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## The Effect of Heat Stress on Oxidative Stress and Antioxidant Status in Local Quail Hens Supplemented with Onion and Garlic Oils

### ABSTRACT

This study was conducted to investigate the potential of onion and garlic oils and their mixture supplementation under heat stress on the egg production, egg weight, total lipid concentration and antioxidant activity of local quail hens. Sixty birds at 42 weeks' age were evenly divided randomly into 4 treatments: feed in control (T0) free of onion and garlic oil, T1 and T2 were supplemented 0.2% in each onion or garlic oil, T3 was supplemented mixture of (0.1% onion + 0.1% garlic oil) respectively. The birds reared in temperature-controlled room (35-38°C) at afternoon.

The results of enzymatically analysis of catalase, superoxide dismutase, Glutathion-Px activity concentrations, egg production percentage, egg weight and egg mass had significantly higher ( $P \leq 0.05$ ) in the most treatments of onion and garlic oils and their mixture supplemented diet. While, significantly lower ( $P \leq 0.05$ ) in total cholesterol, hydrogen peroxide ( $H_2O_2$ ) and Malondialdehyde (MDA) in onion and garlic oils and their mixture supplemented diet compared with control of blood serum, liver tissue and egg yolk of quail hens under heat stress.

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## INTRODUCTION

The metabolic oxidation capacity of skeletal muscle enhanced by heat stress causing releasing of corticosterone, this begin lipid peroxidation in cell membranes, (Azad *et al.*, 2009). Li *et al.* (2007) was defined antioxidant as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate. Antioxidants are playing a very important role in the body defense system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells. Increasing the intake of dietary antioxidant to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system. According to Tepe *et al.* (2005), antioxidants have great importance in terms of preventing oxidative stress that may cause several generative diseases. Allium species are revered to contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries (Benkeblia, 2005).

Garlic in different forms has antioxidant properties, these properties are shown to be due to the existence of compounds such as water-soluble organosulfur compounds, S-allylcysteine and lipidsoluble compounds such as diallyl sulfide (Bhagyalakshmi *et al.*, 2005). Garlic allicin is the

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most predominant thiosulphate in garlic that is responsible for the characteristic odor and has an antibacterial effect (Cunha *et al.*, 2012). One milligram of alliin is considered equivalent to 0.45 mg of allicin (WHO, 1999).

According to Munday *et al.* (2003) onion is rich in two chemical groups are the flavonoids and the alkane cysteine sulfoxides (ACSOs), both being strong antioxidants.

The aim of our study is the determination of serum, liver and egg yolk total cholesterol and antioxidants enzymes activity in local quail hens feeding on diets supplemented with onion and garlic oils under heat stress then aid toward improving egg production efficiency and egg weight.

## MATERIAL AND METHODS

In this study, 60 local quail hens (40 weeks of age) of similar weight about (210g±5) were randomly assigned to four treatments. Hens were obtained from the College of Agriculture/ Animal Resources Dept., University of Salahaddin-Erbil/Iraq.

The quail hens were reared in special cages 65cm×60cm×50cm length, width and height respectively, designed for quails in a temperature-controlled room 35-38°C at afternoon, 28-30 C° at night and morning. Each treatment was containing three replicates and there were 5 quail hens per replicate. The feed in control (T0) free of onion and garlic oil, T1 and T2 were supplemented 0.2% in each onion and garlic oil, T3 was supplemented mixture of (0.1% onion + 0.1% garlic oil) respectively. The garlic and onion oils produced in Turkey, the GC-MS analysis of garlic essential oil were content the major components diallyl disulfide, diallyl trisulfide, allyl methyl, diallyl sulfide, diallyl tetrasulfide and allyl methyl disulfide. Whereas, the main components of onion oil were dipropyl disulfide, dipropyl trisulfide, 1-propenyl propyl disulfide and methyl propyl trisulfide. The diets were formulated according to the NRC (1994), the basal composition of the diet is shown in Table 1.

**Table 1. Ingredient and chemical composition of quail production ration.**

Ingredients	%	Calculated chemical contents of diet (per kg) <sup>3</sup>
Corn	48.43	Metabolizable Energy (kcal) 2800
Soybean meal	27.6	Crude protein 18.52
Wheat	15.00	Crude fiber 4.30
Sunflower oil	1.00	Methionine 0.450
Bay leaf powder	.000	Lysine 1.02
Bay leaf oil	.000	Ca 2.61
Limestone	6.00	Available P 0.354
DCP	1.15	Na 0.160
Vitamine premix <sup>1</sup>	0.10	
Mineral premix <sup>2</sup>	0.10	
Methionine	0.17	
Lysine	0.45	

<sup>1</sup> Vitamine premix/ kg diet: vit.A12,000 IU; vit.D3 60 µg; vit.E 32.96 IU; vit.K3 3 mg; vit.B1 3 mg; vit.B2 7 mg; vit.B6 4 mg; vit.B12 0.02 mg; nicotinic acid 40 mg; pantothenate 8 mg; folic acid 1 mg; biotin 0.045 mg; vit.C 50 mg; choline chloride 125 mg. phosphate. <sup>2</sup> Minerals premix:/ kg diet: Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; Co, 0.1 mg; Se 0.15 mg. DCP= Di-calcium phosphate <sup>3</sup> NRC: Chemical analysis of ingredients depending on NRC (1994).

The trial lasted for 60 days. Quail had *ad libitum* fed and water. The birds had 16-h photoperiod. Daily egg production was recorded and feed intake, FCR, egg weight were weekly determined. At the end of the study blood samples were taken for biochemical analyses from their brachial veins, each time from the same 20 birds in every group liver tissue samples taken and 20 egg from each treatment were collected then used to determine total cholesterol and catalase, glutathione peroxidase, superoxide dismutase, malondialdehyde and H<sub>2</sub>O<sub>2</sub> peroxidase concentrations by spectrophotometer. They were determined by using a commercial kit (Bio-Medical Co.). The method and principle to determine antioxidant indicators using in the kits were described.

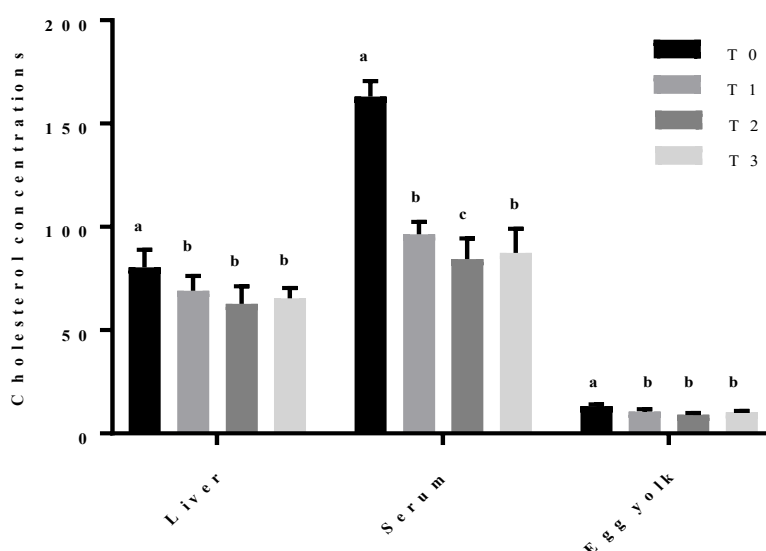
The experiments executed as a complete randomized design (CRD), all data analyzed using the SAS (2005) at 0.05 depending on the statistical model.

## RESULTS AND DISCUSSION

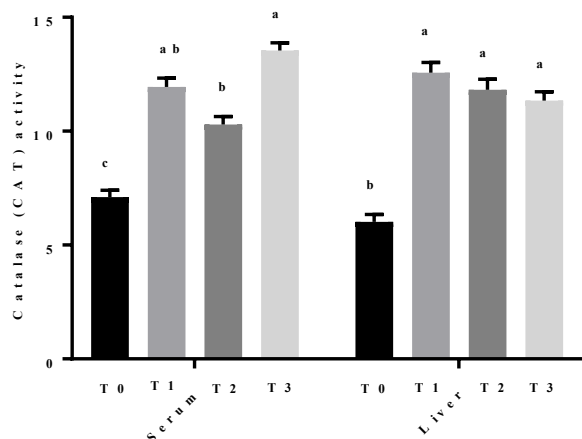
The percentage of total cholesterol in figure 1. performs significantly lower ( $P \leq 0.05$ ) in the all treatments of supplementation onion or garlic oils and their mixture in liver (62.7, 68.3, 69.0 mg/g tissue), serum (80.4, 84.7, 97.0 mg/dl) and egg yolk (9.13, 10.33, 10.52 mg/g yolk) in the treatments T2 (0.2% garlic oil), T3 (0.1% mixture oil of each onion & garlic), T1 (0.2% onion oil) respectively compared with control T0 liver (80.3), serum (163) and egg yolk (13.13) when quail hens exposed to heat stress. However, garlic group shows more lower in serum plasma compared with the others. The mechanism of action was suggested by Sodimu *et al.*, (1984) who indicated that garlic oil prevented an increase of cholesterol by inactivation of thiol group enzymes as HMG-CoA reductase and CoASH, the rate limiting enzyme for cholesterol biosynthesis and the multi-enzyme complex for fatty acid biosynthesis. Thus garlic allicin in garlic and onion oils potentially active component it regards unstable and poorly absorbed from the digestive tract (Lawson *et al.*, 1992 and Mathew *et al.*, 2003).

**Figure1.** The total cholesterol concentrations in the liver (mg/g), serum (mg/dl) & egg yolk (mg/g yolk) of quail hens on a diet supplemented with onion & garlic oil under heat stress.

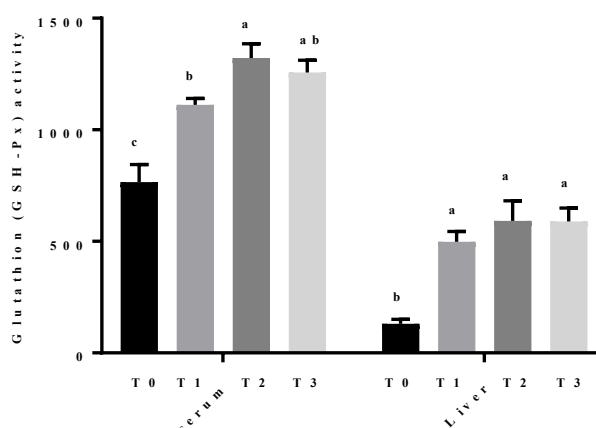
T0= standard diet, T1=0.2% onion oil, T2= 0.2% garlic oil, T3 mixture of (0.1% onion + 0.1% garlic) oils. Mean values without letters means non-significant. a, b, c, Mean values within different letters were significantly ( $P < 0.05$ ).



Figures 2, 3 & 4. show the supplementation of onion or garlic oils and their mixture were significantly higher ( $P \leq 0.05$ ) in the all treatments in serum and liver of catalase (CT), Glutathion-Px (GSH-Px), superoxide dismutase (SOD) activities concentrations compared with controls T0 of quail hens under heat stress. Bang and Kim (2009) demonstrated that catalase activity was higher in rats injected with 7 % onion oils. Garlic and onion have been shown to exert its action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and inhibiting lipid peroxidation, thus protecting endothelial cells from the injury by the oxidized molecules, which contributes to arteriosclerosis (Barile *et al.*, 2005), of the quails liver and serum of T1, T2 and T3 treated groups compared with non-supplemented control T0.



**Figure 2.** The activity of catalase concentrations in the serum (U/mL) & liver (U/mg pro) of liver quail hens on a diet supplemented with onion supplemented& garlic oil under heat stress.



**Figure 3.** The activity of Glutathion-Px concentrations in the serum (U/mL) & (U/g pro) of quail hens on a diet with onion & garlic oil under heat stress

**Figure 4.** The activity of superoxide dismutase concentrations in the serum (U/mL) & liver (U/g pro) of quail hens on a diet supplemented with onion & garlic oil under heat stress.

T0= standard diet, T1=0.2% onion oil, T2= 0.2% garlic oil, T3 mixture of (0.1% onion + 0.1% garlic) oils  
a, b, c, Mean values within different letters were significantly ( $P < 0.05$ ).

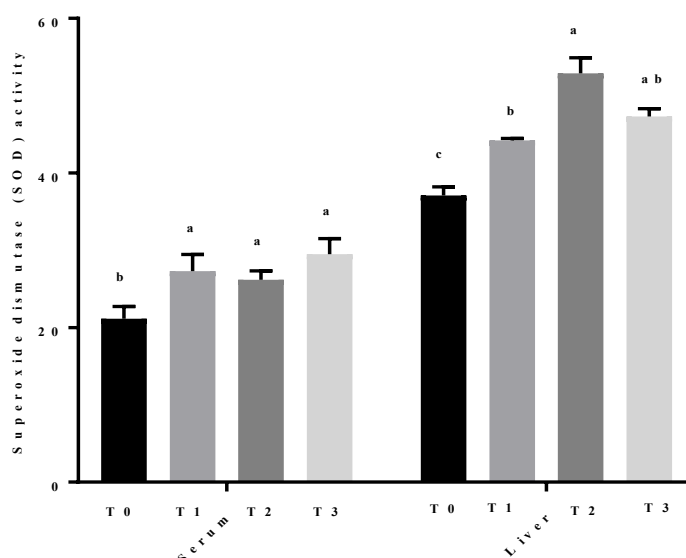
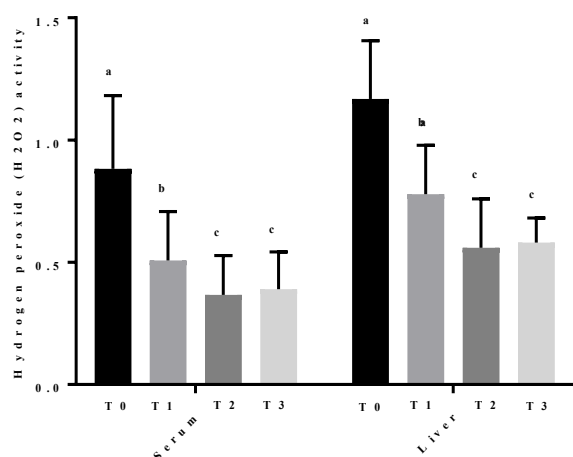
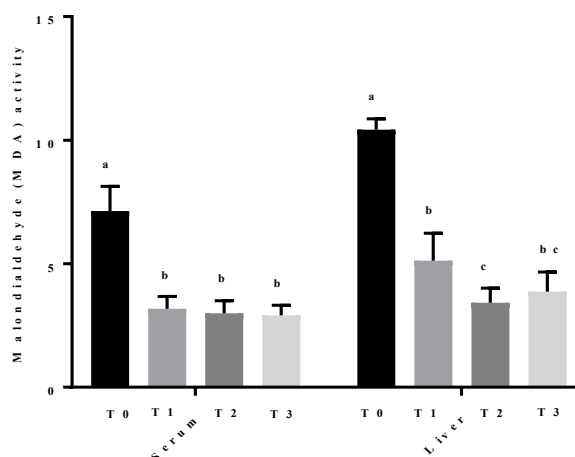


Figure 5 & 6. shows the activity of hydrogen peroxide ( $H_2O_2$ ) and Malondialdehyde (MDA) activities concentrations were significantly lower ( $P \leq 0.05$ ) in the treatments of onion and garlic oils and their mixture supplementation in serum and liver compared with control T0 of quail hens under heat stress. According to the previous studies the low levels of  $H_2O_2$  in the experimented groups feeding on the supplemented onion and garlic oils is due to increased activities of GPx and catalase enzymes which may convert those  $H_2O_2$  into  $H_2O$  but the two enzymes work in different pathways to minimize these free radicals (Hussain *et al.*, 2004).



**Figure 5.** The activity of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> concentrations in the serum (μmol/L) & liver ) (μmol /g pro) of quail hens on a diet supplemented with onion & garlic oil under heat stress.



**Figure 6.** The activity of Malondialdehyde concentrations in the serum (nmol/L) and liver (nmol/g) of quail hens on a diet supplemented with onion & garlic oil under heat stress.

T0= standard diet, T1=0.2% onion oil, T2= 0.2% garlic oil, T3 mixture of (0.1% onion + 0.1% garlic) oils

a, b, c, Mean values within different letters were significantly (P<0.05).

Table 2. Represent the supplementation of onion or garlic oils in quail hens diet were significantly higher (P≤0.05) in the activity of antioxidant enzymes concentrations: catalase (CT), superoxide dismutase (SOD) and Glutathion-Px compared with the control T0, while, Malondialdehyde (MDA) activities concentrations was significantly lower (P≤0.05) in the all treatments supplementation compared with control T0 in the egg yolk under heat stress. Garlic and onion oils can influence on normalization of cellular metabolism and functions by their powerful antioxidant effect against oxidative damage in caused by heat stress (Hussein *et al.*, 2007). These results shows that dietary garlic is more effective as a hypocholesterolemic agent than onion and has additional effects in reducing the activity of antioxidative enzymes. These effects are similar with disulfide intake from onion or garlic during heat stress (Sklan *et al.*, 1992). Antioxidants can protect against the damage induced by free radicals (Aher *et al.*, 2011) acting of using garlic onion oils. Antioxidant ability to interact with free radicals such as peroxy radicals and to scavenge and quench singlet oxygen is well documented (Farombi and Britton, 1999). Defense mechanisms against free radical-induced oxidative damage include the catalytic removal of free radicals and reactive species by factors such as Catalase (CAT), Superoxide dismutase (SOD) and reduction of free radicals by electron donors (Limon-Pacheco and Gonsebatt, 2009).

**Table 2. Effect of supplementation onion or garlic oils in quail hens diet on the activity of antioxidant enzymes concentrations in egg yolk.**

Traits	T0	T1	T2	T3
CAT (μg/g)	25.05±2.33 <sup>d</sup>	39.11±1.91 <sup>c</sup>	51.45±2.88 <sup>a</sup>	43.87±2.33 <sup>b</sup>
SOD (U/g)	46.33±4.37 <sup>d</sup>	52.25±3.47 <sup>c</sup>	72.05 ±3.12 <sup>a</sup>	58.75 ±3.16 <sup>b</sup>
GSH-px (U/g)	14.07±2.50 <sup>b</sup>	17.01±2.11 <sup>a</sup>	18.61±1.55 <sup>a</sup>	17.33±1.63 <sup>a</sup>
MDA (μg/g)	4.30±0.511 <sup>a</sup>	3.17±0.370 <sup>b</sup>	1.53±1.234 <sup>c</sup>	1.91±0.142 <sup>bc</sup>

T0= standard diet, T1=0.2% onion oil, T2= 0.2% garlic oil, T3 mixture of (0.1% onion + 0.1% garlic) oils

a, b, c, Mean values within different letters were significantly (P<0.05).

Table 3. Clarify the supplementation of onion or garlic oils in quail hens diet were significantly higher ( $P \leq 0.05$ ) in egg production (H.D %), egg weight, egg mass and improvement in feed conversion ratio (FCR) compared with the control T0, but was not significant differences among all treatments of onion or garlic oils and their mixture supplementation in feed intake (FI) under heat stress. The improvement in egg production, feed conversion ratio (FCR) and egg weight specially in garlic oil group related to contain allicin, free fatty acid (oleic acid: 8.40%), Iodine value (126.9 g/100g), saponification value (192 mg/KOH/g), Acid value (41.7 mg/KOH/g) and (mg/L) of: Ca (6.77), P (0.32), K (62.22), Mg (27.60), Mn (0.060), Cu (0.030) (Bagudo and Acheme, 2014) all these nutrients enhanced egg production, egg weight and improved FCR. Conclusively the reduction of cholesterol concentration in the groups of onion and garlic oil supplementation improved antioxidant statues by decreasing heat stress effect upon quail hens that due high egg production and egg weight, also improved FCR against lower performances in the control group without supplemented onion and garlic oils as a result of heat stress effectiveness.

**Table 3. Effect of supplementation onion or garlic oils in quail hens diet on production performance under heat stress.**

Traits	T0	T1	T2	T3
Egg Production (H.D %)	75.83±3.05 <sup>b</sup>	82.33±2.92 <sup>a</sup>	84.75±3.10 <sup>a</sup>	83.28±3.14 <sup>a</sup>
FI(g/day/bird)	27.52±1.74	27.88±1.56	28.19±1.48	27.93±1.60
FCR (g feed / g egg)	3.232±0.36 <sup>a</sup>	2.882±0.41 <sup>b</sup>	2.731±0.39 <sup>b</sup>	2.811±0.35 <sup>b</sup>
Egg Weight (g)	11.23±0.79 <sup>b</sup>	11.75±1.10 <sup>a</sup>	12.18±0.82 <sup>a</sup>	11.93±0.77 <sup>a</sup>
Egg mass (g)	851.8±72 <sup>b</sup>	967.4±58 <sup>a</sup>	1032.3±61 <sup>a</sup>	993.5±53 <sup>a</sup>

T0= standard diet, T1=0.2% onion oil, T2= 0.2% garlic oil, T3 mixture of (0.1% onion + 0.1% garlic) oils

<sup>a, b</sup> Mean values within different letters were significantly ( $P < 0.05$ ). Mean values without letters means non-significant.

H,D = Hen day, FI= feed intake, FCR= feed conversion ratio.

## CONCLUSIONS

Garlic and onion oils contain different phytochemicals that can be fortified in dietary supplementation as an antioxidant defense mechanism against free radicals in laying quails, depending on the degree of oxidative damage caused by heat stress. All supplemented groups had a promotional effect on the antioxidant capacity of quail hens compared with the control (free of onion and garlic oil). The results of enzymatically analysis of catalase, superoxide dismutase and Glutathion-Px activities concentrations were significantly higher ( $P \leq 0.05$ ) while, cholesterol,  $H_2O_2$  and MDA were significantly lower ( $P \leq 0.05$ ) in supplemented treatments compared with control of blood serum and liver tissue. Also egg production, feed conversion ratio and egg weight were higher in the supplemented treatments compared with the control. The garlic oil role more effectiveness as antioxidant as compared with onion oil.

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## تأثير الإجهاد الحراري للإجهاد التأكسدي والحالة التأكسدية بإضافة زيتي البصل أو الثوم لعليقة إناث السمان المحلي

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## المستخلص

أجريت هذه الدراسة لبحث إمكانية إضافة زيتي البصل والثوم وخليطهما تحت ظروف الإجهاد الحراري في التركيز الكلي للكلسترول ونشاط انزيمات الأكسدة في الكبد، مصل الدم وصفار البيض، كذلك على إنتاج البيض، وزن وكتلة بيض إناث السمان المحلي. وزعت 60 أنثى السمان وبعمر 42 اسبوعاً عشوائياً على أربع معاملات: T0 معاملة السيطرة ( بدون أية إضافة لزيتي البصل والثوم)، T1 و T2 أضيفت اليهما 0.2% زيت البصل و زيت الثوم على التوالي والمعاملة T3 أضيفت إليها 0.1% مزيج لكل من زيتي البصل والثوم. ربيت الطيور في غرفة بدرجة حرارة (35-38) °م.

نتائج التحليل لنشاط أنزيمات CAT، SOD و GSH-Px وإنتاج البيض ووزنه ارتفعوا معنوياً ( $P \leq 0.05$ ) في معظم معاملات إضافة زيتي البصل والثوم وخليطهما على معاملة السيطرة. في حين تركيز الكولسترول الكلي ونشاط انزيمي  $H_2O_2$  و MDA انخفضوا معنوياً ( $P \leq 0.05$ ) في معظم معاملات إضافة زيتي البصل والثوم وخليطهما على معاملة السيطرة في الكبد، مصل الدم وصفار البيض في إناث السمان تحت ظروف الإجهاد الحراري. لوحظ تفوق معاملة إضافة زيت الثوم على البصل في معظم الصفات.

الكلمات المفتاحية: زيتي البصل والثوم، انزيمات الأكسدة، الكولسترول، إنتاج البيض ووزنه، الأجهاد الحراري، السمان المحلي.