 2022; Sajid *et al*., 2021).
Fatty liver syndrome (FLS) is a dietary and metabolic disorder that mostly impacts commercial caged layers (Trott *et al*., 2014). FLS is a noninfectious disease characterized by the excessive accumulation of fat in liver and abdominal cavity of hens. This condition leads to liver rupture, hemorrhage and ultimately, sudden death of laying hens. Flocks affected by Fatty Liver Hemorrhagic Syndrome can cause considerable economic losses due to bird mortality and reduced egg production (Crespo and Shivaprasad 2003). Previous studies have shown that high-energy diets can lead to Fatty Liver Syndrome (FLS) due to excessive fat deposition in the liver (Choi *et al*., 2012; Jiang *et al*., 2013). This metabolic disease is difficult to identify in live birds. However, yellowish livers coloration with many hemorrhages and increased liver size are seen after postmortem (Rozenboim *et al*., 2016). A high-energy diet either from fat or carbohydrates was found to induce FLS, but an average high hemorrhagic score was observed in the group in which fat was used as an energy source (Rozenboim *et al*., 2016; Zhang *et al*., 2008).
Fat and oil inclusion is a common practice in modern poultry production to increase energy content of feed (Abd El-Moneim and Sabic, 2019; Attia *et al*., 2020). Oils play a crucial role in promoting a healthy diet for layer hens. They serve as essential sources of fatty acids, which are vital for

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their well-being and can be incorporated into the diet to enhance production performance and optimize nutrient absorption. Their proper inclusion in the diet is key to supporting optimal health and productivity in layers (Shahryar *et al*., 2019). In fact, a study has shown that the addition of oils in a layer's diet improved production performance, egg weight, FCR, and egg quality (Gao *et al*., 2021). Another study has shown that supplementing layer birds' diet with fats and oils increased egg mass production, feed efficiency and digestibility of other dietary components (Zhang *et al*., 2021).

Fats and oils play essential roles in the digestive process of birds. They promote secretion of intracellular digestive substances, such as enzymes, bile and mucus, which aid in the breakdown and absorption of nutrients in the gut. Additionally, fats and oils contribute to maintenance of structural integrity of gut epithelial cells. However, excessive consumption of fats and oils in high-energy diets, can lead to alterations in lipid metabolism. This can result in deposition of excessive fat in liver, leading to Fatty Liver Syndrome, as mentioned earlier (Zhang *et al*., 2008). Currently, the main oils and fats used are vegetable and animal oils to meet the energy demands of layer birds (Ravindran *et al*., 2016). Animal origin by-products, such as lard, tallow and poultry oil are inexpensive sources and are mostly included in poultry diets to fulfill energy requirements (Sanz *et al*., 2000).

Nowadays poultry oil is mostly used in layer feed industry because of its high nutritional value and an inexpensive energy source. Poultry oil is produced by extraction of chicken fat through rendering. It is reported that broiler skin adipose tissues contain about 30% oil (Lin and Tan, 2017). It is one of the most unsaturated animal fats because it has the greatest amount of oleic acid (49%) and the least linoleic acid content (22%) (Suresh *et al*., 2019). Due to high cost of alternative energy sources, poultry oil is reintroduced in poultry industry, having high nutritional contents and low cost (Korantenga *et al*., 2022). It has the highest percentage of oleic acid (49%) and the lowest amount of linoleic acid (22%), making it the most unsaturated terrestrial animal fat. However, rare studies have been done on the effect of poultry oil on fatty liver syndrome in laying hens. Hence, this study was designed with the aim to gain insights into potential effects of poultry oil on the occurrence of fatty liver syndrome and its implications for the health and productivity of laying hens.

MATERIAL AND METHODS

Experimental animals, design and treatments: 120 Lohmann Single Comb Layer (LSL-Lite) 25-week-old were purchased from a commercial layer farm and randomly assigned to 4 experimental treatment groups (T1, T2, T3 and T4). There were 30 birds in one treatment group and ten birds per replicate. Poultry oil used in this experiment was obtained through a rendering process from chicken fat and was procured from a commercial source. Increasing amounts of poultry oil (0, 1.5, 3.0 and 4.5%) were used in the experimental diets. Experimental diets were prepared in an Animal Nutrition Feed Mill, University of Agriculture, Faisalabad Pakistan. Hens were fed various diets for a duration of 20 weeks. The birds received 16 hours of light per day throughout the experimental period. All the diets were examined for their proximate composition before the experiment's commencement using the method outlined by AOAC (2010).

Table 1. Ingredient composition of experimental diets.

Ingredients (%)	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Maize	52.2	60.7	54.3	45.5
Soybean meal 44%	23.2	19.4	18.5	15.7
Limestone	8.55	8.40	8.56	8.75
Rice Polishing	0.00	0.12	4.00	10.0
Rice tips	10.4	0.00	0.00	0.00
Canola meal 36 %	0.00	4.47	6.00	10.0
Guar meal	3.00	3.00	3.00	3.00
Dicalcium Phosphate	1.54	1.52	1.62	1.43
Poultry oil	0.00	1.50	3.00	4.50
Sodium bicarbonate	0.33	0.47	0.47	0.47
NaCl	0.27	0.30	0.24	0.24
Premix *	0.22	0.22	0.22	0.22
DL- Methionine	0.74	0.15	0.15	0.15
Lysine sulphate 55%	0.05	0.06	0.06	0.06
Phytase 10000C	0.005	0.005	0.005	0.005

*Each kg of premix will provide per kg of diet: vitamin A 3000 IU, Vitamin D_3 300 IU, Vitamin E 5 IU, Vitamin K 0.5 IU, Riboflavin 2.5 mg, Pantothenic acid 2 mg, Niacin 10 mg, Vitamin B_{12} 0.004 mg, Choline 1050 mg, Folacin 0.95 mg, Biotin 0.1 mg, Thiamine 0.7 mg, Pyridoxin 2.5 mg, Potassium 0.15%, Sodium 0.15%, Chlorine 0.13%, Magnesium 500 mg, Manganese 20 mg, Zinc 35 mg, Iron 45 mg and Iodine 0.035 mg.

Table 2. Nutrient composition of experimental diets.

Nutrient (%)	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Metabolizable energy (kcal/kg)	2750	2750	2750	2750
Crude Protein	16.0	16.0	16.0	16.0
Calcium	3.75	3.75	3.75	3.75
Avail. Phosphorus	0.39	0.39	0.39	0.39
Lysine	0.82	0.82	0.82	0.82
Methionine	0.40	0.40	0.40	0.40
Meth + Cyst	0.68	0.68	0.68	0.68
Arginine	1.08	1.08	1.08	1.08
Tryptophan	0.18	0.18	0.18	0.18
Threonine	0.60	0.60	0.60	0.60

[1]T_1: diet containing 0% poultry oil, T_2: diet containing 1.5% poultry oil, T_3: diet containing 3% poultry oil, T_4: diet containing 4.5% poultry oil.

***Collection of tissue samples*:** For determination of FLS, after slaughtering, liver was collected for liver hemorrhage score on a scale of 0-5. Zero (0) indicated no bleeding and number 5 indicated most hemorrhages (Shini *et al*., 2019). Dry matter contents and fat contents of dried liver samples were determined by using the ether extraction method (Seong *et al*., 2015). For the determination of lipid fractions, blood samples were obtained from wing veins using sterile needles for the measurement of different blood parameters. The serum of birds was evaluated for total cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) contents. These fractions were measured by using the spectrophotometric method (Cruz *et al*., 2018). The enzymatic GOP-Pap technique was used to measure the serum triglyceride concentration.

Sample preparation for histopathology investigations: For further histo-pathological analysis, liver samples from different parts were collected and preserved in a 10% neutral buffered formalin solution. Using the paraffin sectioning technique, tissue was removed from the liver. Using a semi-automated rotary microtome, sections were made (AMOS Scientific AEM-450, Austria) and stained using Hematoxylin and Eosin staining technique (Bancroft and Gamble, 2008). Three independent images/views from the liver were collected to determine the histopathology of the liver. Prepared slides were examined with different magnifications under the light microscope.

***Data analysis*:** One-way ANOVA under CRD with GLM procedure of SAS (SAS Institute Inc., Cary, NC) was applied to analyze data collected and means were separated through Duncan's Multiple Range test at probability level of 5%, considering each pen as an experimental unit.

RESULTS

The addition of poultry oil in layers diet showed a significant ($p<0.05$) effect on liver fat content. The T4 group, in which cage layer feed included 4.5% poultry oil, had the highest fat content (36.33%). The T2 group, which utilized 1.5% poultry oil, had the lowest value (26.50%). However, adding poultry oil to the diet of caged hens significantly impacted the liver's dry matter content. When 4.5% poultry oil (T4 group) was used in feed, layers showed the highest dry matter contents in the liver (35.22%) as compared to the control group (30.44%).

Fatty liver syndrome: FLS of layers is indicated by liver score (0-5). Poultry oil exhibited a significant ($p<0.05$) impact on birds' liver scores. A higher liver score revealed a higher incidence of fatty liver syndrome. A higher liver score (3.667) was seen in layers fed feed with 4.5% poultry oil (T4 group).

T1 (control) T2 (1.5% poultry oil), T3 (3.0% poultry oil), T4 (4.5% poultry oil)

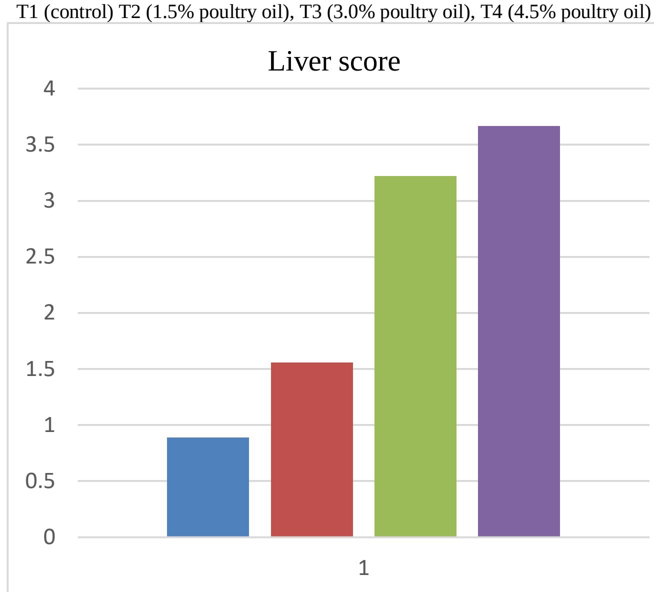

Figure 1: Liver score graph

Figure 2. Effect of PO on the liver of leghorn white cage layers (T1 (control) T2 (1.5% poultry oil), T3 (3.0% poultry oil), T4 (4.5% poultry oil))

Table 2. Effect of poultry oil on liver fat contents, dry matter and liver score.

Variables	Treatment				SEM	p-value
	T1	T2	T3	T4		
Liver fat (%)	31.17^{b}	26.50^{c}	31.00^{b}	36.33^{a}	1.449	0.032
Dry matter in liver (%)	30.44^{c}	31.89^{b}	31.33^{b}	35.22^{a}	0.349	0.046
Liver score	0.889^{d}	1.556^{c}	3.222^{b}	3.667^{a}	0.644	0.022

Mean values with different superscript are significant ($p<0.05$)

***Histopathology of the liver*:** In the control group, hepatic parenchyma was normal in appearance and hepatocytes were arranged in hepatic cords with normal nuclei having nucleolus with chromatin material. Whereas treatment group T2, in which 1.5% poultry oil was used, showed a mild to moderate degree of vacuolar degeneration in the hepatic parenchyma of the liver. A mild degree of congestion was also present throughout the parenchyma of the liver. In group T3, 3.0% poultry oil effect on the liver of layer birds because hepatic parenchyma indicated a mild degree of vacuolar degeneration in the cytoplasm of hepatocytes at some places. Individual cell necrosis of the liver also presents in this group, indicating the condensed nuclei. However, a mild degree of congestion was also present in this group. At a higher dose of poultry oil (4.5%), group T4 showed congestion, a severe degree of degeneration and moderate cell necrosis throughout the parenchyma of the liver.

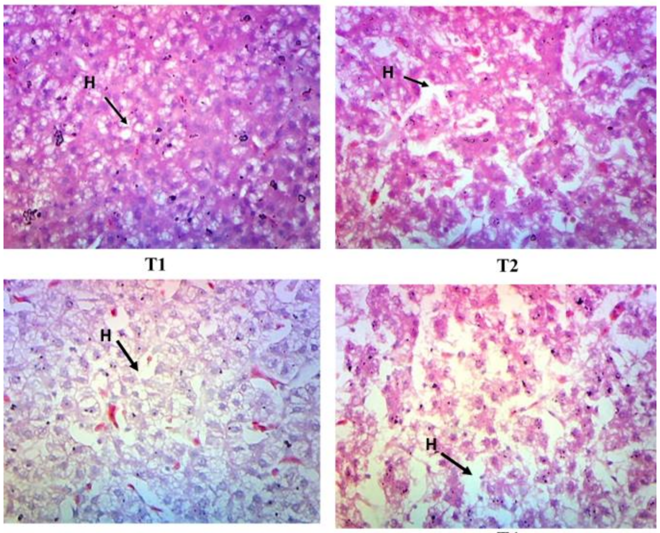

Figure 3. Effect of Poultry oil on histopathology of liver. H (Hepatocytes) T1 (control) normal in appearance, while T2 (1.5% poultry oil) showed mild to moderate degree of vacuolar degeneration in hepatic parenchyma. In group T3 (3% poultry oil) seen mild degree of congestion with vacuolar degeneration of parenchyma and in T4 (4.5% poultry oil) group showed severe congestion, cell necrosis and vacuolar degeneration in throughout the parenchyma of liver. (H & E stain, 40X)

***Serum lipid profile**:* The blood serum lipid profile was significantly affected ($p<0.05$) by the inclusion of poultry oil in caged layers (Table 4). Serum total cholesterol (TC) concentrations were significantly ($p<0.05$) higher in the treatment groups. T3 group (3.0% poultry oil) birds had the highest (156.0 mg/dl) TC levels as compared to other groups. Triglyceride (TG) levels were higher in the poultry oil group than in the control group. Layer birds in group T1 (control) showed the lowest TG (1313.7 mg/ dl). On the other hand, the T4 group had triglyceride levels that attained their maximum (1560.5 mg/ dl) when 4.5% poultry oil was added to the diet. Serum high-density lipoprotein (HDL) levels were highest (33 mg/dl) in the T4 group, which used 4.5% poultry oil. Serum low-density lipoprotein (LDL) levels showed a significant ($p<0.05$) effect on layer birds fed diets with poultry oil. The T2 group in which 1.5% poultry oil was used in feed showed a maximum (236.3 mg/dl) level of serum LDL.

Table 3. Effect of poultry oil on plasma lipid profile (mg/dl) in cage layers.

Variables	Treatments				SEM	p-value
	T1	T2	T3	T4		
TC	128.0^{d}	224.0^{a}	156.0^{b}	145.5^{c}	1.989	0.023
TG	1313.7^{d}	1520.7^{b}	1471.0^{c}	1560.5^{a}	2.639	0.043
HDL	31.0^{b}	28.3^{c}	28.3^{c}	33.0^{a}	1.939	0.046
LDL	198.7^{d}	236.3^{a}	224.0^{b}	220.5^{c}	3.349	0.034

Mean values with different superscript are significant ($p<0.05$)

Serum liver enzymes: Alanine Aminotransferase (ALT) was significantly ($p<0.05$) higher in blood serum of layers as compared to the control group. The T4 group, which used poultry oil at a 4.5% concentration, had the highest level of ALT (37.5 u/l). Aspartate aminotransferase (AST) in the blood serum of layers is greatly affected by poultry oil. The T4 group, which utilized 4.5% poultry oil, had the highest value of AST (67.5 u/ l). Alkaline phosphatase (ALP) By including poultry oil in a layer diet, significant variations in alkaline phosphatase (ALP) levels in blood serum were discovered. Lactate dehydrogenase (LDH) levels in the blood serum of layers were significantly affected ($p<0.05$) by the addition of poultry oil in feed as compared to the control group. The T4 group, which utilized 4.5% poultry oil, had the highest LDH value (725.5 u/ l). The lowest value (478.7 u/l) was seen in the T2 group, which used 1.5% poultry oil.

Table 4. Effect of poultry oil on serum liver enzymes (U/L) in cage layers.

Variables	Treatments				SEM	p-value
	T1	T2	T3	T4		
ALT	14.3^{d}	21.3^{c}	28.3^{b}	37.5^{a}	2.959	0.033
AST	45.3^{d}	52.3^{c}	60.0^{b}	67.5^{a}	1.589	0.049
ALP	2053.3^{a}	1225.7^{d}	1482.7^{c}	1718.0^{b}	2.239	0.029
LDH	557.0^{c}	478.7^{d}	724.0^{ab}	725.5^{a}	2.159	0.110

DISCUSSION

The birds that received higher levels of poultry oil (3% and 4.5%) exhibited elevated hemorrhagic liver scores (ranging from 0 to 5). These higher liver scores indicated the presence of Fatty Liver Syndrome (FLS), a finding consistent with the study conducted by Shini *et al*. (2019) investigating the causes of FLS in layer birds. According to this study, high energy diet is the most typical reason for FLS in cage layers. Nevertheless, FLS is a disease of lipid metabolism. Factors like nutrition, genetics, feeding practices, environmental circumstances, toxic chemicals and hormones all had a role in the development of FLS in layer birds (Xianquan *et al*., 2012). Similar results were obtained by Rozenboim *et al*. (2016), who found increased liver hemorrhagic scores in layers that fed a diet with low protein and high energy. When 4.5% poultry oil was used, the liver's dry matter and fat levels were increased in layers. The presence of FLS in layer birds was suggested by the liver's high-fat content (Choi *et al*., 2012;

Zhang *et al.,* 2008). From a histological standpoint, it was clear that fatty degeneration and hemorrhages, which are the critical signs of FLS in layer birds and are the main symptoms observed in our investigations at higher levels of poultry oil (Trott *et al*., 2014).

Poultry oil had a significant effect ($p<0.05$) on blood serum lipid profile, including total cholesterol (TC), High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Triglycerides (TG). Laying hens fed diets containing poultry oil showed higher TC and TG serum levels. HDL was significantly lower than LDL in treatment groups. However, these findings were in line with Khatun *et al*. (2017) and Hosseini *et al*. (2008) who observed a significant effect ($p<0.05$) of different sources of oil on serum lipid profile in broilers. According to Naviglio *et al*. (2011), higher levels of fatty acid synthesis and production of bile acids from cholesterol resulted in an increase in the concentration of serum triglycerides. Liver enzymes (ALT, AST and LDH) were also significantly higher in the treatment group. ALP level was higher in the control group as compared to the treatment group. These enzymes are used for indication of liver leakage. AST and ALT are two important transaminases of hepatocytes. These transaminases could be released when hepatocytes have been destroyed (Shini *et al*., 2019). ALP significantly decreased by increasing the level of poultry oil in layer feed. ALP also indicated liver damage. LDH significantly ($p<0.05$) increased at a higher level of poultry oil in layer feed. Similarly, higher LDH indicated inflammation of the liver (Zhang *et al.,* 2008). A similar effect has been observed by Attia *et al*. (2020), who observed the effect of different oil sources on the blood lipid profile of broilers. On the other hand, insignificant results were observed by Saleh *et al*. (2021) who observed that poultry fat did not affect production performance, or plasma lipid profile in broilers.

The incorporation of poultry oil has an impact on the liver histology of layer birds. When the amount of poultry oil in the layer diet increased, pathological lesions in the poultry oil liver were seen. Hepatocytes in the control group were grouped in clusters and the liver seemed normal. A high proportion of poultry oil (4.5%) resulted in significant liver damage, degeneration and cell necrosis throughout the liver parenchyma. Layers with the standard layer diet did not gain weight or develop hepatic liver contents (Zhang *et al*., 2008). Our findings are consistent with those of Liu *et al.* (2017) who looked at the impact of a soybean phospholipids supplement on hepatic and serum indices associated with fatty liver hemorrhagic syndrome in layer birds. The results of the current study are consistent with those of Shaniko *et al.* (2020) who found that layer birds may develop FLS due to greater feed consumption and positive energy intake.

Conflict of interest: There is no conflict of interest.

Conclusions: It was concluded that the dietary addition of 4.5% poultry oil led to an increase in serum lipid levels, elevated liver enzymes, and adverse changes in liver tissue structure, collectively suggesting a negative impact on the liver health of caged layers. These findings highlight the importance of carefully managing the inclusion of poultry oil in layer diets to ensure optimal health and productivity in poultry farming. Proper diet formulation and consideration of the appropriate level of poultry oil are essential to prevent potential health issues and maintain the well-being of the birds.

Authors contribution: MY designed the project and finalized the manuscript. SL conducted a research trial and analyzed data. FA applied statistics and interpreted results. MKS do histopathology of the liver and review the manuscript.

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