

EFFECT OF COENZYME Q10 AND L-CARNITINE ON GROWTH PERFORMANCE, PHYSICAL AND CHEMICAL BLOOD INDICES, ANTIOXIDANT STATUS AND IMMUNE RESPONSE OF NEWBORN EGYPTIAN BUFFALO CALVES

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SUMMARY

In an experimental trial lasted 102 days started from the age of 4 days after birth to the weaning age (105 days) conducted to compare the effect of Co-enzyme Q10 (CoQ10) and L-Carnitine (L-Ca) on growth, physical and chemical blood indices immunity, and antioxidant status of newborn Egyptian buffalo calves. Fifteen calves with 36.13 ± 0.86 Kg initial live body weight (LBW) were allocated into three groups where calves in the first group received no treatment (control group, Ctrl). The calves in the second and the third groups received 40 mg L-Ca/kg LBW and 10 mg CoQ10/kg LBW, respectively as daily oral doses. Significant differences in weight gain among treatments starting from the age of 60 days where the CoQ10 and L-Ca groups were higher than the control. Blood physical parameters of all experimental animals show no abnormal cases and no alteration in hematological indices among groups were recorded. Meanwhile, blood biochemical contents were varied significantly ($P < 0.05$) among groups, where the control group gave the highest values of glucose, total lipids, total cholesterol, triglyceride, ALT, AST, ALP, T3, and T4 compared to the other groups. Moreover, the CoQ10 group exhibited the highest significant ($p < 0.05$) contents of TP and the lowest T3 and T4 values. Furthermore, the highest values of lysozyme and bactericidal activities were recorded in the CoQ10 group compared to the other groups. Both CoQ10 and L-Ca groups showed higher superoxide dismutase (SOD) and catalase (CAT) contents than control. In conclusion, CoQ10 or L-Ca supplements improve growth, immunity and antioxidant contents.

Keywords: *Egyptian buffalo, co-enzyme Q10, l-carnitine, immune response and oxidative status.*

INTRODUCTION

Intensification as an ideal option for the production of an enormous amount of animal protein has led to increased focus of studies on overcoming the adverse effects and stressful conditions of intensive production (Fraser, 2008; Korthals and Thompson, 2008).

Disease-free and stress-resistant calves are the basis of good production (Lensink *et al.*, 2001). Chemotherapy and/or antibiotics were the most prevalent ways of dealing with stresses and diseases, however, with increasing apprehension about the consequences of the chronic use such as the development of resistant pathogen strains, accumulation of toxic residues, suppression of immune system and environmental risks, their use in many countries has been banned (Papatsiros *et al.*, 2013). Eco-friendly natural strategies and/or functional feed additives such as fermentation, vaccines, and pro-, pre-, synbiotics, have been in great demand in the livestock sector (Musa *et al.*, 2009). Coenzyme Q10 (CoQ10) and L-carnitine (L-Ca) are an ideal additives that labor as an antioxidant, regulator of immunity and metabolism (Ghanbarzadeh *et al.*, 2013). Both CoQ10 and L-Ca are naturally present in mammals, plants and some bacteria's biological system but in insufficient quantities, especially under stressful conditions and therefore need to be obtained from an external sources (Hernández-Camacho *et al.*, 2018).

CoQ10 or ubiquinone, a hydrophobic molecule (Nepal *et al.*, 2010), which occur in the mitochondrial inner membrane where function a significant part in the electron transport chain (ETC) and generates ATP (Shukla and Dubey, 2018). CoQ10 is an antioxidant and free radical eliminator and considered as a super-vitamin (vitamin Q) that helps in the protection of DNA, cells membrane lipids and proteins from the

hazards of the oxidative damage, assists regenerating of vitamin E and reliefs healthy energy levels (Bentinger *et al.*, 2007; Linnane *et al.*, 2007). The addition of CoQ10 aids to improve human and animal health in the case of many diseases such as obesity, diabetes, heart disease, muscle atrophy, aging, cancer, Alzheimer's disease, etc. (Adarsh *et al.*, 2008; Prakash *et al.*, 2010; Roffe *et al.*, 2004; Sohet *et al.*, 2009; Varela-López *et al.*, 2016). Also, in vitro results indicate its anti-inflammatory role (Schmelzer *et al.*, 2009).

Carnitine is produced naturally in the body from the amino acids lysine and methionine and the form of L-carnitine (L-Ca) is considered a biologically active substance. Its vital role is an essential co-factor in transporting long-chain fatty acids to the inner membrane of mitochondria (lipids metabolism) and thus in energy production as well as improve neuronal metabolism which helps in the treatment of certain diseases such as Alzheimer's, obesity, diabetes and heart disease (Bloomer *et al.*, 2009). Due to the multiple benefits of CoQ10 and L-Ca, the potential therapeutic advantages and the ease of synthesis, whether by biological or chemical methods, have made it one of the special substances of high demand in the market along with other compounds such as alpha lipoic acid, vitamin C and vitamin E (Golbidi *et al.*, 2011; Shukla and Dubey, 2018).

This study was designed to evaluate the effect of CoQ10 and L-Ca on the growth rate and health status of Egyptian buffalo calves.

MATERIALS AND METHODS

Animals and experimental design:

This study was performed at Animal Production Research Gemmieza station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Fifteen Egyptian buffalo calves with 36.13 ± 0.86 Kg live body weight (LBW) were allocated into three groups, 5 animals each and were individually placed in a separate pen. Calves in the first group received no treatment and served as a control group (Ctrl). The calves in the second and third groups received 40 mg L-Carnitine (L-Ca)/kg LBW and 10 mg coenzyme Q10 (CoQ10)/kg LBW, respectively as daily oral administration from the fourth day to 105 days of age (weaning age).

Feeding system:

Animals were separately fed twice daily (6:00 and 18:00 h) at the rate of 10% of LBW on dam colostrum for the first three days (with no treatments) and whole cow milk until weaning with the gradual use of starter feed from day 15 after birth (Berseem hay, BH 3rd cut and rice straw at free choice). Calves were given a free access to feeds and clean water during the experimental period.

Growth performance:

Animal's weights were recorded at birth and then measured at 30, 60, 90 and 105 days of age (weaning age) before serving the morning meal. The growth parameters were calculated according to the following formulae:

$$\text{Weight Gain (WG, Kg)} = W_T - W_0$$

$$\text{Average Daily Gain (ADG, Kg/day)} = \frac{W_T - W_0}{T}$$

Where:

W_T = body weight at a specific time; W_0 = birth body weight; T= the trial period in days (d).

Blood sampling:

Blood was sampled after 2 hour of feeding from all calves at the end of the experimental period by jugular venipuncture using sterile syringes with or without anticoagulant (heparin) and samples were centrifuged undercooling (4°C) at a speed of 3000 rpm for 10 minutes to obtain plasma and serum, respectively. Both of plasma and serum samples were kept at -20°C until analysis. Blood hematological parameters [red blood cells (RBCs), white blood cells (WBCs), packed cell volume (PCV %), and haemoglobin (Hb)] and biochemical contents [glucose, total protein, total lipids, cholesterol, triglycerides,

alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP)] were measured using Semi-automatic analyzer (Model 2000 Evolution, EMEG) for clinical chemistry and hematology tests using Bayer Diagnostics Reagents strips following the manufactory guidelines. The concentration of triiodothyronine (T3) and thyroxin (T4) in blood serum were measured using radioimmunoassay (RIA) commercial kits (Coat-A-Count®-TKT31) by Automatic Mini-Gamma Counter (LKB-1275). Immunoglobulin G and M concentrations in serum samples were determined with the quantitative ELISA (Bovine IgG and IgM, ELISA Quantitative kit, Bethyl laboratories, UK).

Non-specific immune responses:

Serum lysozyme activity (LYZ) was assessed spectrophotometrically at 450 nm with 96-well microplate turbidimetric assay as described by Lygren et al. (1999). Bactericidal activity (BA) of serum was diagnosed spectrophotometrically at 570 nm according to the modified methods of Gallage et al. (2016) as described by Wang et al. (2018).

Antioxidant potential assessment:

Enzymes in serum samples were measured according to the method of Paoletti and Mocali (1990) (superoxide dismutase SOD) and Aebi (1984) (catalase CAT).

Statistical analysis:

Data were processed with the SPSS analysis program (SPSS version 15 for Windows) using one-way analysis of variance (ANOVA) and expressed as mean ± SE of five replicates. To detect significance and to compare the means of treatments, Duncan multiple range tests were performed at $P < 0.05$ level (Duncan, 1955).

RESULTS AND DISCUSSION

Growth characteristics:

Egyptian buffalo calves' growth during the trial period is shown in Table (1). From the age of 60 days until weaning, significant differences in weight were observed between treatments and CoQ10 and L-Ca treatments gave the highest body weights and average daily gain (ADG) compared to the control at weaning.

Table (1): Body weight gain of Egyptian buffalo calves during the experimental period.

Item	Treatment			P-Value
	Ctrl	L-Ca	CoQ10	
Body weight (BW, Kg):				
Birth (BW0)	35.80±1.07	36.20±1.36	36.40±2.16	0.895
30 d	46.20±1.86	49.80±2.89	50.20±2.42	0.782
60 d	63.00±2.12 ^b	68.80±1.63 ^{ab}	69.80±2.15 ^a	0.045
90 d	77.60±2.02 ^b	86.60±1.36 ^a	89.40±2.38 ^a	0.038
105 d (Weaning)	91.00±2.92 ^b	104.20±3.61 ^a	110.20±2.78 ^a	0.020
Average total gain (Kg):				
Birth – 30 d	10.40±1.08	13.60±2.16	13.80±0.97	0.910
30 d – 60 d	16.80±.86	19.00±1.67	19.60±0.40	0.784
60 d – 90 d	14.60±0.40 ^b	17.80±0.37 ^a	19.60±1.03 ^a	0.042
90 d – weaning	13.40±1.50 ^b	17.60±2.32 ^{ab}	20.80±0.58 ^a	0.016
Birth – weaning	55.20±2.15 ^b	68.00±2.35 ^a	73.80±1.16 ^a	0.012
Average daily gain (ADG, Kg/d):				
Birth – 30 d	0.347±0.04	0.453±0.07	0.46±0.03	0.880
30 d – 60 d	0.560±0.03	0.634±0.06	0.654±0.01	0.721
60 d – 90 d	0.487±0.01 ^b	0.593±0.01 ^a	0.653±0.03 ^a	0.039
90 d – weaning	0.447±0.05 ^b	0.587±0.08 ^{ab}	0.693±0.02 ^a	0.022
Birth – weaning	0.526±0.02 ^b	0.648±0.02 ^a	0.703±0.01 ^a	0.017

Values represent Means ± S.E. (n=5). Means in the same row with different superscripts differ significantly ($P < 0.05$).

The results of calves' growth during the trial period showed an improvement with the addition of CoQ10 and L-Ca. The increase in growth may be due to improve utilization of feed due to the role of CoQ10 and L-Ca in improving the condition of the intestine by modulating intestine microflora or anti-inflammatory properties (Fortin *et al.*, 2009; Lee *et al.*, 2016). Moreover, this improvement in growth may be due to the indirect effect of CoQ10 and L-Ca on vitamin E re-synthesis (Bentinger *et al.*, 2007; Linnane *et al.*, 2007). Furthermore, CoQ10 plays a pivot in the metabolism of protein, lipids, and carbohydrates (Shukla and Dubey, 2018). In addition, the positive impacts of CoQ10 and L-Ca treatments on insulin, glucagon and cortisol hormones support its role in improving the performance of animal (Butler *et al.*, 2003; Ruggenenti *et al.*, 2009).

Blood hematological and biochemical metrics:

Table (2) represents the blood indices of Egyptian buffalo calves at the end of the trial period. Blood statuses of all experimental animals show no satisfactory cases and were within normal rates. The hematological indices of the treated groups showed no significant variation ($P>0.05$). Meanwhile, the biochemical contents are varied significantly in terms of glucose, total lipids, T-Chol, triglyceride, ALT, AST, and ALP. The control group gave the highest values of glucose, total lipids, T-Chol, triglyceride, ALT, AST, and ALP compared to the other groups. Moreover, the CoQ10 group exhibited the highest significant ($p<0.05$) contents of TP.

Blood status is an accurate indicator that reflects the health status of the animal as well as the response to stressors and external stimuli (Puppel and Kuczyńska, 2016). Overall, the value of the blood indices recorded in the present study is within the normal ranges. Glucose is an accurate evidence of the presence of stress conditions as it increase with stress and decreases in well-being (Chen *et al.*, 2015; Garner *et al.*, 2017). Interestingly, levels of glucose have been decreased in calves fed CoQ10 and L-Ca when compared with un-supplemented calves; this could be linked either to hypoglycemic hormone stimulation (insulin) or reduction in glucose absorption (Modi *et al.*, 2006). Also, total lipids, cholesterol and triglyceride levels were decreased and this may due to the hypocholesterolemic impact of CoQ10 and L-Ca (Huang *et al.*, 2013; Lee *et al.*, 2016; Raeisi-Zeydabad *et al.*, 2017). Conversely, the higher total protein (TP) contents observed in CoQ10 group may indicate an improvement in calves' health or could be a post-injury or infection adaptation response (Gerwick *et al.*, 2002; Panigrahi *et al.*, 2010). Also, the noticed alterations in the activities of liver metabolic enzymes (ALT, AST, and ALP) reported in the present study reflect the absence of CoQ10 and L-Ca toxicity.

Table (2): Hematological and biochemical blood indices of Egyptian buffalo calves fed experimental additives.

Item	Treatment			P-Value
	Ctrl	L-Ca	CoQ10	
RBCs ¹ (10 ⁶ /μl)	7.19 ± 0.40	7.32±0.18	8.06±0.61	0.108
WBCs ² (10 ⁶ /μl)	9.47±0.50	10.13±0.41	10.88±0.62	0.215
PCV ³ %	31.42±0.88	32.75±0.71	33.20±0.62	0.342
Hb ⁴ (g/dl)	10.68±0.55	11.02±0.78	12.01±0.59	0.448
Glucose (mg/dl)	101.04±2.06 ^a	81.56±2.32 ^b	77.38±1.84 ^b	0.033
TP ⁵ (g/dl)	6.30 ±0.18 ^c	7.94±0.15 ^b	8.89±0.12 ^a	0.042
Total lipids (mg/dl)	697.60±6.78 ^a	653.48±7.18 ^b	544.04±6.60 ^c	0.000
T-Chol ⁶ (mg/dl)	102.74±2.02 ^a	92.58 ±1.47 ^b	77.95 ±1.68 ^c	0.000
Triglyceride (mg/dl)	56.03±0.76 ^a	51.89±0.63 ^b	42.15±0.79 ^c	0.030
ALT ⁷ (IU/l)	13.64±0.29 ^a	10.90±0.25 ^b	9.45±0.22 ^c	0.027
AST ⁸ (IU/l)	35.14 ±0.69 ^a	30.55 ±0.84 ^b	26.86±0.62 ^c	0.018
ALP ⁹ (IU/l)	94.52±1.34 ^a	81.93±2.50 ^c	88.19±1.77 ^b	0.039
Creatinine (mg/dl)	1.46±0.22	1.24±0.26	1.22±0.19	0.384
Urea (mg/dl)	19.18±0.60	18.23±0.53	18.20±0.49	0.432

Results are expressed as Means ± S.E. (n=5). Means in the same row bearing different superscript are significantly different at ($P < 0.05$).

¹ RBCs: Red blood cells; ² WBCs: White blood cells; ³ PCV: Packed cell volume; ⁴ Hb: Hemoglobin; ⁵ TP: Total protein; ⁶ T-Chol: Total cholesterol; ⁷ ALT: Alanine transaminase; ⁸ AST: Aspartate transaminase; ⁹ ALP: Alkaline phosphatase.

Serum triiodothyronine (T3), Thyroxine (T4), and Immunoglobulin G and M concentrations at 105 day of age of calves were presented in Fig. (1). No differences were found between the experimental groups in blood IgG and IgM contents. The highest T3 and T4 values were observed in the control group followed by L-Ca group and the lowest values were seen in the CoQ10 group.

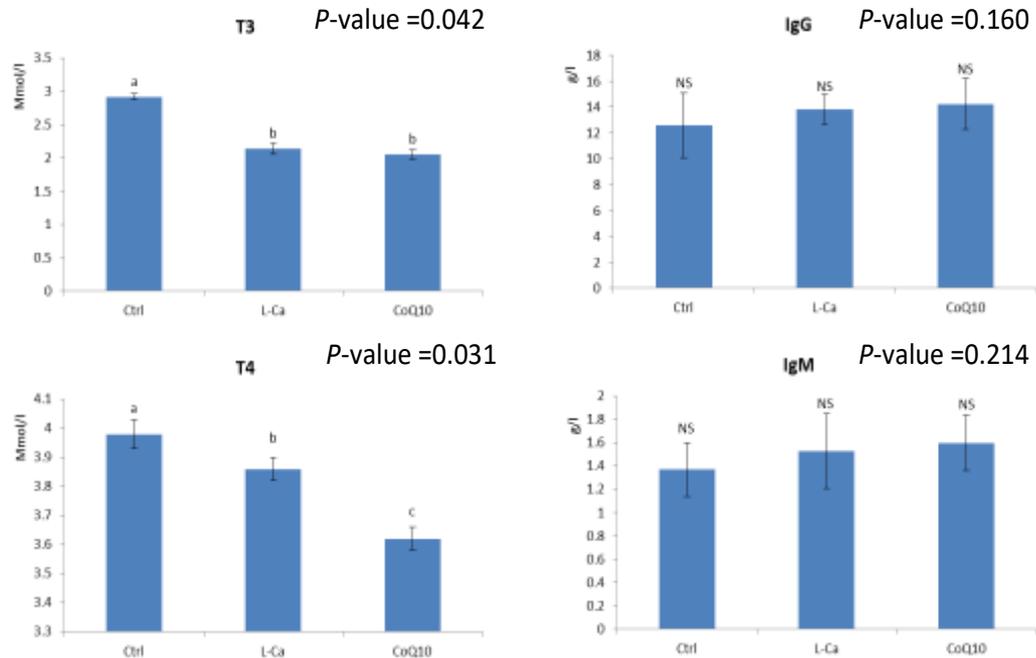


Fig. (1): Egyptian buffalo calves serum triiodothyronine (T3), Thyroxine (T4), and Immunoglobulin G and M concentrations at 105 day of age.

Non-specific immune responses:

Serum lysozyme (LYZ) and bactericidal activities (BA) of Egyptian buffalo calves are presented in Fig. (2). The lowest values of LYZ and BA activities were recorded in the control group and the highest values were observed in group supplemented with CoQ10.

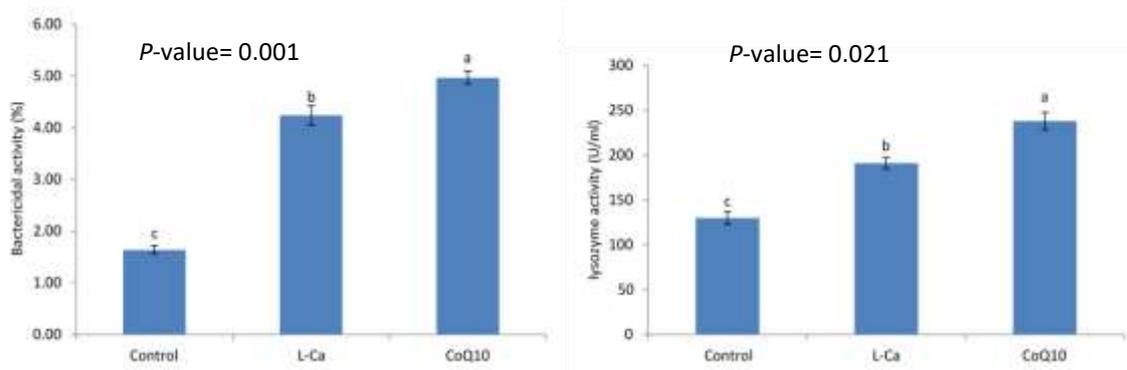


Fig. (2): Egyptian buffalo calves' serum bactericidal and lysozyme activities.

Immune responses in animals can be altered by feed additives treatments by stimulating immune cells directly or indirectly through metabolic, neurological, or endocrine pathways (Selvaraj, 2012). Phagocytosis is one of the main defense mechanisms in animals which is used in the activities of lysozyme and anti-microbes (bactericidal activity)(Janeway *et al.*, 2001; Karavitis and Kovacs, 2011). The activity of lysozyme used to assess the ability of the immune system in different animals (Cecchini *et al.*, 2000). Bactericidal activity (BA) serves as a main tool of the host to withstand pathogens (Kawakami *et al.*, 2000).CoQ10's result in raising body immunity can be attributed to improved body energy, enhanced mitochondrial respiration, or / and maximized vitamin E level (Feher *et al.*, 2007; Raeisi-Zeydabad *et al.*, 2017).

Antioxidant potential assessment:

Data on serum SOD and CA contents are presented in Fig. 3. The highest SOD and CA contents were recorded in groups supplemented with CoQ10 and L-Ca compared to the control group.

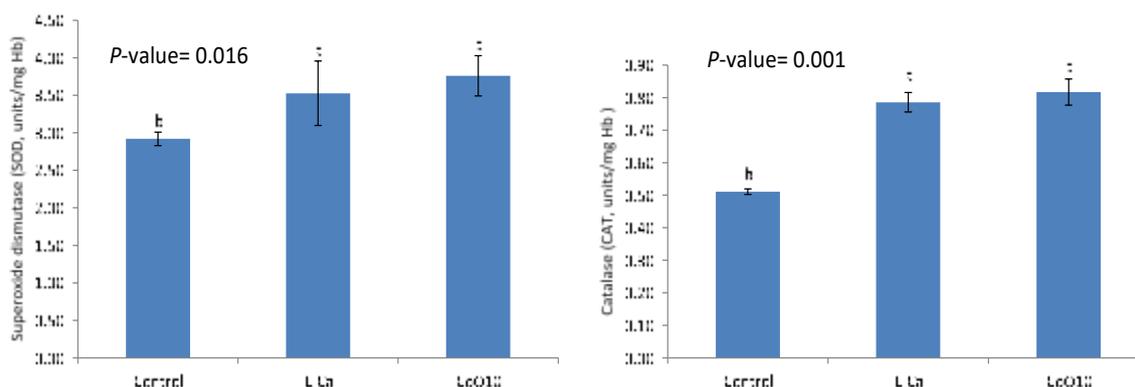


Fig. (3): Egyptian buffalo calves serum superoxide dismutase (SOD) and catalase (CAT) contents at 105 day of age.

There is a strong correlation between oxidative state and the immunity (Alhidary *et al.*, 2016; Tomasi *et al.*, 2018). Oxidative stress increases as a result of an imbalance between the production and disposal of reactive oxygen species (ROS) (Castro and Tafalla, 2015; Sharbidre *et al.*, 2011). SOD and CAT as major part of the antioxidant defense line play vital roles in removing excessive ROS, sustaining the homeostasis of the cell (Aruoma, 1998). SOD and CAT values were significantly higher in groups supplemented with CoQ10 and L-Ca compared with the control group emphasizing the antioxidant capacities of CoQ10 and L-Ca. CoQ10 is well known as a super-vitamin (vitamin Q) that exhibits potent antioxidant ability, free radical scavenger, helps regenerating of vitamin E, supports healthy energy levels, improves health in case of many disease (Varela-López *et al.*, 2016). Furthermore, L-carnitine (L-Ca) exhibits potent antioxidant ability, free radical scavenger, helps in energies body, and the treatment of certain diseases (Bloomer *et al.*, 2009).

In conclusion, CoQ10 or L-Ca supplements improve the growth, health being of Egyptian buffalo calves. CoQ10 is similar to L-Ca in its antioxidant capacity while CoQ10 outperforms L-Ca to boost immunity.

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تأثير مساعد الانزيم Q10 والكارتين علي أداء النمو وصحة الدم وحالة مضادات الأوكسدة والاستجابة المناعية لعجول الجاموس المصري حديثة الولادة

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تم اجراء التجربة بهدف مقارنة تأثير Co-enzyme Q10 و L-Carnitine على النمو والمناعة وحالة مضادات الأوكسدة لعجول الجاموس المصري حديثة الولادة. تم استخدام خمسة عشر عجلاً بمتوسط وزن 0.86 ± 36.13 كيلوجرام قسمت الى ثلاث مجموعات. لم يتلق العجول في المجموعة الأولى أي معاملة (المجموعة الضابطة Ctrl). تلقت العجول في المجموعة الثانية 40 ملجم L-Ca لكل كيلوجرام من وزن الجسم و المجموعة الثالثة 10ملجم CoQ10 لكل كيلو جرام من وزن الجسم كجرعات يومية عن طريق الفم. اظهرت النتائج فروق معنوية في وزن العجول بين المعاملات بداية من عمر 60 يوماً وكانت مجموعة CoQ10 ومجموعة L-Ca أعلى من المجموعة الضابطة. اما عن حالة مكونات الدم، أعطت المجموعة الضابطة أعلى قيم للجلوكوز، الدهون الكلية، الكوليسترول الكلي، الدهون الثلاثية و ALT و AST و ALP و T3 و T4 مقارنة بالمجموعات الأخرى. علاوة على ذلك، كانت مجموعة CoQ10 أعلى بمعنوية عند مستوى ($P < 0.05$) في قيمة TP وأدنى قيم T3 و T4. ايضاً فان أعلى قيم Iysozyme وأنشطة مبيد الجراثيم تم تسجيلها في مجموعة CoQ10 مقارنة بالمجموعات الأخرى. بينما أظهرت كلتا المجموعتين CoQ10 و L-Ca أعلى محتوى من SOD و CAT. وفي الملخص يتضح من النتائج ان CoQ10 أو L-Ca لها القدرة على تحسين نمو وصحة عجول الجاموس المصري و لها قدرة متماثلة كمضادات للأوكسدة بينما يتفوق CoQ10 على L-Ca في تعزيز المناعة.