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Research Article
Effect of Soy Lecithin on Growth Performance, Nutrient Digestibility and Hepatic Antioxidant Parameters of Broiler Chickens

[1]Farman Ali Siyal, [2]Mohamed Ezzat Abd El-Hack, [2]Mahmoud Alagawany, [1]Chao Wang, [1]Xioli Wan, [1]Jintian He, [3]Mingfa Wang, [1]Lili Zhang, [1]Xiang Zhong, [1]Tian Wang and [4]Kuldeep Dhama

[1]College of Animal Science and Technology, Nanjing Agricultural University, 210095 Nanjing Jiangsu, China
[2]Department of Poultry, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt
[3]Henan Academy of Agricultural Sciences, Institute for Animal Husbandry and Veterinary Research Zhengzhou, Henan, China
[4]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, 243122 Uttar Pradesh, India

Abstract
Background and Objective: Lecithin is a feed supplement and dietary source of several active compounds; therefore, this study evaluated the Soybean Lecithin (SL) in broiler diets by measuring performance, nutrient utilization, serum parameters and hepatic antioxidant status. **Methodology:** About 216 days old Arbor Acre broiler chicks were allotted into three groups as follow: the first group was fed a Basal Diet (BD) without emulsifier; the second and third groups were fed basal diet supplemented with 0.05 (SL0.05) and 0.1% (SL0.10) of SL, respectively. **Results:** During starter, grower and overall period, chicken fed with SL has better daily gain and feed intake compared with control, while feed conversion was improved in SL0.10 throughout the experiment compared to SL0.05 and control. At 21 and 42 days old, SL0.10 showed highest relative liver weight compared to SL0.05 and control ($p<0.05$). On day 21, digestibility of dry matter, ether extract and protein in chickens fed diet with SL0.10 was significantly improved in comparison with those fed SL0.05 and control. Cholesterol, triglyceride and low density lipoprotein concentrations were decreased in SL0.10 group in comparison with control. Serum glucose was higher in SL0.10 group compared to SL0.05 and control. Feeding SL0.10 resulted in the decreased hepatic malondialdehyde content and remarkably increased catalase, total superoxide dismutase and total antioxidant capacity enzyme activities. **Conclusion:** Feeding soy lecithin at 0.10% improved performance, reduced cholesterol and triglyceride, LDL-cholesterol concentrations in serum broilers. In addition, soy lecithin is suitable for improving antioxidant status and has ability to protect against oxidative stress.

Key words: Soy lecithin, performance, antioxidant capacity, serum parameters, broilers

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Corresponding Author: Tian Wang, College of Animal Science and Technology, Nanjing Agricultural University, 210095 Nanjing Jiangsu, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lipids are important components and play vital functions in animal's body. Feed contains oils and fats that are rich in energy with highest number of energy bonds per unit of weight[1]. The oils and fats of natural resources are incorporated in poultry feed to enhance the energy contents as a result growth performance can be attained along with industry needs[2]. However, fat utilization, their proper level and its digestibility varies in poultry with the age of the birds due to lack of several digestive enzymes. Fats are water-insoluble; therefore an emulsion step is required in the absorption of fat. Emulsifier is a molecule which maintains the oil droplets in the emulsion distributed which is better for the utilization and absorption of lipids.

In previous studies, using bile salt was reported to improve emulsions and digestibility of fat in broilers[3,4]. Similarly, soy-lecithin not only provides energy to broilers but also serves as an emulsifier to improve digestibility of dietary fats. Hertrampf [5] claimed that nutrient digestibility in poultry improved by feeding diets enriched with lecithin. Moreover, soy-lecithin is also very popular for its beneficial effects in lowering blood cholesterol[6,7]. While others reported that lecithin did not bring any significant change in the performance of broiler[8]. However, the relevant study for the effect of soy lecithin on antioxidant enzymes activities of broiler chickens is scarce. Therefore, the objectives of this study were to estimate the impacts of soy lecithin (emulsifier) supplementation on growth performance, relative weight organs, nutrient digestibility, blood constituents, lipids metabolism and hepatic antioxidant capacity parameters of broiler chickens.

MATERIALS AND METHODS

A total of 216 day-old Arbor Acre broilers were used in a 42 days experiment. Broilers were allotted into three groups with six replicates per group (12 chicks/replicate). The three groups as follow: the first group was fed a Basal Diet (BD) without emulsifier; the second and third groups were fed basal diet supplemented with 0.05 (SL0.05) and 0.1% (SL0.10) of Soy Lecithin (SL), respectively. The basal diet was formulated to meet broiler requirements (Table 1). Each treatment group contained 50% male and 50% female. The chickens were given starter feed til 21 days and grower feed from 21-42 days. Both feed and water were accessible to the birds on an *ad libitum* basis in a controlled room (34-36°C) temperature during 1-14 days and later on decreased up to 26°C till the end of the trial with a 12 h light-dark cycle (06:00-18:00 h light). The experiment was handled according to the guidelines of Animal Care and Use Committee of Nanjing Agricultural University.

Table 1: Formulation and calculated composition of basal diets (as-fed basis)

Items	Starter (1-21days)	Finisher (21-42 days)
Ingredients (%)		
Corn (7.90%)	54.10	64.15
SBM dh (49.8)	35.64	27.75
Palm oil	5.51	4.16
Mono-calcium phosphate	1.87	1.45
Limestone (39%)	1.24	1.01
Salt	0.38	0.28
Sodium bicarbonate	0.15	0.15
Choline chloride (60%)	0.08	0.05
BS premix	0.20	0.20
L-lysine HCl	0.16	0.18
DL-methionine	0.26	0.20
L-threonine	0.01	0.02
Pellet binder	0.30	0.30
Toxin binder	0.05	0.05
Cygro	0.05	0.05
Total	100.00	100.00
Calculated value		
Crude protein (%)	22.10	20.30
Ether tract (%)	5.48	6.13
Dry matter (%)	89.74	88.12

Provided per kg of diet; Iron: 60 mg, Copper: 7.5 mg, Zinc: 65 mg, Manganese: 110 mg, Iodine: 1.1 mg, Selenium: 0.4 mg, Bacitracin zinc: 30 mg, Vitamin A: 4500 IU, Vitamin D3: 1,000 IU, Vitamin E: 20 mg, Vitamin K: 1.3 mg, Vitamin B1: 2.2 mg, Vitamin B2: 10 mg, Vitamin B3: 10 mg, Choline chloride: 400 mg, Vitamin B5: 50 mg, Vitamin B6: 4 mg, Biotin: 0.04 mg, Vitamin B11: 1 mg and Vitamin B12: 1.013 mg

Growth performance: On 21 and 42 days of age, broilers were weighed and Feed Intake (FI) was recorded to evaluate Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) for all phases of feeding. At the end of 3rd and 6th weeks of feeding trial, the birds were restricted from feed for 12 h but had free access to water, later on, one bird per replicate was randomly chosen, weighed and sacrificed by bleeding of jugular vein. After decapitation, the visceral organs (liver, thymus, bursa, pancreas and spleen) were collected and weighed. The relative weights of mentioned organs were immediately calculated and expressed as relative to BW (g of organ/kg of BW).

Serum measurements: At 42nd day of the trial, one bird per replicate was randomly selected and sacrificed to get blood. The samples were centrifuged at 1,500×g 4°C for 15 min to get the serum and frozen at -20°C for further analysis. The concentrations of serum triglyceride (TG), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) were determined by using the corresponding diagnostic kits obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China, whereas the concentrations of total protein, glucose, urea and creatinine were calculated enzymatically by using an automatic biochemical analyzer (Olympus AU-800, Olympus, Tokyo, Japan).

Nutrient digestibility: On day 19-21, birds (6 chickens from each group) were kept into individual cages, trays were placed under each cage and 3-day total collection of excreta was performed. Before the collection, birds were restricted from feed for 12 h with free access to water and were fed with experimental diets *ad libitum* for 3 days. To avoid contamination, scales and feathers were carefully removed from the trays. The fecal samples were collected and stored at -20°C for each day. After 3 days excreta collection period, feed intakes were recorded and excreta of each cage were mixed. Mixture of samples were taken and dried in an oven at 65°C for 24 h and grounded with a laboratory mill fitted through 1 mm screen. On day 40-42, another 3 days total collection period for excreta was conducted in the same way. Samples of feed and excreta were analyzed for Gross Energy (GE), Dry Matter (DM), Crude Protein (CP) and Ether Extract (EE) according to standard method of AOAC[9].

Determination of hepatic antioxidant enzymes: About 0.3 g liver was taken to make homogenate. The minced liver tissue sample was homogenized in ice-cold 0.9% sodium chloride buffer (w/v, 1:9) using an Ultra-Turrax homogenizer and then centrifuged at 4000 rpm for 10 min to obtain the supernatant and was frozen at -80°C for further analysis. The activities of total antioxidant capacity (T-AOC), catalase (CAT), total superoxide dismutase (T-SOD) enzymes and the content of malondialdehyde (MDA) in the liver was determined by using kits (Nanjing Jiancheng Bioengineering Institute, China). The enzyme activities of liver T-SOD, T-AOC and CAT were expressed in U per milligram of protein and liver MDA content was expressed as Nano moles per milligram of protein[10,11]. Bicinchoninic Acid Assay (BCA) was used to determine the protein concentration in the liver homogenates.

Statistical analysis: Data were analyzed by one-way ANOVA. Duncan's test was used to identify significant difference among the means at p<0.05. All the analysis were executed using SPSS software (Version. 20). Cage means were used as experimental units in growth experiment, while individual observations were used as experimental units in other experiments.

RESULTS

Growth performance and relative organ weights: The effects of soy lecithin on broiler performance are summarized in Table 2. During starter (0-21 days), grower (21-42 days) and overall period (0-42 days) of chickens fed diets with soy lecithin has better (p<0.05) ADG and ADFI compared with control. However, FCR remained unaffected between the different treatments from days 0-21 and 21-42, while, during the overall period was improved (p<0.05) in SL0.10 compared to SL0.05 and control. Effect of soy lecithin on relative weight of liver, bursa, spleen, pancreas and thymus are shown in Table 3. During 21 and 42 day, SL0.10 recorded the highest relative liver weight compared to SL0.05 and control (p<0.05). Relative weights of bursa, spleen, pancreas and thymus were not affected among the different dietary treatments during days 21 and 42.

Table 2: Effect of soy lecithin on growth performance of broilers (n = 6)

	Treatments*			
Items	BD (control)	SL0.05	SL0.10	SEM
Starter phase (0-21days)				
0-21ADG (g day^{-1})	31.23^b	33.55^a	33.08^a	0.299
0-21 ADFI (g day^{-1})	45.61^b	48.55^a	48.14^a	0.405
0-21 FCR (F:G)	1.46	1.44	1.45	0.012
Grower phase (21-42 days)				
21-42 ADG (g day^{-1})	56.97^b	66.33^a	69.98^a	2.061
21-42 ADFI (g day^{-1})	120.4^b	1.373^a	139.2^a	2.534
21-42 FCR (F:G)	2.13	2.07	1.99	0.055
Overall (0-42 day)				
0-42 ADG (g day^{-1})	44.10^b	49.94^a	51.53^a	0.657
0-42 ADFI (g day^{-1})	83.05^b	92.95^a	93.70^a	1.201
0-42 FCR (F:G)	1.88^a	1.86^{ab}	1.82^b	0.014^{abc}

Means in the same row with different superscripts significantly (p<0.05). SEM: Standard error of mean, *Treatments: BD: Basal diet, SL0.05: BD+0.05% soy lecithin, SL2: BD+0.1% soy lecithin, ADG: Average daily gain, ADFI: Average daily feed intake and FCR: Feed conversion ratio

Table 3: Effect of soy lecithin on relative weight of organs of broiler (n = 6)

	Treatments*			
Items	BD	SL0.05	SL0.10	SEM
21 days of age				
Liver (g kg^{-1} b.wt.)	26.06^b	25.34^b	28.72^a	0.681
Bursa (g kg^{-1} b.wt.)	2.04	2.07	2.10	0.028
Thymus (g kg^{-1} b.wt.)	2.82	2.65	2.53	0.225
Pancreas (g kg^{-1} b.wt.)	3.80	3.97	4.09	0.203
Spleen (g kg^{-1} b.wt.)	1.14	1.13	1.21	0.039
42 days of age				
Liver (g kg^{-1} b.wt.)	46.95^b	46.97^b	50.74^a	1.072
Bursa (g kg^{-1} b.wt.)	2.25	2.28	2.26	0.375
Thymus (g kg^{-1} b.wt.)	5.27	5.89	6.75	0.321
Pancreas (g kg^{-1} b.wt.)	4.76	4.75	4.55	0.070
Spleen (g kg^{-1} b.wt.)	3.85	3.76	3.58	0.594^{abc}

Means in the same row with different superscripts significantly (p<0.05). SEM: Standard error of mean, *Treatments: BD: Basal diet, SL0.05: BD+0.05% soy lecithin and SL2: BD+0.1% soy lecithin

Table 4: Effect of soy lecithin on nutrient digestibility of broiler (n = 6)

Items	Treatments*			
	BD	SL0.05	SL0.10	SEM
19-21 days				
GE (%)	67.94[b]	71.31[ab]	76.96[a]	1.951
DM (%)	79.25[b]	81.02[b]	86.60[a]	1.733
CP (%)	71.21[b]	73.68[ab]	77.04[a]	1.416
EE (%)	71.61[b]	74.93[ab]	78.55[a]	2.150
40-42 days				
GE (%)	68.04[b]	73.16[ab]	76.29[a]	1.962
DM (%)	82.15	83.77	85.83	2.507
CP (%)	74.67	78.13	82.95	3.383
EE (%)	68.52[b]	72.00[ab]	77.73[a]	2.698[abc]

Means in the same row with different superscripts significantly (p<0.05). SEM: Standard error of mean, *Treatments: BD: Basal diet, SL0.05: BD+0.05% soy lecithin, SL2: BD+0.1% soy lecithin, GE: Gross energy, DM: Dry matter, CP: Crude protein and EE: Ether extract

Table 5: Effect of soy lecithin on serum parameters of broiler at 42 days of age (n = 6)

Items	Treatments*			
	BD	SL0.05	SL0.10	SEM
Total cholesterol (mmol L^{-1})	2.67[a]	2.55[b]	2.30[c]	0.013
Triglyceride (mmol L^{-1})	0.68[a]	0.53[b]	0.45[c]	0.023
HDL-C (mmol L^{-1})	2.33	2.27	2.29	0.013
LDL-C (mmol L^{-1})	1.20[a]	1.05[a]	0.75[b]	0.060
Glucose (mmol L^{-1})	10.72[b]	11.22[b]	12.91[a]	0.280
Total bilirubin (umol L^{-1})	2.09	2.44	2.43	0.396
Total protein (g L^{-1})	36.36	37.88	37.78	0.660
Albumin (g L^{-1})	26.70	26.73	26.30	0.420[abc]

Means in the same row with different superscripts differ (p<0.05). SEM: Standard error of mean, *Treatments: BD: Basal diet, SL0.05: BD+0.05% soy lecithin and SL2: BD+0.1% soy lecithin

Table 6: Effect of soy lecithin on hepatic antioxidant enzymes of broiler (n = 6)

Items	Treatments*			
	BD	SL0.05	SL0.10	SEM
MDA (U mg^{-1} protein)	7.15[a]	6.83[a]	5.64[b]	0.280
CAT (U mg^{-1} protein)	57.88[b]	59.78[ab]	61.99[a]	0.913
T-SOD (U mg^{-1} protein)	274.43[b]	280.23[ab]	287.59[a]	3.781
TAOC (U mg^{-1} protein)	10.33[b]	11.40[b]	12.76[a]	0.378[abc]

Means in the same row with different superscripts differ (p<0.05). SEM: Standard error of mean, *Treatments: BD: Basal diet, SL0.05: BD+0.05% soy lecithin, SL2: BD+0.1% soy lecithin, T-SOD: Total superoxide dismutase, T-AOC: Total antioxidant capacity, CAT: Catalase and MDA: Malondialdehyde

Effects on nutrients digestibility: The effects of soy lecithin on nutrients digestibility are shown in Table 4. On day 21, the digestibility of DM, GE, CP and ether extract in chicks fed with SL0.10 diet were increased (p<0.05) compared to SL1 and control groups. On day 42, utilization of GE and ether extract were relatively higher (p<0.05) compared to those fed with SL0.05 and control. However, there was no difference in DM and CP utilization on day 42.

Effects on serum parameters: Data presented in Table 5 show that serum total cholesterol and triglyceride concentrations were significantly (p<0.05) decreased in SL0.05 and SL0.10 treatments compared with control, while LDL cholesterol decreased (p<0.05) in SL0.10. However, no difference was seen in HDL cholesterol on d 42 day. Furthermore, elevated (p<0.05) serum glucose was observed in SL0.10 compared to SL0.05 and control groups. In addition, concentrations of total protein, albumin and total bilirubin were unaffected among different treatments.

Effect on hepatic antioxidant enzymes: Table 6 shows data of hepatic antioxidant enzymes and lipid peroxidation in broilers at day 42. Compared with control and SL0.05, chicken diet supplemented with SL0.10 resulted in the decreased MDA content and increased hepatic enzyme activities of CAT, T-SOD and TAOC (p<0.05).

DISCUSSION

Growth performance and relative organs weights: Average daily gain and ADFI of broilers fed with the diet containing 0.1% soy lecithin were improved during starter (0-21 day), grower (21-42 days) and overall (0-42 days) periods, but FCR was improved during the overall period only. Results of our study coincided with the findings of Roy *et al.*[3] and Zosangpuii *et al.*[12] with exogenous emulsifiers were reported having beneficial effects on BW, feed intake and FCR in broilers. The positive effects with the addition of emulsifier to the diets on growth performance might be due to improved palatability which leads to higher feed and energy intake[13]. On the contrary, Azman and Ciftci[8] demonstrated that the body weight was not affected between the groups supplemented with lecithin and control diet at 21 and 35 days of age. Zhao *et al.*[7] reported that emulsifiers enhanced digestibility of nutrients in animals fed a diet with low energy using beef tallow as fat source. No impact of emulsifiers on feed intake was previously reported by Aguilar *et al.*[14]. Therefore, inconsistent results on growth performance of the birds fed with palm oil and emulsifier might be associated with fatty-acid composition of fat source and its effects on fat digestion and absorption so that the birds extracted more nutrients and sustained their growth rate even under low energy density diet.

In birds, liver is principal place involved in lipids metabolism of the body which accounts for 95% of the de novo fatty acid synthesis[15]. In present study, relative liver weight was improved in the broiler fed with 0.1% soy lecithin diet on days 21 and 42, respectively. Current results are in line with the previous findings of Huang *et al.*[16] and Nagargoje *et al.*[17] who observed better weight of liver by adding soy lecithin into the diet of broilers. Therefore, improved weight of liver possibly associated with the increased lipid metabolism within the organ.

Serum parameters: Blood measurements (protein and its fractions, lipid indices, renal and hepatic enzymes) are usually related to health status and they are good indicators of the nutritional status of the animals. Jones *et al.*[18] observed lower content of serum triglycerides in weaning piglets given tallow with emulsifiers (lecithin or lysolecithin) compared to those fed without emulsifiers. This is according to our findings that triglycerides were lower in broilers fed SL compared to those groups without SL. Similarly, De Rodas *et al.*[19] found decreasing trends of serum triglycerides by supplementing emulsifiers into the diet of pigs. Soy stanol-lecithin powder reduces cholesterol absorption[20] and increased fecal sterol excretion was observed with addition of polyunsaturated phosphatidylcholine (PC) to diet[21], which is consistent with our findings that serum cholesterol and LDL were decreased in broiler diet supplemented with SL as compared to diet without SL. On the contrary, Guerreiro Neto *et al.*[22] found non-significant differences on serum total cholesterol, LDL or triglyceride levels with emulsifier addition. The reduction mechanism of serum cholesterol by soy lecithin is still not clear, but this action may be returned to increase digestibility of fat as a consequence of which chylomicrons are rapidly cleared from the blood or secreted into the blood at a slower speed[18]. In the present study, serum glucose was increased with the birds fed SL0.10 diet. Such improvement in serum glucose might be reflected that sufficiently glucose was consumed by SL0.10 supplemented group for growth and performance.

Nutrient digestibility: Some previous studies indicated that the activities of lipase increase with age[23]. On the other hand, bile salts also play important role as a limiting factor for the digestion of lipids during the first week after hatching[24]. The digestibility of fat is limited in young chickens when secretion of lipase is not enough. In current study, the digestibility of DM, GE, CP and EE were increased on day 21, while utilization of GE and EE were higher in the chickens fed SL0.10 vs. SL0.05 and control diets. The same results were reported by Huang *et al.*[16] who stated that increased nutrient utilization in chickens fed diets supplemented with soy-oil and soy-lecithin in a proportion of 25/75 during 19-21 day. Findings of our study are also supported by the earlier reports[25-28] who speculated that increased nutrient digestibility in the broilers fed diet incorporated with fat emulsifier and low dietary fat level. On the contrary, linear decrease in the ATTD of DM, CP or GE was also reported[13] and with LPL supplementation in weanling piglets[29]. Inconsistency in the obtained results can be regarded due to different sources of lipids and various levels of emulsifiers used in the diet[14,30]. Furthermore, vegetable oil are more digestible than animal oils documented by Tan *et al.*[31] and Li *et al.*[32], which may be one of causes that no effects were seen in proceeding research conducts.

Hepatic antioxidant enzyme activities: Oxidative stress is harmful to animals that may alleviate their immune response and affects their performance by generating Reactive Oxygen Species (ROS). Obtained results indicated that supplementation of SL0.10 decreased hepatic MDA content and increased TAOC, T-SOD and CAT enzymes activities compared to those fed control and SL0.05. Outcomes in our study are accordance with Attia and Kamel[33] who pointed out that activities of GSH, GPx, SOD and GST was increased, but TBARS decreased in blood and seminal plasma with increasing soy lecithin level in rabbits. Such improvements in current study are also in line with Al-Daraji *et al.*[34] and Das *et al.*[35]. Similarly, soy lecithin have antioxidant and neuroprotective properties and it decreases liver injuries and improves oxidative strength[36-38]. It can improve the oxidative stability of oils and fats and its effect may be due to phospholipids, the main components of lecithin. According to King *et al.*[39] this property was characterized to the fact that phospholipids can contribute a hydrogen atom from amino group which triggering the oxidized phenolic molecule of the true antioxidant. Furthermore, Judde *et al.*[40] depicted that antioxidant effect of lecithin rely on the type of oil, tocopherol and fatty acids composition. Therefore, this can imagine that the effect of soy lecithin occurred with the γ- and δ-forms of tocopherols or tocotrienols that are naturally found in palm oil. From the previous findings, it was found that soy lecithin is a natural source, superior feed ingredient that is an important source of dietary phospholipids. Supplementation of lecithin in poultry or animal feed improves feed utilization and growth rates.

SIGNIFICANCE STATEMENTS

- The present study was planned to evaluate the effects of the soybean lecithin emulsifier in broilers diets
- Feeding soy lecithin at 0.10% improved growth performance, feed utilization and health status
- Using soy lecithin in boiler diets reduced cholesterol and triglyceride, LDL concentration in serum broilers
- Soy lecithin as an emulsifier is suitable for improving antioxidant status and has ability to protect against oxidative stress

CONCLUSION

Soy lecithin can be used as fat replacer in poultry diet; supplementation of soy lecithin (0.10%) led to improvement in growth performance and nutrient digestibility. In addition, broilers fed diet supplemented with 0.10% of soy lecithin had a lower lipid profile or lipid peroxidation rate in serum. Furthermore, dietary soy lecithin improved the activities of hepatic antioxidant enzymes and has ability to protect against oxidative stress.

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