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LAYING PERFORMANCE, FAT DIGESTIBILITY AND LIVER CONDITION OF LAYING HENS SUPPLEMENTED WITH VITAMIN B_{12} OR BIOTIN AND/ OR BILE ACIDS IN DIET

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Abstract: For 8 weeks feeding trial, two hundred and seventy, 53 weeks old laying hens were used to investigate the effects of dietary supplementation of vitamin B_{12} or biotin and/or bile acids on performance, egg quality, fat digestibility and liver composition and histopathology. Birds were randomly divided into 6 groups (three replicates) and fed the experimental diets; group1 (G1) fed on the basal diet without additives as control while G2 and G3 supplemented with 0.02 ppm vitamin B_{12} and 0.3 mg biotin/kg diet respectively, groups 4-6 fed as the previous detailed design of G1- G3 with the addition of 400 g of dried bile acid (DBA) /tone feed. Biotin supplementation significantly (P<0.05) increased body weight losses of laying hens, both vitamins significantly (P<0.05) decreased daily feed intake (FI) and improved FCR. DBA addition alone or with biotin reduced these weight losses along with significant (P<0.05) increase in daily FI. Vitamin B_{12} supplementation alone or with DBA increased average egg production (P<0.05) while was reduced with biotin supplementation. Fat digestibility was non-significantly improved (P≥0.05) with both vitamins supplementation without or with DBA. Biotin significantly (P<0.05) reduced the average yolk relative weight, which was increased when mixed with DBA, while significantly increased average egg albumin relative weight. Vitamin B_{12} or biotin addition without or with DBA non-significantly increased blood serum GOT and GPT activities, non-significantly reduced (P≥0.05) fat content of liver tissue (on dry matter or fresh basis) and serum lipid profile parameters except serum HDL concentration, was increased, with no histopathological changes in hepatic tissue. It could be summarized that vitamin B_{12} supplementation without or with DBA is recommended in layer diet as it gave the best performance, reduced serum lipid profile and improved fat digestibility and the hepatic health.

Key words: layers; egg quality; liver; vitamin B12; biotin; bile acids

Introduction

Recently, laying hens facing many challenges influencing egg production industry resulting in significant economic losses. Such

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challenges as environmental changes, imbalanced or poor-quality feed, frequent diseases affect the physiological condition of layers and bring out pathological burden to hen body especially to sensitive and important organs such as liver. Therefore, liver is in continuous overloaded condition, which disturbs its function. One of the common disorders influences liver in layers is the fatty liver hemorrhagic syndrome (FLHS), negatively affecting their performance. FLHS is a metabolic or nutritional disorder of layers characterized by excessive accumulation of fat depots in the hepatic tissue and abdominal cavity as liver become enlarged, pale and ends up with rupture and hemorrhage causing death of the affected birds (1).This syndrome occurs when the lipogenesis process exceeds the capacity of lipoprotein synthesis and secretion (2). The actual cause of FLHS has not been fully defined, but could be a combination of nutritional, genetic, environmental, and hormonal factors (3). Different studies have been done to follow up the actual factors inducing this problem in laying hens with the development of some managemental and nutritional approaches to reduce its occurrence. One of these strategies is dietary supplementation of some feed additives such as some lipotropic factors and antioxidants, which could control the lipid metabolism, reduce the production of free radicals and protect the liver from damage.

Methionine and choline as lipotropic factors are used to treat or prevent this syndrome as their inadequate dietary levels increase oleic acid absorption and decreased triacylglycerol secretion (4, 5). Other nutrients deficiency such as vitamin B_{12} and vitamin E increased hepatic triacylglycerol accumulation in laying hens (6, 7), increasing the incidence of occurrence. Earlier studies reported that biotin deficiency is an important dietary factor involved in inducing fatty liver in birds (8, 9).

Recently, the poultry production sector is continuously searching for new feed additives to improve feed efficiency and physiological condition of layers for optimal performance and egg production. Bile acids are natural components synthesized in human and animal body from cholesterol, which have a critical role in dietary fat digestion and absorption through the intestinal wall (10). Previous studies have been done investigating impacts of B-vitamins supplementation and emulsifiers as bile acids on broilers health and productivity (11-14), however, little literature is available regarding their effect on laying hens productive performance and egg quality parameters.

We hypothesized that dietary inclusion of these additives could help in the digestion of fat, maintain the healthy condition of the liver of laying hens. Therefore, the main objectives of the present study to investigate the effects of vitamin B_{12} or biotin supplementation without or with dried bile acids on productive performance, egg quality, fat digestibility, serum biochemistry and liver histopathology of laying hens.

Material and methods

Birds, experimental design and feeding program

Management procedures for all birds used during the course of experiment followed the guidelines approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Alexandria University. A total of two hundred and seventy, 53 weeks old of *Isa brown* laying hens purchased from local company and used in this experiment. Hens were weighed separately, then randomly divided into 6 separate groups (45 bird /group) with three replicates each (15 bird/ replicate). Birds were kept in wire cages, and each cage was provided with feeder and water troughs. The control group (Group 1) was fed the basal diet (BD) without any additives, group 2 and 3 were fed the BD supplemented with 0.02 ppm vitamin B_{12} and 0.3 mg biotin/kg diet respectively, while group 4-6 were fed as the previous detailed design of the first three groups but, with the addition of 400 g bile acid/tone diet. BD were formulated to meet bird nutrient requirements according to NRC (15). BD composition and its chemical analysis is presented in table 1, which was analyzed according to AOAC (16). Additives used in this experiment included; vitamin B_{12} and biotin produced by Allgäu Vet. Co., China; the dried bile acids (DBA) (Shangdong Longchang

Animal Health Product Co., Ltd, Jinan, China). All the experimental groups were fed the designated diets with free access to fresh water for 8 weeks experimental period. Hens were weighed individually at the beginning and end of experiment and the live body weight (BW) changes were taken with recording of feed intake (FI). Feed conversion ratio (FCR**)** was calculated based on feed consumed (g) and produced egg mass (g).

Egg production and Egg quality

Eggs produced from various laying hens were collected daily for calculating the average daily egg production % (EP) and egg weight (g) for each group for 14 days period for four successive periods. Egg mass was calculated by multiplying egg weight by egg production. At the end of the experiment, 20 eggs from each group were collected to estimate the egg quality parameters (yolk weight and relative weight to total egg weight, yolk index, albumin weight, and its relative weight, albumen index, shell thickness and weight as well as its relative weight were recorded) according to Card and Nesheim (17). Six samples of egg yolk from each group were collected for egg yolk cholesterol determination according to Rotenberg and Christensen (18).

Fat digestibility

During the last week of the feeding trail, the excreta were quantitatively collected for 5 successive days in addition to recording the amount of feed consumed. The excreta then dried in hot air oven, ground and stored until chemical analysis of fat. Ether extract of feed, fecal and liver samples was determined according to AOAC (16)**.**

Serum biochemical parameters

Blood samples were collected (n= 6/group), then left to coagulate at room temperature. Separation of serum was done by centrifugation at 3000 rpm for 10 minutes. The clear serum samples were kept in freezer (-20° C) until analysis of the following blood serum indices; total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). All these parameters were determined by spectrophotometer using specific commercial kits produced by Biodiagnostic, Co. (Diagnostic and Research reagents).

Liver Histopathology

At the end of the experiment (61 week of hen age), three hens from each group (one hen from each replicate) were collected randomly and used for liver tissue collection. Samples were fixed in 10% formalin for at least 2 days then slides were prepared according to Bancroft et al. (19), and stained with Hematoxylin and Eosin (H&E) for general inspection.

Statistical analysis

Results were analyzed using analysis of variance (one-way ANOVA) using IBM SPSS Statistics 22 statistical package (SPSS Inc., Chicago, IL, USA) to measure the significant differences between the means of different variables. Differences among experimental groups were considered significant at ($P<0.05$).

Results and discussion

Layer Performance

Biotin supplementation significantly ($P<0.05$) increased BW losses of laying hens. Both vitamins significantly ($P<0.05$) decreased average daily FI, but, improved FCR compared with control. On the other hand, DBA addition alone or with biotin reduced these losses in BW along with significant ($P<0.05$) increase in daily FI compared with laying hens without DBA addition (table 2). Moreover, FCR was significantly improved with DBA inclusion with vitamin B_{12} and biotin compared with birds fed the same diet without DBA or control group.

Unlike the obtained results, earlier studies examined the effects of vitamin B_{12} (20, 21), concluded that it had no clear effect on FI of commercial laying hens, while increased FI with Halle and Ebrahem (22). Improvement of FCR with vitamin B_{12} supplementation in the present study is in line with Halle and Ebrahem (22), while inconsistent with Kato et al. (20). Additionally, Abdel-Mageed and Shabaan (23) documented another different result as similar

FI was obtained in laying hens groups fed biotin-supplemented diets while, improved FCR with increasing its level from 162.5 to 325.5 μg/kg. Additionally, Whitehead (24), found that biotin supplementation during lay did not have any beneficial effect upon FI and FCR of laying hens.

Bile acids are the principal constituents of bile, playing a significant role in emulsification, digestion, and absorption of fat and lipid-soluble vitamins (25). Best FCR was obtained with birds fed BD contained DBA plus vitamin B_{12} (2.08), while the worst value with birds fed BD supplemented with DBA (2.49). Previous reports documented improvements of broiler performance (weight gain, higher FI and FCR) with the dietary inclusion of bile acids (13, 26-28), improved FCR in quail laying hens (29). The variation in response may be related to different breeds considered in these trials.

Regarding EP (table 2), vitamin B_{12} supplementation alone or with DBA increased average EP ($P<0.05$) with the highest EP (86.34%) in birds supplemented with the 2 additives suggesting that they could have a synergistic effect. On the other hand, biotin supplementation showed dissimilar result as it reduced EP (72.71%), even when added with DBA (78.22%), compared with control birds. Unlike the obtained result, Kato et al. (20) found that vitamin B_{12} supplementation had no effect on EP, attributing it to short experimental period. Additionally, Bunchasak and Kachana (30) reported that supplemental vitamin B_{12} didn't alter egg production or FI. However, Daryabari et al. (31) reported that 0.3g biotin/L water supplementation improved EP of breeding hens, as well as Abdel-Mageed and Shabaan (23) who found that biotin inclusion in diet enhanced it. Biotin concentration in the blood has been reported to be lower in young broiler breeder hens and increases linearly with age (32), therefore, the lower or unaffected EP in the biotin supplemented groups in the present study could be attributed to the higher level of biotin in birds. Response due to bile acids addition is inconsistent with the finding obtained with (29) as 1.2% supplemental ox bile addition in quail laying hens diet significantly improved EP %.

Both vitamins supplementation non-significantly ($P\geq0.05$) reduced average egg weight (table 2). The same response was obtained when diet was supplemented with DBA alone or combined with vitamin B_{12} while, when DBA mixed with biotin showed slightly higher egg weight compared with birds fed on the same diet without DBA. On contrary, Kato et al. (20) found increased egg weight of laying hens with vitamin B_{12} supplementation.

Fat digestibility

Fat digestibility showed non-significant improvement ($P\geq0.05$) with vitamin B_{12} or biotin compared with control (table 2). The same result was obtained when DBA was supplemented alone or with both vitamins. Fat digestibility improvement especially in laying hens supplemented with DBA being the highest was supported by Alzawqari et al. (28), who found that supplemental increased levels of bile in diet linearly increased fat digestibility in broiler chicks. This improvement with the addition of DBA might be attributed to inadequate amount of bile salts secreted by bird, so more emulsification of fats occur by the exogenous bile acids or for replacement of the active catabolism of bile salts occur by gut microflora (26). Moreover, Lai et al. (12) indicated that supplementation with 60 and 80 mg/kg bile acids significantly increased the activity of intestinal lipase and lipoprotein lipase on day 21 and 42. The present study revealed a close relationship between dietary DBA supplementation and fat digestibility. Most of the previous studies reported, were concerned with investigation of the effects of these exogenous emulsifiers in broiler diet especially at younger age of chicks due to the physiological limitation of the digestive tract to produce endogenous emulsifiers. However, literature on their application in layers diets is scarce.

Egg quality (External and internal quality)

Average egg shell relative weight was non-significantly increased ($P\geq0.05$) with vitamin B_{12} or biotin compared with the reference group (table 3). DBA addition single or combined with vitamin B_{12} increased ($P\geq0.05$) egg shell relative weight, while, was reduced ($P \geq 0.05$) when biotin was added to diet. Regarding egg shell thickness, no changes were found among different treatments. In support, Keshavarz (33) concluded that vitamin B_{12} supplementation without or with methionine improved egg shell quality, Rajalekshmy (34) reported that shell weight was decreased at higher levels of folic acid (4 ppm) when vitamin B_{12} was added (0.01 ppm). However, Kato et al. (20) found another different result as egg shell thickness and weight per unit of surface area were higher ($p<0.01$) for birds fed diet without vitamin B_{12} supplementation.

In terms of internal egg quality (table 3), biotin supplementation significantly reduced ($P<0.05$) average egg yolk relative weight, but this effect was reversed when DBA was added with biotin. On the other hand, average egg albumin relative weight was significantly increased with biotin supplementation, though the lowest relative weight was recorded with birds received diet supplemented with DBA only. Regarding average yolk index, it was significantly reduced in birds supplemented with biotin and DBA, with no significant difference between the other experimental groups. The present data are in line with El-Husseiny et al. (35), who found that dietary supplementation with 0.01-0.02 ppm vitamin B_{12} did not have any significant effect on egg shell thickness, shell percentage, Haugh units and yolk index. Moreover, Whitehead (24), reported that albumen height was slightly improved with biotin supplementation. Additionally, Abdel-Mageed and Shabaan (23), showed that biotin inclusion at 325.5 μg/kg diet gave the highest increase in egg shape index, yolk index and Haugh unit. Furthermore, Soltan (29) concluded that ox bile addition in quail laying hens had no significant influence on internal and external egg quality.

Egg yolk triglyceride and cholesterol content were non-significantly ($P \geq 0.05$) reduced with BDA supplementation compared with birds fed the same diets without DBA addition. These finding could support that DBA has an important role in fat metabolism through enhancing its digestibility and utilization. The egg is a highly nutritious food for human consumption, however, its higher content of cholesterol associated with cardiovascular diseases restricts its consumption. So, previous studies were concerned with the production of egg with low cholesterol content through genetic selection; dietary interventions or using some drugs such as bile acids sequestrants in the diet of laying hens (36, 37).

Serum biochemical parameters

Blood serum lipid profile (table 4) was non-significantly altered with additives used. However, serum total cholesterol, TG, LDL, and VLDL concentrations were non-significantly ($P \geq 0.05$) reduced with DBA addition single or in combination with vitamins used except for serum HDL concentration which was increased. The higher reduction was observed in birds supplemented with DBA.

Serum TG, LDL, and HDL concentrations considered an important diagnostic indicators of lipid metabolism. The synthesis of adipose tissue, fat deposition, and formation of yolk in poultry is dependent on available serum TG. Most of fatty acids are produced in the liver and transported via LDL or chylomicrons for storage in adipose tissue as TG (2). On the other hand, HDL enhances the uptake and transport of cholesterol from extra hepatic tissues to hepatic tissue for catabolism (38), which was confirmed in the current study as it was non significantly increased especially with DBA addition. Therefore, these findings suggesting that the used feed additives (vitamin B_{12}, biotin and DBA) could have a hypolipidemic effect by affecting lipid metabolism. On contrary, Alzawqari et al. (13), reported that dietary inclusion of DBA significantly increased serum components of cholesterol, TG, HDL and LDL concentrations at days 21 and 42 of age, however, serum cholesterol concentration was numerically decreased (28). Furthermore, Lai et al. (12) documented that bile acid inclusion in broiler diets showed no differences in serum TG, HDL, and LDL concentrations among all groups at days 21 and 42. Inconsistency in results between experiments maybe related to differences in breed, age of birds and feeding experimental design.

Vitamin B12 or biotin supplementation without or with DBA non-significantly increased blood serum GOT and GPT activities compared with their reference birds (table 4). Serum GOT and GPT activity are traditional biochemical indices of liver function and are used clinically for diagnosis of liver injury. Yousefi et al. (5), documented that AST enzyme activity (GOT) could be used as diagnostic indicator for FLHS in laying hens. All liver function related parameters in the present study were within normal range (39)**,** which could confirm that these supplemental additives in laying hen diet had no adverse effects on liver function.

Liver composition and histopathology

As presented in table 4, both vitamin supplementation without DBA addition non-significantly decreased liver moisture % while significantly increased ($P<0.05$) it when combined with DBA compared with their reference birds. On the other hand, the fat content of the liver tissues (on dry matter or fresh basis), was non-significantly reduced ($P \geq 0.05$) with both vitamins supplementation without or with DBA. Fatty liver is associated with the reductions in egg production with poor egg shell quality (40), which is particularly a serious problem during the late period of laying. With the long-term accumulation of fat and various toxins in the hepatic tissue, hepatocytes are injured with impaired function, reducing absorption and utilization of fat-soluble vitamins which consequently affecting the assimilation and deposition of calcium resulting in poor egg shell quality. So, liver health is closely associated with egg production and egg shell quality. Dietary inclusion of bile acids enhances the clearance function of very low-density lipoproteins, which transports fat from liver to systemic tissues, and reduces fat deposition in hepatocytes. Additionally, it stimulates the thyroid hormone activity (41), increases energy expenditure, reducing excessive accumulation of fat inside the liver and other body tissues of laying hens.

The examined liver of laying hens fed on the BD without any supplemental additives showed centrilobular vacuolation represented with clear vacuoles with round border consistent with fatty changes (Fig.1A). Most of the hepatic sinusoids were congested in addition to multifocal lymphocytic cells aggregation. Vitamin B_{12} or biotin supplementation markedly decreased these pathological vacuolations with nearly normal hepatocytes (Fig.2 B and C). DBA inclusion in the diet without or with vitamin B_{12} or biotin supplementation showed mild vacuolation of hepatocytes or even normal hepatocytes (Fig.1D, E, and F). The present findings are in line with Lai et al. (42), who concluded that liver of broiler chickens supplemented with bile acids was normal and no histological changes. Therefore, these findings revealed that these feed additives especially bile acids could help in maintaining liver health by reducing fat deposition in the hepatic tissue.

Table 1: Ingredients composition and chemical analysis of experimental diet

Ingredients	%
Yellow corn ground	56.83
Soybean meal (44% CP)	20.0
Corn gluten meal	5.0
Wheat bran	5.0
Vegetable oils (sunflower oil)	1.25
Ground lime stone[1]	9.80
Monocalcium phosphate[2]	1.30
Common salt	0.25
Vitamin premix [3]	0.15
Mineral premix [4]	0.15
Lysine[5]	0.02
Methionine[6]	0.10
Choline[7]	0.10
Mycotoxin adsorbent	0.05
Chemical analysis (%)	
Moisture	11.65
Crude protein	17.07
Ether extract	4.87
Ash	12.76
Crude fiber	4.43
NFE[8]	49.22
Calcium	3.72
Phosphorus	0.63
ME (Kcal/kg)[9]	2746.88

[1]Lime stone contains 37 % calcium and locally produced. [2]Mono calcium phosphate contains 21% phosphorus and 17 % calcium. [3]Vitamin premix: Each 1.5 kg contains: Vit. A (12000000 IU), vit. D (2000000 IU), vit. E (10 g), vit. K3 (2 g), vit. B1 (1 g), vit. B2 (5 g), vit. B6 (1.5 g), vit. B12 (10 g), nicotinic acid (30 g), pantothenic acid (10 g), folic acid (1g), biotin (50 mg), produced by Archar Daniels Midland Co., LL., USA. [4]Mineral Premix: Each 1kg contain, Manganese 100000 mg, Zinc 600000 mg, Iron 30000mg, Copper 10000 mg, Iodine 1000 mg, Selenium 200 mg, Cobalt 100 mg. [5]Lysine: 98% lysine hydrochloride, Shandong Longue Co., China. [6]DL-methionine, Evonik Co. (guaranteed analysis 99.5% DL- methionine).[7]Choline: choline chloride 60 % with vegetable carrier (corn powder) produced by Shandong pharmaceutical Co., China. [8]Nitrogen free extract was calculated by difference. [9]ME were calculated according to NRC (1994).

Table 2: Effect of dietary vitamin B_{12} or biotin supplementation without or with dried bile acids on performance and fat digestibility of laying hens

Parameters	Supplementation					
	Control	Vitamin B_{12}	Biotin	Control	Vitamin B_{12}	Biotin
	Without DBA[1]			With DBA		
Initial body weight (g/hen)	1851.82 ±21.07^{a}	1826.55 ±19.19^{a}	1815.66 ±23.77^{a}	1798.47 ±22.20^{a}	1844.27 ±19.63^{a}	1829.60 ±24.50^{a}
Final body weight (g/hen)	1827.74 ±35.02^{a}	1802.24 ±29.49^{ab}	1731.11 ±31.14^{b}	1794.15 ±30.66^{ab}	1815.27 ±28.93^{ab}	1795.38 ±26.79^{ab}
Weight changes (g/hen)	-24.08 ±20.02^{b}	-24.31 ±16.06^{b}	-84.55 ±19.26^{a}	-4.32 ±17.40^{c}	-29.00 ±17.35^{b}	-34.22 ±10.38^{b}
Average Feed Intake (g/hen/day)	113.41 ±0.07^{b}	109.99 ±0.19^{d}	96.90 ±0.13^{f}	120.21 ±0.06^{a}	111.35 ±0.12^{c}	107.34 ±0.16^{e}
Average FCR	2.35 ±0.03^{b}	2.14 ±0.03^{c}	2.14 ±0.06^{c}	2.49 ±0.03^{a}	2.10 ±0.03^{d}	2.19 ±0.03^{c}
Average egg production (%) 53 – 61	76.65 ±0.69^{bc}	81.56 ±0.74^{ab}	72.17 ±1.37^{c}	78.60 ±0.79^{b}	86.34 ±4.27^{a}	78.22 ±0.76^{b}
Average egg wt. (g)	62.83 ±0.22^{a}	62.74 ±0.15^{a}	62.48 ±0.15^{a}	61.38 ±0.16^{a}	61.75 ±0.14^{b}	62.65 ±0.14^{a}
Average egg mass (g/hen)	48.17 ±0.46^{bc}	51.21 ±0.50^{ab}	45.20 ±0.89^{c}	48.21 ±0.48^{bc}	53.33 ±2.63^{a}	49.02 ±0.50^{b}
Fat digestibility %	75.07 ±2.84^{a}	81.67 ±2.40^{a}	78.97 ±4.65^{a}	84.40 ±0.60^{a}	82.30 ±1.95^{a}	79.20 ±6.45^{a}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).[1] DBA: Dried bile acids

Table 3: Effect of dietary vitamin B_{12} or biotin supplementation without or with dried bile acids on egg quality parameters

Parameters	Supplementation					
	Control	Vitamin B_{12}	Biotin	Control	Vitamin B_{12}	Biotin
	Without DBA[1]			With DBA		
External egg quality						
Average eggshell relative weight	12.86 ±0.49^{a}	13.25 ±0.20^{a}	12.90 ±0.39^{a}	13.43 ±0.34^{a}	12.97 ±0.13^{a}	12.23 ±0.29^{a}
Average eggshell thickness (mm)	38.78 ±0.36^{a}	37.56 ±0.47^{a}	37.04 ±0.70^{a}	37.19 ±0.64^{a}	37.94 ±1.42^{a}	37.33 ±0.94^{a}
Internal egg quality						
Average yolk relative wt.	29.99 ±0.89^{ab}	29.82 ±0.50^{ab}	29.22 ±0.66^{b}	30.06 ±0.58^{ab}	30.74 ±0.69^{ab}	31.47 ±0.76^{a}
Average yolk index	0.43 ±0.01^{a}	0.41 ±0.01^{ab}	0.43 ±0.00^{a}	0.43 ±0.00^{a}	0.41 ±0.01^{ab}	0.40 ±0.01^{b}
Average albumin relative wt.	57.15 ±0.60^{ab}	57.17 ±0.39^{ab}	58.24 ±0.64^{a}	54.84 ±2.02^{b}	56.51 ±0.50^{ab}	55.17 ±0.82^{ab}
Average albumin index	8.89 ±0.24^{a}	8.71 ±0.09^{a}	8.78 ±0.23^{a}	8.68 ±0.20^{a}	8.81 ±0.16^{a}	8.84 ±0.15^{a}
Yolk cholesterol						
(mg/g yolk)	9.27 ±0.41^{a}	8.93 ±0.89^{a}	10.11 ±1.44^{a}	8.85 ±0.25^{a}	8.85 ±0.33^{a}	8.89 ±0.34^{a}
(mg/whole yolk)	181.48 ±3.65^{a}	165.58 ±20.61^{a}	181.54 ±35.65^{a}	170.35 ±6.36^{a}	161.63 ±11.76^{a}	162.76 ±6.47^{a}
Yolk triglycerides						
(mg/g yolk)	10.11 ±0.49^{a}	10.31 ±1.55^{a}	9.74 ±0.13^{a}	9.75 ±0.52^{a}	9.95 ±0.66^{a}	9.56 ±0.48^{a}
(mg/whole yolk)	197.86 ±3.73^{a}	189.29 ±23.47^{a}	176.66 ±11.79^{a}	187.14 ±4.53^{a}	181.00 ±11.40^{a}	174.69 ±5.72^{a}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$). [1] DBA: Dried bile acids

Table 4: Effect of dietary vitamin B_{12} or biotin supplementation without or with dried bile acids on blood serum parameters of laying hens

	Supplementation					
	Control	Vitamin B_{12}	Biotin	Control	Vitamin B_{12}	Biotin
	Without DBA[6]			With DBA		
Blood serum lipid profile parameters						
Cholesterol (mg/dl)	198.15 ±0.70 [a]	191.23 ±6.12[a]	196.30 ±2.37[a]	195.53 ±3.43[a]	190.69 ±2.31[a]	190.77 ±2.75[a]
Triglyceride (mg/dl)	205.14 ±1.14[a]	205.86 ±3.62[a]	206.91 ±1.70[a]	203.61 ±3.06[a]	201.08 ±2.29[a]	204.90 ±0.58[a]
HDL[1] (mg/dl)	47.17 ±2.06[a]	48.43 ±2.10[a]	47.23 ±1.57[a]	49.80 ±1.42[a]	50.80 ±2.60[a]	51.63 ±1.49[a]
LDL[2] (mg/dl)	109.95 ±1.13[a]	101.62 ±5.46[a]	107.69 ±3.61[a]	105.01 ±3.24[a]	99.67 ±0.75[a]	98.15 ±3.06[a]
VLDL[3] (mg/dl)	41.03 ±0.23[a]	41.17 ±0.72[a]	41.38 ±0.34[a]	40.72 ±0.61[a]	40.22 ±0.46[a]	40.98 ±0.12[a]
Blood serum parameters related to liver functions						
GOT[4] (u/L)	65.33 ±17.9[a]	96.33 ±3.18[a]	71.33 ±16.51[a]	94.00 ±2.08[a]	82.50 ±12.50[a]	77.00 ±10.50[a]
GPT [5](u/L)	20.90 ±3.51[a]	24.67 ±1.45[a]	21.50 ±0.50[a]	21.67 ±1.45[a]	22.50 ±0.50[a]	24.67 ±1.67[a]
Liver tissue composition (%)						
Moisture	70.37 ±0.53[ab]	70.23 ±0.43[ab]	69.99 ±2.54[ab]	69.08 ±1.59[b]	73.03 ±0.71[a]	71.13 ±0.93[ab]
Fat (DM basis)	25.43 ±3.30[a]	22.78 ±2.47[a]	19.62 ±3.50[a]	22.86 ±4.18[a]	21.51 ±1.67[a]	23.61 ±0.99[a]
Fat (fresh basis)	7.55 ±1.05[a]	6.08 ±0.81[a]	5.96 ±1.27[a]	7.06 ±1.72[a]	5.82 ±0.59[a]	6.80 ±0.12[a]

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P<0.05$). [1]HDL: high-density lipoprotein, [2]LDL: low-density lipoprotein (LDL), [3]VLDL: very low-density lipoprotein, [4] GOT: glutamic oxaloacetic transaminase, [5]GPT: glutamic pyruvic transaminase. [6] DBA: Dried bile acids

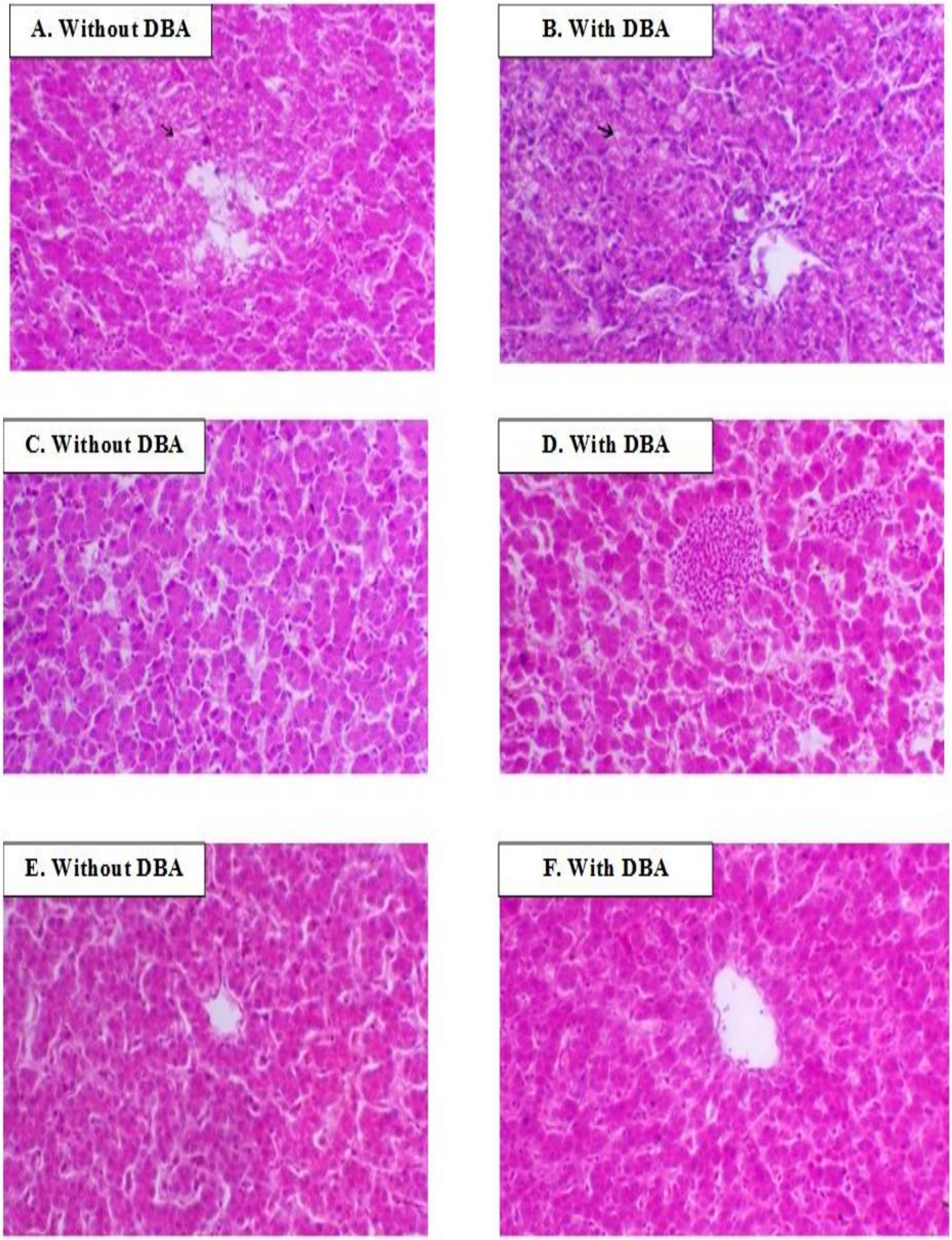

Figure 1: Histopathological changes of hepatic tissue of laying hens fed on control (without DBA) showing centrilobular hepatic vacuolation with fatty changes (arrow) (A); or with DBA mild degree of hepatic vacuolation consistent with fatty changes (arrow) (B); hepatic tissues of birds supplemented with vitamin B_{12} supplementation and without DBA or with DBA addition showing normal hepatocytes (C and D); Birds supplemented with biotin without or with DBA addition showing normal hepatocytes (E and F)

Conclusion

Under the conditions of the present study, vitamin B_{12} supplementation single or combined with dried bile acids is recommended in laying hen diet as it gave the best productive performance and improved fat digestibility, hepatic health and reduced the serum lipid profile. On the other hand, biotin supplementation is not recommended for laying hens except if combined with DBA.

Conflict of interest

All authors declared that no conflicts of interest, as they are responsible for the content and writing this article.

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