Egyptian J. Nutrition and Feeds (2008) 11 (2) : 353-364

PROTECTIVE ROLE OF LIPOIC ACID AGAINST PEROXIDATIVE CELL DAMAGE AND ITS EFFECT ON PERFORMANCE PARAMETERS IN RABBITS.

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(Received 15/2/2008, Accepted 20/5/2008)

SUMMARY

The effects of lipoic acid (LA) on muscle growth, metabolic response and hepatocellular antioxidant in rabbits received 10, 50 or 100 mg/kg body intra peritoneal were examined. 60 apparently healthy male newly weaned Newzealand White (NZW) rabbits were divided randomly into 4 similar groups (15 each) reared under the same environmental and hygienic conditions. Group1 kept as a control received i.p. (intra peritoneal) injection of saline; group 2,3 and 4 injected i.p. with lipoic acid either 10, 50 or 100 mg/kg body weight respectively. After 8-weeks of experiment, rabbits show a significant increase on body weight gain and tissue weight in all groups than control one. The absolute mass of the abdominal fat weight showed a significant decrease in the groups 2, 3 and 4 respectively than control group. While, concentration of tissue protein in the breast muscle showed a significant increase in the same groups.

The data revealed significant decrease in serum glucose and serum TAG (trigacylglycerol),While the level of serum NEFA (non estritified fatty acids), blood glutathione, glutathione peroxidase, ceruloplasmin and transferrin significantly increased in all groups than control one. At the same time, superoxide dismutase showed a non significant changes in all experimental groups. But, serum iron and copper revealed a non significant decreased in all experimental groups than control group.

The results indicated that there was an effective role of lipoic acid in improving the performance of rabbits and could be considered an ideal antioxidant.

Keywords: *rabbits, Lipoic acid, Body weight gain, NEFA, TAG, blood glutathione, glutathione peroxidase, superoxide dismutase*

INTRODUCTION

Lipoic acid, also known as α-lipoic acid (alpha lipoic acid) or thioctic acid, has formula $C_8H_{14}S_2O_2$ and systematic name 5- (1, 2-dithiolan-3-yl)pentanoic acid. Alpha-lipoic acid is the lipophilic endogenous disulfide, generally classified with the B group vitamin. It occurs naturally in every cell of the body and is essential to the chemical reactions that allow our bodies to produce energy. As a supplement, it is rapidly absorbed into the blood and the cells where it can prevent free-radical damage. It is vital for the creation of energy in every organ of the body, Perham RN (2000); Kayali, *et al.* (2007); Cakatay and Kayali (2005). The endogenous production of alpha lipoic acid serves as a

cofactor for two reactions involved in energy production of the Kreb's cycle. It is a cofactor for the enzymes pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase. Each of these reactions is carried out in the mitochondria. It also enhances energy production by the mitochondria in various tissues throughout the body. Alpha lipoic acid also serves as a cofactor for the production of the branched chain amino acids leucine, isoleucine, and valine, Kayali, *et al.* (2007).

Lipoate, or its reduced form, dihydrolipoate, reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen. It also protects membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E. In addition to its antioxidant activities, dihydrolipoate may exert prooxidant actions through reduction of iron. Furthermore, lipoate can function as a redox regulator of proteins such as myoglobin, prolactin, thioredoxin and NF-kappa B transcription factor. We review the properties of lipoate in terms of (1) reactions with reactive oxygen species; (2) interactions with other antioxidants; (3) beneficial effects in oxidative stress models or clinical conditions, Packer *et al.* (1995); Adil and Jacques (2002).

In lipid metabolism, lipoic acid (LA) supplementation reduces serum levels of total cholesterol and B-lipoproteins in rabbits with experimental atherosclerosis, Ivanov (1974). Segermann *et al.* (1991) also demonstrated that the serum triacylglycerol (TAG) level of rats is lowered by intraperitoneal injection of LA. These metabolic effects of LA on energy metabolism may be available and beneficial to energy redistribution and the control of adipose tissue acceretion in meat –producing animals. However, there has been little investigation of the effect on growth performance or metabolic response in domestic animals, Yoshio (2002).

Metalloprotein being abundant proteins containing certain metals such as Cu, Zn and Fe are involved in most biological activities such as oxidative tissue damage due to free radical formation Underwood (1977). Mackenzie,*et al.* (2006) revealed that α-Lipoic acid and N-acetyl cysteine prevent zinc deficiency-induced activation of neuroblastoma. The metalloprotein ceruloplasmin (CP) is $\acute{\alpha}_2$ glycoprotein has been recognized as the major Cu containing component in mammalian plasma and serum Baraboj *et al.* (1994).

The metaloprotein transferrin (TF) is B_1 glycoprotein. It is a major carrier of iron to its storage sites (liver and reticuloendothelial system) as well as to the cells that synthesize iron containing compounds e.g. haemoglobin, myoglobin and cytochromes, Soejima *et al.* (1995).TF and CP are considered important extracellular antioxidants (Frieden,1993).

The purpose of this study was to determine whether receiving of lipoic acid intraperitoneal increased cellular and general metabolic activity in rabbits by its effects on growth performance and metabolic response by estimating the levels of glucose, non esterified fatty acids, triacylglycerols, iron, copper and glycoprotein in serum and examine whether this supplementation affected hepatocellular antioxidant status by estimating the level of glutathione, glutathione peroxidase and superoxide dismutase in whole blood.

MATERIALS AND METHODS

Animals and experimental diets:

60 apparently healthy male Newzeland White (NZW) newly weaned rabbits with average live body weight/head 550–600 g. The animals were housed in specially designed cages, Rabbits were divided randomly into 4 similar groups (15 each) reared under the

same environmental and hygienic conditions. Group1 kept as a control received i.p. injection of saline; group 2 injected i.p. with lipoic acid 10 mg/kg B.w group 3 received 50 mg /kg while group 4 received 100 mg/kg body weight, once every week for 8 week.

The rations were given to animals as mixed ration and formulated to satisfy the nutrient requirements of the intensively reared rabbit according to NRC (1989) and Lebas *et al.* (1998) for 8 weeks experimental period. The ration consists of crude protein 17% fat not more than (2.94) crude fiber not more than (13.8), digestible energy (not less than) 2700 Kcal /kg.

Lipoic acid was purchased from Sigma Chemical Comp. St Lowis, was dissolved in 1M $NaHCO_3$ and diluted to the desired concentrations with sterile saline. The pH was adjusted to 7.4 before i.p. administration to rabbits interapertoneal. At the end of experiment (8 weeks) final body weight was recorded and the body gain was calculated.

Slaughter, sample collection and chemical analysis:

At the end of the experiment (8 weeks), the animals were sacrificed for blood which centrifuged to separate serum the breast muscle and abdominal fat pad were removed and weighted. The breast muscle was stored at -20C until protein determination. Protein concentration in the breast muscle was determined with the modified lowry method using bovine serum albumin as the standard, Markwell *et al.* (1987).

Serum samples were mixed with 10% trichloroacetic acid. After centrifugation the diluted supernatant (10%) was used for estimation of metals. Estimation of metals (Fe and Cu) was carried out using atomic absorption spectrophotometer (Pye – Unicom).

Serum glucose was assayed spectrophotometrically using commercial chemical reagent kits (Diamond, Diagnostic) as described by Trinder (1969) using Boehring Mannheium kits.

Enzymatic assay kits were used in the determination of serum triglycerides according to Wahlefeled (1974) and non esterified fatty acids, according to Radwan (1978) by using Gas liquid Chromatography.

Total protein content was determined in serum according to the procedure described by Doumas (1975) to calculate glycoproteins. The serum glycoproteins were fractionated using non-denaturing polyacrylamide gel electrophoresis, Davis (1964) the apparatus was vertical polyacrylamide gel obtained from Sigma USA.

Glutathione (GSH) content was estimated chemically in whole blood by measuring the optical density (OD) of the yellow color that developed when 5,5 dithiol – bis (2-nitrobenzoic acid) was added to sulfhydral compounds, the absorbs light at 412 nm, according to Beutler, *et al.* (1963).

Estimation of glutathione peroxidase (GSH-Px) chemically in whole blood was based on the measurement of the amount of residual GSH left after exposure to enzyme activity for a fixed time, the absorbs light at 412 nm, Gross *et al.* (1967).

Superoxide dismutase activity was estimated chemically in whole blood by the method that depends on detection superoxide anions by nitroblue tetrazoluim formazan color development, the absorbs light at 412 nm, according to Minami and Yoshikawa (1979).

Statistical analysis:

The results were presented as mean values ± standred error for groups were compared using one – way analysis of variance (ANOVA) according to Perrie and Watson (1999).

RESULTS AND DISCUSSION

Effects of dietary LA on growth performance:

The effects of LA on BW gain and tissue weights in the rabbits are shown in Table (1). Supplementation of LA shows a significant increase on body weight gain or tissue weight. The absolute mass of the abdominal fat weight showed a significant decrease in the groups 2, 3 and 4 which received 10 mg/kg B.W lipoic acid, 50 mg/kg B.W lipoic acid and 100 mg/kg B.W lipoic acid respectively than in control group. Also, concentration of tissue protein in the breast muscle showed a significant increase in the groups 2, 3 and 4 respectively than in control group.

Metabolic responses of LA on rabbits:

The effect of LA on serum metabolites in rabbits is shown in Table (2). When rabbits injected with LA show a significant decrease in serum glucose in all groups. While, serum NEFA values of the experimental groups were significantly greater than that of the controls. But LA causes a decrease in serum TAG than control group.

Table (1): Effect of lipoic acid administration on Live body weight and tissue weight in experimental rabbit groups.

Item	Experimental groups				LSD at 0.05
	Control	10 mg LA/kg BW	50 mg LA/kg BW	100mg LA/kg BW	
Initial BW, g/h	570.0± 33.50	571.5±35.70	570.5±35.31	571.1±34.41	NS
Final BW, g/h	2209±221.20	2238±213.40	2287±182.20	2355±173.20	40.25***
BW gain, g/animal	1639±143.30	1666.5±145.40	1716.5±152.30	1783.9±147.60	34.9***
Total feed consumption, g	5430.00	5485.25	5580.50	5730.50	40.25***
Feed conversion, kg/kg gain	3.313	3.291	3.251	3.212	0.021*
Breast muscle weight, g/h	130.50±12.24	142.34±13.55	165.25±15.21	180.25±17.22	1.15***
Abdominal fat, g/bird	60.50±5.22	50.55±4.21	45.28±3.50	39.85±3.32	5.50***
Conc. of breast muscle protein, mg/g	213.25±18.12	222.10±20.15	242.20±22.50	261.50±25.43	7.52***

NS= non significant difference **=(P<.0.05)* **** = (P<.0.01)*

Table (2): Effect of LA administration on energy metabolites in serum of experimental rabbit groups.

Item	Experimental groups				LSD at 0.05
	Control	10 mg LA/kg BW	50 mg LA/kg BW	100mg LA/kg BW	
Glucose mmol/L	10.42±1.23	9.05±0.87	8.80±0.73	7.35±0.65	0.75***
NEFA µmol/L	577±57.54	598±58.55	652±60.43	669±66.23	18.12***
TAG µmol/L	599±57.44	450±44.75	368±34.56	357±34.60	65.50***

**** = (P<.0.01)*

Effect of LA administration on serum iron, copper in rabbits:

Table (3) revealed a non significant decrease in serum iron and copper in all groups than control one

Effect of LA administration on blood glutathion, glutathione peroxidase and superoxide dismutase content in rabbits:

The level of blood glutathione and glutathione peroxidase, Table (4) significantly increased in all groups than control one. Where in superoxide dismutase there is a non significant changes in all groups.

Effect of LA administration on ceruloplasmin and transferrin concentrations of serum of rabbits:

Table (5) recorded a significant increase of ceruloplasmin and transferrin in all groups than control group.

Table (3): Effect of lipoic acid administration on serum iron and copper content in different of experimental rabbit group.

Groups	Serum iron mg/100ml	Serum copper mg/100ml
1st (control)	0.350±0.023	0.360±0.033
2nd (10 mg/kg BW lipoic acid)	0.343±0.030	0.334±0.31
3rd (50 mg/kg BW lipoic acid)	0.320±0.021	0.327±0.030
4th (100 mg/kg BW lipoic acid)	0.305±0.025	0.311±0.22
LSD at 0.05	0.025 ns	0.028 ns

NS= non significant difference

Table(4): Effect of lipoic acid administration on blood glutathion,glutathione peroxidase and Superoxide dismutase content in different rabbit groups.

Groups	GSH mg/dl	GSH-px U/ml	SOD U/ml
1st (control)	82.44 ±7.52	89.30±7.8	8.90±0.90
2nd (10 mg/kg BW lipoic acid)	88.65±8.24	92.33±9.6	8.77±0.82
3rd (50 mg/kg BW lipoic acid)	95.30±8.54	95.50±10.5	8.95±0.0.95
4th (100 mg/kg BW lipoic acid)	105.58±8.77	100.9±13.2	9.10±0.93
LSD at 0.05	4.55 ***	1.97***	0.35^{NS}

NS= non significant difference *** = *highly significant difference*

Table (5): Effect of lipoic acid administration on ceruloplasmin and transferrin concentrations of serum of different experimental rabbit groups.

Groups	Ceruloplasm mg/dl	Transferrin mg/dl
1st (control)	375.60±35.70	370.80±33.57
2nd (10 mg/kg BW lipoic acid)	380.52±36.35	374.94±35.44
3rd (50 mg/kg BW lipoic acid)	386.33±37.52	381.54±36.25
4th (100 mg/kg BW lipoic acid)	392.54±40.54	388.62±37.50
LSD at 0.05	3.15 ***	2.25 ***

*** = *highly significant differences*

Effect of Lipoic acid (LA) on growth performance:

A previous study observed no effect on BW gain or tissue weight in broilers fed 5 or 50 mg/kg LA. Yoshio,(2002) and Hamano *et al.* (1999). But,the present experiment (table-1) indicates that LA had a significant increase in body growth, body gain or tissue mass and low feed conversion ratio (more efficient), this result indicate that supplementation of LA cause increase muscle mass and protein deposition or decreases adipose tissue accretion,Yoshio,(2002). Also, Shen *et al.* (2005) show that supplementing of ALA may decrease fat accumulation and influence growth performance of animal The significant decrease of the abdominal fat (table-1) may be a result of alteration in the adipose tissue accretion due to different modes of action of LA as to fatty acid metabolism. This could be attributed to the fact that α-Lipoic acid is a strong antioxidant in foods (Packer *et al.*., 1995). Very recently, it was reported that ALA decreased the AMPK (activated protein kinase) activity in the hypothalamus and caused profound weight loss in rats by decreasing food intake and enhancing ß-oxidation of fatty acid. It is quite possible that ALA also alters AMPK activity in skeletal muscle and thereby affects the postmortem glycolysis and fat accumulation (Kim *et al.*, 2004).In agreement with the results of Kim *et al.*,(2004); Andersson *et al.* (2004) and Minokoshi *et al.*., (2004), Shen *et al.*,(2005), recorded that, supplementing mice diets with 1.0% ALA caused a net loss in fat and it had detrimental effects on mouse performance, Nonetheless, 0.5% ALA supplement only induced a loss in fat; thus, it might be a good level of supplement to decrease fat accumulation. In addition, LA administration stimulate muscle protein acceretion and cause a significant increase in concentration of muscle protein (table-1).Many studies have noted that the muscle protein deposition induced by LA is attributed to a lowered fractional rate of protein degradation rather than to a stimulated fractional protein synthesis, Yoshio (2002).

Effect of LA administration on serum metabolites of different experimental rabbit groups:

Table (2) revealed a significant decrease in serum glucose in experimental groups than control one, in this experiment, a decrease in serum glucose might be attributed to enhanced glucose uptake by peripheral tissue in the LA-treated rabbits. Previous studies found that LA treatment decreases hyperglycemia and enhances glucose uptake, a result of stimulated insulin action in skeletal muscle of obese rats, Yoshio (2002). While, Daniel *et al.* (2001) revealed that, In animal models, α-lipoic acid restored insulin-stimulated glucose uptake into insulin-resistant skeletal muscle of rats. Treatment of streptozotocin-induced diabetic rats with α-lipoic acid caused a significant reduction in plasma glucose levels and enhanced insulin-stimulated glucose uptake into muscle, Khamaisi *et al.* (1997). Also, Estrada *et al.* (1996) and Yaworsky *et al.* (2000) show that α-lipoic acid stimulates glucose uptake by rapid translocation of the glucose transporters and from an internal membrane fraction to the plasma membrane. At the same time, α-lipoic acid differs from other stimuli that increase glucose uptake, such as contraction and hypoxia, which stimulate glucose uptake by a kinase–independent mechanism,Yeh *et al.* (1995) and Lee *et al.* (1995).While, Midaoui *et al.* (2003) conclude that Lipoic acid prevents hypertension, hyperglycemia, and the increase in heart mitochondrial superoxide production.

Table (2) revealed a significant increase in the level of non esterified fatty acid (NEFA).With regard to fatty acid mobilization, plasma NEFA is generally considered as an index of adipose tissue lipolysis. This result agree with Yoshio (2002) who conclude that LA supplementation enhanced the plasma non esterified fatty acid (NEFA) level and

the rate of in vitro hepatic oxygen consumption in chickens at the level of 50 mg/kg diet. An increase in blood NEFA will also occur when the rate of fatty acid utilization or uptake in peripheral tissue is below the releasing rate of NEFA with lipolysis, Hamano *et al.* (1999) At the same time, LA decreased serum triglycerides (table-2) and this effect would result from lowered neutral fat transport (lipoprotein) or reduced fat synthesis in the liver.LA has also been reported to decrease serum β-lipoproteins in rabbits with experimental atherosclerosis, Ivanov (1974). In addition, a similar reduction of serum TAG has been previously observed in 6-week old broiler chickens with LA (50mg/kg BW) but not in 4 week old birds,Yoshio,(2002).While, Segermann, *et al.* (1991) demonstrated that the serum triacylglycerol (TAG) level of rats is lowered by intraperitoneal injection of LA. These metabolic effects of LA on energy metabolism may be available and beneficial to energy redistribution and the control of adipose tissue acceretion in meat-producing animals.

Effect of LA on serum Iron and copper of rabbits:

Data in Table (3) show a non significant decrease in iron and copper level of serum, our result indicate that LA may reduce the risk of Fe and Cu induced oxidative damage and also might be useful as a treatment of Fe and Cu overload. This result agree with Suh *et al.* (2005) who revealed that lipoic acid did not lower the iron content in young rat, suggesting that lipoic acid mainly removes the excess iron accumulated with age. While, Goralska *et al.* (2003), showed that. The cells convert lipoc acid (LA) into dihydroplipoic acid (DHLA), which in the presence of iron can act as a prooxidant. In vitro DHLA reduces Fe (+3) to Fe (+2) and removes iron from ferritin, increasing the risk of Fe catalyzed free radical formation and found that LA decreases Fe uptake from transferrin, increases Fe deposition into ferritin and increases the concentration of this protein. When administered together with ascorbic acid, lipoic acid changes the characteristic heavy to light chain ratio of ferritin make up. The decreased Fe uptake and increased storage diminishes the size of the cytosolic highly reactive Fe pool. These changes are associated with increased cell resistance to H_2O_2 challenge. At the same time, Gregus *et al.* (1992) showed that LA (150 mumol/kg,iv) did not increase, but rather decreased, the biliary excretion of methyl mercury, cadmium, zinc, and copper, which are transported into bile in a glutathione-dependent manner. But, Yamamoto *et al.* (2001) revealed that,although at the highest dose, LA slightly suppressed the accumulation of Cu in crude mitochondrial fraction and it had no effect on the accumulation of Cu in cytosolic fraction. Morever, Ou *et al.* (1995),found that lipoic acid had a profound dose-dependent inhibitory effect upon Cu (2+) and also increased the partition of Cu2+ into n-octanol from an aqueous solution suggesting that lipoic acid forms a lipophilic complex with Cu2+. lipoic acid also inhibited Cu (2+)-catalysed liposomal peroxidation. Furthermore, lipoic acid inhibited intracellular H_2O_2 production in erythrocytes challenged with ascorbate, a process thought to be mediated by loosely chelated Cu2+ within the erythrocyte. These data, taken together, suggest that prior intracellular reduction of lipoic acid to dihydrolipoic acid is not an obligatory mechanism for an antioxidant effect of the drug, which may also operate via Cu (2+)-chelation. On the other hand, Kayali *et al.* (2007) concluded that LA may exhibit prooxidant effect depending on the altered trace element homeostasis and their results emphasize the importance of monitoring the dose of LA supplementation and duration of treatment.

Effect of LA administration on blood glutathion, glutathione peroxidase and superoxide dismutase content of the different experimental rabbit groups:

The level of blood glutathione and glutathione peroxidase significantly increased in all treated groups than control one while in superoxide dismutase there is a non significant changes in all groups, Table (4) this result may be due to a fact that alpha-Lipoic acid raises GSH values by increasing cysteine availability which is the rate-limiting factor in its biosynthesis and plays an essential role in mitochondrial dehydrogenase reactions and has recently gained considerable attention as an antioxidant. Lipoate, or its reduced form, dihydrolipoate, reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen., Packer *et al.* (1995) and Konrad (2005). While, Busse,*et al.* (1992) ;Han, *et al.* (1995) and Han *et al.* (1997), revealed that,the addition of exogenous alpha-lipoic acid causes a rapid increase of intracellular unbound thiols,the rise of cellular thiols is a result of the cellular uptake and reduction of lipoic acid to dihydrolipoic acid and a rise in intracellular glutathione and rises in glutathione correlate with the levels of intracellular dihydrolipoic acid ($P<0.01$). On the other hand,Gregus *et al.* (1992) conclude that following injection of LA, the concentrations of endogenous disulfides in arterial blood plasma (e.g., cystine, glutathione disulfide, cysteine-glutathione, protein-cysteine, and protein-glutathione mixed disulfides) were considerably diminished, while the levels of endogenous thiols (e.g., glutathione and cysteine) were increased. Also, Lass *et al.* (1999) and Hagen *et al.* (1999) pointed that lipoic acid (LA), increased endogenous antioxidants or mitochondrial bioenergetics.

Effect of LA administration on ceruloplasmin and transferrin concentrations of serum of of the different experimental rabbit groups:

Table (4) recorded a significant increase of ceruloplasmin (TF) and transferrin(CP) in all groups than control group. This result may be due to the antioxidant effect of lipoic acid which increase the metalloproteins like ceruloplasmin and transferring. This result agree with Goralska *et al.* (2003) who revealed that TF and CP are considered important extracellular antioxidants and LA decreases Fe uptake from transferrin, increases Fe deposition into ferritin and increases the concentration of this protein. While, Frieden,(1993) conclude that ceruloplasmin catalyzes the oxidation of Fe^{2+} to Fe^{3+} which would enable it to attach to and be transported by transferrin thus, increasing transferrin-iron concentration.

CONCLUSION

Results of this study indicate that lipoic acid cause increase in body growth, body gain or tissue mass, stimulate muscle protein acceretion and cause a significant increase in concentration of muscle protein Furthermore, α-lipoic acid supplementation dramatically decreased carcass fat accumulation and content, which might be a practical method of improving carcass composition. Also, its hepatocellular antioxidant effect by increasing blood glutathione, glutathione peroxidase and glycoprotein.

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الدور الوقائي للحمض الدهني ضد تأثير الأكسدة الضارة و تدمير الخلية و تأثيرها على معدلات الأداء فى الأرانب

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يعتبر الحمض الدهنى من المركبات المحبة للدهون ثنائية الكبريت و يقسم عموماً مع مجموعة فيتامين ب. و قد تم فى هذة الدراسة إجراء البحث على عدد ٦٠ أرنب حديث الفطام وزن(٥٥٠ ـ٦٠٠جم) تم تقسيمهم إلى ٤ مجاميع متساوية المجموعة الأولى ضابطة و المجموعة الثانية و الثالثة و الرابعة يتم حقنها فى البريتونيا (IP) بنسب ١٠ و ٥٠ و ١٠٠ ملجم/كجم وزن على التوالى بالحمض الدهنى إسبوعياً لمدة شهرين.

و قد تم تقدير أوزان الأرانب بعد ٨ أسابيع وقد وجد زيادة معنوية بالأوزان مع زيادة بوزن عضلة الصدر و نقص بوزن دهون البطن كما وجد أيضاً زيادة بنسبة البروتينات بأنسجة الصدرفى المجموعات التجريبية عن المجموعة الضابطة.

كما دلت النتائج المتحصل عليها إلى نقص معنوى بمستوى الجلوكوز و الدهون الثلاثية بمصل الدم مع زيادة فى الأحماض الدهنية غير المشبعة و الجلوتاثيون و الجلوتاثيون بيروكسيديز كما وجد زيادة ملحوظة بنسبة السيريولوبلازمين و الترانسفيرين بجميع المجاميع عن المجموعة الضابطة. أما السوبر أكسيد ديسميوتيز لم يتأثربجميع المجاميع. مع نقص غير معنوى بنسبة الحديد و النحاس بمصل الدم.

من هنا يتضح لنا دورالحامض الدهنى المهم فى تحسين معدلات الأداء الإنتاجى و زيادة الوزن و تقليل نسبة الدهون الضارة كما تلعب دوراً هاماً ضد الأكسدة الضارة و تدمير الخلايا الحية و ذلك بزيادة نسبة الجليكوبروتينات (السيريولوبلازمين و الترانسفيرين) و إنزيمات الأكسدة كما وجد انة يستخدم لمعالجة سمية المعادن.