

## DIETARY SUPPLEMENTATION OF VITAMIN E AND SELENIUM ALLEVIATE THE NEGATIVE EFFECTS OF HIGH STOKING DENSITY IN GROWING JAPANESE QUAIL

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### SUMMARY

In poultry intensive production system, stoking density is an inevitable practice. Thus, this study was designed to investigate the negative effects of high stoking density on quail growth performance, blood constituents and gut microbial count, and whether vitamin E and selenium supplementations have potential effects on enhancing the bird performance under high stoking density. A total of 225 Japanese quail at eight days of age were randomly allocated to five experimental groups (45 chicks each), with three replicates per group (15 chicks each). The groups formed low stoking control group (80 birds/m<sup>2</sup>) as the negative control (N-Control), the high stoking control group (100 birds/m<sup>2</sup>) as the positive control (P-Control), and three groups having high stoking density (100 birds/m<sup>2</sup>) and supplemented with either 200 mg vitamin E/kg diet or 0.3 mg selenium/kg diet or 100 mg vitamin E and 0.15 mg selenium/kg diet. High stoking density negatively affected body weight gain and feed conversion ratio compared to the low stoking density. Blood calcium, phosphorus and tibia ash and tibia calcium decreased significantly with high stoking density. The supplementation of diet with vitamin E and selenium separately or combined, under high stoking density, improved quail body weight gain and feed conversion. Blood calcium and phosphorus levels and tibia calcium and phosphorus contents significantly increased with different diet supplementations compared to the non-supplemented high stoking density control. Vitamin E and selenium supplementations significantly reduced ileum total microbial count and E-coli count. The result revealed that high stoking density impaired quail performance, but vitamin E and selenium and their combination can be used to improve quail performance under intensive production system.

**Keywords:** *Stoking density, quail, vitamin E, selenium, growth performance.*

### INTRODUCTION

Birds under different stress conditions suffer from oxidative stress and consequently the bird performance declines. Harsini *et al.* (2012) reported significant increases in antioxidant enzymes in both blood and skeleton muscles of broilers subjected to cyclic heat stress when the feed was supplemented with a combination of vitamin E and selenium. Attia *et al.* (2017) reported that vitamin E at 100 mg/kg diet alleviate the negative effect of heat stress on body weight gain and dressing percentage of broilers. The improvement in egg production was reported by Biswas *et al.* (2010) when vitamin E was supplemented to the feed of native chicken in India. Sahin *et al.* (2002) reported the improvement of egg production and egg quality in heat stressed quail fed diet supplemented with 250 mg vitamin E/kg.

On the other hand, selenium is one of the important micronutrients in poultry diet that maintains antioxidant defenses when birds are under oxidative stress conditions (Surai, 2002; Fan *et al.*, 2009; Habibian *et al.*, 2015). Oliveira *et al.* (2014) recommended the supplementation of broiler diet by selenium at 0.15 ppm/kg in order to maintain bird normal performance. Broiler viability under heat stress condition increased significantly with selenium supplementation at the level of 0.3 mg/kg diet (Albuquerque *et al.*, 2017). In

quail and during heat stress condition, egg production and antioxidant status improved significantly when diet was supplemented with organic-form selenium (Sahin *et al.*, 2008). Vesco *et al.* (2017) reported a significant effect of selenium supplementation, at 0.33 mg/kg diet, on gene expression of natural antioxidant enzymes in quail reared under heat stress.

A synergistic effect of vitamin E and selenium was reported to improve the natural activities of antioxidant enzymes in the chicken superficial pectoralis muscle (Avanzo *et al.*, 2001). Vitamin E and organic selenium supplementation improve the productivity and immune response of laying hen (Ziaei *et al.*, 2013). Singh *et al.* (2006) also reported high immune response of broilers when feed was supplemented with 200 mg vitamin E/kg and 0.2 mg selenium/kg diet. Sahin *et al.* (2003) recommended a combined supplementation of 250 mg vitamin E and 0.2 mg selenium/kg diet in order to reduce the negative effects of cold stress on quail egg production and quality.

Early studies revealed positive relationship between high stoking density of broilers and profitability (Shanawany, 1988; Cravener *et al.*, 1992). In this concern, significant reduction in body weight gain, feed intake, feed conversion and immune response were common negative effects of high stoking density in broiler chickens (Dozier *et al.*, 2006; Abudabos *et al.*, 2013a; El-Gogary *et al.*, 2015; Qaid *et al.*, 2016; Selvam *et al.*, 2017). Also, egg production was impaired by high stoking density in laying hens (Asghar Saki *et al.*, 2012; Jahanian and Mirfendereski, 2015; Mirfendereski and Jahanian 2015). Nagarajan *et al.* (1991) reported significant improvement in body weight gain, egg production and food conversion with the increase in cage space per laying hen. In Egypt, laying quail showed declines in fertility, hatchability, egg production, egg quality and immune response when reared under high stoking density (143 cm<sup>2</sup>/bird) compared to low stoking density (200 cm<sup>2</sup>/bird) (EL-Tarabany *et al.*, 2015; El-Tarabany, 2016). The various effects of high stoking density on quail meat production are not intensively studied.

The present study was designed to investigate the negative effects of high and low stoking density imposed on quail performance, blood metabolites and ileum bacterial count, and to investigate the potential effects of vitamin E and selenium dietary supplementation on elevating quail performance under high stoking density.

## **MATERIALS AND METHODS**

### ***Management and experimental design:***

A total of 225 unsexed Japanese quail at eight days of age were randomly allocated to five experimental groups (45 chicks each) with three replicates (15 birds each) in each group. Each experimental group was assigned randomly to one of five treatments. Two groups were fed the basal diet (Table 1) and served as the negative and positive stoking density control with 80 and 100 birds/m<sup>2</sup>, respectively. The remaining three groups were fed the basal diet and assigned to one of the following diet supplementations: a diet supplemented with 200 mg vitamin E/kg (Vit E), a diet supplemented with 0.3 mg selenium/kg (Se), or a diet supplemented with 100 mg vitamin E/kg and 0.15 mg selenium/kg (Vit E+Se) with a stoking density of 100 birds/m<sup>2</sup>. Quail were reared in wired cages of 30 × 50 cm<sup>2</sup>. The feed was offered *ad libitum* and fresh water was available at all time.

### ***Production and physiological parameters:***

Body weights were recorded at 8, 21, 35 and 42 days of age and body weight gain was calculated for each group. Feed intake was recorded and feed conversion was calculated for each group.

At the end of the experiment, nine birds from each group were slaughtered and blood samples were collected in heparinized tube. Plasma was separated and stored at -20 °C until further analysis. Plasma total protein, albumin, cholesterol, triglycerides, ALT, AST, calcium and phosphorus were measured using kits (Salucea, Haansberg, Netherlands).

**Table (1): Composition and calculated analysis of the basal diet (%).**

Ingredients	8-42 days
Yellow corn	54.62
Soybean meal	30.88
Meat-bone meal	2.80
Fish meal	9.58
Dicalcium phosphate*	0.96
DL-methionine	0.15
Salt	0.12
Vitamin premix**	0.25
Mineral premix***	0.25
Lysine	0.17
Choline	0.22
Calculated analysis	
Crude protein	23.61
Metabolizable energy, kcal/kg	3066.79
Calcium	0.89
Phosphorus	0.67
L-lysine	1.42
Methionine+cysteine	0.95

\*Contains 24% Ca and 17.5% P.

\*\*Provided per kg of diet: vitamin A, 12,000 IU; vitamin D3, 1,500 IU; vitamin E, 50 IU; vitamin K, 5 mg; vitamin B<sub>1</sub>, 3 mg; vitamin B<sub>2</sub>, 3 mg; pyroxidine, 30 mg; vitamin B<sub>12</sub>, 0.3 mg; pantothenic acid, 12 mg; niacin, 25mg; D biotin, 0.5 mg; folic acid, 1mg; choline chloride, 400 mg.

\*\*\*Provided per kg of diet: iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

Tibia ash percentage determination was performed according to AOAC International (2005), where tibia pieces were collected, defatted, and ashed at 600°C for 16 h to determine ash percentage. Total phosphorus in the samples was determined according to Onyango *et al.* (2003), using a colorimetric method. Briefly, samples were ashed and boiled in acid to dissolve all phosphorus. The concentration of phosphorus in the supernatant was determined using available kits. Ammonium molybdate was added to the supernatant to form phosphomolybdate, which was then reduced to form a blue phosphomolybdenum complex. Color intensity of the complex was proportional to the phosphorus concentration and was determined with a spectrophotometer using absorption at 620 nm. Calcium was determined by flame atomic absorption spectroscopy.

**Microbial count:**

At the end of the experiment, nine birds from each group were slaughtered (3 birds from each replicate) and a part of ileum was taken. Total bacterial count, salmonella, E-coli and clostridium were counted in each ileum part using the procedure of AOAC (2005).

**Statistical analysis:**

Data were statistically analyzed by one-way analysis of variance for treatment effect, using the general linear model (GLM) procedure of SAS (2006). When the model was significant, Duncan's test (Duncan, 1955) was used to separate treatment means at P<0.05.

## RESULTS AND DISCUSSION

### *Production performance:*

The performance of Japanese quail under different stoking densities and fed diet supplemented with vitamin E and selenium are represented in Table (2). High stoking density negatively affected body weight gain (BWG) and feed conversion ratio (FCR) compared to the low stoking density at all periods under study. Similar results were obtained in broiler chickens reared under low and high stoking densities with impaired BWG and FCR in the high stoking density group compared to the low stoking density group (Guardia *et al.*, 2011; Zuowei *et al.*, 2011; Houshmand *et al.*, 2012; Tong *et al.*, 2012; El-Gogary *et al.*, 2015). Feddes *et al.* (2002) reported a reduction in body weight and carcass weight in broilers reared under high stoking density.

On the other hand, different experimental supplementations with vitamin E and selenium had positive effects on BWG, FCR and feed intake but with different magnitude, being more pronounced for Vit E+Se followed by Vit E, Se, the negative control and then the positive control group. On the other hand, feed intake changed significantly, and was the highest for Vit E+Se group and the lowest was for the negative control group at the first and the third periods. The second period showed adverse change in feed intake where the P-Control group had the highest feed intake followed by the Vit E group and Se group and then the Vit E+Se group and finally the N-Control group. Sahin and Kucuk (2001b) reported positive effects of 250 mg vitamin E and 0.2 mg selenium supplementation/kg diet on final body weight, feed intake and feed conversion to heat stressed quail. In the study of Sahin and Kucuk (2001a), vitamin E supplementation showed a beneficial effect on feed intake, final body weight and feed efficiency when was supplemented to the diet of heat stressed quail.

**Table (2): The performance of quail under different stoking densities and fed diet supplemented with vitamin E and selenium.**

Performance	N-Control	P-Control	Vit E	Se	Vit E+Se
Initial weight, g	32.5±0.31	31.4±0.30	32.7±0.32	33.2±0.25	32.8±0.27
Final weight, g	245.6±2.44 <sup>c</sup>	229.4±2.46 <sup>d</sup>	262.8±2.42 <sup>b</sup>	261.4±2.57 <sup>b</sup>	279.7±2.40 <sup>a</sup>
Weight gain, g					
8-21d	80.1±1.40 <sup>d</sup>	72.0±1.60 <sup>e</sup>	92.9±1.43 <sup>b</sup>	89.6±1.57 <sup>c</sup>	101.6±1.44 <sup>a</sup>
22-35d	108.2±0.76 <sup>d</sup>	102.9±2.04 <sup>e</sup>	110.8±2.41 <sup>b</sup>	108.8±2.04 <sup>c</sup>	114.0±2.29 <sup>a</sup>
36-42d	24.80±0.39 <sup>d</sup>	23.07±2.12 <sup>e</sup>	26.44±3.08 <sup>c</sup>	29.80±3.07 <sup>b</sup>	31.22±2.87 <sup>a</sup>
Feed intake, g/d					
8-21d	169.8±1.43 <sup>e</sup>	177.1±1.14 <sup>d</sup>	211.8±0.92 <sup>b</sup>	205.2±0.85 <sup>c</sup>	224.5±0.98 <sup>a</sup>
22-35d	309.5±1.34 <sup>d</sup>	351.9±1.62 <sup>a</sup>	345.7±1.28 <sup>b</sup>	343.8±1.13 <sup>b</sup>	337.4±1.00 <sup>c</sup>
36-42d	84.6±0.82 <sup>c</sup>	87.9±1.75 <sup>c</sup>	92.7±0.88 <sup>b</sup>	105.8±1.26 <sup>a</sup>	106.7±0.85 <sup>a</sup>
Feed conversion					
8-21d	2.12±0.02 <sup>d</sup>	2.46±0.02 <sup>a</sup>	2.28±0.01 <sup>b</sup>	2.29±0.01 <sup>b</sup>	2.21±0.01 <sup>c</sup>
22-35d	2.86±0.01 <sup>d</sup>	3.42±0.02 <sup>a</sup>	3.12±0.01 <sup>b</sup>	3.16±0.01 <sup>b</sup>	2.96±0.01 <sup>c</sup>
36-42d	3.41±0.03 <sup>b</sup>	3.81±0.08 <sup>a</sup>	3.51±0.03 <sup>b</sup>	3.55±0.041 <sup>b</sup>	3.42±0.03 <sup>b</sup>

*N-Control: negative control, P-Control: positive control.*

*Means within the same raw having different superscripts differ (P<0.05).*

### *Blood constituents and tibia composition:*

Blood constituents and tibia ash composition are presented in Table (3). Plasma total protein, albumin, ALT and AST were not differed in different treatments or stoking densities. Although there was a noticeable change in liver enzymes which decreased and plasma total protein and albumin levels which increased due to the supplementation of feed with vitamin E and selenium compared to the negative and the positive control

groups. Abudabos *et al.* (2013a) reported no effect of different stoking densities on broiler blood total protein but AST increased significantly in high stoking density.

**Table (3): Blood plasma constituents and tibia ash, calcium and phosphorus of Japanese quail under different stoking density and fed diet supplemented with vitamin E and selenium.**

Parameter	N-Control	P-Control	Vit E	Se	Vit E+Se
TP, g/dl	5.23±0.19	5.25±0.15	5.62±0.15	5.59±0.15	5.66±0.15
Alb, g/dl	2.64±0.14	2.67±0.12	2.91±0.12	2.89±0.12	2.98±0.12
Chole, mg/dl	147.2±2.53 <sup>ab</sup>	149.3±2.49 <sup>a</sup>	137.4±2.89 <sup>c</sup>	139.2±2.84 <sup>bc</sup>	136.3±3.53 <sup>c</sup>
TL, mg/dl	269.5±5.59 <sup>a</sup>	265.4±5.58 <sup>a</sup>	231.7±4.03 <sup>b</sup>	230.6±4.12 <sup>b</sup>	229.5±4.27 <sup>b</sup>
ALT, U/l	23.58±1.14	23.64±1.03	21.82±1.06	22.04±1.15	21.72±1.06
AST, U/l	34.33±0.92	34.42±0.97	32.47±0.89	32.51±0.84	32.39±0.65
Ca, mg/dl	8.46±0.22 <sup>a</sup>	7.41±0.27 <sup>b</sup>	8.59±0.28 <sup>a</sup>	8.48±0.26 <sup>a</sup>	8.76±0.25 <sup>a</sup>
P, mg/dl	4.96±0.13 <sup>a</sup>	4.32±0.14 <sup>b</sup>	4.88±0.17 <sup>a</sup>	4.76±0.13 <sup>a</sup>	4.95±0.15 <sup>a</sup>
Tibia ash, %	45.34±0.47 <sup>c</sup>	43.64±0.42 <sup>d</sup>	49.53±0.42 <sup>b</sup>	48.63±0.35 <sup>b</sup>	51.22±0.43 <sup>a</sup>
Tibia Ca, %	12.87±0.59 <sup>a</sup>	10.74±0.56 <sup>b</sup>	13.83±0.44 <sup>a</sup>	13.62±0.31 <sup>a</sup>	14.00±0.25 <sup>a</sup>
Tibia P, %	8.21±0.39 <sup>b</sup>	7.46±0.36 <sup>b</sup>	10.00±0.39 <sup>a</sup>	9.64±0.36 <sup>a</sup>	10.16±0.28 <sup>a</sup>

*N-Control: negative control, P-Control: positive control, TP: total protein, Alb: albumin, Chole: cholesterol, TL: total lipid, Ca: calcium, P: phosphorus. Tibia ash, Ca and P are calculated as a percentage of tibia dry matter.*

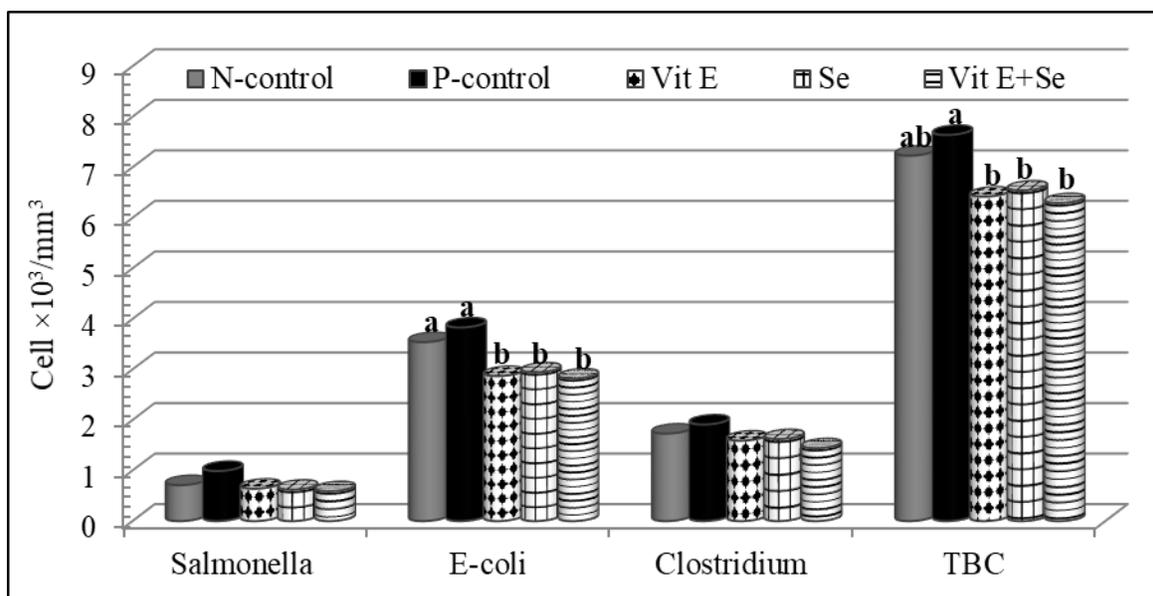
*Means within the same raw having different superscripts differ (P<0.05).*

Vitamin E supplementation decreased plasma cholesterol level compared to the negative and positive control groups. Plasma total lipid decreased due to different supplementation treatments. Plasma calcium and phosphorus levels also were significantly increased due to different supplementations compared to positive control group. Houshmand *et al.* (2012) found no effect of different stoking densities on blood cholesterol level for broiler chickens. Also, serum calcium tended to decrease in laying hens under high stoking rate compared to under the low stoking rate (Kang *et al.*, 2016). The abdominal fat relative weight in broilers reared under cyclic heat stress decreased linearly when selenium was supplemented at the level of 0.1 mg/kg and vitamin E supplementation level increased from 300 to 500 mg/kg diet (Albuquerque *et al.*, 2017). This result suggests a modulation in lipid metabolism which could be the reason for the reduction in plasma total lipid and cholesterol observed with these supplementations.

Tibia ash and calcium percentage significantly decreased in the high stoking density control (P-Control) compared to the low stoking density control (N-Control). Vitamin E and selenium supplementations significantly increased tibia ash and phosphorus percentages compared to the P-Control group. Tibia calcium percentage was significantly the highest in the positive control group and was not differed in the different supplementation groups compared to N-Control group. The availability of calcium and phosphorus in the blood pool observed in supplemented groups may explain the significant differences in tibia calcium and phosphorus percentages. Kang *et al.* (2016) reported significant reduction of bone mineral densities in laying hens under high stoking density.

**Microbial count:**

The results of microbial counts in the ileum reveal that counts of salmonella and clostridium microbes did not differ among different groups (Fig. 1). Vitamin E and selenium supplementations and their combination reduced the total bacterial count (TBC) compared to P-Control group and E-coli count compared to N-Control and P-Control groups. The changes in the digestive microbial counts in birds under different stoking densities have been reported by Guardia *et al.* (2011). They suggested that the changes in the digestive microbiota in high stoking density chickens may be involved in the consequent growth reduction observed. Abudabos *et al.* (2013b) reported significant increases in ileal Clostridium (*C. perfringens*) and gram-negative Bacilli counts in the broilers under the high stoking density compared to those under the low stoking density.



**Fig. (1):** Different ileum bacterial counts and total bacterial count (TBC) of Japanese quail under different stoking densities and fed diet supplemented with vitamin E and selenium.

## CONCLUSION

The results revealed that high stoking density impaired quail production performance. Vitamin E and selenium and their combination supplementation to quail diet can alleviate the negative effects of stoking density and improve the production performance, plasma calcium and phosphorus levels and tibia bone calcium and phosphorus contents. Vitamin E and selenium supplementation can be a good management practice to improve quail performance under high stoking conditions.

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### إضافة فيتامين هـ والسيلينيوم إلى العليقة تعمل على تخفيف الآثار السلبية لزيادة الكثافة في السمان الياباني النامي

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تعتبر زيادة الكثافة أمر أساسى تحت نظم الإنتاج المكثف فى الدواجن. صممت التجربة لدراسة تأثير زيادة الكثافة على النمو ومكونات الدم وميكروبات الأمعاء وقدرة فيتامين هـ والسيلينيوم على تخفيف الآثار السلبية لزيادة الكثافة على أداء السمان. تم استخدام عدد 225 طائر من السمان الياباني على عمر 8 أيام وتم قُسمت عشوائياً على خمس مجموعات تجريبية وتم تقسيم كل مجموعة إلى ثلاث مكررات. شكلت المجموعات على أن تكون المجموعة الأولى منخفضة الكثافة (80 طائر/متر<sup>2</sup>)، والمجموعة الثانية مرتفعة الكثافة (100 طائر/متر<sup>2</sup>) والثلاث مجموعات المتبقية كانت ذات كثافة عالية (100 طائر/متر<sup>2</sup>) مع إضافة أما فيتامين هـ بواقع 200 ملجم/كجم عليقة أو إضافة السيلينيوم بواقع 0,3 ملجم/كجم عليقة أو إضافة كلاً من 100 ملجم فيتامين هـ و0,15 ملجم سيلينيوم/كجم عليقة. أدت زيادة الكثافة إلى انخفاض وزن الجسم وكفاءة تحويل الغذاء مقارنة بالكثافة المنخفضة. بينما إنخفضت نسبة كالسيوم وفسفور الدم ونسبة الرماد والكالسيوم في عظمة الفخذ معنوياً مع زيادة الكثافة. أدت إضافة فيتامين هـ والسيلينيوم للعليقة كلاً على حدا أو مجتمعان على تحسين زيادة الوزن وكفاءة تحويل الغذاء تحت ظروف زيادة الكثافة كما أرتفع كل من مستوى الكالسيوم والفسفور فى الدم ونسبة الكالسيوم والفسفور فى عظمة الفخذ نتيجة للإضافات المختلفة مقارنة بمجموعة الكثافة العالية. كما أدت إضافة فيتامين هـ والسيلينيوم إلى خفض العدد الكلى للميكروبات وعدد بكتريا الإيكولاى فى الأمعاء. أظهرت النتائج أن زيادة الكثافة أدت إلى خفض الأداء الإنتاجى للسمان وأن إضافة كل من فيتامين هـ والسيلينيوم يمكن أن يستخدم لتحسين أداء السمان تحت ظروف الإنتاج المكثف.