



Effects of Lipotropic Products on Productive Performance, Liver Lipid and Enzymes Activity in Broiler Chickens

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Abstract

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In a 42-d experiment, 576 one-day-old Vencobb 308 broiler chicks were used to investigate the effects of lecithin extract (0.5 g/kg), choline chloride 60% (1 g/kg) and Bio choline (1 g/kg) in diets of moderate and high energy in a 4 × 2 factorial arrangement on performance and certain physiological traits in broiler chickens. The inclusion of Bio choline and lecithin extract in the diet significantly increased average daily gain and improved feed conversion ratio in overall (1 to 42 d) period ($P < 0.05$). Performance efficiency index was improved in the birds fed with Bio choline compared to those fed control diet. Broilers fed diets containing Bio choline and lecithin extract had less abdominal fat percentage than those fed choline chloride or control diet. Regardless of dietary energy level, supplementation of diet with Bio choline, choline chloride and lecithin extract significantly decreased liver lipid concentration ($P < 0.05$). Aspartate aminotransferase activity increased in the serum of broilers fed high energy diets while it was decreased in the birds received diets containing choline chloride. Lipotropic compounds decreased serum aspartate aminotransferase activity in the birds fed on high energy diets. The addition of Bio choline and lecithin extract to diet significantly decreased serum γ -glutamyltransferase activity ($P < 0.05$). Results of the present study revealed that dietary supplementation of commercial lipotropic compounds could remove potential detrimental effects from high energy diets through reducing liver fat and maintaining liver health.

Introduction

The use of high energy diets aim at shortening the rearing period may increase metabolic disorders such as fatty liver syndrome (FLS) in broiler chickens (Leeson *et al.*, 1995). Increased abdominal fat pad (Corduk *et al.*, 2007), the incidence of leg problems (van Emous *et al.*, 2015) and hypertension (Gopi *et al.*, 2014) are some other detrimental responses associated

with high energy diets (Buyse *et al.*, 2001). Fatty liver syndrome is a condition that generally affects fast growing broilers fed high energy diets and caged layers with an inadequate chance to move and exercise freely (Jiang *et al.*, 2013). FLS is described as a metabolic disorder caused by a deficiency of methyl group donors in feed and decreased gluconeogenesis in the

liver due to biotin deficiency in commercial chicken (Jiang *et al.*, 2013). Death may be triggered by insufficient levels of the key biotin-dependent enzyme pyruvate carboxylase. Therefore, the inclusion of choline and biotin in commercial poultry diets may noticeably reduce the risk of FLS in birds.

Many studies have revealed that broiler health and performance and in a broad sense economy of production could be influenced by dietary supplementation of commercially available herbal and synthetic choline-containing compounds or lipotropic feed additives (Gujral *et al.*, 2002; Singh *et al.*, 2003; Waldroup *et al.*, 2006). Dietary supplementations of such compounds provide an effective nutritional strategy to decrease the adverse metabolic consequences of a high energy diet in broiler chicken (Leeson and Summers, 2005). Lipotropic agents are compounds serving broiler chicken to efficiently utilize the high energy diets (Corduk *et al.*, 2007). These additives may also decrease fat deposition in liver, in part, due to stimulating the liberation of lipids from liver (Wen *et al.*, 2014). However, different lipotropic agents may exert dissimilar metabolic supports in broiler depending on diet composition, bird's metabolic status as well as environmental conditions (Azadmanesh and Jahanian, 2014). Research on the efficacy of lipotropic agents for efficient utilization of high energy diets are not scanty but provide contradictory and confusing results, thus warranting further characterization of the broilers performance and liver function with the dietary administration of different lipotropic agents. This study was conducted to evaluate the responses in liver function and productive performance to dietary supplementation of three commercial lipotropic compounds in broiler chickens fed diets with moderate and high energy levels.

Materials and Methods

Birds and diets

Five hundred seventy-six one-day-old Vencobb mixed-sex broiler chicks were randomly allocated to eight experimental groups, comprising of four replicates of 18 birds. The birds were reared in floor pens furnished with rice husk in a grow-out broiler house under standard management practices. The environmental temperature was kept about 32°C during the first week and then gradually reduced by 2°C weekly to reach about 24°C

during the fourth week. A 1 : 23 darkness to lighting regimen was followed throughout the experimental period. Two corn-soybean basal diets with moderate and high energy levels were formulated based on Vencobb breeder company recommendations for starter (1 to 14 days) and grower (15 to 42 days) periods in broiler chicks. Chemical composition and the proximate analysis of the experimental diets are shown in Table 1. Each basal diet was supplemented with lecithin extract (0.5 g/kg), choline chloride 60% (1 g/kg) and Bio choline (1 g/kg) and provided to the birds in mash form for *ad libitum* consumption. All the lipotropic additives were provided from Peekay Agencies Pvt. Ltd. Kolkata - 700071, West Bengal, India, and supplemented in the diets based on the doses recommended by the company.

Growth performance

Considering pen as the experimental unit, data on body weight (BW) and feed intake (FI) were collected weekly for starter and grower periods and data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Mortality was recorded upon occurrence. Performance efficiency index (PEI) was calculated based on the following equation: $PEI = [(LW \times S) / (FCR \times AS)] \times 100$, where LW is live weight (kg), S is survival rate (%), FCR is feed conversion ratio and AS is age of slaughter (day) (Euribrid, 1994)

Blood and carcass measurements

At 42 d of age, 5 mL blood were collected from four birds in each replicate by the brachial vein puncture in non-heparinised tubes and kept on slush-ice until they were subjected to serum collection by centrifuging the whole blood sample at $2,500 \times g$ for 10 min. The serum samples were analyzed for activity of γ -Glutamyltransferase (GGT, E.C.2.3.2.2), and aspartate aminotransferase (AST, E.C.2.6.1.1) (as the indicators of liver health) using the Express Plus (Ciba-Corning Diagnostics Corp., Medfield, MA) automated clinical chemistry analyzer according to the manufacturer's directions (Elliott, 1984).

Four randomly selected birds from each replicate were sacrificed by cervical dislocation at day 42 of age to collect the data on carcass yield (%) and abdominal fat percentage. Liver total lipids were measured in all the sacrificed birds using the method of Folch *et al.* (1957).

Statistical analysis

Collected data were subjected to two-way ANOVA using GLM procedure of SAS® software (SAS, 1996). Means were partitioned

using Duncan's multiple range tests. All the values expressed as significant were tested at $P < 0.05$ level.

Table 1. Ingredients and nutrient composition of basal diets

Ingredients (%)	Starter(d 1-14 of age)		Grower(d 15-42 of age)	
	Moderate energy	High energy	Moderate energy	High energy
Yellow maize	52.30	47.64	56.67	52.17
Soybean meal	38.77	39.62	36.22	37.04
Corn gluten meal	1.98	1.98	-	-
Soybean oil	2.06	5.89	2.58	6.27
CaCO ₃	1.75	1.74	1.60	1.60
Monocalcium phosphate	1.42	1.42	1.29	1.30
Sodium bicarbonate	0.15	0.15	0.15	0.15
Mineral Premix ¹	0.25	0.25	0.25	0.25
Vitamin Premix ²	0.25	0.25	0.25	0.25
Salt	0.24	0.24	0.24	0.24
DL-Methionine	0.18	0.19	0.16	0.17
L-Lysine HCL	0.09	0.08	0.05	0.03
L-Threonine	0.06	0.05	0.04	0.03
Zeolite	0.50	0.50	0.50	0.50
<i>Nutrient composition</i>				
ME (Kcal/kg)	2950	3250	2974	3286
Crude protein (%)	22.25	22.25	20.10	20.15
Lysine (%)	1.19	1.19	1.11	1.11
Threonine (%)	0.88	0.88	0.79	0.79
Methionine + Cystine (%)	0.92	0.92	0.83	0.83
Calcium (%)	1.00	1.00	0.98	0.98
Available P (%)	0.48	0.48	0.44	0.44

¹Vitamin premix provided the following per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,500 IU; vitamin E (dl- α -tocopheryl acetate), 60 mg; vitamin K (menadione), 3mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B₁₂ (cyanocobalamin), 0.01 mg; niacin, 45 mg; pantothenic acid (d-calcium pantothenate), 11 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 500 mg; ethoxyquin (antioxidant), 150 mg.

²Mineral premix provided the following per kilogram of diet: Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg.

Results

Broiler chickens fed on high energy diets gained more and approached greater body weight than those birds received moderate energy diets at day 28 and 42 of age (Table 2). Supplemented diets with Bio choline, and Lecithin extract, irrespective of energy level, improved body weight of the birds compared to the control diet at day 42 of age (Table 2). The inclusion of Bio choline and Lecithin extract significantly increased ADG in overall (1 to 42 d) experimental period. Lipotropic agents were more effective in increasing growth rate of broilers fed moderate energy level compared to those fed high energy level.

Supplementation of Bio choline, choline chloride and Lecithin extract to moderate and

high energy diets caused no significant difference in ADFI when compared to their corresponding control diets (Table 3). However, ADFI increased ($P < 0.05$) in the birds fed high energy diets when compared to moderate energy diets during the grower (15 to 42 d) and overall (1 to 42 d) periods. FCR was lower in the birds fed Bio choline and Lecithin extract than the control birds. Dietary energy level had no significant effect on FCR (Table 3).

Performance efficiency index was improved in the birds fed diets supplemented by Bio choline compared to those fed on control diet (Figure 1). The addition of Lipotropic compounds to the high energy diet increased PEI, but this effect was not observed with moderate energy diet.

Table 2. Effect of different lipotropic compounds with moderate and high energy diets on body weight (BW) and average daily gain (ADG) in broiler chickens

Main effects/levels	BW (g)			ADG (g/b/d)		
	14 d	28 d	42 d	1 to 14	15 to 42	1 to 42
Energy level						
Moderate	280.7	952.3 ^b	1921.8 ^b	16.2	58.6 ^b	44.5 ^b
High	285.5	969.4 ^a	1965.1 ^a	16.5	60.0 ^a	45.5 ^a
SEM	6.37	10.37	22.93	0.23	0.56	0.42
Lipotropic compound						
None	282.9	945.1 ^b	1902.2 ^b	16.3	57.8 ^b	44.0 ^b
Bio choline	283.9	975.6 ^a	1981.9 ^a	16.4	60.6 ^a	45.9 ^a
Choline chloride	281.6	954.7 ^{ab}	1915.7 ^b	16.2	58.4 ^{ab}	44.3 ^b
Lecithin extract	284.1	968.1 ^{ab}	1974.0 ^a	16.4	60.3 ^{ab}	45.7 ^a
SEM	6.74	10.83	24.18	0.28	0.93	0.54
Energy level × Lipotropic compound						
Moderate Control	283.4	938.7	1866.6	16.4	56.4 ^b	43.1 ^b
Moderate Bio choline	277.7	975.6	1979.2	16.0	60.7 ^a	45.8 ^a
Moderate Choline chloride	277.4	929.7	1895.9	15.9	65.7 ^a	43.8 ^b
Moderate Lecithin extract	284.3	965.6	1945.4	16.4	59.3 ^{ab}	45.0 ^{ab}
High Control	282.4	951.6	1937.9	16.3	59.1 ^{ab}	44.8 ^a
High Bio choline	290.0	975.7	1984.6	16.8	60.6 ^a	46.0 ^a
High Choline chloride	285.7	979.8	1935.5	16.5	59.0 ^{ab}	44.8 ^a
High Lecithin extract	283.9	970.7	2002.5	16.4	61.4 ^a	46.4 ^a
SEM	7.04	11.22	26.93	0.17	0.49	0.38
Probabilities						
Energy level	0.398	0.014	0.039	0.398	0.010	0.001
Lipotropic compound	0.987	0.012	0.009	0.987	0.041	0.001
Energy level × Lipotropic compound	0.791	0.053	0.791	0.792	0.035	0.048

^{a,b}Means in the same column without same superscript differ significantly ($P < 0.05$).

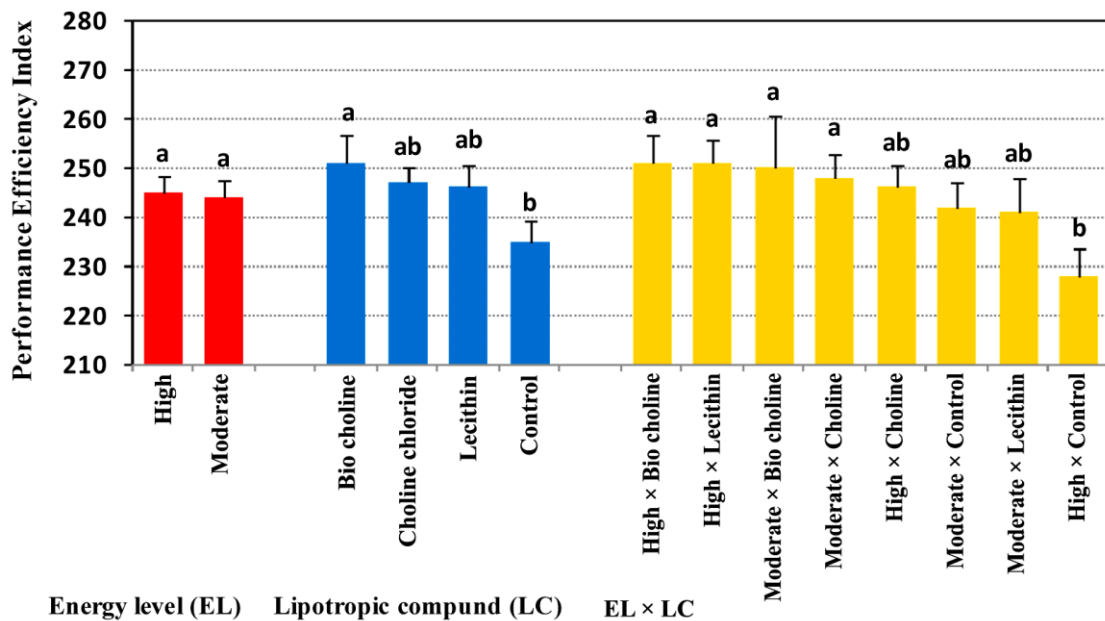


Figure 1. Effect of high and moderate energy levels of diet ($SEM=3.35$ and $P=0.874$), lipotropic compounds ($SEM= 4.22$ and $P=0.031$) and their interaction ($SEM=5.85$ and $P=0.043$) on performance efficiency index in broiler chickens through day 1 to 42 of age.

Table 3. Effect of different lipotropic compounds with moderate and high energy diets on average daily feed intake (ADFI) and feed conversion ratio (FCR) in broiler chickens

Main effects/levels	ADFI (g/b/d)			FCR (g/g)			
	1-14 d	15-42 d	1-42 d	1- 14 d	15-42 d	1-42d	
Energy level							
Moderate	24.6	119.4 ^a	88.1 ^a	1.52	2.03	1.97	
High	24.7	114.6 ^b	85.0 ^b	1.49	1.91	1.87	
SEM	0.44	3.11	2.09	0.02	0.01	0.02	
Lipotropic compound							
None	25.0	117.8	86.9	1.53	2.04	1.97 ^a	
Bio choline	24.7	117.7	86.7	1.50	1.94	1.88 ^b	
Choline chloride	24.6	117.3	86.4	1.51	2.00	1.95 ^{ab}	
Lecithin extract	24.6	117.2	86.3	1.50	1.94	1.85 ^b	
SEM	0.32	2.01	1.87	0.04	0.03	0.02	
Energy level × Lipotropic compound							
Moderate	Control	25.3	114.1 ^b	84.5 ^b	1.54	2.02	1.96
Moderate	Bio choline	24.6	117.3 ^{ab}	86.4 ^b	1.53	1.93	1.88
Moderate	Cholinechloride	24.2	115.4 ^{ab}	85.0 ^b	1.52	1.75	1.94
Moderate	Lecithin extract	24.5	114.6 ^b	84.6 ^b	1.49	1.93	1.88
High	Control	24.6	121.7 ^a	89.3 ^a	1.51	2.06	1.99
High	Bio choline	24.8	117.9 ^{ab}	86.9 ^b	1.47	1.94	1.88
High	Cholinechloride	24.9	119.2 ^a	87.8 ^{ab}	1.51	2.02	1.95
High	Lecithin extract	24.6	119.8 ^a	88.1 ^a	1.50	1.95	1.89
SEM		0.48	2.08	1.20	0.03	0.05	0.07
Probabilities							
Energy level		0.845	0.048	0.001	0.449	0.604	0.277
Lipotropic compound		0.887	0.985	0.928	0.867	0.335	0.046
Energy level × Lipotropic compound		0.636	0.037	0.041	0.719	0.804	0.959

^{a,b}Means in the same column without same superscript differ significantly ($P < 0.05$).

Carcass yield percentage did not change by supplementation of Bio choline, choline chloride and Lecithin extract to the moderate and also high energy diets (Table 4). The birds received diets containing Bio choline and Lecithin extract had lower abdominal fat percentage than those fed on choline chloride or control diets (Table 4). Abdominal fat was increased in the birds fed high energy diet by 13.4% compared to those birds fed moderate energy diet. Supplementation of the diet with Bio choline, choline chloride and Lecithin extract decreased liver lipid concentration (LLC) by 21.9, 16.1 and 22.8%, respectively (Table 4).

Aspartate aminotransferase activity increased in the broilers fed high energy diets while it was decreased in the birds received diets containing choline chloride (Table 4). The addition of Bio choline, choline chloride and Lecithin extract to diet decreased serum GGT activity in broiler chicken fed on moderate, but not on high energy diets (Table 4).

Discussion

The results of this study with respect to increased growth performance in the birds fed

on diets supplemented with lipotropic agents agreed with the findings of Cengiz *et al.* (2012) and Jiang *et al.* (2013) and disagreed with the findings of Waldroup *et al.* (2006). The improved body weight observed in the broilers received Bio choline and Lecithin extract supplemented diets may be attributed to efficient carbohydrate and fat metabolism supported by increased biotin and choline concentration in serum and liver cells. Lecithin extract supplemented to moderate and high energy diets exhibited greater growth promotion effect. Similar results were reported by Jahanian and Rahmani (2008) who observed greater weight gain in birds fed by Lecithin extract as compared to those fed with a choline chloride-added diet. The pronounced growth encouraging effect of Lecithin extract may be attributed to lecithin content providing more freely available biotin and choline vitamins.

In contrast to the results of current study, Waldroup *et al.* (2006) observed improved FI in birds fed on a choline-supplemented diet. The marginal improvement in FI observed in Bio choline groups could be reasoned by its medicinal plants ingredient containing a broad

spectrum of vitamins, acids and alkaloids among many other active compounds mainly supposed to increase bile flow and improve feed intake. Natural biotin in Bio choline and lecithin and related constituents in Lecithin extract might have enhanced the assimilation of dietary

nutrients (Cengiz *et al.*, 2012). Increased weight gain with no change in FI and minute improvements in FCR by experimental diets suggest that lipotropic agents (especially Bio choline and choline chloride) may improve the utilization of energy in the diet.

Table 4. Effect of different lipotropic compounds with moderate and high energy diets on abdominal fat (% BW), liver lipid concentration (LLC), carcass yield and serum enzyme activity

Main effects/ levels	Abdominal fat (%BW)	LLC (mg/g)	Carcass Yield (%)	Enzyme activity		
				AST (IU/L)	GGT (IU/L)	
Energy level (EL)						
Moderate	1.95 ^b	41.23 ^b	71.77	189 ^b	18.3	
High	2.21 ^a	56.87 ^a	71.05	212 ^a	17.1	
SEM	0.17	4.32	0.87	5.14	2.81	
Lipotrophic compound (LC)						
None	2.21 ^a	57.85 ^a	71.85	205 ^a	21.3 ^a	
Bio choline	1.97 ^b	45.20 ^b	71.62	202 ^{ab}	16.9 ^b	
Choline chloride	2.08 ^{ab}	48.52 ^b	71.01	198 ^b	18.6 ^{ab}	
Lecithin extract	1.92 ^b	44.65 ^b	71.24	198 ^{ab}	14.2 ^c	
SEM	0.13	3.11	0.75	3.26	2.11	
Energy level × Lipotropic compound						
Moderate	Control	1.90 ^{bc}	49.41 ^c	72.03	190 ^{cd}	19.6 ^{bc}
Moderate	Bio choline	1.92 ^c	35.10 ^d	71.21	185 ^d	19.2 ^{bc}
Moderate	Choline chloride	1.96 ^{bc}	41.0 ^{cd}	70.90	195 ^{cd}	19.1 ^{bc}
Moderate	Lecithin extract	1.93 ^{bc}	39.31 ^d	73.08	185 ^d	15.5 ^{cd}
High	Control	2.51 ^a	66.32 ^a	71.72	220 ^a	23.0 ^a
High	Bio choline	2.03 ^b	55.30 ^{bc}	72.06	218 ^a	14.5 ^d
High	Choline chloride	2.20 ^{ab}	55.91 ^b	71.11	200 ^{bc}	18.1 ^c
High	Lecithin extract	1.92 ^{bc}	50.07 ^c	69.45	211 ^{ab}	13.1 ^d
SEM		0.11	2.43	0.96	3.64	1.95
Probabilities						
Energy level		0.012	0.001	0.346	0.030	0.108
Lipotrophic additive		0.039	0.018	0.731	0.045	0.001
EL × LC		0.002	0.031	0.213	0.021	0.013

^{a-d}Means in the same column without same superscript differ significantly (P < 0.05).

No single production indication entirely demonstrates the economic output of a broiler flock. Researchers, therefore, suggested pooling of the fractional influence of the major production parameters in an index to compare the performance of different flocks or the birds assigned to different treatments (Lup *et al.*, 2010; Samarakoon and Samarasinghe, 2012). The PEI created by Euribrid (1994) calculated based on final body weight, FCR and mortality. The calculated index for the treated birds with 1 g/kg dietary supplementation of bio choline was greater than the index calculated for the control birds. The trace advantages of the diets supplemented with 1 g/kg bio choline as increased weight gain and improved FCR in 1 to 42 days of age and, in particular, lower mortality rate accumulated in the index and suggested bio

choline as a lipotropic feed additive of economic interest.

Decreased liver lipid concomitant with increased abdominal fat in the current study support the results reported by Cengiz *et al.* (2012) who observed efficient carbohydrate and lipid metabolism by incorporating lipotropic agents into broiler diets. Biotin and choline play an essential role in the transportation of lipids from liver toward peripheral tissues and organs in the form of lipoproteins (Quarantelli *et al.*, 2007). Rama Rao *et al.* (2001) observed choline supplementation of broiler breeder's diets reduced liver fat, the results which confirmed by Waldroup *et al.* (2006) who also showed choline deficiency in quails increased liver fat and supplementing choline into quail diet decreased liver fat percentage. Zhai *et al.* (2013) in laying

hens and Jahanian and Rahmani (2008) with broilers chicks reported decreased liver fat content when choline was added to the diet. Choline is responsible for lipoproteins formation (Kettunen *et al.*, 2001), therefore, choline mobilizes the liver fat in the form of lipoproteins toward extra-hepatic tissues where that may be metabolized or deposited.

Change in carcass percentage was anticipated as fat metabolism and increased abdominal fat was observed in this study. However, no difference was observed in carcass yield of the birds fed on diets with and without lipotropic agents. These results were in accordance with Wen *et al.* (2014) results and disagreed with Cengiz *et al.* (2012) findings. Liver as a principal organ in avian metabolism is susceptible to nutritional alterations. Serum ALT and AST low activity are usually interpreted as satisfactory liver health and functionality (Corduk *et al.*, 2007). Live cell damages caused by metabolic pressure and hypertension ease the liberation of these cellular enzymes into the serum. As the results of the current study showed, using high energy diets to meet the broiler's energy demand caused liver damages, phenomena which happen frequently with commercial broiler flock to facilitate the growth rate and shorten the raising period. In this study, no hepatic damage demonstrated by increased enzyme activity observed in the birds fed on moderate energy diets. Feeding diets with high energy elevated activity of circulatory AST and GGT (Walzem *et al.*, 1993), which could be attributed to hepatic biliary damage caused by fat accumulation. In contrast with our results, Corduk *et al.* (2007) found elevated serum AST activity in the birds fed on a high-energy diet compared to those received low energy diets.

The inclusion of Bio choline and Lecithin extract was effective in normalizing the increased activities of enzymes caused by high energy diets as indicated by normal GGT activity in supplemented groups as compared to their corresponding control and choline chloride supplemented groups. These findings indicate the restoration of the liver to a fully functional status by dietary administration of Bio choline and Lecithin extract in broiler chicken grown on high energy diets. However, no consistent positive effects with a single lipotropic compound for all examined variables in the current study suggest plausible encouraging

effect for the inclusion of three agents in a practical diet to trace the effects of all agents in a single diet.

Conclusion

Results of the study reveal that high energy diets improve growth performance of broiler chicks and dietary supplementation of commercial lipotropic compounds can remove potential detrimental effects from such diets through reducing liver fat and maintaining liver health.

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