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THE EFFECT OF URSOLIC ACID ADDITION INTO HİGH-ENERGY LAYING HEN DIET ON PERFORMANCE, EGG QUALITY PARAMETERS, SERUM LIPID PROFILE AND LIVER FAT RATE

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Abstract: This study was conducted to determine the effect of ursolic acid (UA) at different ratios (0, 0.5, 1 and 1.5%) supplementation into high-energy laying hen diet on performance, egg quality parameters, serum lipid profile, some liver enzymes and liver fat ratio.
A total of 120 Lohman LSL laying hens, 70 weeks old, were used in present study. The animals were divided into 5 groups and each group consisted of six subgroups. In the experiment, the control group was fed with basal feed, and the treatment groups were fed with high-energy (HE) diets including 0, 0.5, 1 and 1.5% UA, respectively. Experiment lasted for 8 weeks. Egg yield decreased in high energy feed groups except HE + 1.5% UA group. Egg weight was found to be highest in the HE + 1.5% UA group. Addition of UA into feed improved the feed conversion ratio (FCR). It was determined that liver fat ratio was higher in the group fed with HE feed (P<0.01) than other groups fed with diets including UA, but the addition of UA decreased the liver fat rate significantly. The addition of UA to feed increased blood plasma MDA and NEFA values, and decreased GSH and GPx values (P<0.01). The addition of 1.5% UA to high-energy feed increased ALT and total cholesterol, while lowering glucose. The highest VLDL, TG and LDL values were found for YE + 0% UA and YE + 1.5% UA groups. Conclusion, high-energy feed adversely affected performance values and liver fat ratio, but the addition of ursolic acid improved FCR and decreased liver fat ratio. Positive effects of ursolic acid have been seen, but more studies are needed.

Keywords: Fatty liver, Ursolic acid, Non-esterified fatty acid, Feed conversion ratio, Hypolipidemic

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1. Introduction

In parallel with the rapidly increasing world population, the demand for eggs is increasing day by day. In order to meet this increasing demand, it has been inevitable to make conventional animal production. More production depends on increasing the number of animals as well as good care and feeding. Therefore, it is inevitable to have more animals per unit area. The most advantageous system in laying hen is cage breeding.

However, in laying hens raised in cages, the restriction in the movement area and the high energy value of the feeds may cause fatty liver. Fatty liver syndrome is one of the important causes of death in commercial layers (Leeson, 2007). In many studies conducted in the past years, it has been reported that fatty liver syndrome is detected at a higher rate in animals housed in cages than those raised on the ground (Butler, 1976; Shini et al., 2006; Squires and Leeson, 1988).

Fatty liver is a disease characterized by an accumulation of fat in the abdominal cavity and liver. Excessive fatty liver adversely affects animal health and causes a significant decrease in egg production. Although there is no known method for the treatment of fatty liver syndrome, its development could be prevented by balanced nutrition techniques. One of them is ursolic acid. Phytosterolic ursolic acid is in the pentacyclic triterpenes group of triterpenes and is found in free or glycoside structure in plants, vegetables and fruits such as apple, basil, olive, oregano, rosemary and thyme (Babalola and Shode, 2013; Mendes Leal, 2012; Ikeda et al., 2008). In several recent studies on mice and rats, it has been reported that the addition of ursolic acid to feed has a hypolipidemic and hypoglycemic effect. It has been proven by studies that ursolic acid regulates lipid metabolism and has a protective effect on the liver (Azevedo et al., 2010; Liu et al., 1995).

Research to date has shown that older laying hens are more vulnerable to internal and external stimuli than younger hens, and that bioactive additives can have improving effects on chickens' performance and physiological function (Jiang et al., 2020; Liu et al., 2020). As a result of our research, no study was found that examined the effect of ursolic acid on fatty liver syndrome in layer hens.

In this study, it was aimed to determine the effect of high energy feed and ursolic acid on performance, egg quality criteria, mortality rate, some antioxidant enzymes and liver fat ratio in laying hens

2. Materials and Methods

In the study, 120 Lohmann (LSL) white laying hens of 70 weeks were weighed and divided into five groups depending on chance, and each group consisted of six subgroups. Chickens were placed in 4-storey battery-type cages (60 * 59 * 61 cm) with 24 animals in each group, 6 replications and 4 animals in each repetition. First group (control group) was fed basal diet (Table 1), and the treatment groups were fed with high-energy (3020 kcal / kg ME) diets including 0, 0.5, 1 and 1.5% ursolic acid, respectively. The feed and water were given to animals as ad-libitum during the 8 weeks' experimental period. Ursolic acid (99.9% purity) was obtained from a commercial company.

Table 1. Composition of feeds used in the trial (%)

Item	Basal diet (control)	High energy diet
Corn 8.5	63	64.17
Soybean meal 44-46	16.39	12.50
Corn gluten 60	8.48	10.64
Limestone	9.68	7.65
DCP 18	1.44	1.44
Soybean oil	0.17	2.68
Vitamin-Mineral mixture[1]	0.25	0.25
Salt	0.22	0.33
Sodium bicarbonate	0.16	0.16
L-Lysine	0.11	0.10
D-L Methionine %99	0.10	0.08
Calculated composition (%)		
Dry matter	88.41	88.54
Crude protein	17.52	17.20
Ether extracte	2.20	4.84
Crude ash	11.87	10.35
Crude fiber	2.78	2.57
D Methionine	0.38	0.38
Methionine	0.40	0.41
Lysine	0.76	0.70
ME Kkal/Kg	2726	3000
Analysed composition (%)		
Dry matter	88.78	88.37
Crude protein	17.12	16.92
Ether extracte	2.43	5.03
Crude ash	11.24	11.09
Crude fiber	3.18	2.91

[1]Per kg diet added 12 000 IU vitamin A; 2500 IU vitamin D3; 30 IU vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 30 mg niasin; 10 mg calcium D-pantothenate; 5 mg vitamin B6; 0.015 mg vitamin B12; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganase; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 0.2 mg iodine; 0.15 mg selenium.

The chemical composition of the diets used in the study was determined according to the Weende analysis method (Kutlu, 2008). As performance values in the study, daily feed consumption, feed conversion rate (kg feed/ kg egg), egg weight and egg production were determined by measurements made every two weeks. The numbers of the animals that died during the trial were recorded daily.

Egg quality criteria (shell thickness, breaking strength, white ratio, yellow ratio, shell ratio, shape index and Haugh unit) were performed every two weeks on one randomly selected egg sample from each subgroup.

At the end of the experiment, blood samples taken from the sub-wing vein of 6 animals from each group into heparinized tubes were centrifuged at 3000 rpm for 10 minutes and their plasma was extracted and stored at -80 ° C for examination. MDA level in plasma (Yoshioka et al., 1979), SOD activity (Sun et al., 1988), GSH level (Tietze, 1969), GPx activity (Matkovics et al., 1988) CAT activity (Goth, 1991), TP levels (Lowry, 1951) and NEFA levels (Biont Chicken NEFA ELISA Kit, Cat No: YLA0179CH) were measured with Biotek Elisa Reader (Bio Tek µQuant MQX200 Elisa reader / USA). TP levels were used to calculate the SOD and GPx activity. Plasma glucose, cholesterol, VLDL, LDL, HDL, AST, ALT and TG values were analyzed in a special laboratory.

At the end of the experiment, 6 animals from each group were slaughtered and their livers were removed and their wet weights were determined. Then the livers were dried at 105 °C and their dry weight was determined.

Later, samples were taken from dried livers and their fat percentage was determined (Kutlu 2008).

2.1. Statistical Analyses

Performance values, egg quality criteria, some blood parameters and antioxidant enzyme values variance analysis were performed by the General Linear Model procedure, and the importance controls of the important data were performed using the SPSS 17 package program. Mortality was determined by the X^2 independence test. Differences between groups were foun d by Duncan multiple comparison test (Düzgüneş et al., 1983).

3. Results and Discussion

The effect of adding different levels of ursolic acid to high energy feeds on feed consumption, egg production, egg weight and feed conversion ratio is given in Table 2. It was determined that there were no significant differences in feed consumption between the groups. It found that egg production decreased significantly (P<0.05) in HE + 0% UA, HE + 0.5% UA and HE + 1% UA groups. The highest egg weight was found only in YE + 1.5% UA group. The best feed injury rate was seen in control, HE + 1% UA and HE + 1.5% UA groups. There was no significant difference between the groups in terms of mortality.

Table 2. Effects of high energy feed and ursolic acid supplements on performance values

Groups	Feed Intake (g)	Egg Production (%)	Egg Weight (g)	Feed Convertion Ratio (g:g)	Mortality (%)
Control	111.87	79.80^a	60.42^b	2.39^c	4.2
HE+ 0 % UA	111.18	73.44^b	61.35^b	2.56^b	20.8
HE+ 0.5 % UA	118.11	73.59^b	61.96^b	2.90^a	20.8
HE+1 % UA	110.63	75.26^b	61.55^b	2.38^c	8.3
HE+ 1.5 %UA	113.22	77.32^a	63.80^a	2.35^c	8.3
SE	2.45	1.51	0.34	0.091	X^2=5.22
P	ns	*	*	*	ns

$^{a, b}$ The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1%, UA= high energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE= standard error, NS= not significant, *P<0.05.

Some researchers observed that the feed consumption of laying hens fed a high energy diet decreased compared to the control group (Harms et al., 2000; Jiang et al., 2013; Valkonen et al., 2008; Yousefi et al., 2005; Zhang et al., 2008). Contrary to these reports, Grobas et al. (1999) found that feed consumption of laying hens fed with feed containing 2680 kcal / kg ME was higher than those containing 2810 kcal / kg ME. Plavnik et al. (1997) reported that as dietary energy increases, feed intake decreases. One of the main reasons for this is that energy content plays a key role in controlling feed intake (McNab and Boorman, 2002).

It has been reported that there are large economic losses due not only to animal deaths but also to reduced egg production due to fatty liver syndrome in caiged-raised chickens (Squires and Leeson, 1988). The metabolic activity of the liver is quite high in poultry, especially during egg production where lipogenesis is stimulated (Nesheim and Ivy, 1970). Butler (1976) reported that animals with fatty liver syndrome may experience sudden decreases in egg production. Smilarly, in many previous studies (Hansen and Walzem, 1993; Julian, 2005; Thomson et al., 2003), it was reported that egg productivity decreased suddenly due to fatty liver syndrome. It has been reported that as dietary energy increases, feed consumption decreases and thus egg production decreases (Plavnik et al., 1997). In this study, it was determined that egg production decreased in high energy groups, but there was no negative change in egg production in the group where 1.5% ursolic acid was added to high energy feed. However, Grobas et al. (1999) reported that there is no significant difference between egg yields of animals fed with feed containing 2680 and 2810 kcal / kg ME. Similarly, Rozenboim et al. (2016) reported that high energy feed does not affect egg yield.

In the present study, it was determined that the addition of 1.5% ursolic acid to a high-energy diet increased egg weight compared to the control group. In previous studies, some of the researchers reported that egg weight was not affected by the energy content of the feed (Summers and Leeson, 1993; Keshavarz and Nakajima, 1995; Grobas et al., 1999; Mathlouthi et al., 2002; Valkonen et al., 2008; Zhang et al., 2008), some reported significant increases in egg weight (Marsden et al., 1987; Peguri and Coon, 1991).

In the current study, it was observed that high energy feed negatively affected the ratio of feed conversion, but the feed conversion ratio values in the groups that added 1% and 1.5% ursolic acid to the feed were similar to the control group.

Unlike this study, Grobas et al. (1999) reported that the group fed with high energy feed had a better feed conversion value. However, in another study conducted on laying hens fed with feed containing different levels of energy, it was reported that there was no significant difference between the groups in terms of feed efficiency (Zhang et al., 2008).

Leeson, (2007) reported that fatty liver syndrome causes significant mortality in commercial layer flocks. Shini et al. (2006) reported that 74% of the cause of death in laying hens in cages in Australia was caused by fatty liver syndrome. Similar to the current study, Valkonen et al. (2008) found that the mortality rate in laying hens fed with high energy feed was higher than the control group, but the difference was not found to be significant.

The effects of ursolic acid addition to high energy feed on egg quality criteria are given in Table 3. No significant difference was detected between groups in terms of shell breaking strength, shell thickness, ratio of egg shell, yolk, albumen and Hough units' values. Similar to this study, Valkonen et al. (2008) reported that the energy value of the feed does not affect eggshell breaking strength, egg shell ratio, yolk ratio, albumen ratio and Haugh unit.

Table 3. Effects of high energy feed and ursolic acid supplements on egg quality of the laying hens

Groups	SBS (kg cm^2)	ST (µm)	Egg shell (%)	Yolk (%)	Albumen (%)	Hough units
Control	3.27	0.459	12.42	30.22	57.27	81.97
HE+ 0 % UA	3.01	0.414	11.94	31.38	56.63	83.69
HE+ 0.5 % UA	3.16	0.434	12.84	30.34	56.81	78.32
HE+1 % UA	3.01	0.413	12.83	30.99	56.17	82.90
HE+ 1.5 %UA	3.32	0.450	12.94	30.56	56.49	80.95
SE	0.14	0.006	0.13	0.19	0.24	0.64
P	ns	ns	ns	ns	ns	ns

$^{a, b}$ The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0%, UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SBS= shell breaking strength, ST= shell thickness SE= standard error, NS= not significant

In many previous studies, it has been reported that high energy feed has no effect on egg shell thickness (Yousefi et al., 2005), yolk weight (Keshavarz and Nakajima, 1995) and albumen weight (Keshavarz and Nakajima, 1995; Grobas et al., 2001). However, Whitehead et al. (1991) reported that the addition of fat to the ration increases the albumen ratio. The liver is a central organ for lipid metabolism. The liver synthesizes cholesterol and triglyceride and produces lipoproteins. Generally, the hepatic lipid content is low (wet liver contains less than 5% fat of its weight) and fatty liver syndrome occurs when the liver lipid stores exceed this value.

The results of the wet weight, dry weight and fat ratio of the livers are given in Table 4. When Table 4 was examined, it was determined that using high energy feed had a significant effect (P<0.05) on the wet weight and dry weight of the liver and the lowest wet and dry liver weight was in the control group. However, it was observed that the liver wet weight was lower in the groups that added ursolic acid to the food compared to the YE + 0% UA group. It was determined that there was a significant difference (P<0.01) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Table 4. Wet weight (g), dry weight (g) and fat ratio (%) of liver

Groups	Wet weight of liver (g)	Dry weight og liver (g)	Fat ratio of liver % (DM)
Control	20.32^c	6.05^b	27.43^c
HE+ 0 % UA	39.13^a	14.12^a	48.26^a
HE+ 0.5 % UA	35.27^b	11.60^a	30.49^c
HE+1 % UA	28.18^b	11.78^a	33.03^c
HE+ 1.5 %UA	34.91^b	13.93^a	40.89^b
SE	2.32	1.18	2.62
P	*	*	**

$^{a, b}$ The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SE= standard error, *P<0.05, **P<0.01.

However, it was observed that the liver age weight was lower in the groups in which ursolic acid was added to the high-energy diet than the group without ursolic acid (YE + 0% UA). It was determined that there was a significant difference (P<0.01) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Ivy and Nesheim, (1973) reported that liver fat ratio exceeds 40% of dry weight and can even reach up to 70% in fatty liver. In the present study, it was determined that the liver fat ratio was 56.8% higher in the group fed with high energy feed (YE + 0% UA) compared to the control group.

Akkılıç and Tanyolaç, (1975) reported that both liver weight and liver fat ratio increased when feed containing high levels of energy was given to the laying hens raised in the cage system. Similar to this study, many studies conducted in previous years reported that the liver fat ratio increased with the increase in the energy value of the feed (Splitgerber et al., 1969; Jensen et al., 1970).

Rozenboim et al. (2016) reported that the liver fat ratio was not affected by the diet in young animals in laying hens fed a high-fat diet, but the liver fat ratio in older animals was lower in than in the control group. Jia et al. (2015) investigated the effects of adding 50 and 200 mg / kg ursolic acid to a high-energy diet on the liver in mice, and found that the liver fat ratio decreased significantly in the group that added 200 mg ursolic acid compared to the high-energy feed group without ursolic acid.

Previous studies have reported that ursolic acid has an anti-obesity effect by decreasing lipid accumulation in adipose tissues. According to these studies, ursolic acid acts as a phosphodiesterase inhibitor that increases lipolysis in adipocytes (Jia et al., 2011; Kim et al., 2009; Rao et al., 2011). Jayaprakasam et al. (2006) reported that the addition of ursolic acid to a high-fat diet reduced the amount of liver fat in mice.

As summarized above, some studies have suggested that liver fat is primarily due to increased lipogenesis rather than dietary lipids, while others have suggested that the condition may be due to excess energy. In this study, it was concluded that the fat in the liver developed due to the high energy in the diet. However, it was determined that the addition of ursolic acid to the diet significantly reduced the liver fat rate. This situation can be explained by the hypolipidemic effect of ursolic acid.

Differences among groups were found to be significant in terms of MDA, GSH, SOD, CAT, GPx and NEFA (Table 5). The highest MDA value was found in HE + 1% UA group. It was determined that the addition of ursolic acid to the ration significantly increased the amount of GSH (P<0.01). Superoxide dismutase (SOD) and catalase (CAT) values were significantly lower in the HE + 1% UA and HE + 1.5% UA groups. The lowest GPx value was detected in the YE + 1% UA group. Non esterated fatty acids (NEFA) concentrations increased significantly (P<0.01) in the high energy feed groups and the highest value was found in the YE + 1% UA group.

Table 5. Non-esterified fatty acids (NEFA) and some enzyme activity of liver of laying hen

Groups	MDA (nmol/L)	GSH (nmol/L)	SOD (U/L)	CAT (KU/L)	GPx (U/L)	NEFA (ng/L)
Control	7.79^{d}	2.33^{a}	57.43^{a}	146.65^{a}	1.46^{a}	0.219^{c}
HE+ 0 % UA	7.56^{d}	2.47^{a}	59.05^{a}	151.41^{a}	1.48^{a}	0.299^{b}
HE+ 0.5 % UA	10.15^{c}	1.92^{b}	57.20^{a}	139.21^{a}	1.40^{b}	0.262^{b}
HE+1 % UA	18.37^{a}	1.62^{c}	52.79^{b}	113.20^{b}	1.22^{d}	0.465^{a}
HE+ 1.5 %UA	15.93^{b}	1.77^{bc}	53.07^{b}	120.24^{b}	1.38^{c}	0.306^{b}
SE	1.18	0.09	0.82	4.23	0.02	0.02
P	**	**	*	**	**	**

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SE= standard error, MDA= malondialdehyde, GSH= glutathione, SOD= superoxide dismutase, CAT= catalase, GPx= glutathione peroxidase, NEFA= non-esterified fatty acids, **P<0.01.

Similar to this study, Yang et al. (2017) observed that serum MDA and NEFA levels increased in laying hens fed with high energy and protein feed. Sundaresan et al. (2014), in their study on mice that induced fatty liver syndrome with a high-energy diet, reported that free fatty acid levels increased significantly in mice fed high-energy ration compared to the control group, and the addition of ursolic acid to the diet significantly reduced these values.

Li et al. (2014) found that ursolic acid supplementation significantly lowered serum NEFA levels and increased SOD, MDA, CAT and GSH-PX values in mice with high-fat diet obesity. Researchers have suggested that the addition of ursolic acid increases the levels of b-hydroxybutyrate in the blood and, based on this result, ursolic acid may increase the oxidation of free fatty acids.

Average values of some plasma parameters are given in Table 6. As can be seen from Table 6, AST and HDL values were not affected by the treatment. Plasma VLDL, triglyceride and LDL values were significantly higher (P<0.01) in the HE + 0% UA and HE + 1.5% UA groups. The highest ALT and total cholesterol values were found in the HE + 1.5% UA group. Plasma glucose ratio decreased significantly (P<0.05) in the HE + 1.5% UA group.

In the present study, it was determined that the VLDL, TG and LDL values increased significantly in the high energy feed group, but the addition of 0.5% and 1% ursolic acid in the high energy feed decreased these rates, while the addition of 1.5% ursolic acid did not affect them.

Jia et al. (2015) reported that 200 mg of ursolic acid supplementation decreased plasma triglyceride and VLDL concentrations, increased HDL concentration, and did not affect the total cholesterol ratio in mice fed a high-fat diet. The high estrogen concentration in laying hens also promotes liver-transported TG synthesis in the form of VLDL (Zhu et al., 2013).

Table 6. Some blood plasma biochemistry parameters of laying hen

Groups	VLDL mg/dl	ALT U/L	AST U/L	Glucose mg/dl	Total cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Control	71.66[b]	3.00[b]	244.66	260.66[a]	124.00[b]	142.50[b]	46.00	89.00[b]
HE+ 0 % UA	329.50[a]	7.00[b]	265.66	248.00[a]	135.00[b]	1567.00[a]	42.66	206.50[a]
HE+ 0.5 % UA	52.00[b]	2.33[b]	186.50	264.00[a]	113.00[b]	258.50[b]	44.33	93.00[b]
HE+1 % UA	67.33[b]	2.00[b]	252.00	278.66[a]	115.00[b]	337.66[b]	53.66	105.56[b]
HE+ 1.5 %UA	337.66[a]	17.50[a]	236.00	235.66[b]	278.50[a]	1687.66[a]	45.66	183.00[a]
SE	39.07	1.92	15.53	5.73	19.25	199.98	3.38	24.85
P	**	*	ns	*	**	**	ns	**

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, VLDL= very low density lipoprotein, ALT= alanine aminotransferase, AST= aspartate aminotransferase, TG= triglyceride, HDL= high density lipoprotein, LD= low density lipoprotein, SE= standard error, *P<0.05, **P<0.01, NS= not significant

High-fat diet increases the amount of free fatty acids in plasma and leads to triglyceride accumulation in the liver (Yki-Järvinen, 2005). The liver plays a central role in maintaining systemic lipid homeostasis. Lipid balance is maintained by the regulation of lipogenesis and lipid oxidation, which are regulated by the cooperative effect of various enzymes and transcription factors found in the liver (Yki-Järvinen, 2005).

Peroxisome proliferator activated receptor (PPAR) is a major regulator of genes involved in fatty acid transport and utilization in the liver and mitochondrial and peroxisomal fatty acid-oxidation (Aoyama et al., 1998; Reddy, 2001). Activation of PPAR with synthetic or natural compounds increases cellular fatty acid uptake and subsequent oxidation rate (Motojima et al., 1998). Jia et al. (2011) reported that ursolic acid activates the nuclear receptor of the PPAR, and there is a decrease in lipid accumulations in hepatocytes through gelation of PPAR-responsive genes in hepatic lipid metabolism. Therefore, administration of PPAR agonists simultaneously improves lipid and glucose metabolism, decreases both plasma and hepatic triglyceride accumulation, increases glucose tolerance, and increases HDL cholesterol concentrations (Harano et al., 2006; Nakajima et al., 2009).

Yang et al. (2017) observed that the serum triglyceride, total cholesterol and LDL-cholesterol ratio increased significantly, while the HDL-cholesterol ratio decreased slightly in laying hens fed with high-energy feed. Jayaprakasam et al. (2006) reported that the addition of ursolic acid to a high-fat diet reduced serum triglyceride levels in mice.

Sundaresan et al. (2014) reported that ALT and AST values were significantly higher in mice fed with a high-energy diet with fatty liver syndrome compared to the control group, and ursolic acid added to the diet significantly reduced these values.

In the present study, it was determined that the addition of 1.5% ursolic acid to high-energy food significantly lowered the plasma glucose ratio. Jayaprakasam et al. (2006) reported that a high-fat diet increased glucose levels in mice, while the addition of ursolic acid significantly reduced this value. The same researchers stated that the passage of glucose into the blood is delayed by ursolic acid.

Due to the lack of a study on ursolic acid on laying hens and also the lack of up-to-date studies on fatty liver in laying hens in recent years, this study has not been discussed sufficiently.

4. Conclusion

In conclusion, it was determined that fatty liver syndrome occurs in laying hens fed with high energy feed. While 1.5% ursolic acid addition to high-energy diet positively affected performance values, it was determined that 0.5 and 1% ursolic acid supplementation significantly reduced liver fat ratio and plasma TG, LDL and VLDL values. As a result, it was concluded that the addition of ursolic acid to the feeds was successful in overcoming the diseases that may occur due to lipid metabolism in laying hens, as well as improving the performance values. In addition, this study will be a source for future studies on fatty liver disease.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	F.P	Ş.C.B.A
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The research protocol was approved and applied in accordance with the Animal Ethics Committee Guidelines of Atatürk University (protocol code: 2018/61 and date: 15 April 2018).

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