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# USING THREONINE, CANTHAXANTHINAND SODIUM SULPHATE AS DIETARY SUPPLEMENTATION FOR IMPROVING EGG PRODUCTION IN LAYING HENS IN POST PEAK PERIOD

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**ABSTRACT:** This study examines the hypothesis that threonine(THR),canthaxanthin (CAN)and sodium sulphate (SS)supplementation can improve the performance of commercial Lohmann Brown (LB) laying hens in late egg production period (44 – 56 wks).A total number of 120hens at 44 weeks old were separated into eight groups, each with five replicates (3 hens) and kept in wire cages. The experimental hens were fed a control diet without or with 2 g THR /kg, 3 ppm CAN, 2 g THR/ kg + 3ppm CAN, 5 g SS/kg, 2 g THR/ kg +5 g SS/kg, 3 ppm CAN+5 g SS/kg and 2 g THR/ kg+ 3ppm CAN+5 g SS/kg from 44 to 56 weeks of age. All feed additives used in this study numerically increased egg number (egg/hen/day) compared to hen fed the control diet. The addition of THR+CAN+SS significantly increased egg mass /day by 8.10% compared to the control diet. All feed additives used in this study increased egg shell thickness, serum total protein and antioxidants capacity compared to the control diet. The mixture of the three feed additives increased shell thickness by 15.15% compared to the control diet. The mixture of THR+CAN+SS was the most successful additive in this study.

**Key words:** Laying hens, threonine, canthaxanthin and post peak period.

## INTRODUCTION

After hens reach the age of 480 days, egg production begins to decline rapidly (Joyner et al., 1987). The drop in egg production in older hens was mostly due to ovarian ageing, which was accompanied by endocrine alterations. (Buyuk et al., 2010).Due to aging, breeder hens have declined reproductive performance in the late laying period, often manifesting as significantly decreased egg-laying rate and poor eggshell quality (Sirri et al., 2018). The amino acid L-threonine (THR) is the third-limiting amino acid. THR supplementation has been shown to improve egg production in laying hens and ducks in previous research (Azzam et al., 2014, 2017;Fouad et al., 2017). L-THR appears to play an active role in antioxidant defence mechanisms, and it is one of the amino acids that carries a small amount of copper in the blood. (Shils et al. 2006).Li et al. 2016 found with laying hens that serum concentrations of total superoxide dismutase ,total antioxidant capacity and malondialdehyderesponse to supplemental L-THR were quadratic. Canthaxanthin(CAN) is a carotenoid that has a highantioxidant activity, alleviating lipid peroxidation inseveral tissues, including embryos, whose developmentis associated with a high oxidative activity (Surai et al., 2003).Canthaxanthin is able to recycle vitamin E by donating an electron to the $\alpha$-tocopherol radical (Surai et al., 2003).On the other hand, study reported that exogenous antioxidants are also a double-edged sword, highlighting that antioxidants at physiological levels are generally safe, while higher levels are detrimental in cellular redox state (Bouayed and Bohn 2010).

Unfortunately, the maximum level of synthetic antioxidants that can be used in animal feeds is controlled in actuality due to the potential for adverse consequences. In animal feeds, for example, the US Food and Drug Administration set a maximum inclusion level of 150 ppm for ethoxyquin and 200 ppm for both butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA). Many other countries have likewise implemented similar government regulations. (Salami et al. 2015).To achieve the best poultry performance, we must use dietary antioxidants with higher maximum level in diets and lower toxicity of its oxidized products(Ali, 2016). Correia-Da-Silva et al., (2014) showed that sulfated small molecules could be of value in therapeutics due to their hydrophobic nature that can contribute to improve the bioavailability. Ress*et al*. (2008) indicated that sulfation confers resistance to oxidation. Sulfation occurs as a common enzymatic modification of endogenous substances including proteins, carbohydrates, catecholamines, and estrogenic steroids as well as xenobiotic chemicals (Strott, 2002).

Ali et al (2012) indicated that SS increased the activity of hydrophobic antioxidants and/ or protect it from free radicals attack during circulation in the blood. Ali et al (2018) found that L tyrosine 0.5 g/kg diet alone or with sodium sulphate increased egg production in local laying hen from 39 to 58 weeks old and indicated that L tyrosine may help birds to elimination of free radicals and sulphate increase its activity. Therefore, this study testifies the hypothesis that THR,CAN, or SS alone or in combination can improve the

commercial laying hen productive performance in post-peak period.

## MATERIALS AND METHODS

**Experimental treatments:**

This study was carried out at Poultry Experimental Station belonging to Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, during the summer season from the first of June to the beginning of September 2018. Total number of 120 aged Lohmann Brown (LB) layers were distributed randomly into 8 treatments groups, where each group contained 15 hens and each group divided into five replicates with 3 hens per each. To achieve the experimental purpose one inorganic compound (sodium sulphate) and two organic compounds (threonine and canthaxanthin) were supplemented to experimental basal diet. Anhydrous Sodium Sulphate was supplied by the Egyptian Salt and Mineral Company. Commercial canthaxanthin 10% was provided by BASF Germany.

A corn-soybean mash meal as a basal experimental layer diet was formulated (Table, 1) to satisfy nutrient requirements (iso-nitrogenous and iso-caloric) of Lohmann Brown (LB) laying hens (18% CP; 2800 kcal ME/kg diet). The 2nd group fed basal diet supplemented with 2 g threonine amino acid/ kg diet and the 3rd group fed basal diet supplemented with 30 mg commercial canthaxanthin(3mg)/ kg diet. Group 4 fed control diet supplemented with mixture of 2 g threonine amino acid and 30 mg canthaxanthin(3mg)/ kg diet together. Group 5 fed control diet supplemented with 5 g anhydrous sodium sulphate/ kg diet. Group 6 fed control diet supplemented with 2 g threonine amino acid and 5 g anhydrous sodium sulphate/ kg diet. Group 7 fed control diet supplemented with 30 mg canthaxanthin(3mg) and 5 g anhydrous sodium sulphate/ kg diet. Group 8 fed control diet supplemented with 2 g threonine amino acid, 30 mg canthaxanthin(3mg) and 5 g anhydrous sodium sulphate/ kg diet.

**Management and performance parameters:**

Day light was completed using additional morning artificial light before sunrise to reach 16 hours continuous light. Clean water was available continuously. Temperature and relative humidity inside the layer house recorded daily at 12 pm. Selected hens kept for 12 wks during period extended from 44 to 56 wks of age and fed previous diets under the same conditions. Feed provided at the beginning of each week where the remaining diets weighed at week end and feed intake was calculated.

Daily produced eggs were counted and weighed separately for each replicate to obtain egg mass. Egg production percent (EP percent/hen/day) calculated by dividing egg number on number of alive hens. Egg mass per hen per day (EM/H) was calculated by dividing total egg mass of each replicate on number of alive hens. Feed conversion ratio was calculated by dividing feed intake on egg mass (gm feed per gm egg mass).

Eggs were examined for exterior and interior quality. Egg components were monthly determined using 8 fresh eggs from each replicate. Eggs were weighed, then egg length and width were determined before breaking. The egg was carefully broken on a glass plate (35×25 cm) to measure both external and internal egg quality characteristics. Yolk was separated from albumen, and eggshell was cleaned from any adhering albumen. Albumen weight was calculated by

subtracting yolk weight and shell weight from the whole egg weight. Egg shape indices were calculated as the ratio of egg width to the length (Awosanya*et al.,* 1998). Yolk index was computed according to Funk *et al.,* (1958), as average yolk height divided by yolk diameter (mm) following removal of the yolk from the albumen. Yolk height was measured by means of tripod micrometer reading to the nearest 0.01 mm, while yolk diameter was measured by vernier caliper to the nearest 0.05 mm. The eggs were examined for shell quality via shell thickness of the eggs using micrometer. Shell thickness was a mean value of measurements at three regions on the eggs (air cell, equator, and sharp end).

**Hepatic cellular and shell gland oxygen consumption:**

Samples of liver and epithelium lining of shell gland were obtained to measure oxygen consumption. Oxygen consumption was measured using constant volume manometry technique by Warburg apparatus. Birds were slaughtered. Apex of right liver lobe was sampled and the epithelium lining of shell gland were scraped by scalpel. All tissue samples were in contact with ice until analysis(Al-Gamal, 2009). The analysis was done within 30 minutes of tissue sampling. A total volume of 2.5 ml of Hanks media (Wasley, 1972) and tissue sample was placed in the flask of Warburg apparatus and a strap of filter paper saturated with 30% KOH was put in the well of the flask. The reading of the manometer was recorded after one hour of incubation on 30°C to determine the O2 consumed by the tested tissue, according to Umbreit*et al.,* (1972). For the sake of standardizing physiological status of oviduct, all data of birds with hard shells ova were considered otherwise data were discarded. All measurements were calculated on the dry sample basis.

**Blood Sampling:**

At the end of the experimental period, blood samples withdrawn from 5 birds of each group and were taken randomly to blood analysis. Birds were fasted overnight before bleeding via jugular vein and blood was collected in unheparinzed tubes to determine the blood profiles and serum was separated and stored frozen at –20 $^{O}$C until analyzed.

**Blood Biochemical Parameters:**

Blood Packed Cell Volume (PCV) was determined by centrifuging the capillary tubes and Blood hemoglobin (Hb) was determined by Cyanomethemoglobin method (Beutler, 1984). Serum total protein was determined according to Weichselbaum (1946). Albumin was measured according to Doumas, (1971). The globulin values were obtained by subtracting the values of albumin from the corresponding values of total proteins. Serum glucose was determined enzymaticly by commercial kit purchased from Bio-Merieux (MotcylEtiosCharbonMierels Rains/ France). Total lipids and total cholesterol, were colorimetrically determined in serum according to Zollner and Kirsch (1962). Serum Triiodothyronine (T3) and Thyroxine (T4) concentrations were analyzed by Radioimmunoassay (RIA) method using RIA kits (Amersham International Ltd., Amersham, United Kingdom). Serum total antioxidant capacity (TAC) was determined by using a kit (Antioxidant Capacity Assay Kit, Randox Chemical Co. Ann Arbor, MI, USA). Serum immunoglobulin G (IgG) concentration was measured using single radial immuno diffusion technique, as

described by Fahey and Mckelvey, (1965).

**Statistical analysis:**

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package (SPSS, 2001, version 11.0). All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significant. Data were analyzed by one way method using the following model.

Yij = u + Ni + eij Where

Yij= the observed value,

u = population means,

Ni = the effect of treatment,

eij= the standard error.

## RESULTS AND DISSCUSION

**Performance:**

The effects of dietary treatments on final body weight, feed intake and feed conversion ratio of laying hens during the experimental from (44 to 56) weeks of age are shown in Table (2). There were significant differences between BW of hens fed different dietary treatments. The hens fed the control diet recorded the lowest values while those fed the diet supplemented with CAN +SS recorded the highest value. These results disagree with those obtained by Ali et al.,(2018)who found with local hens that dietary feed additives affect significantly body weight and hen fed CAN+SS recorded the lowest value. However, the differences between this study and the work done by Ali et al.,(2018) may be due to differences between local strain and commercial laying hens used in this study. In this respect, McDaniel *et al*., (1981) reported that excessive body weight in broiler breeders hens reduced egg production.

Also, significant differences were detected between values of daily feed intake recorded by different dietary treatments. The hens fed CAN+ SS recorded the highest value while those fed THR recorded the lowest values. Moreover, the results indicated that there were no significant differences between values of feed conversion ratio(g feed / g egg) recorded by hens fed different treatments. These results disagree with those reported by Ali et al.,(2018) who found with local hens that feed additives (as antioxidants) improved significantly feed conversion (g feed / g egg ). The effect of dietary treatments on egg number and egg mass of laying hens during the experimental period at (44 – 56) weeks of age is shown in Table (3).All feed additives used in this study numerically increased egg number (egg/hen/day) compared to hens fed the control diet. These results agree with study has been done by Jiang et al.,(2020) who found that diet supplemented with 0.25 g/kg Stevioside powder (as antioxidants ) improved the daily egg production. Also, Ali et al.,(2012)found that antioxidants like commercial canthaxanthin(CAN)with or without sodium sulphate increased egg production. There were significant differences between values of egg mass/ day recorded by hens fed different treatments. The hen fed the control diet recorded the lowest value while those fed CAN+SS+THR recorded the highest value. Compared to control diet, the hens fed THR or CAN recorded higher egg mass/day values by 4.10% and 4.41%, respectively. These results disagree with those obtained by Azzam et al. (2014, 2017) who reported that supplementing

laying hens' diets with THR enhanced egg production but had no effect on egg weight. The beneficial effect of CAN have been reported before. For example, supplementing brown egg layers with CAN at a dose of 2.1 mg/kg improved egg production compared to a dose of 1.1 mg/kg corn-based control diet (Cho et al., 2013).

The hens fed CAN+THR recorded higher egg mass by 5.23% compared to hen fed control diet meaning synergist effect between two additives. It was surprise that addition SS alone increased egg mass/ day by 5.48% compared to control diet .The beneficial effect of SS on egg production have been reported before by Ali et al.,(2012). They indicated that SS may play a role in sexual hormones protection from free radical attack. In this respect, human serum levels of estrone sulphate are as much as 10 times higher than those of unconjugated estrone and estradiol, and the half life of estrone sulphate is much longer than the half-life of unconjugated estrogen (Bhattacharyya and Tobacman 2007).

The addition of THR +SS significantly increased egg mass/day by 6.88 % compared to control diet meaning synergist effect between THR and SS .The amino acids in vivo exist in proteins, but they are also present in our body fluids as free forms (Huichun, et al. 2003).

The beneficial effect of THR on egg mass may be to its role in antioxidants capacity when it present in body fluids as free forms. Azzam et al. (2012) discovered that include dietary THR increased the antioxidant capacity of laying hens, as shown by increased superoxide dismutase (SOD) and glutathione peroxidase activities. The beneficial effect of SS with amino acid as antioxidants have been demonstrated in previous work by Ali et al. 2018 who found with local laying hens that addition of SS plus tyrosine (as antioxidants) increased egg mass by 37.71% compared to control diet .The addition of CAN+SS increased egg mass/day by 8.08% compared to control diet .These results agree with those obtained by Ali et al.,(2018) who found with local laying hen that addition of SS+CAN increased egg mass by 48.95% compared to control diet. They indicated that addition of sulphate to laying hen diets increased the utilization of canthaxnthin. The addition of THR+CAN+SS significantly increased egg mass /day by 8.10% compared to control diet. These results agree with those obtained by Ali et al. 2018 who found CAN+ SS + tyrosine increased egg mass by 58.25% compared to control diet .The beneficial effect of antioxidants with SS have been reported by Ali et al 2012who found that antioxidants like commercial canthaxanthin with or without sodium sulphate increased T3 hormone, estrogen, egg production, fertility and hatchability. They indicated that addition of sulphate to laying hen diets increased the utilization of canthaxnthin. The differences between response to antioxidants with sulphate in these study and response in experiment done by Ali et al. 2018 may be due to differences between local hens (low egg production) and commercial laying hens (high egg production) used in this study. However, Correia-Da-Silva et al., (2014) showed that sulfated small molecules could be of value in therapeutics due to their hydrophobic nature that can contribute to improve the bioavailability. The same trend was found between egg mass/ week values recorded by different treatments.

The equilibrium between ROS generation and antioxidant systems, on the other hand, might be interrupted as antioxidant levels decline with age (Tong et al., 2012). We may deduce from the performance data that a combination of three additions (THR+CAN+SS) was the most successful additive under the conditions of this investigation.

**Egg quality:**

The effect of dietary treatments on egg weight, yolk weight, albumen weight and egg shell weight of laying hens during the experimental period at (44 – 56) weeks of age are shown in Table (4).All feed additives significantly increased egg weight compared to control diet. The hens fed THR+CAN+SS recorded the highest value while those fed control diet recorded the lowest value. The feed additives used in this study did not affect the yolk weight but increased albumin weight are detected compared to control diet .Addition THR alone numerically increased albumin weight compared to control diet. However, Azzam et al. (2014)found that the addition of dietary THR (from56 to 64 wk of age) increased the albumen height in laying hens. The birds fed CAN+SS recorded the highest value while birds fed control diet recorded the lowest value. Analysis of variance indicated that differences between shell weight values are significant among dietary treatments .The hens fed the mixture of three feed additive recorded the highest value while hens fed CAN+SS recorded the lowest value. These results disagree with those obtained by Ali et al.,(2018) who found with local hens that feed additives (as antioxidants) did not affect shell weight significantly compared to control diet. The effect of dietary treatments on eggshell thickness, shape index, yolk index and yolk color of laying hens during the experimental period at 56 weeks of age is shown in Table (5).All feed additives used in this study increased egg shell thickness compared to control diet. Addition of THR numerically increased egg shell thickness compared to control diet. These results agree with those obtained by Ali et al., (2007) who found that thyme ( as a nature antioxidants)alone or with SS increased shell thickness. The mixture of three feed additives increased shell thickness by 15.15% compared to control diet. These results agree with work have been done by Ali et al.,(2018) who found that the birds fed Tryptophane +CAN+SS recorded the highest value of shell thickness while birds fed control diet recorded the lowest value and indicating that these additives improved shell thickness. Also, all feed additives increased shape index compared to control diet. However, eggshell strength is influenced by other parameters such as egg shape, egg size, or eggshell thickness (Sapkota et al., 2017). Also, the eggshell play role in protection against the contamination of egg internal content so from the food safety point of view, eggshell quality plays an important role as well (Vlckova et al., 2018).Compared to control diet, all feed additives increased yolk index except SS. There were significant differences between yolk colors core values recorded by different treatments. The hens fed the mixture of three feed additives recorded the highest value while the control diet recorded the lowest value. The addition of CAN, CAN+ THR, CAN+SS or CAN+SS+THR significantly increased yolk color score compared to control diet . The increase yolk score may be due to increase the CAN in the yolk .These

results agree with those obtained by Ali et al.,(2018) who found that addition of CAN significantly increased yolk score .In this respect, Johnson-Dahl et al. (2017) found that dietary CX (6 mg/kg) supplementation increased egg yolk CX content from 0 to 300 $\mu$g/egg in 7 days.

**Hepatic cellular and shell gland oxygen consumption:**

The effect of dietary treatments on hepatic cellular and shell gland oxygen consumptions shown in Table (6). All feed additives used in this study except SS significantly increased oxygen consumption by hepatic cellular and shell gland compared to control diet. The hens fed CAN+SS recorded higher hepatic cellular oxygen consumption by 100%compared to control diet. However, the process of egg production, the liver is responsible of synthesizing most egg yolk precursors, which are subsequently transferred into oocytes (Bourin et al., 2012).These results clearly demonstrated that these feed additives succeeded in improving metabolism in hepatic and shell gland.

**Blood Biochemical parameters**:

The effect of dietary treatments on serum parameters is shown in Table (7).There were significant differences between serum glucose values recorded by hens fed different dietary treatments. The hens fed the control diet recorded the lowest value while those fed THR+ CAN+SS recorded the highest value. As the hen increase in age, the level of glucose decrease. For example, Pavlík et al. (2007) discovered a decrease in glucose at75 wk of age while Onbasilar and Aksoy (2005) at 56 wk of age.

The analysis of variance indicated that differences between T3 values recorded by hens fed different treatments were significant .The hens fed the control diet recorded the lowest value while hens fed the mixture of feed additives recorded the highest value. These results agree with the previous work done by Ali et al., (2012) who found that all feed additives (antioxidants) increase T3 compared to control diet. They indicated that CAN or another natural antioxidants can protect it from free radicals attack (saving effect). The same trend also was observed with T4 values. In this respect, thyroid hormone synthesis was dependent on tyrosine sulfation and hormone synthesis decreased when tyrosine sulfation decreased (Nlend et al.,1999). All feed additives used in this study decreased the serum lipid compared to control diet. The addition of SS with THR, CAN or both significantly decreased serum cholesterol compared to control diet. These results agree with those obtained by Ali et al.,(2012) who found with local laying hens that all feed additives (as antioxidants) decreased plasma cholesterol. The effects of dietary treatment on other serum parameters are shown in Table (8). The birds fed SS with THR, CAN or both significantly recorded higher values of serum IgG compared to hens fed control diet. All feed additives used in this study significantly increased serum antioxidants capacity compared to hens fed control diet. These results agree with those obtained by Ali et al.,(2012) who found that feed additives (as antioxidants) increase antioxidant capacity in plasma compared to control diet. Because antioxidant levels steadily decline with age (Tong et al., 2012), the balance between ROS generation and antioxidant systems might be interrupted, and aged chickens require more antioxidants than young hens. When compared to the control meal, all feed additives applied in this study

significantly increased serum total protein. However, a higher total protein level in the blood indicates that the animal is in better health (Marono et al., 2017).All feed additive except SS in this fed different dietary treatments. The hens fed the control diet recorded the lowest results in this study we can state that feed additives used with aged commercial laying hen improved egg weight, egg mass, shell thickness and shell weight. One the other hand, previous work with local hen(Ali et al. 2012, 2018), reported that feed additives improved egg production and shell thickness .The differences between these experiments may be to differences between local hen and commercial laying hens used in this study. It is clearly that the mixture THR+CAN+SS work together to achieve

study increased significantly serum albumin compared to control diets. There were significant differences between serum globulin values recorded by hens

value while those fed THR+ CAN+SS recorded the highest value. From previous optimum performance under condition of this study. In this respect, Surai (2003) showed that to achieve optimum protection from free radicals, the tissues deploy an integrated antioxidant system that consists of a diverse array of lipid-soluble (e.g. vitamin E, carotenoids), water-soluble (e.g. ascorbic acid, glutathione) and enzymic (e.g. glutathione peroxidase, superoxide dismutase) components. The author showed that these various components act in synergy

## CONCLUSION

In conclusion, the results of this study indicate that feeds supplemented with THR+CAN+SS improved laying hens productive performance and physiological parameters. In addition, layers fed with diet supplemented with THR+CAN+SS improved all blood

serum parameters investigated, such as T3 and T4 hormones, IgG and globulin values which reflect better immunity for these hens. Therefore, it is recommended to applicate THR+CAN+SS in layer chicken diets at levels studied.

**Table (1):**The compositions and calculated analysis of the basal diet.

| Ingredients | (%) |
|---|---|
| Ground yellow corn (8.8%) | 63.23 |
| Soybean meal (44%) | 16.50 |
| Corn gluten meal (60%) | 8.00 |
| Monocalcium phosphate | 1.51 |
| Limestone | 9.80 |
| Premix[1] | 0.30 |
| Sodium Chloride (NaCl) | 0.30 |
| DL-methionine | 0.19 |
| L-lysine-HCl | 0.17 |
| Total | 100 |
| Calculated analysis[2] | |
| Crude protein% | 17.97 |
| ME. Kcal/Kg feed | 2798 |
| Calcium% | 4.00 |
| Available P.% | 0.42 |
| Lysine% | 0.86 |
| Methionine% | 0.46 |
| Methionine + Cystine% | 0.77 |

[1]Each 3Kg of vitamin and minerals mixture contain: Vit. A 10.000.000 IU, Vit.$D_3$ 2.000.000 IU, Vit. E 10.000 mg, Vit.$K_3$ 2.000 mg Vit.$B_1$ 1.000 mg, Vit.$B_2$ 5.000 mg, Vit. $B_6$ 1.500 mg, Vit. $B_{12}$ 10 mg, Niacin 30.000 mg, Pantothenic acid 10.000 mg, Folic acid 1.000 mg, Biotin 50 mg, Choline chloride 500.000 mg, Copper 4.000 mg, Iodine 1.000 mg, Iron 30.000 mg, Manganese 60.000 mg, Zinc 50.000 mg, Cobalt 100 mg and Selenium 100 mg.
[2]According to NRC (1994).

**Table (2):**The effect of dietary threonine, canthaxanthin and sodium sulphate on body weight, feed intake and feed conversion ratio of laying hens during the experimental period at (44 – 56) weeks of age (ẍ ±SE).

| Treatments | Initial body weight (g/bird) at 44 wk of age | Final body weight (g/bird) at 56 wk of age | Feed intake (g/hen. day) | Feed intake (g/hen. week) | Feed conversion ratio (g feed/1g eggs) |
|---|---|---|---|---|---|
| T1 (control) | 1530.3±9.4 | $1690.7^{c}$±41.82 | $114.2^{b}$±1.2 | $799.4^{b}$±12.2 | 2.18±0.01 |
| T2 (THR) | 1544.3±13.4 | $1751.7^{c}$±54.80 | $113.3^{b}$±1.8 | $793.2^{b}$±12.6 | 2.08±0.01 |
| T3 (CAN) | 1538.2±17.3 | $1750.1^{c}$±41.77 | $118.9^{ab}$±1.6 | $832.3^{ab}$±11.4 | 2.18±0.01 |
| T4 (THR+CAN) | 1540.2±13.0 | $1815.5^{bc}$±52.74 | $117.1^{ab}$±1.8 | $819.7^{ab}$±12.9 | 2.13±0.01 |
| T5 (SS) | 1547.2±4.6 | $1726.1^{c}$±29.60 | $121.4^{a}$±1.6 | $850.1^{a}$±12.9 | 2.20±0.02 |
| T6 (THR+SS) | 1563.1±2.1 | $1875.5^{ab}$±27.49 | $113.4^{b}$±1.2 | $793.9^{b}$±12.3 | 2.03±0.01 |
| T7 (CAN+SS) | 1546.7±12.1 | $1946.5^{a}$±36.54 | $122.1^{a}$±1.6 | $854.1^{a}$±12.8 | 2.16±0.01 |
| T8 (THR+CAN+SS) | 1555.7±5.4 | $1889.3^{ab}$±30.85 | $117.1^{ab}$±1.6 | $819.2^{ab}$±10.9 | 2.07±0.01 |

Mean ± Standard Error.
a, b and c = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (3):**The effect of dietary threonine, canthaxanthin and sodium sulphate on egg number and egg mass of laying hens during the experimental period at (44 – 56) weeks of age (ẍ ±SE).

| Treatments | Egg number (egg number/hen. day) | Egg number (egg number/hen. week) | Egg mass (egg mass (g)/hen. day) | Egg mass (egg mass (g)/hen. week) |
|---|---|---|---|---|
| T1 (control) | 0.88±0.02 | 6.13±0.11 | $52.32^{b}$±0.98 | $366.26^{b}$±6.87 |
| T2 (THR) | 0.91±0.02 | 6.34±0.10 | $54.47^{ab}$±0.95 | $381.30^{ab}$±6.63 |
| T3 (CAN) | 0.91±0.0.2 | 6.39±0.12 | $54.63^{ab}$±1.24 | $382.40^{ab}$±8.69 |
| T4 (THR+CAN) | 0.90±0.02 | 6.28±0.11 | $55.06^{ab}$±1.00 | $385.41^{ab}$±6.97 |
| T5 (SS) | 0.90±0.02 | 6.28±0.12 | $55.19^{ab}$±1.08 | $386.35^{ab}$±7.56 |
| T6 (THR+SS) | 0.88±0.02 | 6.17±0.17 | $55.92^{ab}$±1.38 | $391.43^{ab}$±9.89 |
| T7 (CAN+SS) | 0.91±0.02 | 6.36±0.14 | $56.55^{a}$±1.42 | $395.86^{a}$±9.96 |
| T8 (THR+CAN+SS) | 0.93±0.01 | 6.51±0.10 | $56.56^{a}$±0.68 | $395.95^{a}$±4.79 |

Mean ± Standard Error.
a, b and c = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (4):**The effect of dietary threonine, canthaxanthin and sodium sulphate on egg weight, yolk weight, albumen weight and eggshell weight of laying hens during the experimental period at 56 weeks of age(ẍ ±SE).

| Treatments | Egg weight (g) | Yolk weight (g) | Albumen weight (g) | Eggshell weight (g) |
|---|---|---|---|---|
| T1(control) | $55.33^{b}$±0.67 | 14.33±0.88 | $35.70^{b}$±1.61 | $5.30^{b}$±0.15 |
| T2 (THR) | $61.33^{a}$±2.67 | 15.67±0.33 | $39.03^{ab}$±3.03 | $6.63^{a}$±0.19 |
| T3 (CAN) | $61.67^{a}$±2.04 | 15.67±1.45 | $40.63^{ab}$±3.06 | $5.37^{b}$±0.64 |
| T4 (THR+CAN) | $62.00^{a}$±1.53 | 14.87±0.47 | $39.80^{ab}$±2.16 | $7.33^{a}$±0.35 |
| T5 (SS) | $60.67^{a}$±1.45 | 14.67±0.88 | $40.73^{ab}$±1.70 | $5.27^{b}$±0.18 |
| T6 (THR+SS) | $64.67^{a}$±0.88 | 15.67±0.67 | $42.47^{a}$±0.98 | $6.53^{a}$±0.18 |
| T7 (CAN+SS) | $64.33^{a}$±0.33 | 14.33±0.67 | $45.00^{a}$±1.05 | $5.00^{b}$±0.10 |
| T8 (THR+CAN+SS) | $65.67^{a}$±0.33 | 15.33±0.88 | $42.93^{a}$±0.97 | $7.40^{a}$±0.31 |

Mean ± Standard Error.
a, b and c = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (5):**The effect of dietary threonine, canthaxanthin and sodium sulphate on eggshell thickness, shape index, yolk index and yolk color of laying hens during the experimental period at 56 weeks of age(ẍ ±SE).

| Treatments | Eggshell thickness (mm) | Shape index | Yolk index | Yolk color |
|---|---|---|---|---|
| T1 (control) | $0.330^{c}$±0.01 | $0.713^{d}$±0.01 | $0.410^{b}$±0.01 | $9.65^{c}$±0.33 |
| T2 (THR) | $0.350^{bc}$±0.01 | $0.723^{cd}$±0.01 | $0.423^{ab}$±0.01 | $10.67^{bc}$±0.33 |
| T3 (CAN) | $0.347^{bc}$±0.01 | $0.730^{cd}$±0.01 | $0.433^{ab}$±0.01 | $11.67^{ab}$±0.33 |
| T4 (THR+CAN) | $0.350^{bc}$±0.01 | $0.730^{cd}$±0.01 | $0.433^{ab}$±0.01 | $11.66^{ab}$±0.33 |
| T5 (SS) | $0.333^{bc}$±0.01 | $0.737^{bc}$±0.01 | $0.410^{b}$±0.01 | $9.67^{c}$±0.33 |
| T6 (THR+SS) | $0.347^{bc}$±0.02 | $0.767^{a}$±0.01 | $0.430^{ab}$±0.01 | $10.33^{c}$±0.33 |
| T7 (CAN+SS) | $0.357^{b}$±0.01 | $0.757^{ab}$±0.01 | $0.437^{ab}$±0.01 | $11.33^{ab}$±0.33 |
| T8 (THR+CAN+SS) | $0.380^{a}$±0.01 | $0.767^{a}$±0.01 | $0.443^{a}$±0.01 | $12.03^{a}$±0.33 |

Mean ± Standard Error.
a, b and c = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (6) :**The effect of dietary threonine, canthaxanthin and sodium sulphate on hepatic cellular and shell gland oxygen consumption of laying hens at 56 weeks of age(ẍ ±SE).

| Treatments | Oxygen consumption (µl. $h^{-1}$/100 mg dry weight) | |
|---|---|---|
| | Liver | Shell gland |
| T1(control) | $1.58^{d}$±0.12 | $5.82^{d}$±0.10 |
| T2 (THR) | $1.92^{c}$±0.04 | $6.15^{c}$±0.02 |
| T3 (CAN) | $2.09^{bc}$±0.12 | $6.31^{c}$±0.10 |
| T4 (THR+CAN) | $2.31^{b}$±0.03 | $6.54^{b}$±0.03 |
| T5 (SS) | $1.64^{d}$±0.05 | $5.89^{d}$±0.05 |
| T6 (THR+SS) | $3.07^{a}$±0.04 | $7.32^{a}$±0.05 |
| T7 (CAN+SS) | $3.16^{a}$±0.03 | $7.37^{a}$±0.01 |
| T8 (THR+CAN+SS) | $3.13^{a}$±0.07 | $7.35^{a}$±0.01 |

Mean ± Standard Error.

a, b, c and d = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (7):**The effect of dietary threonine, canthaxanthin and sodium sulphate on blood parameters of laying hens at 56 weeks of age(ẍ ±SE).

| Treatments | Serum Glucose (mg/dl) | Serum T3 (ng/ml) | Serum T4(ng/ml) | Serum Total Lipids (mg/dl) | Serum Total Cholesterol (mg/dl) |
|---|---|---|---|---|---|
| T1(control) | $185.00^{c}$±0.60 | $2.27^{f}$±0.05 | $6.09^{f}$±0.03 | $445.67^{a}$±4.98 | $141.33^{a}$±1.76 |
| T2 (THR) | $191.67^{b}$±1.45 | $2.37^{def}$±0.03 | $6.18^{def}$±0.02 | $426.33^{b}$±2.96 | $142.67^{a}$±1.86 |
| T3 (CAN) | $189.33^{bc}$±1.01 | $2.41^{de}$±0.03 | $6.22^{de}$±0.02 | $424.68^{b}$±7.13 | $139.65^{a}$±1.20 |
| T4 (THR+CAN) | $192.00^{b}$±1.73 | $2.45^{d}$±0.04 | $6.25^{d}$±0.03 | $428.67^{ab}$±2.33 | $141.32^{a}$±1.76 |
| T5 (SS) | $186.33^{bc}$±2.19 | $2.34^{ef}$±0.04 | $6.13^{ef}$±0.03 | $438.64^{ab}$±1.76 | $141.66^{a}$±1.45 |
| T6 (THR+SS) | $209.00^{a}$±0.60 | $3.37^{c}$±0.06 | $7.17^{c}$±0.04 | $375.66^{c}$±5.21 | $126.33^{b}$±2.96 |
| T7 (CAN+SS) | $212.66^{a}$±2.73 | $3.56^{b}$±0.07 | $7.34^{b}$±0.06 | $378.01^{c}$±5.77 | $130.00^{b}$±1.53 |
| T8 (THR+CAN+SS) | $213.00^{a}$±2.51 | $3.72^{a}$±0.05 | $7.51^{a}$±0.04 | $371.00^{c}$±5.86 | $129.33^{b}$±2.19 |

Mean ± Standard Error.

a, c, d, e, and f = Means within the same column with different superscripts are significantly different (P≤0.05).

**Table (8):** The effect of dietary threonine, canthaxanthin and sodium sulphate on blood parameters of laying hens at 56 weeks of age(ẍ ±SE).

| Treatments | Blood PCV (%) | Blood Hb (g/dl) | Serum IgG (mg/dl) | Serum Total Antioxidant Capacity ( mM/L) | Serum Total Protein (g/dl) | Serum Albumin (g/dl) | Serum Globulin (g/dl) |
|---|---|---|---|---|---|---|---|
| T1(control) | $30.26^{c}$±0.09 | $12.15^{e}$±0.02 | $8.12^{b}$±0.02 | $0.69^{c}$±0.06 | $5.30^{e}$±0.06 | $2.26^{b}$±0.02 | $3.04^{e}$±0.05 |
| T2 (THR) | $30.86^{c}$±0.22 | $12.27^{c}$±0.03 | $8.15^{b}$±0.01 | $1.02^{ab}$±0.02 | $6.30^{c}$±0.06 | $3.14^{a}$±0.03 | $3.16^{e}$±0.09 |
| T3 (CAN) | $31.06^{bc}$±0.09 | $12.23^{cd}$±0.03 | $8.47^{b}$±0.04 | $1.02^{ab}$±0.02 | $6.33^{c}$±0.09 | $3.17^{a}$±0.02 | $3.16^{e}$±0.09 |
| T4 (THR+CAN) | $31.39^{bc}$±0.37 | $12.17^{de}$±0.01 | $8.80^{b}$±0.03 | $1.09^{a}$±0.02 | $6.47^{c}$±0.03 | $3.18^{a}$±0.02 | $3.28^{de}$±0.05 |
| T5 (SS) | $30.58^{c}$±0.20 | $12.15^{e}$±0.02 | $9.17^{b}$±0.06 | $1.00^{b}$±0.02 | $5.59^{d}$±0.06 | $2.25^{b}$±0.02 | $3.34^{cd}$±0.06 |
| T6 (THR+SS) | $32.11^{ab}$±0.36 | $14.22^{a}$±0.02 | $11.25^{a}$±0.03 | $0.98^{b}$±0.01 | $6.91^{b}$±0.03 | $3.23^{a}$±0.03 | $3.68^{bc}$±0.03 |
| T7 (CAN+SS) | $32.13^{ab}$±0.35 | $14.14^{b}$±0.01 | $11.26^{a}$±0.03 | $1.11^{a}$±0.01 | $7.02^{ab}$±0.04 | $3.05^{a}$±0.03 | $3.98^{ab}$±0.06 |
| T8 (THR+CAN+SS) | $32.81^{a}$±0.31 | $14.24^{a}$±0.03 | $11.22^{a}$±0.06 | $1.05^{ab}$±0.02 | $7.21^{a}$±0.05 | $3.07^{a}$±0.04 | $4.15^{a}$±0.09 |

Mean ± Standard Error.

a, c, d and e = Means within the same column with different superscripts are significantly different (P≤0.05).

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## الملخص العربى

## استخدام الثريونين، والكانثازانثين، وكبريتات الصوديوم؛ لتحسين إنتاج البيض للدجاج البياض فى فترة ما بعد قمة الإنتاج

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تم إجراء هذه الدراسة لاختبار فرضية أن الثريونين، ومسحوق الكانثازانثين، وكبريتات الصوديوم؛ يمكنهما تحسين أداء الدجاج البياض التجاري (سلالة لوهمان البني) وذلك عندنهاية فترة إنتاج البيض (٤٤ – ٥٦ أسبوعًا من العمر)، ولدراسة ذلك تم تقسيم وتوزيع عدد ١٢٠ دجاجة بياضة من سلالة لوهمان البني عند عمر ٤٤ أسبوع عشوائيًاوبالتساوي إلى ٨ مجموعات، حيث احتوت كل مجموعة على ٥ مكررات، وكل مكررة احتوت على ٣ دجاجات.

تم تسكين الدجاجات فى أقفاص سلكية، وتم تغذية الدجاج البياض خلال فترة التجربة التى استمرت من ٤٤ إلى ٥٦ أسبوع على العليقة الكنترول بدون أو بإضافة ٢ جم ثريونين / كجم علف ، ٣ جزء فى المليون من مسحوق الكانثازانثين، ٢ جم ثريونين / كجم علف + ٣ جزء في المليون من مسحوق الكانثازانثين، ٥ جم كبريتات صوديوم / كجم علف ، ٢ جم ثريونين / كجم علف +٥ جم كبريتات صوديوم / كجم علف، ٣ جزء في المليون مسحوق الكانثازانثين +٥ جم كبريتات صوديوم / كجم علف و٢جم ثريونين / كجم علف + ٣ جزء في المليون من مسحوق الكانثازانثين + ٥ جم كبريتات صوديوم / كجم علف.

أشارت الدراسة أن جميع الإضافات الغذائية المستخدمة أدت إلى زيادة عدد البيض (بيضة / دجاجة / يوم) مقارنة بالمجموعة الكنترول، أيضًا أدى إضافة الثريونين + مسحوق الكانثازانثين+ كبريتات الصوديوم إلى زيادة معنوية في كتلة البيض / اليوم بنسبة ٨.١٠% مقارنة بالمجموعة الكنترول، كذلك أدت جميع الإضافات المستخدمة في هذه الدراسة إلى زيادة سمك قشر البيض والبروتين الكلى في سيرم الدم ومضادات الأكسدة مقارنة بالمجموعة الكنترول،وأدى الخليط المكون من الثلاث إضافات معًا إلى زيادة سمك القشرة بنسبة ١٥.١٥% مقارنة بالمجموعة الكنترول،وكان الخليط المكون (الثريونين + مسحوق الكانثازانثين+ كبريتات الصوديوم) أكثر المواد المضافة نجاحًا في هذه الدراسة.