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Impact of N-Acetylcteine on Productive, Reproductive and Physiological Performance of Local Chickens under Egyptian Summer Conditions

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The present study was carried out to evaluate whether N-acetylcysteine (NAC) has a beneficial role in enhancing productive and reproductive performance in Inshas layer hens under Egyptian summer conditions. A total number of 300 Inshas hens and 30Inshas cocks, 34 weeks of age were randomly distributed into 5 groups of 60 hens and 6 cocks each and then subdivided into six replicates (10 hens + 1 cock/ replicate). The first group (G1) was served as the control group without any administrationand was injected with water only. The second and the third groups (G2 and G3)drank water supplemented with 50 and 100 mg NAC / litter, respectively, while the fourth and the fifth groups (G4 and G5) were injected with 50 and 100 mg NAC / kg BW, respectively, first three days every month for three months (the experimental period). The obtained results showed thattreatment with NAC significantly increased egg production percentage, whereas birds injected with 100 mg NAC/ kg BW recorded the highest value, as compared with those in the control group (68.69 vs. 51.39%, respectively, P<0.001). High doses of NAC significantly increasedplasma total protein(TP), albumin (A), globulin (G), and A/G ratio. It could be concluded that N-acetylcysteine (NAC)treatment having the largest impact onInshas laying hens performance under heat stress, particularly with high dose (100 mg) injection or in water. From the economic point of view, 100 mg NAC injection/ kg BW improved feed conversion, increased egg production, and egg mass.

Keywords: N-acetylcysteine, laying hens, blood metabolites, antioxidant, egg production, egg quality, economic efficiency.

Introduction

In the subtropics, heat stress is a major problem that adversely affects poultry's performance and physiological traits. Feed intake reduction is the worst effect of heat stress, which leads to decreased bodyweight, feed efficiency, egg production, and quality (Deng *et al.,* 2012). However, in addition, to feed intake depression, heat stress leads to decreased dietary digestibility, and reduced plasma protein and calcium levels (Mahmoud *et al.,* 1996). Additionally, heat stress has been shown to cause a significant reduction of egg weight (-3.24%), eggshell thickness (-1.2%), eggshell weight (-9.93%), and eggshell percent (-0.66%) (Ebeid*et al.,* 2012). At the same time, heat stress increased lipid peroxidation as a consequence of increased free radical generation as it enhances the formation of reactive oxygen species (ROS) and induced oxidative stress in cells. Oxidative damage may be minimized by antioxidant defense mechanisms that protect the cell against cellular oxidants and repair systems that prevent the accumulation of oxidatively damaged molecules. (Pınar *et al.,* 2009). Many efforts have been done by Egyptian scientists to improve and manage the poultry industry. They used different antioxidants as feed additives through supplementation or administration, one of these antioxidants is N-acetylcysteine (NAC).

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The NAC can be defined as, an intracellular glutathione (GSH) precursor that is currently one of the most studied antioxidants as it is endogenously synthesized basically in all cells. Furthermore, it has the ability to regulation of cell proliferation, regulates immune responses, as well as regulates leukotriene and prostaglandin metabolism precursors (Agostini*et al*., 2014).In addition, NAC is a commercial product has been tested in human by many studies and reported its positive role in many diseases (Cam *et al*., 2008; de Andrade *et al*., 2015 and Gao *et al*., 2017). Furthermore, NAC was used in animal experiments and reported positive results, and was introduced orally(well accepted), intravenously, or by inhalation (Gao *et al*., 2017). Moreover, Ashour *et al.* (2018) reported that NAC capability to improve rabbit's performance particularly with high doses (100 mg/kg BW).

Therefore, the present study was undertaken to evaluate whether N-acetylcysteine (NAC) has a beneficial role in enhancing productive and reproductive performance in Inshas layer hens under Egyptian conditions.

Materials and Methods

Experimental design

The present study was carried out in Poultry Research Unit at Sakha Research Station located in Kafr El-Shiekh governorate, Egypt, which belongs to Animal Production Research Institute (APRI), Agricultural Research Center (ARC). The fieldwork was executed during the summer months in Egypt from June to August.A total number of 300Inshas hens and 30Inshas cocks, 34 weeks of age (at 44 % egg production) were randomly distributed into 5 groups of 60 hens and 6 cocks each and then subdivided into six replicates (10 hens + 1 cock/ replicate).Inshas strain is developed from a cross between Sina and Plymoth Rock breeds, which characterized 22 wks at sexual maturity and 170-190 average egg production/ season (Saleh *et al.,* 2006). The first group (G1)was served as the control group without any administration and was injected with water only, to exert any mechanical effects the injection might have (Mohammadi *et al.,* 2015). The second and the third groups (G2 and G3) drank water supplemented with 50 and 100 mg N-acetylcysteine (NAC)/ litter, respectively, while the fourth and the fifth groups (G4 and G5) weresubcutaneous injected with 50 and 100 mg N-acetylcysteine (NAC)/ kg BW, respectively, first three days every month for three months (the experimental period) according to Omer *et al*. (2012) and Ashour *et al.* (2018).The NAC was purchased from a commercial pharmacy. The birds were individually injected subcutaneously at the base of the neck (the injections were one-off). Each hen received its specific NAC dose. All experimental groups fed basal diet (Table 1), which was formulated to be at least satisfying the nutrient requirements according to Agriculture Ministry Decree (1996). The birds were reared under the same managerial conditions in an open-sided house on the floor and photoperiod of 17 hours daily. The birds were fed *ad libitum* and the water was available all the time.

Means of ambient temperature, relative humidity, and temperature-humidity index (THI) inside the building were 32.6±4.3 °C, 72.9±4.5%, and 31.07, respectively, which indicate severe heat stress. According to Marai*et al.* (2002), there is very severe heat stress when THI is higher than 30.0. The THI was calculated according to Marai*et al.* (2001):

$$THI = db°C–[(0.31–0.31RH) \times (db°C–14.4)]$$

Where db°C is dry bulb temperature in Celsius, and RH is the relative humidity as a percentage.Feed intake (FI), egg production (%), and egg weight were recorded daily. Five representative eggs from each treatment were collected monthly at 30, 60, and 90 days of starting experimental period(S1, S2, and S3) throughout the experimental period to determine egg and shell quality. Shape index and yolk index were determined according to Romanoff and Romanoff (1949) as follows:

Shape index (%) = (width / length) X 100
Yolk index (%) = (height / diameter) X 100

Eggshell thickness, including shell membranes, was measured using a micrometer at the equator. The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the "1961, Roche Improved Yolk Color Fan. The previous analyses were done at Lab. of Food Science, Faculty of Agriculture, Cairo Univ., Giza.

TABLE 1. Composition and chemical analysis of the basal diet.

Ingredient	%	Chemical analysis	%
Yellow corn	59.70	Crude protein	16.0
Soybean meal (44% CP)	24.02	Metabolizable energy (ME, kcal/kg)	2700
Wheat bran	5.40	Crude fiber	3.72
Corn oil	1.00	Available phosphorus	0.40
Limestone	7.77	Calcium	3.30
Di-calcium phosphate	1.45	Lysine	0.90
Sodium chloride	0.30	Methionine	0.35
Mineral-vitamin premix[1]	0.30	Methionine +Cystine	0.62
DL-Methionine	0.06	Sodium	0.14

[1] Each 3 kg of Vit. & Min. Mixture contains: Vit. A, 10000,000 IU; Vit. D3, 2000,000 IU; Vit. E, 10,000 mg; Vit. k3, 1000 mg; Vit. B1, 1000 mg; Vit. B2, 5000 mg; Vit. B6, 1500 mg; Vit. B12, 10 mg; Pantothenic acid, 10,000 mg; Niacin, 30,000 mg; Folic acid, 1000 mg; Biotin, 50 mg; Choline, 250,000 mg; Manganese, 60,000 mg; Zinc, 50,000 mg; Copper, 10,000 mg; Iron, 30,000; Iodine, 1000 mg; Selenium, 100 mg; Cobalt, 100 mg; Ca CO3 to 3,000 gm.

Rectal temperature (RT) of birds was taken with a digital thermometer by the rectal probe (0.1% accuracy) as previously described and the respiratory rate (RR) of the birds was taken as the number of breaths per minute.Blood samples (about 3 ml) were collected at30, 60, and 90daysof starting experimental period (S1, S2, and S3) according to Wahab *et al*. (2016). The samples were collected in heparinized tubes from each doe and were centrifuged at 3000 rpm. to get blood plasma stored under -20°C until biochemical analysis. A colorimetric method was used to determine total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), andmalondialdehyde (MDA) and by using kits obtained from Bio-Diagnostic Company, Dokki, Giza, Egypt. Quantitative colorimetric determination of total protein (TP), albumin (A), triglycerides (TG), cholesterol (CHO), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were executed by using kits of Stanbio Laboratory Inc, procedure No. 0280. (San Antonio, Texas, USA). Globulin concentration (G) was calculated by subtracting A values from TP values. Albumin/ Globulin ratio (A/G ratio) was calculated. Kits from EGY- CHEM for lab technology (Badr City, IndustrialArea Piece 170 - Egypt) were used in the determination of concentrations (mg/dl) of blood urea nitrogenand creatinine as indicators for kidney functions. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators for liver functionswere determined calorimetrically using kits supplied by Sentinel Ch. Spa- Via Robert Koch, 2-20125 Milan- Italy. All determinations were performed according to the procedures outlined by the respective manufacturers.

Luteinizing hormone (LH), follicular stimulating hormone (FSH), triiodothyronine (T3), thyroid-stimulating hormone (T4), and adrenocorticotropic hormone (ACTH) were detected using commercially available ELISA kits (Jianglai Biological Technology Co. Ltd., Shanghai, China).

At the end of the experimental period, economic efficiency was performed as:

Net revenue = price of total egg/ hen (L.E.) – Total feed cost/ hen (L.E.)

Statistical analyses

The collected data of egg quality, rectal temperature, respiratory rate, and blood parameters were subjected to a two-way analysis of variance to detect the effects of treatment(T) and time of collecting blood samples (sample date, SD) and their interaction (T*SD), while data of egg production subjected to one-way analysis of variance to detect the effect of treatment (T) using the general linear model (GLM) procedure of SAS (SAS, 1999). The differences among treatments, time, and interaction means were separated according to Duncan's Multiple Range Test (Duncan, 1955). The significance level was set at 5%.

Results and Discussion

Egg production

Treatment with NAC significantly increased egg production percentage (Table 2), whereas birds in the G5 group recorded the highest value, followed by those in the G3 group, as compared with those in the G1 group (68.69 and 61.03 vs. 51.39%, respectively, P<0.001).The same trend was observed with egg mass, which was significantly increased with NAC treatment in water or injection, while feed intake did not significantly affect.Feed conversion was significantly affected by NAC treatment, whereas the G5 group recorded the best value, followed by the G3 group, as compared with the G1 group (3.012 and 3.568 vs. 4.254 g feed/ g egg, respectively, P<0.001).

Heat stress impairs overall poultry and egg production by modifying the bird's neuroendocrine profile both by decreased feed intake and by activation of the HPA axis. In general, birds react similarly to heat stress but express individual variation of intensity and duration of responses, which may also be affected by the intensity and duration of the heat stress event (Lara and Rostagno, 2013).Decreased feed intake is very likely the starting point of most detrimental effects of heat stress on production, leading to decreased bodyweight, feed efficiency, egg production, and quality (Deng *et al.,* 2012 and Mashaly*et al.,* 2004). However, in addition to decreased feed intake, it has been shown that heat stress leads to reduced dietary digestibility, and decreased plasma protein and calcium levels (Mahmoud *et al.,* 1997 and Zhou *et al.,* 1998).

Star *et al.* (2009) reported a reduction of 31.6% in feed conversion, 36.4% in egg production, and 3.41% in egg weight in laying hens subjected to heat stress. Moreover, He *et al.* (2019) reported that heat stress treatment for broiler significantly impaired growth performance (P< 0.01, P< 0.05), when compared to those of the control group over the entire experimental period. The added supplementation of NAC in the diet (1%) of broilers limits effectively adverse effects of HS by improving performance and redox status, which indicate that NAC is potent in counteracting chronic heat stress. Also, Afifi *et al.* (2010) observed that oral administration of NAC significantly decreased the feed conversion rate and increase body weight in broiler chickens. On contrary, Valdivia *et al.* (2001) found daily treatment with NAC (400 to 800 mg/kg BW for 7 d) did not alter BW, DWG, or FCI.

Egg quality

Data in Table (3) showed that NAC administration had a positive effect on egg weight. Birds in the G5 group were significantly increased by 15.1% as compared with the control group. No significant differences could be observed due to sampling time or interaction between treatment with NAC and sampling time concerning egg weight. High level of NAC (100 mg) in water or injection (G3 and G5) significantly increased egg width, albumin diameter, yolk weight, and shell weight, while only the G5 group significantly increased yolk diameter, albumin height, and shell thickness, whereas egg length did not significantly affect as compared with other treatment groups.

TABLE 2. Effect of N-acetylcysteine (NAC) level on egg production of Inshas laying hens.

Item	Egg production (%)	Average egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed/g egg)
NAC level:					
G1	51.39d	44.80c	23.03d	97.88	4.254a
G2	53.69c	44.88c	24.11cd	97.29	4.045ab
G3	61.03b	47.31a	28.87b	97.21	3.368c
G4	55.68c	45.49bc	25.32c	97.61	3.856b
G5	68.69a	47.06ab	32.33a	97.36	3.012d
SEM	0.727	0.351	0.416	0.364	0.048
P-value	0.0001	0.0184	0.0001	0.7871	0.0001

[a, b, c.]Means in the same row with different superscripts are significantly different at P<0.05, SEM = Standard error of the mean, G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW.

TABLE 3. Effect of N-acetylcysteine (NAC) level and time on egg quality of Inshas laying hens.

Item	Egg Weight (g)	Egg width (mm)	Egg length (mm)	Yolk diameter (mm)	Albumin diameter (mm)	Albumin height (g)	Yolk height (g)	Yolk weight (g)	Shell weight (g)	Shell thickness (µm)	Yolk color score	Shape index (%)	Yolk index (%)
NAC level:													
G1	44.32^{c}	39.58^{b}	52.50^{ab}	35.33^{b}	60.42^{b}	7.50^{b}	16.92^{b}	13.19^{c}	4.46^{c}	35.00^{bc}	6.42^{c}	75.45^{b}	48.24
G2	47.68^{b}	40.25^{ab}	51.58^{b}	36.83^{ab}	59.00^{b}	7.83^{b}	17.42^{b}	14.65^{ab}	4.83^{bc}	33.67^{c}	6.58^{c}	78.09^{a}	47.39
G3	47.61^{b}	40.50^{a}	53.50^{a}	35.83^{b}	73.58^{a}	7.50^{b}	16.75^{b}	15.25^{a}	5.73^{a}	36.58^{b}	8.00^{ab}	75.77^{ab}	46.84
G4	47.27^{b}	39.42^{b}	53.33^{a}	35.92^{b}	60.92^{b}	7.58^{b}	17.08^{b}	13.92^{bc}	5.04^{b}	36.67^{b}	7.75^{b}	74.02^{b}	47.66
G5	51.03^{a}	40.08^{ab}	52.75^{ab}	37.92^{a}	69.08^{a}	9.83^{a}	18.25^{a}	15.42^{a}	5.53^{a}	39.17^{a}	8.42^{a}	76.04^{ab}	48.20
SEM	0.701	0.289	0.515	0.575	1.681	0.289	0.250	0.267	0.133	0.607	0.213	0.832	1.104
Sampling time:													
S1	47.63	40.25	53.00	36.85	65.65	7.80	16.95^{b}	14.59	5.11	36.25	7.40	76.02^{ab}	46.07^{b}
S2	47.64	39.90	52.00	36.50	62.50	8.15	17.15^{b}	14.30	5.08	35.90	7.35	76.80^{a}	47.08^{b}
S3	47.48	39.75	53.20	35.75	65.65	8.20	17.75^{a}	14.57	5.17	36.50	7.55	74.80^{b}	49.85^{a}
SEM	0.736	0.271	0.445	0.442	1.836	0.302	0.246	0.321	0.156	0.580	0.256	0.690	0.704
P-value of:													
NAC level	0.0001	0.0614	0.1275	0.0487	0.0001	0.0001	0.0022	0.0001	0.0001	0.0001	0.0001	0.0211	0.8827
Time	0.9804	0.2858	0.1174	0.2799	0.1308	0.3168	0.0246	0.6617	0.8643	0.6192	0.6740	0.0912	0.0079
NAC level x time	0.6725	0.5241	0.7124	0.9987	0.2608	0.2897	0.8368	0.3249	0.8843	0.0536	0.2257	0.8759	0.9906

[a, b, c.]Means in the same row with different superscripts are significantly different at $P<0.05$, SEM = Standard error of the mean.

G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW, S1: egg quality at 30 days of starting experimental period, S2: egg quality at 60 days of starting experimental period, S3: egg quality at 90 days of starting experimental period.

No significant differences could be observed due to sampling time or interaction between treatment with NAC and sampling time concerning egg width, albumin diameter, yolk weight, shell weight, yolk diameter, albumin height, shell thickness, and egg length. Treatment with NAC injection in high dose (100 mg/kg BW) recorded significantly higher values of yolk height and yolk color score by 7.9 and 31.2%, respectively, as compared with the control group. Yolk height significantly increased with the progressive sampling time after treatment with NAC, while no significant effect could be observed due to interaction between treatment with NAC and sampling time. No significant differences could be observed due to sampling time or interaction between treatment with NAC and sampling time concerning yolk color score.

Only treatment with 50 mg NAC/ liter drinking water significantly increased shape index (%), as compared with other treatment groups, while yolk index (%) did not significantly affect NAC treatment. Sampling time after NAC treatment significantly influenced shape index and yolk index (%), whereas the highest values were recorded at 60 and 90 days of treatment, respectively. The interaction between treatment with NAC and sampling time cleared that; values of shape index and yolk index (%) did not differ.

Under high-temperature conditions, birds alter their behavior and physiological homeostasis seeking thermoregulation, thereby decreasing body temperature. In general, different types of birds react similarly to heat stress, expressing some individual variation in the intensity and duration of their responses (Lara and Rostagno, 2013). Animals utilize multiple ways for maintaining thermoregulation and homeostasis when subjected to high environmental temperatures, including increasing radiant, convective, and evaporative heat loss by vasodilatation and perspiration (Mustaf *et al.,* 2009). Birds have an

additional mechanism to promote heat exchange between their body and the environment, which are the air sacs. Air sacs are very useful during panting, as they promote air circulation on surfaces contributing to increase gas exchanges with the air, and consequently, the evaporative loss of heat (Fedde, 1998). However, it is worth noting that increased panting under heat stress conditions leads to increased carbon dioxide levels and higher blood pH (i.e., alkalosis), which in turn hampers blood bicarbonate availability for eggshell mineralization and induces increased organic acid availability, also decreasing free calcium levels in the blood. This process is very important in breeders and laying hens, as it affects the eggshell quality (Marder and Arad, 1989). However, although many studies have attempted to characterize the physiological mechanisms associated with the egg quality decrease in heat-stressed birds, there is no definitive knowledge, and several potential pathways are still under investigation, including changes in reproductive hormones levels and intestinal calcium uptake (Elnagar*et al.,* 2010 and Ebeid*et al.,* 2012). Heat stress can affect the reproductive function of poultry in different ways. In females, heat stress can disrupt the normal status of reproductive hormones at the hypothalamus, and at the ovary, leading to reduced systemic levels and functions (Donoghue *et al.,* 1989 and Elnagar*et al.,* 2010). In another study (Lin *et al.,* 2004), heat stress caused decreased production performance, as well as reduced eggshell thickness, and increased egg breakage. Additionally, heat stress has been shown to cause a significant reduction of egg weight (-3.24%), eggshell thickness (-1.2%), eggshell weight (-9.93%), and eggshell percent (-0.66%) (Ebeid*et al.,* 2012). Corroborating these reports, Mack *et al.* (2013) also observed decreased egg production, egg weight, and eggshell thickness in laying hens subjected to heat stress.

Rectal temperature and respiratory rate

The effect of N-acetylcysteine (NAC) level and time on rectal temperature (ºC) and respiratory rate (breaths/ minute) of Inshas laying hensare shown in Table 4. Treatment with a high dose of NAC by injection (100 mg/ kg BW) significantly reduced rectal temperature, as compared with the control group (41.67 vs. 42.30 ºC, respectively, P<0.05), while other treatment groups did not significantly affect rectal temperature. Treatment with NAC by injection in G4 and G5 groups decreased (P<0.001) respiratory rate than those of the control group by -14.3 and -21.0%, respectively, while treatment with NAC in drinking water in G2 and G3 groups decreased (P<0.001) respiratory rate by -10.4 and -16.6%, respectively.Sampling time after NAC treatment did not significantly influence rectal temperature and respiratory rate. The interaction between treatment with NAC and sampling time cleared that; values of rectal temperature and the respiratory rate did not differ.

The reduction in rectal temperature and respiratory rate means that laying hens treatment by NAC decrease or erase the effect of heat stress, which recorded by Rizk*et al.* (2017) who found that rectal temperature and respiratory rate were significantly (P<0.05 and 0.01) higher for Sinai hens reared under heat stress than for those reared in thermo-neutral at 24 and 34 weeks of age. The present results agree with those of He *et al.* (2019) who reported that heat stress treatment for broiler significantly and increased rectal temperature, respiratory rate (P< 0.01, P< 0.05) when compared to those of the control group over the entire experimental period. The added supplementation of NAC in the diet (1%) of broilers limits effectively adverse effects of HS by decreased RR and improved redox status, which indicate that NAC is potent in counteracting chronic heat stress.

Physiological parameters

Antioxidant status

Treatment with NAC by injection in G4 and G5 groups increased (P<0.001) TAC than those of the control group by 72.8 and 150.9%, respectively, while treatment with NAC in drinking water in G2 and G3 groups increased (P<0.001) TAC by 64.0 and 145.6%, respectively (Table 5). The same trend was observed with SOD, GSH, and CAT, which were significantly increased with increasing NAC levels in water or injection (P<0.001). It is important to mention that during summer conditions in Egypt birds suffer from heat stress, which reduces serum total antioxidant capacity, activities of superoxide dismutase and glutathione peroxidase, ability to inhibit hydroxyl radical and sulfhydryl group, whereas NAC supplementation significantly reversed the adverse effects by significantly increasing partial antioxidant values (He et al., 2019).

Cellular oxidative stress is often seen as a GSH deficiency that is characteristic of many pathological conditions. Though primarily seen as

TABLE 4. Effect of N-acetylcysteine (NAC) level and time on rectal temperature (ºC) and respiratory rate (breaths/ minute) of Inshas laying hens.

Item	Rectal temperature (ºC)	Respiratory rate (min^{-1})
NAC level:		
G1	42.30a	65.44a
G2	42.18a	58.67b
G3	41.96ab	54.56bc
G4	41.99ab	56.11b
G5	41.67b	51.67c
SEM	0.118	1.269
Sampling time:		
S1	42.11	57.53
S2	41.99	57.27
S3	41.95	57.07
SEM	0.103	1.608
P-value of:		
NAC level	0.0329	0.0001
Time	0.4907	0.9550
NAC level x time	0.9997	0.9370

[a, b, c,] Means in the same row with different superscripts are significantly different at P<0.05, SEM = Standard error of the mean, G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW, S1: blood samples were collected at 30 days of starting experimental period, S2: blood samples were collected at 60 days of starting experimental period, S3: blood samples were collected at 90 days of starting experimental period.

TABLE 5. Effect of N-acetylcysteine (NAC) level and time on blood antioxidant of Inshas laying hens.

Item	TAC (mmol/l)	SOD (u/l)	GSH (nmole/l)	CAT (u/l)	MDA (nmol/l)
NAC level:					
G1	0.114c	0.146c	0.130c	0.112c	0.409a
G2	0.187b	0.174b	0.174b	0.174b	0.332b
G3	0.280a	0.207a	0.224a	0.218a	0.282c
G4	0.197b	0.172b	0.181b	0.163b	0.344b
G5	0.286a	0.214a	0.237a	0.228a	0.282c
SEM	0.010	0.007	0.006	0.006	0.012
Sampling time:					
S1	0.217	0.183	0.191	0.170b	0.349a
S2	0.206	0.189	0.188	0.189a	0.323b
S3	0.215	0.176	0.189	0.179ab	0.317b
SEM	0.017	0.009	0.011	0.012	0.015
P-value of:					
NAC level	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.3872	0.1833	0.8640	0.0196	0.0224
NAC level x time	0.1110	0.1320	0.0692	0.3457	0.6339

[a, b, c,] Means in the same row with different superscripts are significantly different at P<0.05, SEM = Standard error of the mean, G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW, S1: blood samples were collected at 30 days of starting experimental period, S2: blood samples were collected at 60 days of starting experimental period, S3: blood samples were collected at 90 days of starting experimental period.

an antioxidant, NAC also has pro-oxidative effects. NAC can scavenge several ROS (including HOCl, $ONOO^{-}$, RO2) (Halliwell and Gutteridge, 2007) and hydroxyl radicals (OH) (Aruoma*et al.,* 1989). NAC can behave as an oxidant by undergoing auto-oxidation in high concentrations or the presence of transition metals. NAC can reduce transitional metals and promote the formation of ROS via Fenton-like chemistry or the production of the radical (Salamon*et al.,* 2019). Besides, the products of pro-oxidant reactions mediated by NAC are involved in altering the redox-sensitive NF-B signal transduction pathway activation; mitogen-activated protein kinase p38 (p38MAPK) and c-Jun N-terminal kinase (JNK) phosphorylation (Das *et al.,* 1995 and Chan *et al.,* 2001).

This increase could be attributed to the ability of NAC in increasing glutathione (GSH) synthesis (Kamboj *et al*., 2010). The beneficial effect of NAC supplementation might involve GSH depletion, free radical scavenger action (van Klaveren*et al.* 1997). Altered expression of antioxidant enzymes has been reported in diabetic conditions (Ali and Agha, 2009; Yuan *et al.,* 2009). SOD and CAT are major antioxidant enzymes involved in protection from oxidative stress. SOD offers protection from highly reactive superoxide anions and converts them to H2O2 (Halliwell 1991). Supplementation of NAC to the diabetic animals leads to high SOD activity, which is in the same line with the observed restoration of SOD activity by NAC in the brain (Kamboj et al. 2008), kidney (Beltowski*et al.,* 2008), oocytes (Whitaker and Knight, 2008) and lungs (Teixeira *et al.,* 2008). CAT is responsible for the catalytic decomposition of H2O2 to O2 and H2O. Moreover, NAC ischemic injury enhanced the CAT activity (Demir and Inal-Erden, 1998). The results obtained confirm that NAC protects the sciatic nerve from hyperglycemia-induced damage by restoring the activity of both these enzymes. Glutathione reductase is an important enzyme involved in maintaining high GSH/GSSG ratios (Carlberg and Mannervik, 1985). NAC is a thiol compound that is converted to cysteine, an important precursor of cellular glutathione (Zachwieja*et al.,* 2005). The antioxidant effect of NAC works in two ways. The first way, as a source of sulfhydryl groups that expedites GSH biosynthesis indirectly and, hence, improves GSH supply for glutathione peroxidase. The second way, it reacts with ROS direct (Ocal*et al.,* 2004).

Youssef *et al.* (2009) concluded that vitamin E and NAC addition had significantly reduced free radicals level resulting from oxidation as evidenced by improving GSH-Px level and reducing MDA level. Lowered levels of TBARS and hydroperoxides by oral administration of NAC could be related to its antioxidant capacity to scavenge reactive oxygen species where NAC contains free sulfhydryl groups and it may directly react with electrophilic compounds such as free radicals (Narasimhanaidu*et al.,* 2005).

It was observed that plasma TAC, SOD, and GSH did not significantly affect by sampling time. The interaction between treatment with NAC and sampling time concerning plasma TAC, SOD, and GSH were insignificant. Plasma CAT was significantly influenced by sampling time, whereas the sample at 60 days recorded the highest value, while the sample at 30 days recorded the lowest value. No significant differences could be observed between treatment with NAC and sampling time concerning plasma CAT.

A significant ($P<0.001$) reduction in concentrations of MDA (a key product of lipid peroxidation) by 15.9 and 31.1% for birds in group G4 and G5, respectively, and by 18.8 and 31.1% for birds received G2 and G3 as compared to control group (G1), respectively. Plasma MDA was significantly influenced by sampling time, whereas the sample at 30 days recorded the highest value, while the sample at 90 days recorded the lowest value. No significant differences could be observed between treatment with NAC and sampling time concerning plasma MDA. It is important to notice that heat stress increases serum malondialdehyde content, whereas NAC is potent in counteracting chronic heat stress (He *et al.*, 2019).

It is important to clarify that, few researches have been conducted to determine the activity of GST in laying hens, most of them (Rao *et al*., 2013 and Fedets, 2015) were concerning with measuring its activity in tissues not in blood plasma or during pregnancy period. This is consistent with Fedets (2015) who found little information on GSH- dependent enzymes activity in food-producing animals and stated that most of the data are about rats, mice and humans. The increase in GSH activity was accompanied bya decline in both MDA and H2O2 levels. This relationship, between the three parameters (GSH,

MDA, and H2O2) could be attributed to; NAC is working as an intracellular GSH precursor by increasing enzymes involved in GSH synthesis. Then, the production of GSH increased which in turn increases the activity of GST to support and accelerate free radicals removal then protecting the cell against apoptosis (Eraslan*et al*., 2005).

The present findings agree with Cam *et al*. (2008) who confirmed the safe use of NAC. They used higher doses of NAC reached 500 mg/kg to treat rabbits from aflatoxins and they did not record any abnormal values in blood parameters. Also, Youssef *et al.* (2009) concluded that vitamin E and NAC supplementation hadsignificantly decreased the level of free radicals resulting from oxidation as evidenced byincreasing the level of GSH-Px and lowering the level of MDA.Moreover, Atef *et al*. (2016) treated rabbits with NAC to eliminate the toxic effect of aflatoxins and recorded that, NAC has the ability in reducing MDA and elevating GSH levels.

Relevant blood metabolites

The treatment with high doses of NAC significantly increased TP, A, G, and A/G ratio,as shown in Table (6). It is well known that blood proteins are good indicators of animal health, particularly albumin level which is considered as a reflection of the animal's ability to synthesize and store protein and working as an index of nutrition status (Ashour etal., 2004 and Meineriet al., 2016).The present results disagree with those ofAshour et al. (2018) who found that total protein, albumin, globulin, and A/G ratio did not significantly affect by NAC injection (50 and 100 mg NAC / kg BW) for rabbit does. Also, the study disagrees withAtakisiet al. (2016) they treated New Zealand rabbits with NAC and collected blood samples after 3,6, and 9 hours of NAC injection and found that TP concentration declined from 6.00 before injection to 5.5 g/dl after 9 hours of NAC injection.

Sampling time (30, 60, and 90 days) did not significantly affect TP, A, G concentrations, and A/G ratio. The interaction between treatment with NAC and sampling time cleared that; values of blood proteins did not differ.

TABLE 6. Effect of N-acetylcysteine (NAC) level and time on some blood parameters of Inshas laying hens.

Item	TP (g/dl)	A (g/dl)	G (g/dl)	A/G ratio	TL (mg/dl)	TG (mg/dl)	CHO (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (U/ml)	ALT (U/ml)	Creatinine (mg/dl)	Urea (mg/dl)
NAC level:													
G1	7.17^{c}	2.42^{b}	4.75^{b}	0.51^{b}	463.6^{a}	224.4^{a}	115.2^{a}	39.7^{a}	35.6^{c}	31.4^{a}	21.0^{a}	0.86^{a}	13.0^{a}
G2	7.36^{b}	2.45^{b}	4.91^{a}	0.50^{b}	422.6^{bc}	200.7^{b}	100.3^{b}	32.3^{b}	41.7^{b}	27.7^{b}	17.6^{b}	0.78^{b}	10.3^{bc}
G3	7.49^{a}	2.58^{a}	4.91^{a}	0.53^{a}	409.9^{bc}	194.8^{c}	93.1^{c}	27.1^{c}	46.0^{a}	24.3^{c}	14.0^{c}	0.73^{bc}	9.3^{c}
G4	7.31^{b}	2.45^{b}	4.86^{a}	0.51^{b}	424.4^{b}	201.9^{b}	100.7^{b}	32.1^{b}	41.6^{b}	27.8^{b}	17.4^{b}	0.77^{b}	10.9^{b}
G5	7.50^{a}	2.60^{a}	4.90^{a}	0.53^{a}	407.9^{c}	191.3^{c}	90.8^{c}	25.3^{c}	47.6^{a}	23.4^{c}	13.7^{c}	0.70^{c}	9.1^{c}
SEM	0.018	0.014	0.022	0.005	1.692	1.176	0.866	0.764	0.689	0.741	0.669	0.013	0.423
Sampling time:													
S1	7.37	2.49	4.88	0.51	425.0	204.1	100.1	31.7	41.9	27.6	17.3^{a}	0.79	10.9
S2	7.35	2.51	4.85	0.52	431.3	202.3	98.9	31.6	42.5	26.9	17.1^{ab}	0.75	10.6
S3	7.38	2.51	4.88	0.51	420.7	201.4	101.0	30.7	43.1	26.3	15.9^{b}	0.76	10.1
SEM	0.037	0.024	0.024	0.005	4.635	3.192	2.279	1.406	1.340	0.872	0.839	0.017	0.496
P-value of:													
NAC level	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.3069	0.5238	0.3727	0.4732	0.1592	0.1453	0.1006	0.4510	0.3587	0.2618	0.0527	0.1413	0.2701
NAC level x time	0.1139	0.7101	0.3508	0.6871	0.4027	0.3963	0.2258	0.6124	0.1069	0.2507	0.1181	0.6353	0.1444

$^{a, b, c,}$ Means in the same row with different superscripts are significantly different at P<0.05, SEM = Standard error of the mean, G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW, S1: blood samples were collected at 30 days of starting experimental period, S2: blood samples were collected at 60 days of starting experimental period, S3: blood samples were collected at 90 days of starting experimental period.

Circulating TL and TG showed an opposite trend to that of blood proteins, it was affected significantly by NAC injection or water supplementation. Levels of TL and TG decreased ($P<0.001$) intreated groups than that of the control group by 10.2 and 12.1% (as average), respectively (Table, 6). The difference between the treated groups was significant, whereas100 mg NAC (G3 and G5) had a lower level of TL and TG than that of 50 mg NAC (G2 and G4). TG is the lipids storage in blood plasma, so the body can use it as fuel under physiological adjustment (Wahab *et al*., 2016) and express exactly about the overall metabolism of nutrients. No significant effect could be observed due to the time of sampling. Also, the effect of interaction between NAC injection and all the studied days was insignificant (Table, 2). This could be explained according to the results of Brizzi *et al*. (2003) and Diniz*et al*. (2006). They illustrated that the antioxidant features of NAC may give it the putative mechanism in reducing blood lipids and the ability to enhance cellular lipids uptake from the blood. In addition, Korou*et al*. (2010)reported that NAC has a great ability in decreasing high levels of saturated fat-induced triacylglycerol and cholesterol accumulation in mice liver through restoring the distributed lipid profile. Therefore, all the previous studies assured our findings in the ability of NAC in reducing blood TG to be in normal values.

Plasma content of cholesterol (CHO) and LDL significantly decreased, while plasma content of HDL significantly increased with increasing level of NAC in water or injection, as compared with the control group.It was observed that the plasma content of CHO, HDL, and LDL insignificantly changed during different sampling times. Also, the interaction between treatment with NAC and sampling time was insignificant. The present results agree with those of Ashour *et al*. (2018) who found that level of triglycerides decreased ($P<0.05$) in rabbits does inject subcutaneously with 50 and 100 mg NAC / kg BW, as compared with those in the control group.

Kidney function

Owing to the treatment effect, NAC significantly decreased plasma urea in treated groups (in water and injection) than that in the control group by 24.6 and 23.1 % (as average),respectively (Table 6). This may be due to the improvement of renal function in eliminating nitrogen from blood to be excreted in the urine in treated groups. The concentration of plasma urea in laying hensis influenced by many factors such as; dietary protein level, feed quality, and feed restriction. This indicates that NAC treatment particularly with a high dose (100 mg) may have the ability to reduce the elevated level of plasma urea. This finding agrees with that of Cam et al. (2008) who reported that NAC protects against negative effects on performance, renal and liver damage, and biochemical alterations induced by diseases.

The same trend was obtained for plasma creatinine concentration. It was decreased significantly in both NAC treatment methods (in water and injection) than that of the control group by 12.2 and 14.5 % (as average), respectively (Table 6). This means that NAC treatment had no negative effect on kidney function. Creatinine is the nitrogen waste product of creatine thatis presented in muscles. Any changes in its concentration are pointing to renal functions, for example, renal diseases resulting in an abnormal elevation in creatinine level. When the improvement of renal function takes place, creatinine level returns to its normal value (Verga, 2002).

It was observed that kidney function (urea and creatinine) insignificantly changed during different sampling times. Also, the interaction between treatment with NAC and sampling time was insignificant.

Liver function

Liver function enzymes (ALT and AST) significantly decreased with increasing NAC levels in water or injection (Table 6). Activities of transaminase enzymes in the three groups ranged between 23.4 and 31.4 U/L for AST and between 13.7 and 21.0 U/L for ALT. The present values of these enzymes are within the normal physiological ranges, which ranged from 10 to 98 U/L for AST and from 10 to 55 U/L for ALT as reported by Verga (2002). These findings indicate that NAC treatment had no negative effect on liver function and assured the good health status of laying hens, where an elevation of these enzymes is considered a sign of liver disease (Benson and Paul-Murphy, 1999 and Jenkins, 2000).

It was observed that AST insignificantly changed, while ALT increased significantly with the progressive of sampling time. The interaction between treatment with NAC and sampling time concerning liver function (AST and ALT) was insignificant.

This, again, assure the safe application of NAC treatment in reducing enzymes activity without harmful and complicated effects on liver function. These results were confirmed by Wang *et al*. (2015) who reported that NAC could inhibit and reduce the expression of inflammatory cytokines that caused the difference in serum AST and ALT which is considered to be one metric to assess the severity of the liver injury.

Blood hormones

Adrenocorticotropic hormone (ACTH) significantly decreased with increasing NAC levels in water or injection, while ACTH did not significantly affect by sampling time or interaction between NAC treatment and sampling time (Table 7). Treatment with NAC significantly increased T3 hormone by 37.9% (as average), while T4 hormone significantly decreased by 27.1% (as average), as compared with the control group. Hormones of T3 and T4 were significantly affected by sampling time after NAC treatment, whereas T3 and T4 recorded the lowest values at 30 and 90 days of treatment (S1 and S3), respectively. No significant differences could be observed due to the interaction between treatment with NAC and sampling time concerning plasma T3 and T4 hormones.

Treatment with NAC significantly increased T3/T4 ratio, whereas treatment with high NAC dose (100 mg) in water and injection (G3 and G5) recorded the highest values, as compared with the control group (17.01 and 17.50 vs. 7.69, respectively, $P<0.001$). The ratio of T3/T4 significantly increased with the progressive sampling time after treatment with NAC, while no significant effect could be observed due to interaction between treatment with NAC and sampling time.

TABLE 7. Effect of N-acetylcysteine (NAC) level and time on blood hormones of Inshas laying hens.

Item	ACTH (ug/ L)	T3 (ng/mL)	T4 (ng/mL)	T3/T4 ratio	FSH (ug/ L)	LH (ug/ L)
NAC level:						
G1	21.86^{a}	1.90^{e}	24.88^{a}	7.69^{d}	3.44^{d}	13.35^{c}
G2	18.84^{b}	2.21^{d}	19.77^{b}	11.17^{c}	3.61^{c}	13.92^{c}
G3	14.99^{c}	2.77^{b}	16.30^{d}	17.01^{a}	3.83^{b}	15.87^{b}
G4	17.19^{b}	2.44^{c}	18.74^{bc}	13.10^{b}	3.77^{b}	15.13^{b}
G5	14.21^{c}	3.06^{a}	17.73^{c}	17.50^{a}	4.11^{a}	17.05^{a}
SEM	0.572	0.048	0.607	0.446	0.039	0.271
Sampling time:						
S1	17.69	2.38^{b}	20.24^{a}	12.26^{b}	3.69^{b}	14. 96
S2	17.37	2.52^{a}	19.58^{ab}	13.46^{a}	3.73^{b}	15.07
S3	17.19	2.53^{a}	18.63^{b}	14.17^{a}	3.84^{a}	15.17
SEM	0.827	0.114	0.877	1.048	0.062	0.402
P-value of:						
NAC level	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.7413	0.0209	0.0121	0.0007	0.0006	0.8079
NAC level x time	0.9930	0.7958	0.7866	0.7604	0.8666	0.9667

$^{a, b, c,}$ Means in the same row with different superscripts are significantly different at $P<0.05$, SEM = Standard error of the mean, G1: control group without any administration, G2: birds drink water supplemented with 50 mg N-acetylcysteine (NAC) / litter, G3: birds drink water supplemented with 100 mg N-acetylcysteine (NAC) / litter, G4: birds injected with 50 N-acetylcysteine (NAC) / kg BW, G5: birds injected with 100 mg N-acetylcysteine (NAC) / kg BW, S1: blood samples were collected at 30 days of starting experimental period, S2: blood samples were collected at 60 days of starting experimental period, S3: blood samples were collected at 90 days of starting experimental period.

High environmental temperatures alter the activity of the neuroendocrine system of poultry, resulting in activation of the hypothalamic-pituitary-adrenal (HPA) axis, and elevated plasma corticosterone concentrations (Garriga*et al.,* 2006 and Quinteiro-Filho *et al.,* 2012). Body temperature and metabolic activity are regulated by the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), and their balance. Previous studies report that T3 concentrations consistently decrease in high-temperature conditions (Mack *et al.,* 2013, Elnagar*et al.,* 2010 and Star *et al.,* 2008), whereas results of heat-mediated alterations on T4 concentrations are inconsistent with studies reporting a decrease (Bobek*et al.,* 1980) or increase (Elnagar*et al.,* 2010). Due to the involvement of the thyroid during the onset of puberty and reproductive function in birds, disruption of thyroid activity by heat stress would be expected to affect the reproductive performance of the hens (Elnagar*et al.,* 2010). Moreover, findings reported by Geraert*et al.*(1996) indicate that endocrinological changes caused by chronic heat stress in broilers stimulate lipid accumulation through increased de novo lipogenesis, reduced lipolysis, and enhanced amino acid catabolism. Moreover, Abbas *et al.* (2017) found that heat stress significantly increased serum cortisol levels, whereas serum concentrations of triiodothyronine (T3) and thyroxine (T4), which are important growth-promoter hormones, are adversely affected.

Hormones of FSH and LH significantly increased with NAC treatment, whereas G5 recorded the highest values of both hormones, as compared with the control group (4.11 and 17.05 vs. 3.44 and 13.35 ug/ L, respectively, P<0.001). FSH hormone significantly increased with the progressive sampling time after treatment with NAC, while no significant effect could be observed due to interaction between treatment with NAC and sampling time.No significant differences could be observed due to sampling time or interaction between treatment with NAC and sampling time concerning plasma LH hormone.

The treatment with NAC increased LH hormone that reflects the possible effect of NAC in reducing the bad effect of heat stress on laying hens, which results in a significant increase in egg production by NAC treatment. Whereas, heat stress was found to reduce LH levels and hypothalamic gonadotropin-releasing hormone-I content (Donoghue *et al.,* 1989), and in addition, a reduction of preovulatory surges of LH and progesterone was observed (Novero*et al.,* 1991). A possible mechanism for the reduction of ovarian function might be the reduction in blood flow to the ovary; differential ovarian blood flow pattern was found in hens exposed to high ambient temperature (Wolfenson*et al.,* 1981).

Economic efficiency

Data of the egg production was subjected to economic study (Table 8). The results showed that the total cost of the NAC treatment group (as average) increased by 16.8%, as compared with the control group, which was mainly due to the price of NAC. However, the price of total egg/ henwasincreased by 16.9% (as average)with NAC treatment, as compared with the control group. Also, the net revenue increased by 4.9 and 113.1% for birds that received 100 mg NAC/ liter drinking water and those injected 100 mg/ kg BW, respectively, as compared to the control group. The results showed that the birds injected 100 mg/ kg BW had the highest value of the economic efficiency percentage (213.1%), followed by those who received 100 mg NAC/ liter drinking water (104.9%), while those received 50 mg NAC/ liter drinking water recorded the lowest value (57.1%), as compared to control group (100%).

TABLE 8. Effect of N-acetylcysteine (NAC) level on economic efficiency

Item	Control	N-acetylcysteine level			
		In water		Injection	
		50	100	50	100
Feed intake/ hen (kg)	8.809	8.756	8.749	8.785	8.762
Price/ kg feed (L.E.)	5.50	5.50	5.50	5.50	5.50
Cost of feed intake (L.E.)	44.45	48.16	48.12	48.32	48.19
Price of N-acetylcysteine (L.E.)	-	3.30	6.60	1.65	3.30
Total cost (L.E.)	44.45	51.46	54.72	49.97	51.49
Total egg number/ hen	46	48	55	50	62
Price of total egg/ hen (L.E.)[1]	55.20	57.60	66.00	60.00	74.40
Net revenue[2]	10.75	6.14	11.28	10.03	22.91
Economic efficiency	100	57.1	104.9	93.3	213.1

[1] Price of one egg (L.E.) = 1.20

[2] Net revenue = price of total egg/ hen (L.E.) – Total feed cost/ hen (L.E.)

Conclusions

It could be concluded that N-acetylcysteine (NAC)treatment having the largest impact onInshas laying hens performance under heat stress, particularly with high dose (100 mg) injection or in water. From the economic point of view, 100 mg NAC injection/ kg BW improved feed conversion, increased egg production, and egg mass.

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تأثير المعاملة بالاستيل سستين على الأداء الانتاجى والتناسلى والفسيولوجى للدجاج المحلى تحت ظروف الصيف المصرية

فؤاد أحمد توفيق ، ابراهيم حمدان سالم
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تم اجراء هذه الدراسة لتقييم اذا ما كان لمركب الاستيل سيستين (N-acetylcysteine, NAC)دور مفيد فى تحسين الاداء الانتاجى والتناسلى لسلالة دجاج انشاص البياض تحت ظروف الصيف المصرية، حيث تم استخدام عدد ٣٠٠ دجاجة و٣٠ ديك أنشاص عمر ٣٤أسبوع قسمت بشكل عشوائى الى ٥ مجموعات وكل مجموعة تحتوى ٦٠ دجاجة و ٦ ديك ثم احتوت كل مجموعة على ٦ مكررات (١٠ دجاجة و ١ ديك/ مكرره)، المجموعةالأولي(G1) هيالمجموعةالكنترول)لم تتقبل أى معاملة وحقنت فقط بالماء)،أما المجموعتين الثانية والثالثة (G2 and G3) اضيف الى مياه الشرب بمعدل ٥٠ ، ١٠٠ مليجرام مركب الاستيل سيستين لكل لتر، على التوالى، بينماالمجموعتينالرابعةوالخامسة (G4 and G5) تم حقنهاتحتالجلدبمركبالأستيلسستينبمعدل٥٠ و١٠٠مليجرام/كيلوجراموزنحي،عليالتوالي. كانت بدايةالمعاملة بالمركب أول ثلاثة أيام من كل شهر لمدة ثلاثة أشهر (مدة التجربة).أوضحتالنتائج أن المعاملة بمركب الاستيل سيستين أدت الى زيادة معنوية فى معدل انتاج البيض حيث سجلت الطيور المحقونة ١٠٠ مليجرام من مركب الاستيل سيستين /كيلوجرام وزن حى أعلى قيمة مقارنة بطيور مجموعة الكنترول (٦٨,٦٩٪ مقابل ٥١,٣٩٪ ، على التوالى). أدت الجرعات المرتفعة من مركب الاستيل سيستين الى زيادة معنوية فى محتوى بلازما الدم من البروتين الكلى ، الالبيومين ، الجلوبيولين ، نسبة الالبيومين الى الجلوبيولين.

نستخلص من الدراسة أن المعاملة بمركب الاستيل سيستين (N-acetylcysteine) له تأثير كبير على أداء دجاج أنشاص البياض تحت ظروف الاجهاد الحرارى، خاصة مع الجرعة المرتفعة (١٠٠ مليجرام) بالحقن أو فى المياه. منوجهة النظر الاقتصادية فان الحقن بمركب الاستيل سيستين بمعدل ١٠٠ مليجرام/ كيلوجرام وزن جسم حى ادى الى تحسن فى الكفاءة الغذائية وزيادة فى معدل انتاج البيض وكتلة البيض.