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THE IMPLICATIONS OF L-CARNITINE AND SILYMARIN SUPPLEMENTATION ON GROWTH PERFORMANCE AND SOME BLOOD PARAMETERS OF BROILERS

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ABSTRACT

Chick performance, carcass traits and some blood biochemical parameters of broilers fed basal diet supplemented with L-*carnitine or silymarin* were studied for 42 days in the experiment. This study was conducted on 300 chicks (Ross 308 hybrid) divided randomly into three groups, (100 chicks per each) and each group was subdivided into four replicates (25 chicks for each). Chicks in the first group (C) were fed ad-libitum on the basal control diets (starter from 1 to 21 days of age, and grower- finisher from 22 to 42 days of age) without addition of L-carnitine and silymarin. Birds in the second group (L) received the same basal diet supplemented with L-carnitine (300mg/kg diet) while, chicks in the third group (S) fed on basal diet supplemented with silymarin (160mg/kg diet) during two experimental periods. The results showed that, supplementation of silymarin and L-carnitine improved significantly body weight gain at starting period. The highest body weight gain was recorded with silymarin supplemented group (662±20 g) in comparison with control (593.5±20g). At the end of the experiment (42 days), the best cumulative weight gain and feed conversion recorded in silymarin supplemented group (2163±23g & 1.9) followed by L-carnitine supplemented group (2103.5±16g &1.94) while the birds fed on control diet had the worst values (1893.8±65g & 1.96). Abdominal fat deposition of L and S groups were significantly lower than control group, while no significant differences were observed in dressed carcass and breast muscle weights. From the results of this study, it can be concluded that, the addition of silymarin to broiler diets significantly improved the body weight gain and feed conversion indices all over the period of raising, while L-carnitine improved growth performance of broilers at growing-finishing period (high energy diet). Finally, it is recommended that we can use both silymarin and L-carnitine as feed additives at growing-finishing period.

Key words: *Broilers, silymarin, L-carnitine, growth performance, carcass traits.*

INTRODUCTION

Poultry market is one of the fastest-growing businesses in Egypt. Latterly, meat-type chicks have been intensively selected for body weight gain. This selection strategy has resulted in a greater growth rate with improved feed conversion efficiency. However, excessive fat deposition is one of the undesirable consequences of selection for increased growth of modern broiler chicks. Accumulation of fat in broiler carcasses considers a waste product to consumers, nutritional and health aspects of their food so; nutritionists continuously try to mediate this problem by means of dietary manipulations, in order to achieve the desired characteristics of growth and carcass composition (Tabeidian *et al.,* 2010).

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Carnitine has gained interest in the recent years as a potential feed additive for improving chicken meat production. Carnitine is synthesized from methionine and lysine with magnesium-ATP, vitamins C, B6, niacin and iron, which are all necessary as catalysts (Bremer, 1983). L-carnitine requirement was not considered due to endogenous biosynthesis. However, Parsaeimehr *et al.* (2012) reported that L-carnitine becomes an essential nutrient under certain circumstances, such as limited carnitine biosynthesis in young animals, diets low in carnitine content, conditions of stress, higher performance, and diets rich in fat. Murali *et al.* (2015) stated that L-carnitine plays a key role in energy metabolism of cells, mainly by transferring long-chain acyl groups from cytoplasm to mitochondrial matrix for oxidation by the fatty acid oxidation complex. L-carnitine supplementation may have a beneficial effect on broiler nutrition status, mainly due to its sparing effect on its precursor's

lysine and methionine (Hossininezhad *et al.,* 2011). In addition, Murali *et al.* (2015) investigated the anti-oxidant properties of L-carnitine which increases the levels and activity of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase. L-carnitine has a role in stabilizing cell membranes and in regulating the function of ion channels because of reducing the amount of oxidative damage that happened in membrane phospholipids (Kalaiselvi and Panneerselvam, 1998). Rabie *et al.* (1997) and Parsaeimehr *et al.* (2012) found that adding L-carnitine improved the growth performance of broilers. Some authors provided conclusive evidence that L-carnitine has a beneficial effect on broilers performance, while others held that it has not or even adversely affects production results and mortality (Golzar Adabi *et al.*, 2011). Harpaz *et al.* (1999) demonstrated that adding L-carnitine resulted in protection against cold stress, while it had a significant response to the acute heat stress (Whitehead *et al.*, 1997). In addition, it strengthened chick's immune function by enhancing antibody responses (Deng *et al.*, 2006).

Silymarin exhibits chemopreventive activity against chemical, viral, bacterial and fungal toxins, inhibits lipid peroxidation, and stabilizes the cell membranes of the liver parenchyma (Opletal and Skrivanova, 2010). Silymarin presents a pharmacologically effective substance containing four main constituents: silybin, isosilybin, silychristin and silydianin (Ding *et al.,* 2001). Fraschini *et al.* (2002) and Suchy *et al.* (2008) literatures stated that silymarin acts in four different ways: as an antioxidant, absorber and regulator of the intracellular glutathione**;** as a stabiliser and regulator of cell membrane permeability that prevents the entering of hepatotoxic substances into hepatocytes**;** as the ribosomal RNA synthesis promoter simulating regeneration of the liver**;** and as an inhibitor of the transformation of liver stellate cells into myofibroblasts - the process responsible for deposition of collagen fibres in liver. Furthermore, absorption of free radicals is considered as a key mechanism securing liver protection (Fraschini *et al.,* 2002). Silymarin supplementation increased productive and reproductive performances and improved livestock health status of animals (Tedesco, 2001). Skottova and Krecman (1998); Crocenzi and Roma (2006) pointed out its potent anticholestatic activity and the possibility of a direct impact of silymarin on the cholesterol metabolism by means of its biosynthesis inhibition. L-carnitine and silymarin are known to increase antioxidant status during aging (Arenas *et al.,* 1998 and Fraschini *et al.,* 2002). The present study was conducted to evaluate the effects of supplementation silymarin or L-carnitine to broiler diets on the performance, carcass traits and some blood parameters.

MATERIALS AND METHODS

Experimental chicks, housing, and management:
This experiment was performed on Ross 308 hybrid (n = 300) chicks which fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 1 to day 42 of chick's age. Room temperature and humidity were controlled and lighting system was 24 hours light. Table 1 shows chemical composition of control; and experimental rations. The rations were calculated according to the recommended nutrient requirements (NRC, 1994). Chicks were fed ad-libitum, and health status was evaluated daily. All broilers were vaccinated against Newcastle and infectious bronchitis at hatch and IBD at 15 days old.

Experimental design and feeding:
Three hundred one –day old chicks were divided randomly into three groups, (100 chicks per each) and each group was subdivided into four replicates (25 chicks for each). Chicks in the first group (C) were fed ad-libitum on the basal control diets (starter from 1 to 21 days of age, and grower- finisher from 22 to 42 days of age) without addition of L-carnitine or silymarin. Birds in the second group (L) received the same basal diet supplemented with L- carnitine (300mg/kg diet) while, chicks in the third group (S) fed on basal diet supplemented with silymarin (160mg/kg diet) during two experimental periods. A 3×4 replicates arrangement of treatments was used in Exp. A standard basal diet was formulated from maize, soybean meal, concentrate mixture and sunflower oil along with other ingredients to meet minimum nutrient requirements of broiler, as established by National Research Council (NRC, 1994) as shown in Table 1.

Table 1: Ingredients and chemical composition of basal control diets

Ingredients	Starter diet 0-21 days of age	Grower-finisher diet 22-42 days of age
Corn grain	56	63.95
Soy bean meal	28	21
Conc. mixture	10	10
Sunflower oil	2.2	1.6
Di-calcium phosphate	2	1.8
Ca-carbonate	0.8	0.8
Salt	0.3	0.3
Na bicarbonate	0.25	0.1
Mineral and Vitamins premix*	0.3	0.3
Methionine	0.1	0.1
Lysine	0.05	0.05
Total	100	100
Calculated analysis		
Metabolizable energy (kcal/kg)	2988	3176
Protein (%)	21	18
Calcium (%)	1.0	0.90
phosphorus	0.9	0.8
Lysine (%)	1.4	1.2
Methionine (%)	0.58	0.57

* Premix provided the following per kg of diet: vitamin A (vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B12, 11 µg; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper,7 mg; iodine, 1 mg; selenium, 0.2 mg.

Tested parameters:

Performance measurements:

Live body weight was measured to the nearest gram at the beginning of the experiment and at the end of the starter period (21 days old) and finisher period (42 days old). The amount of feed consumed was weekly recorded in each of the different experiment groups. The average amount of feed intake of each bird was calculated by dividing the weekly consumed food by its respective number of birds in each group at this week.

Body weight gain (BWG) was calculated by subtracti ng the average
initial LBW at a certain period from the average final LBW at the same period. Feed conversion was calculated by dividing feed consumption by total BW gain. Mortality was observed and recorded. Chicks Health status was evaluated daily.

Carcass yields

Prior to slaughter birds were given a feed withdrawal period of 12h. At the end of experiment, 12 birds were randomly selected from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. Selected chickens were deboned and weighed breast muscle and abdominal fat. These values were calculated as a percentages of live weight.

Blood biochemical Parameters

Blood samples from three chicks of each group were collected by puncturing the wing vein. Blood samples were allowed to stand for one hour and centrifuged at a speed of 3.000 rpm for 10 minutes. The clear serum was collected in sterilized disposable plastic tubes and stored at -20 ºC for further analysis. The individual serum sample was analyzed for total proteins, albumin, creatinine, triglyceride, cholesterol, and aspartate amino transferase (AST). Also, the methodology for each parameter was based on recommendations of the manufacturer of the analytical system.

Statistical analysis:

The data was statistically analyzed with the standard procedures of analysis of variance (ANOVA), using completely randomized block design as suggested by Steel and Torrie (1981). The statistical packages SAS (1998) were used to perform the above analysis on computer.

RESULTS

The summary of the growth performance variables is listed in Table 2 & 3. Obtained data revealed that supplementation of silymarin and L-carnitine improved significantly ($p<0.05$) body weight gain at starting period (first three weeks of age) compared with control. The highest body weight gain was recorded by silymarin supplemented group (662±20 g) in comparison with control (593.5±20g). At the end of the experiment (42 days), the best cumulative weight gain and feed conversion recorded in silymarin supplemented group (2163±23g & 1.9) followed by broilers in L-carnitine supplemented group (2103.5±16g &1.94) while the birds fed on control diet had the worst values (1893.8±65g & 1.96).

Table 2: Body weight gain (g/bird) of broilers during the experimental period

Groups	1-3 weeks of age	4-6 weeks of age	1-6 weeks of age
Control group (C)	593.5±20[b]	1300.3±58[b]	1893.8±65[b]
L-carnitine group (L)	624.4±30[b]	1479.1±39[a]	2103.5±16[b]
Silymarine group (S)	662±20[a]	1501±32[a]	2163±23[a]

* Figures in the same column having the same superscripts are not significantly different (P<0.05)

Concerning feed intake at starting period, L-carnitine supplemented group recorded lowest feed intake in comparison with control and silymarin supplemented groups (Table 3). Birds in the two experimental groups recorded numerical feed intake at growing-finishing period when compared with control, with the highest value scorded by silymarin supplemented group (3260±22g). Regarding the cumulative feed consumption during the whole experimental period, silymarin supplemented group recorded numerically higher feed intake (4120±80g) compared to L-carnitine supplemented group (4072±80g).

Table 3: Feed intake(g/bird) and final FCR of broilers during the experimental period

Groups	1-3 weeks of age	4-6 weeks of age	1-6 weeks of age	FCR
Control group (C)	872±40	3197 ±72	4069±65	2.15
L-carnitine group (L)	829±35	3243±61	4072±80	1.94
Silymarine group (S)	860±20	3260±22	4120±74	1.90

* Figures in the same column having the same superscripts are not significantly different (P<0.05)

Abdominal fat deposition of L and S groups were significantly lower than control group, while no significant differences were observed in dressed carcass and breast muscle weights (Table 4). Blood parameters appeared to be of particular interest mainly at the age of 42 days when S and L groups showed higher plasma protein levels (table 5).

Table 4: Carcass traits parameters of broiler at the end of the experiment

Groups	Dressed carcass (% of live body weight)	Breast (% of carcass weight)	Abdominal fat (% of live body weight)
(C)	65.17	37.53	1.96[b]
(L)	653.6	36.3	1.6[a]
(S)	65.49	37.44	1.88[a]

* Figures in the same column having the same superscripts are not significantly different (P<0.05)

Table 5: Blood biochemical parameters of broiler at the end of the experiment

Groups	Protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	AST (U/1000ml)	Cholesterol (mg/100ml)	Triglycerides (mg/100ml)	Creatinine (mg/100ml)
(C)	3.94±0.26	1.57±0.08	2.37±0.1	127.1±12	111.49±11[b]	64.28±28	0.15
(L)	4.17±0.15	1.63±0.09	2.54±0.15	139±23	144±9[a]	87±19	0.15
(S)	4.3±0.34	1.61±0.05	2.69±0.11	144.6±20	108.9±17[b]	83.48±24	0.16

* Figures in the same column having the same superscripts are not significantly different (P<0.05)

DISCUSSION

Growth performance:

The significant improvement of weight gain and higher feed intake of silymarin supplemented group may be attributed to its mechanisms of action that are antioxidant, cell membrane stabilizer and permeability regulator, as well as a promoter of DNA, RNA, and protein synthesis (Magliulo *et al.,* 1973). Similarly, Gowda and Sastry (2000) confirmed a significant improvement of silymarin on body weight gain and attributed its effects to antioxidant activity in the protein synthesis stimulation by the bird's enzymatic system. The higher weight gain was reported by Chakarverty and Parsad (1991). In contrast to our results, Suchy *et al.* (2008) and Wojcik *et al.* (2002) observed that the addition silymarin as Silybum Marianum seed cakes caused a decrease in the weight gain and feed conversion ratio. This explained as the herbal seed be bitter in taste on have another anti-nutritional factor attributed to decrease growth and increase FCR.

Improvement of final body weight and decrease of FCR of L- carnitine supplemented group also attributed to L-carnitine plays an important role in energy metabolism of cells (Murali *et al.,* 2015) and its sparing effect on its precursor's lysine and methionine (Hossininezhad *et al.,* 2011). In addition, Murali *et al.* (2015) found that antioxidant activity of L-carnitine increases the activity of other antioxidant enzymes, L-carnitine has a role in stabilizing cell membranes and in regulating the function of ion channels (Kalaiselvi and Panneerselvam 1998).

The numerical increase of bodyweight gain and decrease feed intake of L group than control at starting period may be explained as l-carnitine helps chicks for better assimilation of dietary protein and energy and has sparing effect of L-carnitine on essential amino acids (methionine and lysine). The significant increase of weight gain at finisher period could be attributed to the protective effects of kidney besides guarding the perfect metabolic pathway of fat and carbohydrate to growth and not for fat deposition. The significant increase of weight gain of S group in coordination with decrease feed intake than C control could be attributed to against toxins in feed and antioxidant, and simulating regeneration of the liver (Opletal and Skrivanova, 2010; Fraschini *et al.,* 2002).

Carcass Traits and blood parameters:

Addition of L-carnitine to the diet did not affect carcass weight, carcass ratio and breast to the carcass weight (Table 5). Similar results obtained by Zhou *et al.* (2009) who found that L carnitine and silymarin supplementation to the diet did not change carcass trait, but decreased abdominal fat ratio. Interestingly, L-carnitine and silymarin addition to the diet induced higher leg ratio than the control group. This is a positive situation regarding the economic condition and this may be attributed to the effect of both L carnitine and silymarin in liver protection and improve of ribosomal enzymes and increase the activity and size of mitochondria in active muscle cells of thigh. Lien and Horng (2001) found negative effect of L-carnitine supplementation to the broiler chicken diet on carcass yield traits. Discrepancies between this study and other studies on carcass yield traits may be related to differences of silymarin source and L-carnitine and their doses.

In addition, a slight, but not significantly increase in triglyceride levels was observed in both treated groups, probably indicating an increase in metabolism, a reduction in hepatic storage of lipids or increase or increased lipid mobilization. Overall serum AST activity was higher for treated groups and a higher value ($p<0.05$) was recorded for S group. AST activity was found to be the most sensitive indicator of liver damage by Lumeij (1997) and was indicated as useful for diagnosis of fatty liver hemorrhagic syndrome by Yousefi *et al.* (2005). Birds normally have been reported to show serum AST activity up to 230 U/L (Coles, 1986). On this basis, we can conclude that all the values observed in the present study were included in the normal range of AST activity. In this study, treatments did not display the hepatoprotective activity effect suggested by others authors (Lang *et al.*, 1990 and Erdogan *et al.,* 2005).

CONCLUSION

From the results of this experiment, it can be concluded that the addition of silymarin to broiler diets significantly improved the body weight gain and feed conversion indices all over the period of raising. L-carnitine improved growth performance of broilers at growing-finishing period (high energy diet). Finally, it is recommended that we can use both silymarin and L-carnitine as feed additives at growing-finishing period.

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اثر اضافة ال-كارنيتين والسيليمارين على كفاءة الأداء وبعض قياسات الدم في بدارى التسمين

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تم دراسة كفاءة الاداء وخصائص الذبائح وبعض من قياسات الدم في بدارى التسمين المغذاه على علائق مضافا اليها ال-كارنيتين وسيليمارين كاضافة علفية لمدة ٤٢ يوم. أجريت الدراسة على عدد ٣٠٠ كتكوت تسمين (روس ٣٠٨ هجين) وقد قسمت عشوائيا إلى ثلاث مجموعات (١٠٠ كتكوت لكل منها) وقسمت كتاكيت كل مجموعة إلى أربعة مكررات (٢٥ كتكوت لكل مكرر). تم تغذية الكتاكيت في المجموعة الأولى (C) تغذية حرة على عليقة اساسية ضابطة بدون اضافة ال-كارنيتين او السيليمارين (باديء من ١-٢١ يوما من العمر، ونامى- ناهى من ٢٢ الى ٤٢ يوما من العمر)، اما فى المجموعة الثانية (L) تم تغذية الكتاكيت من نفس العليقة الاساسية للمجموعة الضابطة مع اضافة ال-كارنيتين بمقدار ٣٠٠مجم / كجم من العليقة ، في حين ان المجموعة الثالثة (S) غذيت الكتاكيت من نفس العليقة الاساسية للمجموعة الضابطة مع اضافة السيليمارين بمقدار ١٦٠ مجم / كجم من العليقة وتم ذلك خلال فترتي الدراسة. أظهرت النتائج أن اضافة سيليمارين وال-كارنيتين قد حسنت بشكل ملحوظ وزن الجسم في الفترة البادئة وتم تسجيل أعلى زيادة لوزن الجسم فى الكتاكيت التى تتغذي على السيليمارين كاضافة علف (٦٦٢ ± ٢٠ جم) بالمقارنة مع المجموعة الضابطة (٥٩٣.٥ ± ٢٠ جم). تم تسجيل افضل وزن تراكمي وكذلك افضل معامل تحويل غذائي في مجموعة السليمارين (٢١٦٣ ± ٢٣جم و ١.٩)، يليه دجاج المجموعة التى تتغذي على ال-كارنيتين كاضافة علف (٢١٠٣.٥ ±١٦ جم و1.94)، في حين أن الكتاكيت التي تتغذى على العليقة الضابطة سجلت اقل القيم في نهاية فترة التجربة (١٨٩٣.٨ ±٦٥ جم و 1.96). بالنسبة للدهون المترسبة في منطقة البطن، فكانت المجموعتين L و S أقل بكثير من المجموعة الضابطة C بينما لم يلاحظ أي فروق معنوية في تصافى الذبيحة وأوزان عضلات الصدر. من نتائج هذه الدراسة، فإنه يمكن أن نخلص إلى أن إضافة سيليمارين إلى علائق بداري التسمين يؤدى الي تحسن كبير في وزن الجسم ومعدلات التحويل الغذائي خلال فترة التربية. واضافة ال- كارنيتين ادت ال تحسين النمو فى مرحلة النامى والناهى لدجاج التسمين (العليقة عالية الطاقة). وأخيرا فمن المستحسن أن نتمكن من استخدام كل سيليمارين و ال-كارنيتين كإضافات غذائية في فترة النامى والناهى لدجاج التسمين.