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Effects of Dandelion (*Taraxacum officinale*) Supplementation on Productive Performance, Egg Quality Traits, Serum Biochemical Parameters and Liver Fat Rate in Laying Hens

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ABSTRACT

Background: This study was conducted to investigate the effects of dandelion (*Taraxacum officinale*) root supplementation on performance, egg quality and some serum antioxidant enzymes capacity with increasing age, after the peak laying stage in hens. This study, also aimed to overcome diseases that occur due to lipid metabolism in layers in the late laying period.

Methods: In total, one hundred and twenty 70-week-old Lohman LSL laying hens were randomly assigned to five groups equally (n = 24). Each treatment was replicated six times (6 replicates × 4 birds). The control group was fed basal diet (2650 kcal/kg ME) with no supplementation. The other groups were fed high-energy diets (2850 kcal/kg ME) to induce fatty liver and also 5, 10 and 15 g/kg dandelion *(taraxacum officinale)* root were supplemented.

Result: The study revealed that the addition of 5 g/kg dandelion root to the high-energy feed positively changed the egg production, decreased lipid profiles (triglyceride and VLDL) and liver fat ratio and played a role in preventing lipid peroxidation.

Key words: Antioxidant enzymes, Dandelion root (*Taraxacum officinale*), Fatty liver, Laying hen, Serum lipid profiles.

INTRODUCTION

Fatty Liver Syndrome (FLS) is a condition that affects primarily caged layers and is characterized as a nutritional and metabolic disease and is a common cause of death in laying hens. Recent studies have reported that increased age, hormone imbalance, a low-protein high-energy diet, heat stress and cage system of rearing predispose birds to Fatty Liver Hemorrhagic Syndrome (FLHS) (Rozenboim *et al*., 2016; Gao *et al*., 2019). The typical characteristics of FLHS are a sudden drop in egg production, which has a devastating effect on the poultry industry (Zhuang *et al*., 2019; Ürüşan 2021).

Medicinal aromatic plants are one of the treatment and preventive measures against diseases, disorders and environmental factors (such as temperature, stress of settlement density) that adversely affect the health of poultry (Bayraktar and Tekce, 2019; Tekce *et al*., 2019). Dandelion (*Taraxacum officinale*) is a medicinal aromatic plant that plays an important role in the treatment of human and animal diseases. It has been used for the treatment of different hepatic disorders and various conditions affecting the gallbladder. The dandelion root, herbs and blossoms constitute the pharmacopeial raw ingredients (Ahmed *et al*., 2013).

In vivo studies in rat models of non-alcoholic steatohepatitis (NAFLD) treated with dandelion extracts showed significant reductions in hepatic lipid accumulation, liver tissue weight, body weight, serum cholesterol levels and serum malondialdehyde (MDA) levels (Cho *et al*., 2002). It has been reported that dandelion suppresses lipid accumulation associated with insulin resistance and lipid reduction *via* AMPK (Davaatseren *et al*., 2013a). It is known that adenosine monophosphate activated protein kinase (AMPK) reduces fatty acid synthase and acetyl CoA carboxylase (ACC) while suppressing SREBP-1c. After the administration of dandelion leaf extracts, insulin resistance was found to be reduced through activation of the AMPK (50 adenosine monophosphate-activated protein kinase) pathway. Dandelion extract has showed benefits in streptozotocin-induced diabetes (Cho *et al*., 2002), carbon tetrachloride (CCL4)-induced liver damages and high-fat diet (HFD)-induced fatty liver disease (NAFLD) (Davaatseren *et al*., 2013a).

Studies to date have shown that older laying hens are more vulnerable to internal and external stimuli than younger birds and that bioactive additives may have curative effects on the performance and physiological function of chickens (Jiang *et al*., 2020; Tao *et al*., 2020). However, little is known about the effects of aging on the organ function or metabolism of laying hens. A literature review on the effect

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of dandelion on fatty liver in laying hens revealed that such studies have not been undertaken. This study was, therefore, conducted to evaluate liver fat ratio, performance, egg quality and serum antioxidant enzymes capacity with increasing age after the peak laying stage of hens. In addition, this study, also aimed to overcome the diseases that occur due to lipid metabolism in layers in the late laying period.

MATERIALS AND METHODS

This study was guided pursuant to the approval (protocol number: 2020/1) of the Local Ethics Board for Animal Experiments of Directorate of Veterinary Control Centre Research Institute in Türkiye.

Animals and experimental design

In this study, in total, one hundred and twenty Lohman LSL hybrid laying hens at 70 weeks old were assigned to five groups (n=24), replicated 6 times with 4 hens per replicate. Laying hens in the control group were given a corn soybean basal diet (2650 kcal/kg ME) and high energy (HE) groups were given a 2850 kcal/kg ME diet without hipolipidemic additives. The remaining three groups respectively were given the HE diet supplemented with dandelion root powder at the rate of 5 g/kg, 10 g/kg and 15 g/kg. During the experiment (12 weeks), hens were fed and watered as *ad libitum.*

Performance and egg quality measurements

Hen day egg production (%) was measured. Feed intake (FI) was recorded and feed conversion ratio (FCR) was calculated daily. FCR was calculated as grams of FI per gram of egg mass produced. Egg quality parameters were evaluated every 15-d. Egg quality characteristic included egg weight, yolk index, specific gravity, shell breaking strength and weight of shell, Haugh units (HU), eggshell thickness, albumen weight and yolk weight were measured every two weeks using eighteen eggs from each dietary treatment.

Serum lipid profile and glucose, ALP, AST and estrogen levels

Blood samples were taken with a 23-gauge needle from the vena brachialis and collected into 10 mL biochemistry tubes (VACUETTE ® TUBE 9 mL Z Serum Clot Activator). At the end of the experiment, blood samples (per group, n=6) were centrifuged at 3000 rpm for 10 min at +20°C. Serum was obtained following centrifugation at 3000 rpm for 10 min at +20°C. Using commercial kits (Roche) in auto-analyzer (Cobas, Japan) with the spectrophotometric method serum cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein (VLDL), total cholesterol, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and estrogen levels were determined.

Analysis of oxidants and antioxidants

Blood collected in heparin tubes was centrifuged at 3000 rpm for 10 minutes and plasma was removed. Plasma malondialdehyde (MDA) levels, Superoxide dismutase (SOD) activity, glutation (GSH) level, glutathione peroxidase (GPx) activity, catalase (CAT) activity, total proteins (TP) levels and NEFA levels were measured with Biotek Elisa Reader (Bio Single ìQuant MQX200 Elisa reader/USA). Total Protein (TP) levels were used to calculate SOD and GPx activity.

Weight and fat ratio of liver

At the end of the experiment, six laying hens randomly selected from each group were slaughtered. Liver wet and dry weight was calculated. After that, it was kept in an oven at 105°C for 24 hours and liver dry weights were determined. By proportioning liver weights to 100 g live weight, the ratio of liver wet and dry weights were determined. Total lipid content from the livers were determined as per Bligh and Dyer (1959).

Analysis of feed and supplement

Feed analysis

The feeds were analysed as per AOAC (2005).

Analysis of dandelion root

The active principles in dandelion root, was identified using GC–MS, as per IUPAC method 2.301. The concentration of phenolic compounds were determined as per Rababah *et al.* (2011) with slight modifications.

Statistical analysis

Kolomogrov-Smirnov normality test was used to determine whether the data were suitable for normal distribution and Levene's statistics were used to test the homogeneity of variances.

Data were analyzed via one-way ANOVA, according to the following general model:

$$Yij = \mu + \alpha i + \varepsilon ij$$

Where,

Yij = Measured dependent variable.

μ = Overall mean.

αi = Effect of treatment.

εij = Random error.

The significance was tested at 0.05 level and SPSS 23.0 program was used in all analyses.

RESULTS AND DISCUSSION

Feed analysis

The ingredient composition and nutrient content of the feeds used in the experiment are given in Table 1.

Analysis of dandelion root

The active principles present in dandelion root is presented in Table 2 and depicts the phenolic compounds present in dandelion root.

Productive performance of birds

The addition of 5 g/kg dandelion to the basal diet significantly increased egg production compared to other groups (Table 3).

Table 1: Ingredient composition and nutrient content of diets.

Ingredients (% inclusion)	Basal diet	High energy diet
Corn	62.2	63.5
Soybean meal (CP-Crude protein 44 %)	17.36	13.15
Corn gluten (CP-60%)	8.48	10.64
Limestone	9.68	7.65
Dicalcium Phosphate (18% P)	1.44	1.44
Soy oil	-	2.7
Vitamin-Mineral premix[1]	0.25	0.25
Common salt	0.22	0.33
Sodium bicarbonate	0.16	0.16
L-Lysine HCL	0.11	0.10
DL-Methionine	0.10	0.08
Analysed nutrientcontent		
Dry matter (g/kg)	88.41	88.54
Crude Protein (g/kg)	17.52	17.20
Crude fat (g/kg)	2.20	4.84
Crude ash (g/kg)	11.87	10.35
Crude fibre (g/kg)	2.78	2.57
DL- Methionine (g/kg)	0.38	0.38
Methionine (g/kg)	0.40	0.41
Lysine (g/kg)	0.76	0.70
ME kcal/kg	2650	2850

[1]The premix provided per kilogram of diet: 12 000 IU vitamin A; 2 500 IU cholecalciferol (vitamin D3); 30 IU α-tocopheryl acetate (vitamin E); 4 mg menadione sodium (vitamin K3); 3 mg thiamine mononitrate (vitamin B1); 6 mg (riboflavin) vitamin B2; 30 mg niacin (vitamin B3); 10 mg calcium D-pantothenate (vitamin B5); 5 mg pyridoxine (vitamin B6); 0.015 mg cyanocobalamin (vitamin B12); 1 mg folic acid; 0.050 mg D-biotin (vitamin H); 50 mg ascorbic acid (vitamin C); 300 mg choline chloride; 80 mg manganese oxide; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 2 mg iodine; 0.15 mg selenium.

Table 2: Phenolic compounds in dandelion root.

Phenolic compounds	Quantity (mg g^{-1})
Apigenin-7-*O*-glycoside	0.016
Caffeic acid	9.21
Chlorogenic acid	3,54
Coumaric acid	11.32
Epicatechin gallate	0.472
Ferulic acid	0.546
Gallic acid	0.756
Luteolin	0.307
Luteolin-7-*O*-glycoside	0.508
Naringenin-7-*O*-glycoside	0.042
Narirutin	0.002
Protocatechuic acid	0.529
Pyrocatechol	0.649
Quinic acid	0.027
Syringic acid	2.678
Vanillic acid	1.721

The reason for this situation is thought to be shaped depending on the dose of dandelion root added to the ration. Canto Saenz *et al*. (2021) also observed that the inclusion of the dandelion (1,2,3,4% levels) and CPB (0.20% of sodium butyrate) in the diet of laying hens improved egg production significantly. Herbal extracts have a stimulating effect on animal digestive systems and these effects could be due to the increased production of digestive enzymes and the improved utilization of digestive products through enhanced liver functions (Jamroz, 2003).

Egg production decreased significantly in the group fed with high-energy feed. This result is confirmed by previous studies showing lower egg production in a HE diet (Ürüşan 2021; Ürüşan 2023). High quantities of fat or energy intake cause metabolic burden with increased fat accumulation in the liver, which leads to a decrease in laying rate (Neijat *et al*., 2014).

Although not statistically significant, the addition of dandelion to the diet decreased feed intake. Smilar findings were reported by Quilcate *et al*. (2018) they observed that dandelion supplementation (4%) in laying hen diets decreased feed intake. It is possible that a high amount of sesquiterpenic lactones (bitter principle) in dandelion that are responsible for the flavor and bitter taste, may have reduced feed consumption in birds. Schleicher *et al*. (1998) observed that herbal plants like chamomile and dandelion had negative impacts on FCR and live body weights (LBW) at 52 days of age in broilers. Limin *et al*. (2016), reported that dietary astragalus polysaccharides and dandelion extracts at the level of 0.1% decreased feed consumption of laying hens. Conversely, Qureshi *et al*. (2015) revealed that a combination of enzyme-treated fenugreek seeds and dandelion leaves supplementation improved FI in broilers. It is believed that this is due to the difference in the type, dose and duration of administration of dandelion added to the diet as a justification for this condition.

In this study, it was determined that the addition of dandelion to the diet did not affect egg weight. But the highest value was for the HE+dandelion 5 g/kg group (62.85 gr). However, Limin *et al*. (2016) reported that egg weight in groups fed astragalus polysaccharides and dandelion extracts were higher than those in control group. However, Quilcate *et al*. (2018) showed that dietary inclusion of dandelion, reduced the weight of the egg. This could be because dandelion was supplemented in the diet at high level.

The addition of dandelion to the diet had no impact on FCR. Consistent with current findings, Quilcate *et al*. (2018) revealed that the addition of dandelion in the laying hen diet did not affect FCR. Similarly, Maljik (2017) showed that dandelion addition did not affect performance parameters.

Egg quality

The experimental results indicated that shell weight was significantly increased by dandelion in high energy diet ($p<0.05$) (Table 4). Similarly, Maljik (2017) showed that dietary dandelion in laying hens improved the shell weight of eggs. Dandelion is a good source of calcium and vitamin K, both of which could have contributed to improvement in shell weight. The addition of 5 and 15 g/kg dandelion to the high energy diet increased significantly egg weight compared to other groups.

For consumers, one of the important purchasing criteria of eggs yolk color (Esfahani-Mashhour 2009). Supplementation of Dandelion in layers has been less studied as source of pigment for egg yolk coloration (Ürüşan 2021; Quilcate *et al*., 2018). This study revealed that supplemental dandelion increased ($p<0.05$) the yolk color score. This was largely due to carotenoids present in dandelion. Consistent with current findings, Maljik (2017) and Quilcate (2018) also observed that the yolk color index was promoted as the result of dietary addition of dandelion. Dandelion also has high antioxidant activity (Hu and Kitts 2003; Sahingil 2019), suggesting that its supplementation could protect fatty acids in egg yolk and thus affect not only color and carotenoid content but also oxidative stability and sensory properties. Thus, it could be a suitable alternative to improve egg quality and provide beneficial bioactive compounds in the human diet.

Shape index values (%) were significantly affected by all the treatments with the values ranging between 68.50 and 75.10%. An important criterion in determining egg quality is the egg shape index. An egg is always oval in shape (72-76). Round eggs and unusually long eggs do not fit well in egg cartons. Hence, they are much more likely to break during the shipment than the eggs of normal shape (Sarýca and Ersayın 2009). The group fed with high energy diet showed the lowest shell thickness. It has been suggested that especially calcium-binding protein synthesis is impaired due to fatty liver in high-energy diets and as a result, Ca++ absorption from the intestines decreases (Sevinç and Aslan

Table 3: Effects of dietary dandelion root on the productive performance of laying hens.

Dietary treatments	Initial body weight (g)	Final body weight (g)	Feed intake (g/day)	Egg weight (g)	Egg production (%)	Feed conversion ratio (g:g)
Control	1550.1	1325.5	107.92	60.01	78.82^{ab}	1.96
High energy (HE)	1558.7	1308.0	109.87	59.58	72.84^{b}	2.30
HE + 5 g/kg dandelion	1540.6	1370.5	104.73	63.10	81.22^{a}	1.96
HE + 10 g/kg dandelion	1515.0	1234.8	106.50	59.95	76.81^{ab}	2.14
HE + 15 g/kg dandelion	1540.8	1291.0	96.66	57.97	72.41^{b}	2.13
SEM	8.56	21.78	2.08	0.63	1.07	0.06
P	0.600	0.405	0.319	0.129	0.024	0.391

Column means with no common superscript letters differ significantly; *Significant at $p<0.05$.

Table 4: Effects of dietary supplementation dandelion root on egg quality (n=12).

Dietary treatments	Albumen weight (%)	Yolk weight (%)	Shell weight (%)	Egg weight (g)	Yolk color score	Haugh units	Shell breaking strenghth (kg cm^3)	Egg shape Index (%)	Shell thickness (µm)
Control	60.65	29.05	10.30^{b}	59.98^{ab}	10.20^{bc}	83.32	2.67	75.10^{a}	0.44^{a}
High energy (HE)	58.90	30.65	10.45^{b}	57.87^{b}	9.80^{c}	84.30	1.61	70.20^{b}	0.37^{b}
HE + 5 g/kg dandelion	57.68	29.97	12.55^{a}	62.72^{a}	11.40^{a}	79.59	2.65	69.54^{b}	0.44^{a}
HE + 10 g/kg dandelion	57.74	29.77	12.49^{a}	58.21^{b}	10.83^{ab}	82.25	2.43	71.04^{b}	0.44^{a}
HE + 15 g/kg dandelion	57.21	30.08	12.72^{a}	63.25^{a}	11.17^{a}	82.88	2.16	68.50^{b}	0.42^{a}
SEM	0.571	0.424	0.289	0.608	0.152	1.011	0.201	0.504	0.008
P	0.339	0.875	0.002	0.005	0.002	0.663	0.481	0.000	0.020

Column means with no common superscript letters differ significantly; *Significant at $p<0.001$.

1998). In the current study, it is thought that there may be a decrease in egg shell thickness due to the decrease in calcium absorption in the HE+group.

Blood serum constituents

Dandelion has many bioactive components, phenolic compounds, glycosides, alkaloids, tannins and flavonoids. It plays a role in reducing cholesterol levels in the blood by intensification of bile secretion (Choi *et al*., 2010). Data showed that serum triglyceride and VLDL concentrations were lower in the control and HE + 5 g/kg dandelion groups than the other groups (p<0.01). Qureshi *et al*. (2015) were found that serum cholesterol was decreased significantly in the groups fed a combination of fenugreek seeds and dandelion leaves with or without enzyme as compared to the control. Similarly, Choi *et al*. (2010) showed that treatment with dandelion root and leaf decreased plasma triglycerides and LDL cholesterol in cholesterol-fed rabbits. Concerning the hypolipidemic effect, many studies have indicated that dandelion reduces the concentrations of cholesterol and triglyceride in the blood (Qureshi *et al*., 2015; Jassim *et al*., 2012; Davaatseren 2013b). The addition of dandelion in the diet of laying hens had no significant differences in ALP, AST, ALT, glucose, total cholesterol, LDL, HDL and estrogen concentration (Table 5).

MDA has been widely used as an indicator of lipid peroxidation. The lowest MDA concentrations were observed in controls, HE 5 g/kg and 10 g/kg dandelion groups. CAT, SOD and GSH-Px are important anti-oxidant enzymes playing a role in reducing toxic effects. They can work together to catalyze free superoxide anion radicals into nontoxic compounds (Xiaohong *et al*., 2016). The GSH value was significantly increased in the control, HE and 10 g/kg dandelion groups (p<0.01) (Table 6). Consistent with present findings, Choi *et al*. (2010) observed that GSH activities were significantly improved in both dandelion root and leaf groups, compared to the control group in cholesterol-fed rabbits. Using 10 g/kg dandelion as feed additive in high energy diet had the highest CAT and GPx enzyme value during the experimental period, compared with other treatment groups. Studies have shown that dandelion has significantly increased hepatic antioxidant enzymes (GPx, GST and GSH) (Choi *et al*. 2010; You *et al*., 2010). The results show that dandelion reduced lipidic superoxide damage, improved the antioxidative status and inhibited free radical formation in laying hens.

Weight and fat ratio of liver

The liver weights (dry and wet) of the laying hens were not influenced significantly (p>0.05) by any of the treatments. The liver fat content decreased in HE+5g/kg, HE+15 g/kg dandelion and control diets compared with other groups (p<0.05) (Table 7). Literature review has revealed that dandelion has protective activity in the liver. It has been

Table 5: Effects of dietary supplementation of dandelion root on serum biochemical parameters in laying hens.

Dietary treatments	ALP (U/L)	AST (U/L)	ALT (U/L)	Trigliseride (mg/dl)	Glucose (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	Estrogen (pg/ml)
Control	247.0	248.5	2.25	321.8^{b}	255.5	113.75	66.5	41.25	76.25^{b}	214.5
high energy (HE)	267.5	264.3	5.25	1525.3^{a}	248.5	207.5	164.75	36.25	312.5^{a}	216.8
HE + 5 g/kg dandelion	376.0	229.0	1.50	682.5^{b}	288.0	89.5	78.5	31.00	136.50^{b}	175.0
HE + 10 g/kg dandelion	655.0	157.5	5.00	1586.5^{a}	287.5	176.5	154.0	13.00	317.50^{a}	205.0
HE + 15 g/kg dandelion	761.5	258.0	1.00	1583.0^{a}	272.5	142.5	206.0	32.00	316.50^{a}	141.5
SEM	74.92	16.27	0.62	167.46	5.84	19.97	19.58	4.10	32.68	17.44
P	0.100	0.361	0.59	0.001	0.69	0.322	0.089	0.336	0.001	0.738

Column means with no common superscript letters differ significantly; *Significant at p<0.001.

Table 6: Effects of dandelion root supplementation at different rates in laying hen ration on antioxidant enzyme parameters (MDA, SOD, CAT, GPx) and NEFA levels.

Dietary treatments	MDA (nmol/L)	GSH (mmol/L)	SOD (U/L)	CAT (KU/L)	GPx (U/L)	NEFA
Control	7.75^{c}	2.37^{a}	57.97	144.09^{abc}	1.48^{b}	0.23
High energy (HE)	13.87^{a}	2.50^{a}	58.13	152.63^{ab}	1.48^{b}	0.22
HE + 5 g/kg dandelion	7.35^{d}	1.73^{b}	53.14	127.28^{c}	1.21^{d}	0.27
HE + 10 g/kg dandelion	7.84^{c}	2.53^{a}	60.30	163.50^{a}	1.63^{a}	0.21
HE + 15 g/kg dandelion	10.16^{b}	1.92^{b}	56.28	136.76^{bc}	1.37^{c}	0.25
SEM	0.82	0.11	0.94	0.04	0.05	0.01
P	0.000	0.002	0.128	0.044	0.000	0.061

Column means with no common superscript letters differ significantly.

MDA- Malondialdehyde; GSH- Glutation, SOD- Superoxide dismutase, CAT- Catalase; GPx- Glutathione peroxidase); NEFA- Nnonesterified fatty acids; *Significant at p<0.001.

Table 7: Effects of of dietary supplementation of dandelion root on liver of laying hens.

Dietary treatments	Liver wet weight (g)	Liver dry weight (g)	Liver fat (%)
Control	23.68	7.13	25.49[c]
High energy (HE)	35.35	13.00	50.72[a]
HE + 5 g/kg dandelion	27.88	8.47	25.62[c]
HE + 10 g/kg dandelion	33.15	10.54	44.73[ab]
HE + 15 g/kg dandelion	22.86	7.68	31.32[bc]
SEM	2.194	0.890	2.816
P	0.243	0.122	0.002

Column means with no common superscript letters differ significantly; *Significant at p<0.001.

reported that the lipid-lowering effect of dandelion is due to the relationship of luteolin with hydroxy-methyl-glutaryl-enzyme A reductase, liver sterol regulatory element-binding proteins and acetyl-CoA C-acetyltransferase (Gebhardt 2002). Although there are several studies with dandelion, none of the research has been conducted to appreciate the inûuence of the hepatoprotective effect of dandelion in laying hens. A search of the literature found only the report by Davaatseren *et al*. (2013a) which recommended that dandelion leaf extract could inhibit hepatic lipid accumulation in high-fat diet-fed mice. In addition, Cho *et al*. (2002) dandelion water extract addition could be used to improve lipid metabolism in rats. At present, hepatoprotection is used in the integrated therapy of liver diseases. A reduction in relative liver fat in laying hens fed dandelion at 5 g/kg with high energy is promising.

CONCLUSION

The use of dandelion in high energy feed of laying hens improved egg production, shell weight and yolk color. Dandelion root supplementation at 5 g/kg feed was useful for reducing the plasma triglyceride, VLDL, MDA concentration and liver fat ratio. Further studies are needed to validate these positive effects.

Conflict of interest: None.

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