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Research article

Effects of dietary mixed herbal extracted product supplementation on fatty liver hemorrhagic syndrome protection and productive performances of broilers

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Abstract

A sample of 880 day-old broilers was used to investigate the efficacy of a mixed herbal extracts product (MHEP) from *Azadiracta indica*, *Eclipta alba*, *Medicago sativa*, *Eleuisine coracana*, *Phyllanthus amarus* and *Solanum nigrum* to protect against the fatty liver hemorrhagic syndrome (FLHS) and its effect on growth performance. Animals were assigned to four groups and four replicates with each consisting of 55 broilers that were fed the same starter diet until 10 days of age (DOA). Subsequently, broilers randomly received one of the experimental diets: the control diet with 3,100 kcal/kg metabolizable energy (ME) and 3,200 kcal/kg ME for the grower and finisher periods, respectively; or a high energy diet with 3,200 kcal/kg ME and 3,300 kcal/kg ME (HE-diet); and the HE-diet supplemented with 250 mg/kg MHEP (HE250) and 500 (HE500) mg/kg MHEP, respectively. The growth performance and the histopathological lesion scores of liver tissue were evaluated and blood was collected for measurement of serum triglyceride, lipid-peroxidation and alanine-aminotransferase (ALT) activity. The results showed that feeding the HE-diet lowered the feed intake in the grower period compared to the control group (1474.4 vs 1566.3 g/bird), leading to a significant decrease in growth performances (1132.2 vs 1213.4 g/bird), but these detrimental effects were not found in finisher period. Feeding with HE250 resulted in growth improvement of broilers in both the grower and finisher periods, but HE500 did not show any advantages. The liver lesion score and ALT activity were not significantly affected, whereas a decrease in serum triglyceride (70.9 mg/dl vs 46.8 mg/dl) and an increase in TBA-MDA adduct (2.56 vs 3.04 nmol/ml) resulted from the MHEP application. In conclusion, 250 mg/kg MHEP supplementation improved the broiler growth performance, but no protective ability against FLHS was evident.

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Introduction

Fatty liver hemorrhagic syndrome (FLHS) is a metabolic condition occurring in poultry, mostly in caged layers, and causes significant losses in production (Aydin, 2005). Shini (2014) reported that 74% of total mortality in caged layers in Queensland, Australia resulted from FLHS. This syndrome is characterized by the accumulation of excess fat in the liver and hemorrhaging from a ruptured liver and sudden death (Rozenboim et al., 2016). However, its etiology is not clear, but a major factor is an excessive intake of dietary energy (Whitehead, 1979; Attia et al., 2009; 2018a). This problem is increasingly causing broiler production losses resulting from the higher feed intake combined with restricted activity (Dey et al., 2018). Several strategies have been developed to alleviate this problem, including dietary energy restriction (Hermier, 1997) and the administration of feed additives including herbs and herbal extracts (Yan et al., 2011). Recently, some potential herbs such as *Azadirachta indica*, *Eclipta Alba*, *Medicago sativa*, *Eleuisine coracana*, *Phyllanthus amarus* and *Solanum nigrum*, have been of interest for solving this problem due to their effective properties, which can decrease fat accumulation in and damage of liver tissue. Examples from the extensive reporting include: hepato-protective and hypolipidemic activities of azadirachtin and nimbolide from *A. indica* (Baligar et al., 2014a,b; Chattopadhyay, 2003), coumestans from *E. Alba* (Tabassum and Agrawal, 2004), saponin from *M. sativa* (Shi et al., 2014; Liang et al., 2015), phenolic compounds from *E. coracana* (Pingle et al., 2011), lignans from *P. amarus* (Ooi et al., 2015; Sethiya et al., 2015) and glycoalkaloids from *S. nigrum* (Elhag et al., 2019). Therefore, a herbal product, namely Himpro®, was developed as a feed additive based on the extraction of these herbs and the current study was conducted to evaluate the protective ability against FLHS of this product and its effect on the productive performances of broilers.

Materials and Methods

A sample of 880 day-old male broilers (Ross 308) was used in a completely randomized design. The chicks were divided into four groups with four replicates each consisting of 55 chickens that were fed the same diet for the starter period. Then. four experimental diets were randomly assigned to the broilers in the grower and finisher periods: diet 1 was the control diet with 3,100 kcal/kg metabolizable energy (ME) and 3,200 kcal/kg ME for the grower and finisher periods, respectively; diet 2 was a high energy diet with 3,200 kcal/kg ME and 3,300 kcal/kg ME (HE-diet); and the HE-diet supplemented with 250 mg/kg MHEP (HE250) for diet 3 and 500 (HE500) mg/kg MHEP for diet 4, respectively. The control and HE diets were formulated to meet the nutrients requirement for each period of growth of Ross 308 broilers recommended by Aviagen (2014). The feed ingredient components and analytical chemical composition are shown in Table 1. Animals were kept in pens in an evaporative cooling house at an ambient temperature of 28–30°C from 14–40 days of age (DOA) with feed and water being provided *ad libitum*. Additionally, all broilers were exposed to 23 hr of light for the first 7 days followed by 16 hr of light to 14 day DOA and an increased day length to 23 hr thereafter. The body weight and feed intake of the broilers were measured at 11 DOA, 24 DOA and 40 DOA to calculate growth performance and feed efficiency in the grower and finisher periods, as well as over the whole period of the experiment (11–40 DOA).

Table 1 Feed ingredients composition and chemical components of control and high energy diets for different stages of growth

Feed ingredient	Starter	Grower		Finisher	
		Control	High energy	Control	High energy
Corn	53.73	55.86	53.66	59.64	57.45
Soybean meal	34.68	29.05	29.39	20.12	20.46
Soybean oil	3.53	4.47	6.32	4.94	6.79
Dried Distillers Grains with Solubles	1.00	3.00	3.00	6.00	6.00
Corn gluten meal	2.00	3.00	3.00	5.00	5.00
Monodicalcium phosphate	2.025	1.792	1.795	1.578	1.580
Limestone	1.217	1.121	1.119	1.036	1.034
Sodium bicarbonate	0.240	0.232	0.227	0.265	0.260
Salt	0.202	0.196	0.200	0.155	0.159
Choline chloride	0.090	0.097	0.097	0.119	0.119
L-Lysine-HCl	0.296	0.293	0.298	0.378	0.373
DL-Methionine	0.310	0.249	0.251	0.199	0.201
L-Threonine	0.132	0.093	0.093	0.077	0.077
Pellex dry	0.300	0.300	0.300	0.300	0.300
*Cygro1%	0.050	0.050	0.050	-	-
**Vitamin/mineral premix	0.200	0.200	0.200	0.200	0.200
Calculated nutrient component					
ME (kcal/kg)	3,000	3,100	3,200	3,200	3,300
Analytical nutrients component (% dry matter basis)					
Dry matter	86.81	87.48	87.31	86.98	87.18
Crude protein	25.83	24.02	23.66	22.71	22.18
Crude fat	6.35	7.73	9.49	8.65	10.46
Crude fiber	2.48	3.05	2.91	2.53	2.35
Calcium	1.07	0.99	0.94	0.86	0.86
Total phosphorus	0.88	0.85	0.86	0.76	0.76

* 1 kg Cygro1% contains Maduramicin ammonium 10 g; **Vitamin and mineral premix per kg diet: vitamin A 10,000 IU; cholecalciferol 3,000 IU; vitamin E 10 mg; vitamin K3 1.5 mg; vitamin B1 2.0 mg; vitamin B2 5 mg; vitamin B6 3 mg; vitamin B12 3 mg; nicotinic acid 12.5 mg; pantothenic acid 25 µg; biotin 10 µg; folic acid 0.5 mg; Mn 60 mg; Zn 50 mg; Fe 40 mg; Cu 10 mg; I 2 mg; Co 2 mg; Se 0.1 mg.

At 24 DOA and 40 DOA, three chickens from each replicate, with body weights nearly the average for each experimental group, were selected for liver tissue sampling to study the protective ability against FLHS of MHEP. The histopathological lesion of the liver samples was scored (0–4) according to Merat et al. (2010) with moderate modification (Fig.1). Furthermore, blood was also collected from the wing vein only at 40 DOA for measurement of serum triglyceride, lipid peroxidation and alanine aminotransferase (ALT) activity.

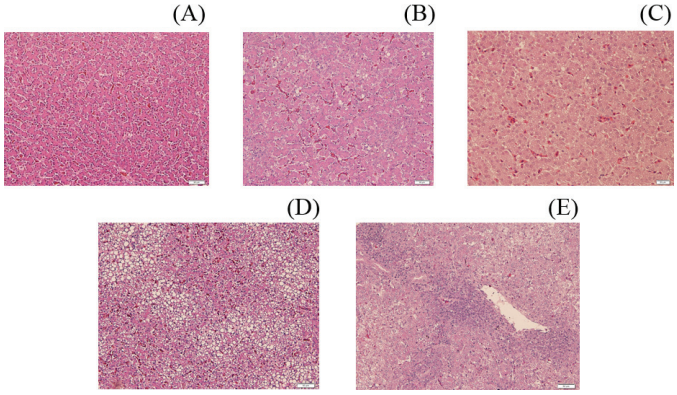

Fig. 1 Histopathological lesions of liver tissues**:** (A) normal liver; (B) fatty infiltration initiating in portal area; (C) fatty infiltration present in portal area and surroundings of central vein, with local hepatitis occuring; (D) fatty infiltration in whole hepatic parenchyma, with focal hepatitis and partial necrosis are present; (E) fatty inflitration and extensive hepatitis present in whole hepatic parenchyma, with fibrosis also present.

The MHEP used in this study was a commercial herbal product, namely Himpro®, composed of crude extracts from *A. indica*, *E. Alba*, *M. sativa*, *E. coracana*, *P. amarus* and *S. nigrum* and distributed by Eurotec Nutrition International Co., Ltd. The recommended dose of MHEP is in the 250–500 mg/kg diet.

Chemical analysis

Feed samples were chemically analyzed according to Association of Official Analytical Chemists (1990), while the serum triglyceride concentration and ALT activity were measured using an IDEXX VetTest® Chemistry Analyzer (IDEXX Laboratories, 2014). Lipid peroxidation was measured using modified methods of thiobarbituric acid reactive substances assays as described by Asakawa and Matsushita (1980) and Uchiyama and Mihara (1978). Thiobarbituric acid with malondialdehyde (TBA-MDA) adduct was detected using fluorescence spectroscopy (PerkinElmer; Walham, MA, USA) with excitation at 515 nm and emission at 553 nm.

Statistical analysis

All data were subjected to analysis by one-way analysis of variance using PROC GLM and where significant differences were observed, means were further subjected to Duncan's new multiple range test (Free Statistic Software, SAS® University Edition; Cary, NC, USA) The results were considered as significant when p-values were less than 0.05 and highly significant when p-values were less than 0.01.

Ethics statements

This study was approved by the Ethics Committee of Kasetsart University, Bangkok, Thailand (Approval no. ACKU61-AGK-009)

Results and Discussion

Effects of mixed herbal extracts product on productive performances of broilers

The HE-diet was fed to the broilers for induction of FLHS as well as applying MHEP in the grower and finisher periods. The responses of the broilers in the grower period indicated that feeding with the HE-diet highly significantly lowered their feed intake, which led to a significant decrease in the body weight gain (Table 2). Normally, a broiler controls its feed intake based on its need for a normalized energy intake (Leeson et al., 1996). Supplementation with 250 mg/kg (HE250) improved the growth of broilers though this was not significantly different to the control group, whereas the larger dose (HE500) did not alleviate the negative effect on the feed intake and body weight gain of the broilers. However, the HE-diet compensated the growth performance in the finisher period, due to the higher dietary fat content (Table 1). It is well known that dietary addition of fat has a greater beneficial effect on growth performance than expected based solely on its energy value (Sugahara, 2003; Attia and Hussan, 2017; Attia et al., 2018b). The greater efficiency of fat utilization as an energy source is due to the reduced heat increment during metabolism compared to carbohydrate; therefore, the broilers could metabolize more energy, especially at the high ambient temperature under heat stress conditions (National Research Council, 1984; Grummer, 1992). Furthermore, the effect of MHEP supplementation was clearly shown in this period of growth. The results indicated that HE250 significantly improved the feed intake, resulting in increased growth performance in tendency (p = 0.051) as shown in Table 2. The higher metabolic rate and more crowed stocking density in this stage of growth led to oxidative stress conditions resulting in heat and free radicals being overproduced and these could detrimentally affect the immune system and intestinal epithelium of birds (Blecha, 2000; Tache et al., 2001). The anti-oxidative property of the phyto-chemicals in MHEP have been reported, such as the isoflavone from *M. sativa* (Elkomy et al., 2014), aradirachtin from *A. indica* (Hossain et al., 2013), lignans from *P. amarus* (Kiemer et al., 2003) and glycoprotein from *S. nigrum* (Lee and Lim, 2003) were confirmed in the current study by the increase in TBA-MDA adduct in the serum of the broilers (Table 3), especially in the HE250 group. This beneficial ability alleviated the damage to epithelium tissue in the gastro-intestinal tract; thus, feed utilization was improved. However, increasing the usage dose of MHEP to 500 mg/kg (HE500) did not produce an advantage compared to the non-supplemented group, probably due to the overloading of detoxification of the excess phyto-chemicals from MHEP.

Table 2 Effect of mixed herbal extracts product supplementation on productive performances of broilers in grower and finisher periods

Performances	Experimental diets				*p-value*
	control	HE	HE250	HE500	
Grower period (11-24 DOA)					
BW (g/b)	1,213.4^{a}±10.4	1,132.2^{b}±40.0	1,154.1^{ab}±29.1	1,116.5^{b}±49.2	0.029
FI (g/b)	1,566.3^{A}±40.2	1,474.4^{B}±21.3	1,489.5^{B}±18.0	1,450.5^{B}±20.6	0.0006
FCR	1.291±0.04	1.302±0.04	1.291±0.03	1.300±0.05	0.974
Live ability (%)	98.8±1.05	98.2±2.10	98.6±1.74	99.1±1.05	0.877
Finisher period (25-40 DOA)					
BW (g/b)	1,509.9±137.0	1,663.2±12.9	1,726.4±110.6	1,639.6±106.8	0.051
FI (g/b)	2,901.7^{b}±70.1	2,969.9^{b}±10.3	3,112.1^{a}±67.2	2,946.0^{b}±108.2	0.028
FCR	1.929±0.13	1.786±0.02	1.807±0.11	1.800±0.07	0.168
Live ability (%)	96.4±3.15	96.8±2.72	97.0±2.78	97.7±1.74	0.913
Whole period of experiment (11-40 DOA)					
BW (g/b)	2,723.3±127.0	2,795.4±32.0	2,888.6±134.4	2,756.1±148.0	0.377
FI (g/b)	4,467.9^{b}±94.1	4,444.3^{b}±11.8	4,609.8^{a}±70.5	4,396.5^{b}±97.2	0.024
FCR	1.642±0.05	1.590±0.02	1.598±0.07	1.598±0.06	0.576
Live ability (%)	95.2±3.78	95.0±4.78	95.2±4.20	96.8±2.29	0.899

MHEP = mixed herbal extracts product; DOA = days of age; HE = high energy; HE250 and HE500 = high energy diet supplemented with 250 mg/kg MHEP (HE250) and 500 (HE500) mg/kg MHEP, respectively.

Means within a row with different lowercase superscripts are significantly ($p < 0.05$) different.

Means within a row with different uppercase superscripts are highly significantly ($p < 0.01$) different.

Table 3 Effects of mixed herbal extracts product supplementation on lipid peroxidation and alanine-aminotransferase (ALT) activity in serum of broilers

Parameter	Experimental group				*p*-value
	Control	HE	HE250	HE500	
TBA-MDA adduct	2.56^{B}±0.20	2.59^{B}±0.23	3.04^{A}±0.24	2.68^{B}±0.25	<0.01
ALT	1.96±1.18	1.88±0.89	1.58±0.62	1.77±1.07	0.250

TBA-MDA = thiobarbituric acid with malondialdehyde; HE = high energy; HE250 and HE500 = high energy diet supplemented with 250 mg/kg MHEP (HE250) and 500 (HE500) mg/kg MHEP, respectively.

Means within a row with different uppercase superscripts are highly significantly ($p < 0.01$) different.

The responses of the broilers to the MHEP supplementation in the HE-diet for the whole experimental period (11–40 DOA) were similar to those in the finisher period. HE250 significantly improved the feed intake of broilers; consequently, compared to the control diet, the body weight gain and feed conversion ratio were improved by 6.07 and 2.68%, respectively.

Effects of mixed herbal extracts product on protection from fatty liver hemorrhagic syndrome

FLHS was found during the grower period in the chicks fed HE-diet and pathogenic changes in the liver tissue were observed based on the lesion scoring. Lesion score changes were evident in broilers at 24 DOA and 40 DOA. Although high dietary fat has been reported to induce macroscopic and microscopic changes in the liver of broilers (Qureshi et al., 2004), feeding the HE-diet did not induce clear signs of FLHS in the current study (lesion scores in treatment birds were not significant from those fed the control diet) as shown in Table 4, probably due to the energy uptake control by the broilers or the relatively small increase in the ME content in the current study. In addition, the serum ALT activity, which is normally related to the function and health status of the liver, confirmed the normal function of liver with no significant differences among the experimental groups (Table 3). Thus, the application of MHEP did not provide any clear protective ability against FLHS at both 24 DOA and 40 DOA.

Table 4 Average lesion scoring of broiler liver tissue ($n = 12$) fed different experimental diets

Experimental diet	Period of growth	
	24 DOA	40 DOA
Control	1.17±1.03	1.25±1.60
HE-diet	1.08±0.79	1.00±1.30
HE-diet+250 mg/kg herbal product	0.83±0.94	1.17±1.19
HE-diet+500 mg/kg herbal product	1.17±0.94	0.92±1.38
p-value	0.780	0.926

DOA = days of age; HE = high energy; Scoring = 0: normal liver, 1: fatty infiltration initiated at portal area, 2: fatty infiltration present in portal area and surroundings of central vein and local hepatitis occurs, 3: fatty infiltration in whole hepatic parenchyma, focal hepatitis and partial necrosis are present, 4: fatty inflitration and extensive hepatitis are present in whole hepatic parenchyma and fibrosis also found.

Although, the MHEP did not produce any obvious fatty liver protection, the hypolipidemic property of the extracts from *A. indica*, *E. alba*, *M. sativa*, *E. coracana*, *P. amarus* and *S. nigrum*, was evident with a decrease in the serum triglyceride concentration (Fig. 2). The serum triglyceride decreased with the increasing dose of MHEP. Normally, triglyceride in serum comes from the dietary fat and *de novo* synthesis from the excess energy uptake from dietary carbohydrate (Polin and Wolford, 1976). However, serum triglyceride can be affected by some herbal active ingredients with different mechanisms. Chattopadhyay and Bandyopadhyay (2005) suggested that quercetin -3-o-β-D-glycoside, myricetin-3-O-rutinoside, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, kaempferol-3-O-β-D-glycoside and quercetin-3-O-α-L-rhamnoside from *A. indica* extract decreased triglyceride synthesis by the inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase), which led to lower cholesterol synthesis in the liver (Hamid, 2014) and the reduction of very low density lipoprotein (VLDL) secretion from liver (Ginsberg, 1998). The decrease in VLDL, which contains more than 90% triglyceride, lowers the serum triglyceride (Griffin et al., 1982). In addition, the flavonoids from *M. sativa* could lower the serum triglyceride content in laying hens by reducing the activities of acetyl-CoA carboxylase, fatty acid synthetase and HMG-CoA reductase, which are related to cholesterol and VLDL synthesis in the liver (Deng et al., 2012). Furthermore, *S. nigrum* extract inhibited the expression of the gene ChREBP (carbohydrate response element-binding protein), which controls the glycolytic and lipogenic genes including the synthesis of acetyl-CoA carboxylase and fatty acid synthetase (Iizuka and Horikawa, 2008).

Increasing the dietary ME content by 100 kcal/kg above the recommendation lowered the feed intake of the broilers only during the grower period, but did not clearly induce FLHS. Thus, MHEP supplementation in the HE-diet did not produce any protective ability against FLHS. However, the anti-oxidative property of the active ingredients in HMEP, especially for the 250 mg/kg diet, may have improved gut health, which is directly related to improved feed utilization; subsequently, the productive performance of broilers would be increased, particularly under stress conditions such as in the finisher period of growth.

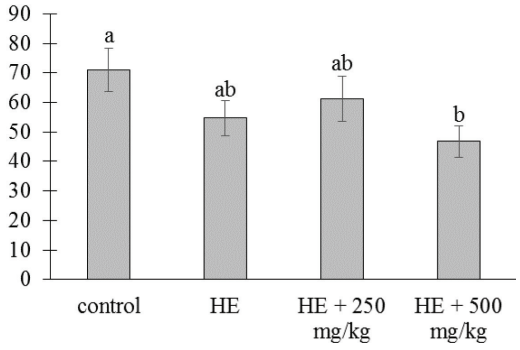

Fig. 2 Effect of mixed herbal extracts product supplementation on serum triglyceride (mg/dl) of broilers aged 40 d, where histograms labeled with different superscripts are significantly ($p < 0.05$) different and error bars indicate ± SD.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Association of Official Analytical Chemists. 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists. Arlington, VA, USA.

Asakawa, T., Matsushita, S. 1980. Coloring condition of thiobarbituric acid test for detecting lipid hydroxide. Lipids 15: 137–140. doi.org/10.1007/BF02540959

Attia, Y.A., Hassan, S.S. 2017. Broiler tolerance to heat stress at various dietary protein/energy levels. Europ. Poult. Sci. 81: 1–15. doi: 10.1399/eps.2017.171.

Attia, Y.A., Hussein, A.S., Tag El-Din, A.E., Qota, E.M., Abed El-Ghany, A.I, El-Sudany, A.M. 2009. Improving productive and reproductive performance of dual-purpose crossbred hens in the tropics by lecithin supplementation. Trop. Anim. Health Prod. 41: 461–475. doi: 10.1007/s11250-008-9209-3

Attia, Y.A., Abd El-Hamid, A.E., Abedalla, A.A., Berika, M.A., El-Gandy, M.F., Sahin, K., Abou-Shehema, B.M. 2018a. Effect of betaine, vitamin C, and vitamin E on egg quality, hatchability, and markers of liver and renal functions in dual-purpose breeding hens exposed to chronic heat stress. Europ. Poult. Sci. 82: 226. doi: 10.1399/eps.2018.226

Attia, Y.A., Al-Harthi, M.A., Elnaggar, A.Sh. 2018b. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. Ital. J. Anim. Sci. 17: 686–697. doi.org/10.1080/1828051X.2017.1416961

Aviagen. 2014. Ross 308 Broiler Nutrition Specifications. Available: https://en.aviagen.com/assets/Tech_Center/Ross_ Broiler/Ross308BroilerNutritionSpecs2014-EN.pdf, September 2, 2019.

Aydin, R. 2005. Type of fatty acids, lipoprotein secretion from liver and fatty liver syndrome in laying hens. Int. J. Poult. Sci. 4: 917–919. doi: 10.3923/ijps.2005.917.919

Baligar, N.S., Aladakatti, R.H., Ahmed, M., Hiremath, M.B. 2014a. Evaluation of acute toxicity of neem active constituent, nimbolide and its hepatoprotective activity against acute dose of carbon tetrachloride treated albino rats. Int. J. Pharm. Sci. Res. 5: 3455–3466. doi: 10.13040/IJPSR.0975-8232.5(8).3455-66

Baligar, N.S., Aladakatti, R.H., Ahmed, M., Hiremath, M.B. 2014b. Hepatoprotective activity of the neem-based constituent azadirachtin-A in carbon tetrachloride intoxicated Wistar rats. Can. J. Physiol. Pharmacol. 92: 267–277. doi: 10.1139/cjpp-2013-0449

Blecha, F. 2000. Neuroendocrine responses to stress. In: Moberg, G.P., Mench, J. A. (Eds.). The Biology of Animal Stress. CABI. New York, NY, USA. pp. 111–119.

Chattopadhyay, R.R. 2003. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II. J. Ethnophamacol. 89: 217–219. doi: 10.1016/j.jep.2003.08.006

Chattopadhyay, R.R., Bandyopadhyay, M. 2005. Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. Afr. J. Biomed. Res. 8: 101–104. doi: 10.4314/ajbr.v8i2.35769

Deng, W., Dong, X.F., Tong, J.M., Xie, T.H., Zhang, Q. 2012. Effects of an aqueous alfafa extract on production performance, egg quality and lipid metabolism of laying hens. J. Anim. Physiol. Anim. Nutr. 96: 85–94. doi: 10.1111/j.1439-0396.2010.01125.x

Dey, S., Pakhira, M.C., Batabyal, K., Isore, D. P., Samanta, I. 2018. Concurrent occurrence of fatty liver haemorrhagic syndrome (FLHS) and Colisepticaemia in a broiler breeder flock. Int. J. Curr. Microbiol. App. Sci. 7: 185–189. doi.org/10.20546/ijcmas.2018.709.023

Elhag, R.A.M., Badwi, S.M.A.E., Bakhiet, A.O., Galal, M. 2019. Hepatoprotective activity of *Solanum nigrum* extracts on chemically induced liver damage in rats. J. Vet. Med. Anim. Health. 3: 45–50.

Elkomy, A.E., Hamid, A.El., Mahmoud, S.S., Dekinesh, S.I., Ahmed, M.A. 2014. *Medicago sativa* seeds as a natural source of isoflavones to counteract the toxicity of contaminated broiler rations. Global J. Pharmacol. 8: 437–443. doi: 10.5829/idosi.gjp.2014.8.3.1118

Ginsberg, H.N. 1998. Effects of statins on triglyceride metabolism. Am. J. Cardiol. 81: 32B–35B. doi: 10.1016/s0002-9149(98)00035-6

Griffin, H., Grant, G., Perry, M. 1982. Hydrolysis of plasma triacylglycerol-rich lipoproteins from immature and laying hens (*Gallus domesticus*) by lipoprotein lipase *in vitro*. Biochem. J. 206: 647–654. doi: 10.1042/bj2060647

Grummer, R.R. 1992. Inedible fats and greases. In: Pearson, A.M., Dutson, T.R. (Eds.). Inedible Meat By-Products. Springer. Dordrecht, the Netherlands. pp. 113–148.

Hamid, O.M.A. 2014. Molecular and biochemical effect of neem extract on experimental diabetes. IOSR Journal of Applied Chemistry 7: 24–29. doi:10.9790/5736-07722429

Hermier, D. 1997. Lipoprotein metabolism and fattening in poultry. J. Nutr. 127: 805s–808s. doi: 10.1093/jn/127.5.805S

Hossain, M.A., Al-Toubi, W.A.S., Weli, A.M., Al-Riyami, Q.A., Al-Sabahi, J.N. 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. J. Taibah Univ. Sci. 7: 181–188. doi.org/10.1016/j.jtusci.2013.05.003

IDEXX Laboratories. 2014. IDEXX VetTest® Chemistry Analyzer. IDEXX Laboratories, Inc. Westbrook, ME, USA.

Iizuka, K., Horikawa, Y. 2008. ChREBP: A glucose-activated transcription factor involved in the development of metabolic syndrome. Endocr. J. 55: 617–624. doi: 10.1507/endocrj.k07e-110

Kiemer, A.K., Hartung, T., Huber, C., Vollmar, A.M. 2003. *Phyllanthus amarus* has anti-inflamatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-KB pathway. J. Hepatol. 38: 289–297. doi: 10.1016/s0168-8278(02)00417-8

Lee, S.J., Lim, K.T. 2003. Antioxidative effects of glycoprotein isolated from *Solanum nigrum* Linn on oxygen radicals and its cytotoxic effects on the MCF-7 cell. J. Food Sci. 68: 466–470. doi.org/10.1111/j.1365-2621.2003.tb05695.x

Leeson, S., Caston, L., Summers, J.D. 1996. Broiler response to diet energy. Poult. Sci. J. 75: 529–535. doi: 10.3382/ps.0750529

Liang, X.P., Zhang, D.Q., Chen, Y.Y., Guo, R., Wang, J., Wang, C.Z., Shi, Y.H. 2015. Effects of alfafa saponin extract on mRNA expression of Ldlr, LXRα and FXR in BRL cells. J. Zhejiang Univ. Sci. B. 16: 479–486. doi: 10.1631/jzus.B1400343

Merat, S., Khadem-Sameni, F., Nouraie, M. 2010. A modification of the Brunt system for scoring liver histology of patients with non-alcoholic fatty liver disease. Arch. Iran. Med. 13: 38–44.

National Research Council. 1984. Nutrient Requirement of Poultry, 8th ed. National Academy Press. Washington, DC, USA.

Ooi, K.L., Loh, S.I., Sattar, M.A., Muhammad, T.S.T., Sulaiman, S.F. 2015. Cytotoxic, caspase-3 induction and *in vivo* hepatoprotective effects of phyllanthin, a major constituent of *Phyllanthus niruri*. J. Funct. Foods. 14: 236–243. doi.org/10.1016/j.jff.2015.01.032

Pingle, B.R., Apte, K.G., Gupta, M., Chakraborthy, G.S. 2011. Hepatoprotective activity of different extracts of grains of *Eleusine coracana*. Pharmacologyonline 2: 279–286.

Polin, D., Wolford, J.H. 1976. Various types of diets, sources of energy, and positive energy balance in the induction of fatty liver hemorrhagic syndrome. Poult. Sci. 55: 325–334. doi: 10.3382/ps.0550325

Qureshi, I.A., Khan, S.A., Chaudhry, Z.I., Mian, N.A., Tipu, M.Y., Rai, M.F. 2004. Effects of high dietary fat on serum cholesterol and fatty liver syndrome in broilers. Pakistan Vet. J. 24: 153–154.

Rozenboim, I., Mahato, J., Cohen, N.A., Tirosh, O. 2016. Low protein and high-energy diet: A possible natural cause of fatty liver hemorrhagic syndrome in caged White Leghorn laying hens. Poult. Sci. 95: 612–621. doi: 10.3382/ps/pev367

Sethiya, N.K., Shah, P., Rajpara, A., Nagar, P.A., Mishra, S.H. 2015. Antioxidant and hepatoprotective effects of mixed micellar lipid formulation of phyllanthin and piperine in carbon tetrachloride- induced liver injury in rodents. Food Funct. 6: 3593–3603. doi: 10.1039/c5fo00947b

Shi, Y., Guo, R., Wang, X., Yuan, D., Zhang, S., Wang, J., Yan, X., Wang, C. 2014. The regulation of alfafa saponin extract on key genes involved in hepatic cholesterol metabolism in hyperlipidemic rats. PLoS One. 9: e88282. doi: 10.1371/journal.pone.0088282

Shini, A. 2014. Fatty liver haemorrhagic syndrome in laying hens: Field and experimental investigations. Ph.D. thesis, School of Agriculture and Food Sciences, The University of Queensland. BNE, Australia. doi: 10.14264/uql.2015.74

Sugahara, K. 2003. Energy utilization of growing chicks in various nutritional conditions. Asian-Australas. J. Anim. Sci. 16: 903–909. doi.org/10.5713/ajas.2003.903

Tabassum, N., Agrawal, S.S. 2004. Hepatoprotective activity of *Eclipta alba* hassk. against paracetamol induced hepatocellular damage in mice. JK Practitioner. 11: 278–280.

Tache, Y., Martinez, V., Million, M., Wang, L. 2001. Stress and the gastrointestinal tract III. Stress related alterations of gut motor function: role of brain corticotrophin releasing factor receptors. Am. J. Physiol. Gastrointest. Liver Physiol. 280: 173–177. doi: 10.1152/ajpgi.2001.280.2.G173

Uchiyama, M., Mihara, M. 1978. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal. Biochem. 86: 271–278. doi.org/10.1016/0003-2697(78)90342-1

Whitehead, C.C. 1979. Nutritional and metabolic aspects of fatty liver disease in poultry. Vet. Quart. 1: 150–157. doi: 10.1080/01652176.1979.9693738

Yan, L., Meng, Q.W., Ao, X., Wang, J.P., Jang, H.D., Kim, I.H. 2011. Evaluation of the dietary wild-ginseng adventitious root meal on egg production, egg quality, hematological profiles and egg yolk fatty acid composition in laying hens. Livest. Sci. 140: 201–205. doi.org/10.1016/j.livsci.2011.03.033