Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2020.17689.1545

Effects of Milk Thistle, Artichoke and Olive Extracts in Comparison with Atorvastatin and Gemfibrozil on Liver Function in Broiler Chicken

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Poultry Science Journal 2020, 8(1): 109-117

Keywords
Atorvastatin
Gemfibrozil
Broiler chicken
Herbal medicine
Hepatoprotection

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Article history
Received: February 7, 2020
Revised: April 13, 2020
Accepted: April 16, 2020

Abstract

One hundred forty four 10-day old male Arbor Acres (320±5g) broiler chicks were used to compare the responses in liver function to dietary supplementation of milk thistle (*Silybum marianum*), artichoke (*Cichorium intybus*) and olive (*Olea europaea*) extracts with gemfibrozil and atorvastatin, in broiler chickens fed with a lipogenic diet. Birds raised in the standard experimental conditions and received a pelleted grower diet (control) supplemented with milk thistle (250 mg/kg), artichoke (200 mg/kg), olive (150 mg/kg) extracts, atorvastatin (20 mg/kg) and gemfibrozil (1800 mg/kg) up to day 42 of age. Liver weight and liver fat parentages were 1.62 and 6.04%, respectively, in the birds fed with the un-supplemented basal diet (control) and modified by +0.21 and -2.13, +0.11 and -1.21, +0.14 and -1.40, -0.07 and -2.36, +0.07 and -0.38% in the birds received milk thistle, artichoke, olive extracts, gemfibrozil, and atorvastatin, respectively. The milk thistle-added diet elicited a significant reduction in serum activity of aspartate aminotransferase and alanine aminotransferase at day 35 of age. Dietary olive extract at 150 mg/kg reduced liver fat at day 35 of age compared with those grown on the basal diet ($P < 0.05$). The serum concentration of triglycerides was lower ($P < 0.05$) in the birds fed with diets enriched with artichoke (34.86 mg/dL) and olive extract (40.63 mg/dL) compared with those fed with the supplemented control diet at day 35 of age. It was concluded that milk thistle exerted greater promising hepatoprotective effects compared with other remedies. Gemfibrozil exhibited a greater hepatoprotective and blood lipid-lowering effect than atorvastatin in broiler chicken.

Introduction

Rudimentary lymphatic system and direct absorption of chylomicrons into the portal blood (Ayala *et al*., 2009) predispose chickens to fat deposition in the liver (Cherian *et al*., 2002). It was shown that chickens develop hepatic alterations consistent with steatohepatitis when they are fed a hyperlipidemic diet (Ayala *et al*., 2009; Makovick *et al*., 2011). A sedentary lifestyle and overfeeding due to free access to feed in commercial broiler flocks are two main common characteristics of chicken life. Xiao Quan *et al*. (2012), showed feeding a low-protein and high-energy diet induced fatty liver syndrome (FLS) in chicken. The same results also confirmed by Önel *et al*. (2017) who reported fatty liver developed in birds not able to move enough to burn the calories received.

Because the nutritional status of the bird influences lipid metabolism in the liver (Hillgartner *et al*., 1995), scientific attempts are mainly directed at identifying potential dietary therapies for FLS. During the last decade, natural products, in particular medicinal plants, have gained increasing consideration to treat various metabolic disorders such as liver diseases, principally because of their multiplex properties and minimal adverse effects on the body. The addition of herbal remedies having lipotropic properties to poultry feed may decrease adverse metabolic consequences of the commonly used high-calorie diets because these additives alleviate fat amassing in the liver (Khosravinia *et al*., 2015). Antioxidant compounds, as another positive aspect of phytogenic extract, which

Please cite this article as Tavakolinasab F, Khosravinia H & Masouri B. 2020. Effects of Milk Thistle, Artichoke and Olive Extracts in Comparison with Atorvastatin and Gemfibrozil on Liver Function in Broiler Chicken. Poult. Sci. J. 8(1): 109-117.

modulates lipid oxidation, peroxidation, and perhaps inflammation, represent another attractive therapeutic approach for animal models suffering from hepatic steatosis (Ferramosca *et al*., 2017). Despite the several reports on the effects of plant-derived products on lipid metabolism and hepatic function, there are few reports on a comparison of their effects with the common synthetic pharmaceuticals prescribed for the same purpose in human clinical cases. Therefore, this study aimed to evaluate the responses in liver function to dietary supplementation of milk thistle (*Silybum marianum),* artichoke (*Cynara scolymus L*.) and olive (*Olea europaea*) extracts in comparison with the well-known synthetic therapeutic medications, gemfibrozil, and atorvastatin, in broiler chickens fed with a high energy and low protein lipogenic diet.

Materials and methods

Birds and diets

A total of 144 healthy and uniform 10-day old male Arbor Acres broiler chicks with an average weight of 320±5 g were randomly distributed to 96 wire cages of three birds each, where they acclimatized for 3 days. At the commencement of the day 14, birds weighed individually and subjected to one of the six experimental treatments concerned up to day 42 of age. Experimental treatments consisted of; 1) a basal grower diet (Table 1; formulated based on the nutrient recommendations by the producer company for strain concerned with slight modifications toward a lipogenic diet) in pelleted form, 2) the basal diet supplemented with milk thistle extract (250 mg/kg), 3, 4, 5 and 6) the same basal diet supplemented with either artichoke extract (200 mg/kg), olive extract (150 mg/kg), atorvastatin (20 mg/kg) or gemfibrozil (1800 mg/kg). The extracts and the pharmaceuticals used were blended with warm water and sprayed on the pelleted feed immediately before feeding twice a day at 6 a.m. and 6 p.m. The effect of each treatment was examined in 12 replicates of two birds each. Ambient temperature was maintained at 28°C for the first week and then gradually reduced by 3°C until a temperature of 22°C was achieved at the end of the fourth week and then maintained constant thereafter. A 23:1h light to darkness lightening regimen was followed throughout the experimentation period. Feed and water were supplied to the birds in each cage using a trough feeder and waterer located on the opposite sides of the cages, for *ad libitum* consumption throughout the experimentation period. During the experiment, no medication or antibiotic was given to the birds. Vaccination was performed against influenza, infectious bursal, and bronchitis viruses in day 21 of age.

Table 1. Ingredients and nutrient composition of basal diets

Ingredients (%)	Starter (1-10 day)	Grower (11-42 day)
Yellow maize	58.34	63.08
Soybean meal	36.38	29.36
Soybean oil	1.50	4.10
Calcium phosphate	1.24	1.40
$CaCO_3$	1.34	1.20
DL-Methionine	0.28	0.18
L-Lysine HCL	0.28	0.07
Salt	0.14	0.19
Mineral Premix[1]	0.25	0.25
Vitamin Premix [2]	0.25	0.25
Nutrient composition		
ME (Kcal/kg)	3000	3176
Crude protein (%)	21.50	17.00
Lysine (%)	1.44	1.00
Methionine (%)	0.56	0.50
Methionine + Cystine (%)	1.08	0.55
Threonine (%)	0.97	0.72
Calcium (%)	096	0.80
Available P (%)	0.48	0.41
Sodium (%)	0.20	0.20
Potassium (%)	0.80	0.76
Chlorine (%)	0.20	0.20

[1 and 2] Each kilogram contains Vitamin A, 12000 international units; vitamin D3; 5000 international units; vitamin E 80 units; vitamin K3; 3.2 mg; vitamin B1; 3.2 mg; vitamin B2; 8.6 mg ; vitamin B3; 20 mg; vitamin B5; , 65 mg; vitamin B6; 4.3 mg; vitamin B9; 2.2 mg; vitamin B12; 0.017 mg; vitamin H2; 0.30 mg; choline chloride; 1700, mg; 1000 mg antioxidant; 120,000 mg manganese; ; Zinc; 110000 mg; copper; 16,000 mg; selenium; 300 mg; iodine; 1250 mg; iron; 20,000 mg.

Extracts preparation

Milk thistle extract provided by Zardband Pharmaceuticals Co, Tehran-Iran. The product was a commercially available extract which contained silybins A (11.52%) and B (13.92%), the isosilybins A (16.43%), and B (17.81%),

silychristin A (6.68%), isosilychristin (5.31%), silydianin (8.51%) and silymarin (80.18%). Artichoke extract was supplied by Soha Jissa Co, Mazandaran-Iran, and its analysis revealed that it comprises caffeoylquinic acids (4.6421%), luteolin-7-glucoside (0.1703%), luteolin (0.1410%), apigenin-7-glucoside (0.7361%), caffeic acid (0.6332%), chlorogenic acid (2.4731%), eugenol (4.8512%) and rosmarinic acid (trace amount). Olive extract (freeze-dried) provided from Dana Kasian Co, Khorammabad, Lorestan, Iran, and contained 41.09% oleuropein as the major polyphenolic compound, followed by oleic acid (28.93%) palmitic acid 10.15%, linoleic acid 7.10%, octadecadienoic acid 5.12%, stearic acid 4.65%, palmitoleic acid 0.98% and tridecanoic acid 1.98%. The capsules of gemfibrozil (300 mg) were produced by Toliddaru Pharmaceutical Co, Tehran-Iran and the atorvastatin tablets (20 mg) provided form Arya Pharmaceutical Co, Tehran-Iran.

Blood collection and analyses

Individual samples of uncoagulated whole blood were collected from the slaughtered birds and centrifuged at 1800× g for 15 min and 5ºC. The average volume of 4 to 5 mL clear and non-haemolysed serum sample was collected for each bird and stored at -20°C pending biochemical assessments. Concentrations of serum biochemical constituents, including glucose (GLU), triglycerides (TG), total cholesterol (TC) and lowdensity lipoprotein cholesterol (LDL), total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB), total protein (TP), high-density lipoprotein cholesterol (LDL), and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) were determined using an autoanalyzer (Clima; Ral. Co, Barcelona, Spain).

Liver parameters and scoring system

At the end of days 35 and 42 of age, one bird was taken randomly from each replicate and slaughtered by puncturing the jaguar veins and carotid arteries. The slaughtered chicken dissected to collect the data on abdominal fat percentage, liver weight, apparent liver score, and liver fat content. The liver color was appraised based on a 4-grade scoring method by assigning a score from 0 to 3; 0 indicating normal liver color and 3 indicating putty-colored livers as described by Trott *et al*. (2014) with slight modifications. Liver lipid content was determined using Folch *et al.* (1956) method. Briefly, a sample of one g liver tissue was homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (1 g in 20 mL of the solvent mixture). After dispersion the whole mixture agitated for 20 min in an orbital shaker at room temperature and then allowed to stand with agitation for 2h. The homogenate was filtered through Whatman #1 filter paper into a 100-mL 54 graduated cylinder, and then 5 mL of 7.3% Potassium chloride solution was added and mixed. After phase separation, the top layer was collected. Total lipids were determined gravimetrically after evaporating the solvent. The sample was then dried and weighed and expressed as the percentage of liver fat against the total liver weight.

Statistical analysis

A complete randomized block design was used to evaluate the response of broiler chickens to six experimental treatments. The blocks were 12 rows of cages perpendicular to airflow direction in the experimental house. All data were analyzed using PROC Mixed in Statistical Analysis System, version 9.1 (2003). The Tukey test was used for multiple treatment comparisons (Kramer, 1956). Liver health scores were subjected to frequency analysis using PROC FERQ in the same statistical analysis software (SAS Institute, 2003). For all tests, the maximum likelihood for type-I error was declared at 5% ($P < 0.05$).

Results

Plasma TG level was declined ($P < 0.05$) in the birds fed with the artichoke- (34.86 mg/dL) and olive-supplemented (40.63 mg/dL) diets compared with those fed with the control diet at 35 days of age. In contrast, feeding the gemfibrozil- containing diet increased plasma TG level by 10.37 mg/dL compared with the control diet ($P < 0.05$). At the same age, the serum concentration of GLU decreased by 23.99 and 26.83 mg/dL in broiler chicken grown on the artichoke- and silymarin–added diets, respectively, compared with those fed with the control diet (191.23 mg/dL) ($P < 0.05$; Table 2).

At 42 days of age, the plasma TG level was lesser in the birds fed gemfibrozil (42.79 mg/dL), while it was greater in silymarin-supplemented diets (44.48 mg/dL) than the birds are grown on the other supplemented diets ($P < 0.05$). At the same age, plasma TC level was greater (239.10 mg/dL) in the birds grown on the control diet and declined in the birds feeding atorvastatin, milk thistle and artichoke diets by 71.54, 60, 57.1 mg/dL, respectively ($P < 0.05$). At day 42 of age, the abdominal fat percentage was greater by 0.52% and 0.45% in the pelleted diet-fed birds, than those received diets containing milk thistle and gemfibrozil, respectively ($P < 0.05$; Figure 1).

Table 2. Mean serum concentration of triglycerides (TG), cholesterol (TC), glucose (GLU) and low-density lipoprotein (LDL) in the chickens received diets supplemented with plant extracts and synthetic lipotropic additives in days 35 and 42 of age

Treatments	35 d				42d	
	TG, mg/dL	TC, mg/dL	GLU, mg/dL	LDL, mg/dL	TG, mg/dL	TC, mg/dL
Control	150.13^{ab}	197.50	191.23^{a}	45.12	133.22^{ab}	239.10^{a}
Silymarin, 250 mg/kg	141.10^{ab}	214.00	164.40^{b}	50.20	177.70^{a}	179.10^{b}
Artichoke, 200 mg/kg	115.27^{b}	194.73	167.64^{b}	42.72	114.20^{bc}	182.00^{ab}
Olive, 150 mg/kg	109.50^{b}	205.30	175.10^{ab}	47.30	110.22^{b}	196.11^{ab}
Gemfibrozil, 1800 mg/kg	160.50^{a}	231.17	196.83^{a}	40.66	90.43^{c}	213.30^{ab}
Atorvastatin, 20 mg/kg	142.44^{ab}	201.56	183.11^{ab}	40.44	152.89^{abc}	167.56^{c}
SEM	16.141	13.368	44.131	4.573	17.071	19.017
P-Value	0.0390	0.3221	0.0213	0.3944	0.0014	0.0013

^{abc} Means within a column, with no common superscript, differ significantly ($P < 0.05$).
SEM= Standard Error of the Mean

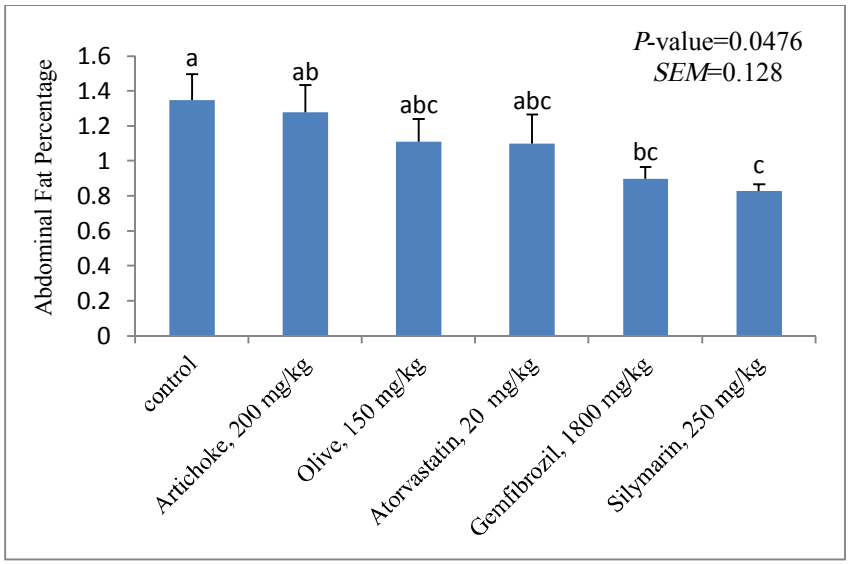

Figure 1. Mean (±SE) abdominal fat percentage in the chickens received diets supplemented with plant extracts and synthetic lipotropic additives in day 42 of age.

Liver fat percentage was 6.04% in the birds grown on control diet in day 35 of age ($P < 0.05$) and dietary olive extract reduced liver fat by 1.58% at the same age ($P < 0.05$). At the 42 days of age, the birds fed with the control diets showed a greater liver fat percentage ($P < 0.05$), but the inclusion of the control diet with silymarin and gemfibrozil significantly decreased liver fat by 2.13 and 2.36%, respectively ($P < 0.05$; Table 3).

Table 3. Mean proportional and absolute liver weight and fat content in the chickens received diets supplemented with plant extracts and synthetic lipotropic additives in days 35 and 42 of age

Treatments	35d			42d		
	Liver, %	Liver, g	Liver fat, %	Liver, %	Liver, g	Liver fat, %
Control	1.71	21.81	6.04^{a}	1.62	25.00	6.40^{a}
Silymarin, 250 mg/kg	2.05	24.99	5.23^{ab}	1.83	27.30	4.27^{b}
Artichoke, 200 mg/kg	2.00	23.52	4.92^{ab}	1.73	28.55	5.19^{ab}
Olive, 150 mg/kg	1.97	22.96	4.46^{b}	1.76	28.04	5.00^{ab}
Gemfibrozil, 1800 mg/kg	1.84	24.14	4.94^{ab}	1.55	24.46	4.04^{b}
Atorvastatin, 20 mg/kg	1.68	22.07	4.79^{ab}	1.69	29.00	6.02^{ab}
SEM	0.125	1.302	0.369	0.136	1.718	0.720
P-Value	0.3447	0.3461	0.0251	0.9748	0.7485	0.0005

$^{a-b}$ Means within a column, with no common superscript letter, differ significantly ($P < 0.05$).
SEM= Standard Error of the Mean.

The mean serum activity of AST and ALT was greater in the broilers fed with the control diet in day 35 of age ($P < 0.05$). Inclusion of silymarin in the control diet significantly reduced serum activities for both enzymes at the same age ($P < 0.05$; Table 4). In day 42, serum AST activity was greater in the birds maintained on the control diet (1.736 U/L) than those received atorvastatin supplemented diet. Feeding the control diet also increased serum LDH activity at the same age ($P < 0.05$). Inclusion of gemfibrozil into the control diet decreased LDH activity by 993 and 964 U/L compared with those fed with control and silymarin containing diets, respectively ($P < 0.05$; Table 4).

Table 4. Mean serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in the chickens received diets supplemented with plant extracts and synthetic lipotropic additives in d ays 35 and 42 of age

Treatments	35d			42d		
	ALT (IU/l)	AST (IU/l)	LDH (IU/l)	ALT (IU/l)	AST (IU/l)	LDH (IU/l)
Control	300.33^{a}	8.000^{a}	4717.5	232.75	6.625^{a}	2788.8^{a}
Silymarin, 250 mg/kg	212.67^{b}	4.538^{b}	3799.7	238.45	5.545^{ab}	2760.1^{a}
Artichoke, 200 mg/kg	246.42^{ab}	6.750^{ab}	4628.0	247.90	6.100^{ab}	2370.5^{ab}
Olive, 150 mg/kg	277.50^{ab}	6.833^{ab}	4300.7	231.22	5.222^{ab}	2215.7^{ab}
Gemfibrozil, 1800 mg/kg	269.42^{ab}	7.333^{ab}	4546.1	247.00	6.000^{ab}	1795.8^{b}
Atorvastatin, 20 mg/kg	254.89^{ab}	6.000^{ab}	4740.8	232.78	4.889^{b}	2567.1^{ab}
SEM	0.803	25.416	319.895	12.760	0.580	256.121
P-Value	0.0205	0.0030	0.4357	0.8519	0.8065	0.0178

$^{a-b}$ Means within a column, with no common superscript letter, differ significantly ($P < 0.05$).
SEM- Standard Error of the Mean.

The serum concentration of TBIL increased in the birds fed with the gemfibrozil-containing diet compared with the other birds at day 35 of age ($P < 0.05$). The serum concentration of DBIL and ALB reduced in the birds receiving the silymarin-supplemented diet, whereas feeding the gemfibrozil–added diet significantly increased both parameters at the same age ($P < 0.05$). At the end of the experiment, serum ALB concentration was further increased in the birds received the control diet ($P < 0.05$).

Supplementation of the diet with artichoke and olive extract as well as atorvastatin significantly decreased serum ALB ($P < 0.05$). Serum TP concentration increased in the birds grown on the control diet compared with those fed with the plant extracts and atorvastatin supplemented diets at day 42 of age (Table 5).

Table 5. Mean serum concentrations of total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB) and total protein (TP) in the chickens received diets supplemented with plant extracts and synthetic lipotropic additives in days 35 and 42 of age

Treatments	35d				42d			
	TBIL, mg/dL	DBIL, mg/dL	ALB, g/dL	TP, g/dL	TBIL, mg/dL	DBIL, mg/dL	ALB, g/dL	TP, g/dL
Control	0.375^{b}	0.079^{ab}	1.838^{ab}	5.61	0.350	0.057	2.262^{a}	5.11^{a}
Silymarin, 250 mg/kg	0.400^{b}	0.061^{b}	1.633^{b}	4.72	0.336	0.056	2.127^{ab}	4.42^{b}
Artichoke, 200 mg/kg	0.433^{b}	0.075^{ab}	1.800^{ab}	4.72	0.440	0.058	2.100^{b}	4.31^{b}
Olive, 150 mg/kg	0.416^{b}	0.070^{ab}	1.791^{ab}	4.53	0.344	0.056	2.077^{b}	4.61^{b}
Gemfibrozil, 1800 mg/kg	0.555^{a}	0.085^{a}	1.988^{a}	5.06	0.400	0.057	2.112^{ab}	4.85^{ab}
Atorvastatin, 20 mg/kg	0.400^{b}	0.085^{a}	1.833^{ab}	4.61	0.333	0.054	2.088^{ab}	4.35^{b}
SEM	0.034	0.007	0.093	0.221	0.045	0.003	0.068	0.390
P-Value	0.0391	0.6719	0.0253	0.3944	0. 0835	0.923	0.0224	0.0001

$^{a-b}$ Means within a column, with no common superscript letter, differ significantly ($P < 0.05$).
SEM: Standard Error of the Mean.

Apparent liver health was appraised based on liver color and scored from 0 to 3 ($P < 0.05$; Table 6). At day 35 of age, the relative frequency of scores 2 and 3, were greater for control birds and significantly reduced in those receiving diets supplemented with all feed additives. Birds receiving silymarin- and Atorvastatin-added diets, in particular, showed improved liver health at the same age indicating by no liver with score 3 for the same birds. At the advanced age (42 d), a prominent hepatoprotective effect was realized for all additives where greater frequency for scores 2 and 3 in control birds significantly minimized in all birds receiving the supplemented diets (Table 6).

Table 6. Frequency of liver scores in broilers chickens received diets supplemented with plant extracts and synthetic lipotropic additives in days 35 and 42 of age

	The liver score, 35 d				The liver score, 42 d			
Treatment	score 0	score 1	score 2	score 3	score 0	score 1	score 2	score 3
Control	20.00	17.86.	22.69	34.33	00.00	10.4	77.78	100.00
Silymarin, 250 mg/kg	00.00	21.42	18.69	00.00	23.08	20.69	00.00	0.00
Artichoke, 200 mg/kg	20.00	25.00	10.34	16.67	19.23	20.69	11.11	0.00
Olive, 150 mg/kg	60.00	14.29	10.34	32.33	15.38	24.14	11.11	0.00
Gemfibrozil, 1800 mg/kg	00.00	10.71	17.24	16.67	19.23	13.79	00.00	0.00
Atorvastatin, 20 mg/kg	00.00	10.71	20.69	00.00	23.08	10.34	00.00	0.00
P-value	0.0404	<0.001	<0.001	0.0352	<0.001	<0.001	<0.012	0.0833
Chai-square	10.00	140.00	145.00	18.00	104.00	145.00	18.00	2

Discussion

Normal fat percentage in broiler chicken liver tissue has been reported in a range of 2 to 5% (Alshamy *et al*., 2019), and a liver fat percentage beyond 5% has been described as FLS (Corey and Chalasani, 2014). In the current study, the formulation used for the basal grower diet commendably induced FLS, evidenced by increased liver fat, serum activity of liver enzymes (ALT and AST) and serum concentration of TP, ALB, TBIL, TG and TC at days 35 and/or 42 of age. All these indications were anticipated in the birds fed with our lipogenic pelleted diet. In other words, we succeed to induce FLS in the experimental flock by feeding the lipogenic grower diet. Therefore, we hypothesized that administration of the selected herbal remedies and two commonly used chemical medications (gemfibrozil and atorvastatin) may modulate the adverse effects of the highly digestible pelleted diet on liver function in broiler chicken. Our expectation realized, however, neither consistent alteration in all parameters evaluated were observed in favor of a single medication nor changes revealed for a selected medication could be manifested in the two recording ages and an obvious pattern.

Supplementation of milk thistle in the basal diet increased LDH, TC, and TG, while significantly decreased liver fat, abdominal fat and simultaneously reduced serum concentrations of ALB, TP, and activity of the enzymes concerned. These outcomes, in part, agrees Gawel *et al*. (2003), findings who observed increased slaughter weight in chicken and turkeys maintained on diets containing silymarin. Metwally *et al*. (2009) also observed reduced serum total lipids, TC, and TG in rats fed on a high cholesterol diet following treatment with silymarin (25, 50, and 100 mg/kg). Previous scientific evidence demonstrated that cholesterol and TG- lowering effects of silymarin may result from its active compounds which act as inhibitors to hepatic 3-hydroxyl, 3-methyglutaryl coenzyme-A reductase (HMG-CoA reductase) enzyme in the mevalonate pathway, where it operates as the rate-limiting step in the biosynthesis of cholesterol and isoprenoids (Nagashima *et al*., 2012). It has been demonstrated that elevated liver enzymes activity is directly associated with higher concentrations of inflammatory markers such as C-reactive protein (Kulkarni *et al*., 2012) and therefore it seems that the impact of silymarin on decreasing liver enzymes can be mediated by its suppressing effects on inflammatory biomarkers. Also, silymarin enhances hepatic glutathione generation by inspiring cysteine availability and inducing cysteine synthesis while inhibiting its catabolism to taurine, the regulation of cysteine synthesis may subsequently contribute to the antioxidant defense (Kwon *et al*., 2013).

In the current study, the inclusion of olive extract in the basal diet decreased liver fat by 1.60% compared with those fed with the basal diet. Moreover, the serum concentration of TP, TG, abdominal fat, and liver enzymes lowered in the same birds, the evidence which encouraged us to declare the possible hepatoprotective effects for olive extract. Tufarelli *et al*. (2016), found increased antioxidant activity indicated by reduced lipid peroxidation in the chicken liver following dietary supplementation of 2.5% olive oil. Our results are in the line with the finding of (Wani *et al.* 2015), who reported that rats maintained on a lipogenic diet possess an increased number of lipid droplets in the liver sections as compared to olive oil-treated animals. In their study, supplementation of olive oil decreased serum TG, normalized the liver enzyme biomarkers, and significantly reduced the fat droplet accretion in the liver by suppressing the inflammation and restoring the abnormal lipid metabolism in experimental animals. Results of the current study suggest olive oil may target energy homeostasis mechanisms particularly in the liver and reduce hepatic lipid droplet amassing. The hepatoprotective and lipid/cholesterol-lowering effects of the olive extract are thought to come from oleuropein, a predominant antioxidant substance in olive leaves (Andrikopoulos *et al*. 2002). Moreover, olive oil can reduce the intestinal absorption of cholesterol, or decrease its synthesis by the liver (Krzeminski *et al*., 2003).

Inclusion of artichoke extract into the basal diet decreased liver fat by about 1.21% and exerted

positive effects on certain serum indicators as specified by decreased serum concentrations of TG, TC, and TP compared with those fed with the non-supplemented control diet. Moreover, artichoke extract had a non-significant positive effect on decreasing liver score and abdominal fat compared with the basal diet. Azcona *et al*. (2005) reported higher metabolizable energy from a diet when it was supplemented with artichoke extract during the first 21 days of the broiler's life. These authors explained such beneficial effects by a higher lipid digestibility in the same birds due to an increased bile secretion due to the metabolic effects of cynarin (a hydroxycinnamic acid) as the major biologically active chemical constituent in artichoke. Tang *et al*. (2017) observed that serum concentration of AST and TG decreased by the inclusion of 1.6 g/kg BW artichoke into a broiler diet. Artichoke extracts mechanism of hepatoprotective and lipid-lowering effect likely results from interactions of luteolin with HMG-coA reductase, liver sterol regulatory element-binding proteins, and acetyl CoA C-acetyltransferase (Gebhardt, 2002), as well as potentially lowering cholesterol effect through increased fecal excretion of bile salts (Qiang *et al*., 2012).

An attractive part of our study was the comparisons between medical remedies with the commonly used synthetic medications in human treatment for their possible hepatoprotective properties. In the present study, the administration of atorvastatin through the basal diet reduced abdominal and liver fat percentage in broiler chicken in day 42 of age. Studies with statins and their effects on poultry species are scanty. In the study of Alvin *et al*. (2012) no significant effect of atorvastatin on BIL, ALB, and TP were observed in patients of hyperlipidemia. However, it has been shown that statins upregulate LDL-receptors in cells therefore even more lipids may enter the cell and subsequently its mitochondria (Arslan et al., 2003). Jafari Golrokh *et al*. (2016) reported a decrease in serum TG and TC concentrations in response to dietary supplementation with atorvastatin (2 g/kg) in broiler chickens.

Inclusion of gemfibrozil into the diet significantly decreased liver and abdominal fat percentage in broiler chicken at days 35 and 45 of age. Gemfibrozil impedes fatty acid metabolism to influence lipid redistribution in favor of increased overall intramuscular fat, a phenomenon that improves carcass quality and nutritive value in broilers (Farrokhyan *et al*., 2014). Additionally, gemfibrozil is known as an activator of peroxisome proliferator-activated receptor-alpha (PPARα), a nuclear receptor involved in the metabolism of carbohydrates and fats, as well as adipose tissue differentiation (Farrokhyan *et al*., 2014). Gemfibrozil is used therapeutically to decline blood TG and TC concentrations in humans. There are evidence displaying gemfibrozil administration can raise serum AST activity in human and rat (Kolovou *et al*., 2004). We do not confirm the same effects in broiler chicken, as no increase in serum ALT and AST activity observed in the birds grown on the gemfibrozil-added diet.

Considering the whole results, milk thistle, olive, and artichoke extracts, as well as gemfibrozil and atorvastatin, demonstrated hepatoprotective, lipid, and cholesterol-lowering effects in broiler chickens fed with a lipogenic diet in a high digestible (pelleted) physical form. However, among plant remedies examined, milk thistle showed greater promising effects evidenced by 2.13, 0.52, and 31.65 reductions in the fat liver, abdominal fat, and serum concentrations of TC compared with the birds receiving the basal diet, respectively. Similarly, gemfibrozil exhibited a greater hepatoprotective and blood lipid-lowering effect than atorvastatin and all three herbal extracts used, except for milk thistle which their effects were comparable. Statins are effective lipid-lowering agents, associated with a depressing the risk of cardiovascular events in several interventional randomized clinical trials with human cases (Pastori *et al*., 2015). However, our results showed no priority for atorvastatin over gemfibrozil for their hepatoprotective effects in broiler chickens grown on a lipogenic diet.

Despite the current therapeutic developments, more attention has presently been shifted towards plant origin therapies as the possible means of alleviating FLS and its associated symptoms (Xiao *et al*., 2013). We agree such ascended concerns phytogenic products are easily available, cost-effective, and convenient and exert minimal side effects compared to the synthetic medications. Our results suggested all three remedies used as potential candidates for the same purpose. However, the outcome acts in favor of milk thistle, commented by many beneficial effects on the reduced-fat liver, serum activity of liver enzymes, and certain serum biochemical markers in the birds maintained on a lipogenic pelleted diet.

Conclusion

Based on the results achieved all herbal remedies used viz. milk thistle, olive, artichoke extracts as well as the two commonly prescribed synthetic medications (gemfibrozil and atorvastatin) demonstrated considerable hepatoprotective, lipid and cholesterol-lowering effects in broiler chickens maintained on a pelleted lipogenic diet. However, milk thistle showed greater promising effects compared with other remedies. Gemfibrozil exhibited a greater hepatoprotective and blood lipid-lowering effect than atorvastatin and all three herbal remedies.

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