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Impact of N-acetyl cysteine (NAC) chit Nanocomposite as antioxidant on broiler chicks

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ABSTRACT

Nutrients nano-forms in livestock's feed are mainly intended to increase the production performance, enhance immunity and antioxidant activities. The current experiment was undertaken by feeding 150 (divided to five group control, N-acetyl cysteine (NAC), NAC chit nano-composite (different levels (30, 60, 120 µg / kg diet)), one day old broiler chicks for 42 days. The effect of these additives as antioxidant on liver and kidney function tests of broiler chicks were investigated. The results were compared with those obtained from feeding another group of broiler chicks on the normal diet but after addition of commercial antioxidant, NAC (5.2g/kg feed). The results showed that chicken in the groups fed on (60,120 µg / kg feed) of NAC chit revealed significant ($P< 0.05$) improved antioxidant status, liver and kidney function indices as compared with control and the other dietary treated groups. Structure of mitochondria in HRTEM examination showed slight to moderate swelling due to increase of NAC chit concentration and found NAC chit appeared inside the mitochondria.

Keywords: Nano-form nutrients, N-acetyl cysteine, antioxidant, broiler performance.

INTRODUCTION

Fullerenes were known to behave like a "radical sponge," as they can sponge-up and neutralize 20 or more free radicals per fullerene molecule. They have shown performance 100 times more effective than current leading antioxidants such as Vitamin E. Fullerene was highly soluble in almond oil and thus it could be used for screening test for ocular tissue toxicity indicating no adverse effect. Fullerenes were powerful antioxidants, reacting readily and at a high rate with free radicals, which were often the cause of cell damage or death. Fullerenes hold great promise in health and personal care applications where prevention of oxidative cell damage or death is desirable, as well as in non-physiological applications where oxidation and radical processes were destructive (food spoilage, plastics deterioration, metal corrosion) (Yadav and Kumar, 2008)

The effect of repeated-dose oral toxicity of fullerene C_{60}, rats were administered fullerene C_{60} by gavage once daily at 0 (vehicle: corn oil), 1, 10, 100,or 1,000 mg/kg/day for 29 days, there were no significant different in total protein, AST,ALT and ALP between treatment groups against control, but creatinine in group100 mg/kg had significant different from control group and other treatment groups (Takahashi *et al*., 2012)

The influence of fullerene C_{60} on lipid peroxidation (POL) and antioxidant protection during the induction of the immune response to heteroantigen. Balb/c mice were immunized intraperitoneal (i.p.) with sheep erythrocytes for the primary immunization. Water dispersion of fullerene C_{60} was injected i.p. once at the dose 50 ng to mice on first, third and sixth days after immunization. During immune response, the increment of malonic dialdehide (MDA) was enhanced in liver, kidneys and heart tissues. Fullerene C_{60} induced POL during the latent phase of immune response, but inhibited this process during progression of immune response. Activities of superoxide dismutase (SOD) and catalase in liver and spleen tissues were induced after injection of fullerene C_{60} to intact mice. Injection of fullerene C_{60} reduced the activities of SOD and catalase in spleen tissues. The fullerene C_{60} can display positive effect on POL processes and antioxidant enzymes activity which was probably due to membrane's stabilization action or the ability of fullerene C_{60} to bind free radicals independently (Vesnina*et al.*, 2012)

The antioxidant status of a cell or tissue was dependent upon a variety of factors that include the presence of a myriad of nonenzymatic and enzymatic antioxidants as well as forces that favor oxidation (Yu *et al.*,2012)

The mechanism of antioxidant activity of buckminsterfullerene C_{60} based on protons absorbing and mild uncoupling of mitochondrial respiration and phosphorylation was postulated. Fullerene's geroprotective activity is sufficiently higher than those of the most powerful reactive oxygen species scavengers that C_{60} has an ability to acquire positive charge by absorbing inside several protons and this complex could penetrate into mitochondria. Such a process allows for mild uncoupling of respiration and phosphorylation. This, in turn, leads to the decrease in ROS production. The proposed ability of C_{60} fullerenes to acquire positive charge allows ascribing them to the mitochondrial-targeted compounds. The key role of mitochondria in the cellular regulation makes such “charge-loaded” fullerenes be of great interest along the route for novel classes of drugs development (Chistyakov *et al.*,2013)

Neither antioxidant source nor level of supplementation had any significant effect on dressing, liver, gizzard, heart, abdominal fat and intestine weight as percentage from life body weight when evaluated the efficiency of aqueous extract of ginger root (GAE), aqueous extract of beetroot (BAE) and tomato puree (TP) as natural antioxidant sources in broiler diets (Selim *et al.*, 2013).

The size distribution and zeta-potential in cell culture RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), the size distribution of FNP nanoparticles was not affected by FBS and/or cell medium and formation of large particles was not induced but caused reduction in zeta-potential of nanoparticles (from −58 mV to −7,9 mV). The influence of FNP on Chinese hamster ovary cells (CHO-K1) survival, as well as antioxidant capacity of FNP in mitomycin C-treated cell line. It had been shown that activity of antioxidative enzymes was increased in dose-dependent manner. The FNP did not induce genotoxic effects; on the contrary, antigenotoxic effects of FNP were confirmed in the experiment done on MMC-damaged CHO-K1 cells in

concentrations of 11–221.6 μM (Srdjenovic *et al*., 2014)

Fullerenol with its antioxidant activity can also mediate oxidative stress-induced senescence. Retinal pigment epithelium (RPE) cells and ARPE-19 cells were exposed to pulsed H2O2 stress for 5 days. Fullerenol protected the RPE cells, as it reduced the number of senescence positive cells, alleviated the depletion of cellular antioxidants, and reduced genomic DNA damage (Zhuge *et al*.,2014).

The present study investigated for the first time the impact of NAC chit nanocomposite as antioxidants on broiler chicks.

MATERIALS AND METHODS

Birds' accommodation:

The present feeding trial was carried out during 6 weeks period using a total of 150 one day old commercial Hubbard chicks in the lab animals unit, Reference Lab. of Quality Control of Poultry Production, Animal Health Research Institute, Dokki, Egypt. The chicks were randomly allotted to six groups (each of 25 birds) and accommodated in batteries under standard hygienic conditions, good ventilation, free access of feed and water, continues lighting and subjected to a prophylactic vaccination and antibiotic program against viral and bacterial diseases.

Preparation of NAC chit nanocomosite:

It was prepared according to Zhen *et al*. (2007). N-acetyl-Cysteine (2.3g) and sodium hydroxide (0.85g) were dissolved in 5ml water, and then 20 ml ethanol was added, the resulting solution was added to a C_{60} toluene solution (60 mg, 60 ml) dropwise, and then five drops 10% cetyltrimethyl-ammonium bromide was added and stirring well.

Preparation of copper with chitosan mixture:

Concentration of 1% chitosan was used with 1% acetic acid and heated in heating checker, then adding copper as concentration 2MM.

Preparation of Final Nanocomposite:

Add $NacC_{60}$ to mixture of copper chitosan (1%, 2MM) and dry by a rotary evaporator.

Biochemical analysis:

Liver function tests:

Serum Glutamic Oxal Acetic Transaminase (GOT) and serum Glutamic-Pyruvic Transaminase (GPT) determination according to Reitman and Frankel (1957) using commercial kits (Biodiagnostic).

Serum Alkaline phosphatase was determined calorimetrically according to Eastman and Bixter (1977) using TECO kit, (TECO, diagnostic, California, U.S.A).

Kidney function tests:

Serum uric acid was determined enzymatically according to Tietz (1990) using commercial kits (Biosub®UA).

Serum Creatinine was determined colorimetrically according to Larsen (1972) using commercial kits (Biodiagnostic).

Antioxidant Biomarker tests:

The total antioxidant capacity was determined calorimetrically according to Koracevic *et al*. (2001);

Glutathione-S-Transferase was determined by U.V method according to Habig *et al*. (1974).

Superoxide Dimutase (SOD) was determined calorimetrically according to Nishikimi *et al*. (1972).

Malondialdehyde (MDA): (lipid peroxidase) was determined calorimetrically according to Ohkawa *et al*. (1979).

Nitric Oxide was determined calorimetrically according to Montgomery (1961) using commercial kits (Biodiagnostic).

HRTEM studies:

Tissue specimens were collected at slaughter day from liver and fixed into a vial containing enough fixative to cover the tissue well. Primary fixative = 2% paraformaldehyde, 2.5% glutar-aldehyde in 0.1 M cacodylate - 0.1 M sucrose, pH 7.4. Let stand at room temperature (RT) for at least 1 hour with occasional agitation. At this point the tissue may be stored overnight or for days at 4° C. Procedure of En Bloc Staining - optional contrast enhancement , dehydration process, transition solvent, infiltration and embedding (Spurr´s low viscosity resin) according to Nowell and Pawley (1980).

The data obtained in this study were analyzed using statistical analysis system software (one way- ANOVA), (SPSS21, 2012) for Windows.

RESULTS AND DISCUSSION:

Analysis of variance of results (Table 1) revealed a non- significant difference at ($P \leq 0.05$) on ALT and ALP levels between groups but AST had significant ($P \leq 0.05$) increase in NAC chit fed groups; and the lower level in that fed NAC (group 3) as compared to control group at end of experimental period.

The total protein showed significant increase in the groups 3 and 4 as compared to group 2 & and control groups. However, the uric acid showed lower significant ($P \leq 0.05$) difference between NAC chit (3&4) groups than other group treatments. While the creatinine showed significant ($P \leq 0.05$) increase in groups 3 &5 than other group treatments.

It was obvious from Table (2) that there were no significant differences between levels of Nitric oxide (NO), total antioxidant capacity (TAC) and superoxide dismutase (SOD) in experimental and the control groups of broiler chicks.

These results were similar to those of Wolff *et al*. (2000) who reported that malonic acid C_{60} derivatives inhibited the catalytic activity of NO syntheses. Also, Mirkov *et al.* (2004) who reported that $C60(OH)_{24}$ (fullerol) was able to quench NO and block its biological activity in vivo. The same results reported by Misirkic *et al*. (2009) who suggested that C_{60} complexes with appropriate host molecules might be plausible candidates for preventing NO-mediated cell injury in inflammatory/ autoimmune disorders. Also Zhen *et al*. (2013) reported that the glutathione C_{60} derivative had the potential to prevent NO-mediated cell death without evident toxicity.

On the other hand, the results of MDA differed with those reported by Tagang *et al.* (2013) who administered yeast probiotic supplement diet to broiler. There was no significant difference in MDA level in all the treatment groups.

Also, the results of MDA level in the present study disagree with those of da Rocha *et al*. (2013) who reported that fullerenol not detect any statistically significant changes in GST activity or TBARS level when the fresh water zebra

fish exposed to fullerenol ($C_{60}(OH)_{18-22}(OK_4)$).

Investigations by HRTEM:

Figure (1A) shows section of normal liver of control group with normal hepatocytes with large spherical nucleus and nucleoli, normal mitochondria, and fibrillo-granular network structure. There was a profuse amount of rough endoplasmic reticulum and there were some fat droplet as development of broiler age.

It was clear from the results of HRTEM (Fig. 1 A,B & C) that there were nanoparticles inside the hepatic cells and mitochondria of broiler chicks fed NAC chit additives.

For chicks feed a dose 30µg/kg diet of NAC chit, the HRTEM of their liver showed that their hepatic cells have mitochondria that had different morphology, with large nucleus, rough endoplasmic reticulum; mitochondrial crista was clear and NAC chit was found inside mitochondria and nucleus as shown in Figure (1 B).

For chicks fed the dose 60µg /kg diet of NAC chit, the HRTEM of their liver showed that mitochondria was spherical in shape with large nucleus, rough endoplasmic reticulum; mitochondrial crista was clear; slight swelling in mitochondria and NAC chit was found inside mitochondria and nucleus as shown in Figure(1C).

While, in chicks fed the dose 120µg /kg diet of NAC chit, the HERTEM of their liver showed that mitochondria was spherical shape with large nucleus, rough endoplasmic reticulum; mitochondrial crista was clear; moderate swelling in mitochondria and NAC chit was found inside mitochondria and nucleus (Fig. 1D).

These results are similar to those of Gharbi *et al*. (2005) who found that characteristic C_{60} particles were detected by TEM in all of the liver sections essentially inside Kupffer cells and some hepatocytes of the capsule as well as inside rare HSCs. However, inside the liver cells, most of the aggregates contained C_{60} crystals with an average size lower than 50 nm and the dissolution of the fullerene inside lipid droplets was sometimes observed, indicating that this fullerene was absorbed well by the organs. However, these results are different from those obtained by Takahashi *et al*. (2012) who explained the effect of repeated- oral dose toxicity of fullerene C_{60}, there were in dose 1000mg/kg; slight granuloma and vacuolation in liver and in kidney there were minimal mineralization but the spleen were normal.

Conclusion:

Innovative synthesis of NAC chit nanocomposite were the work of study to invent a new Nano composite based on the molecular weights, and surface modification to enhance the biological effect of each compound on production performances and improved antioxidant indices of broiler chicks. The first feeding trial was undertaken to study the effect of feeding different levels (30, 60, 120 µg / kg diet) of Nanocomposite of N-acetyl cysteine on 150, one day old broiler chicks fed on a diet for 42 days to be compared with that fed on the same diet but after addition of commercial antioxidant, liver and Kidney function indices had no any significant effect on ALP and ALT while, TP; AST; uric acid and creatinine showed some changes. Antioxidant status of

broiler at the end of the experiment had no significant difference in NO; SOD; CCP and 8-OHdG but significant improvement was recorded in MDA and GST.

Structure of mitochondria in HRTEM examination showed slight to moderate swelling due to increase of NAC chit concentration and found NAC chit appeared inside the mitochondria.

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Table (1): Effect of NAC and Nano composite dietary treatments on liver and kidney function tests.

Group	**Control (1)**	**NAC 2**	**3**	**4**	**5**
ALT (U/ ml)	29.25±4.21 ^{a}	25.13±0.95 ^{a}	29.25±4.21 ^{a}	31. 5±4.91 ^{a}	26.98±5.78 ^{a}
AST (I.U/ ml)	79.0±8.66 ^{c}	71.5±7.5 ^{b}	59.25±15.2 ^{a}	70.5±15.23 ^{b}	81.75±14.57 ^{c}
ALP (I.U/ ml)	243.58±25.65 ^{a}	247.14±10.21 ^{a}	252.96±4.42 ^{a}	249.38±10.99 ^{a}	246.36±4.78 ^{a}
Uric acid (mg/dl)	5.71±0.54 ^{a}	4.21±0.51 ^{bc}	3.47±0.16 ^{c}	3.75±.65 ^{c}	5.3±0.07 ^{ab}
Creatinine (mg/dl)	0.57±0.01 ^{a}	0.69±0.10 ^{b}	0.78±0.25 ^{c}	0.69±0.13 ^{b}	0.74±0.36 ^{c}

Values are means ± SE

Values in the same row with different superscripts are significantly different at $P \leq 0.05$

Table (2): Effect of NAC and Nano composite dietary treatments on antioxidant status at end of experiment. Group

Group	**Control (1)**	**NAC 2**	**3**	**4**	**5**
NO(ummol/l)	16.63±1.02 ^{a}	17.85±2.24 ^{a}	19.05.±3.7 ^{a}	15.91±1.04 ^{a}	16.89±1.28 ^{a}
MDA(nmol/ ml)	7.16±0.74 ^{bc}	8.18±2.42 ^{a}	12.91±1.8 ^{a}	11.08±1.61 ^{ab}	12.53±0.36 ^{a}
TAC (mM/ l)	2.55±0.29 ^{a}	2.89±0.80 ^{a}	2.19±0.24 ^{a}	2.23±0.30 ^{a}	2.29±0.51 ^{a}
SOD (U/ml)	280.99±0.41 ^{a}	268.52±12.6 ^{a}	270.61±9.8 ^{a}	268.06±6.4 ^{a}	269.87±4.31 ^{a}
GST(U/L)	4112.07± 588.2 ^{b}	7747.06±811.1 ^{a}	3415.28±542.8 ^{b}	4883.61±611.44 ^{ab}	7023.07±382.92 ^{a}

Values are means ± SE

Values in the same row with different superscripts are significantly different at $P \leq 0.05$.

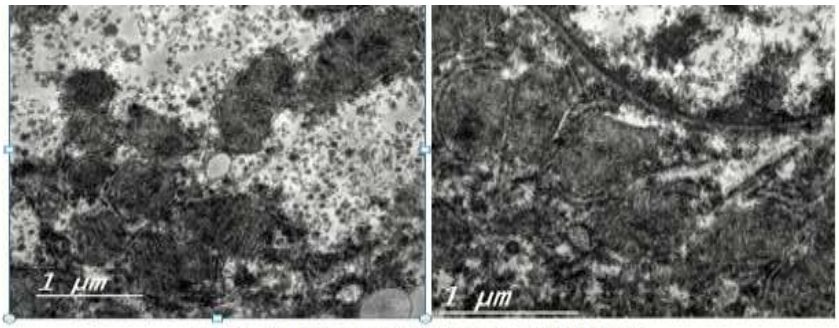

Fig.(1 A) liver cells of control by HRTEM.

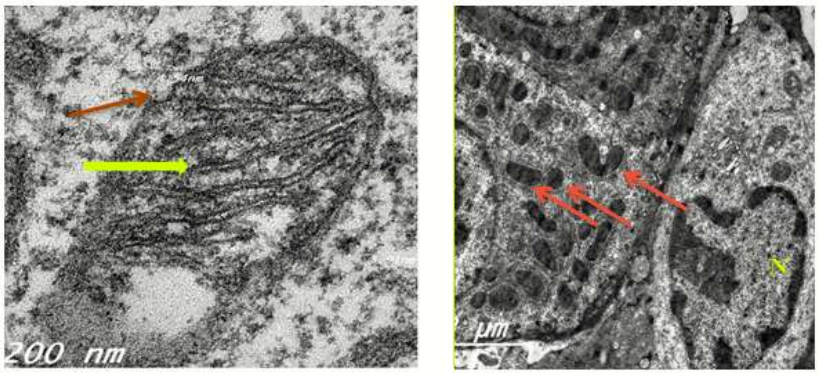

Fig.(1 **B**) Liver cells of gr.3 showing nanoparticle by HRTEM.

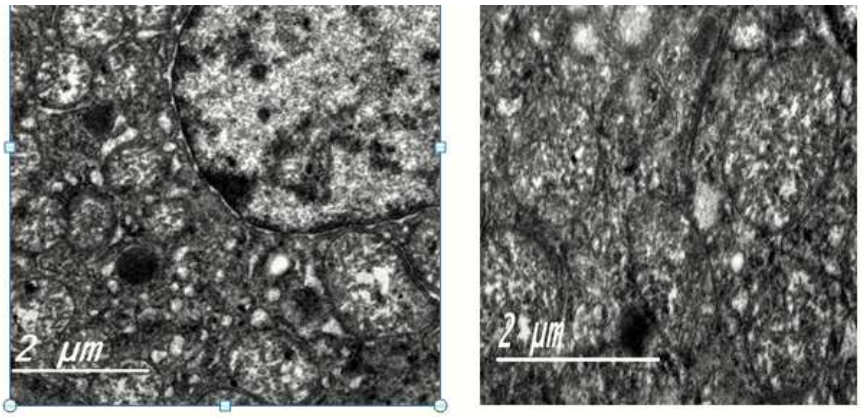

Fig.(1 C) liver cells of gr.4 showing nanoparticle by HRTEM.

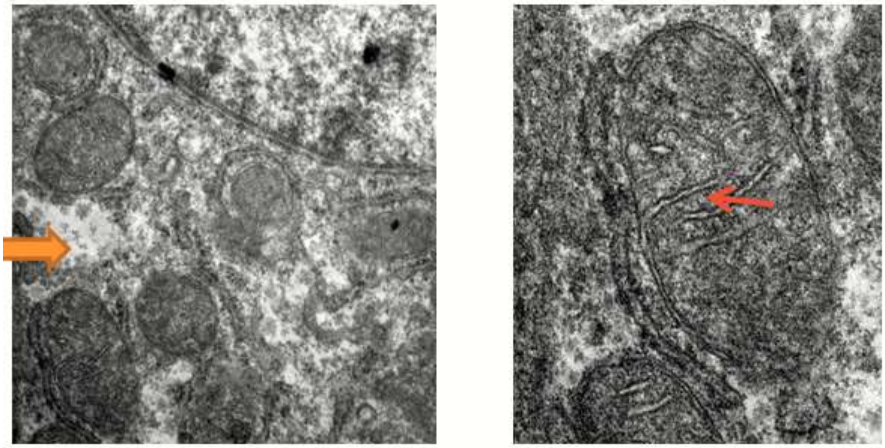

Fig.(1D) Liver cells of gr.5 showing nanoparticle by HRTEM.

تأثير اضافة المركب النانومترى (N-acetyl cysteine (NAC لغذاء كتاكيت التسمين على مستوى مضادات الأكسدة

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المستخلص

الاضافات الغذائية ذات تركيبة نانونية تهدف أساسا لزيادة أداء الإنتاج. فى هذة الدراسة تم تحضيرمركب NAC chit من مشتقات C60 فلورين المحمل عليه الحمض الأمينى ن- أسيتيلسيستين (NAC) والمغلف بالشيتوزان. تمت هذه الدراسة بإجراء تجربة لدراسة تأثير التغذية على مستويات مختلفة (30، 60، 120 ميكروغرام/كغ علف) من مركب NAC chit على عدد 150 كتكوت من كتاكيت الدجاج اللاحم عمره يوم واحد لمدة 42 يوما بالمقارنة مع التي تتغذى على نفس العليقة ولكن بعد إضافة مضاد للأكسدة، ن-أسيتيل سيستين (5.2غ/كغ علف) على وظائف الكبد والكليتين، والبنية الفوقية للكبد ومضادات الاكسدة.

أظهرت النتائج ان الدجاج في المجموعات التي تتغذى على (60,120 ميكروغرام) لاتوجد تغيرات ملموسة في وظائف الكبد والكلى وفي حين اظهر حمض اليوريك والكرياتينين بعض التغييرات. أما الاختبارات السيرولوجية الخاصة بمضادات الاكسدة فلقد أظهرت تحسنا معنويا فى مستواها. ونتائج دراسة التغيرات التي طرات على الميتوكوندريا بخلايا الكبد أظهرت تغير طفيف إلى معتدل بسبب زيادة تركيز التركيبة والعثور NAC chit داخل الميتوكوندريا.