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# EFFICACY OF BENZOIC ACID IN IMPROVING THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF SINAI CHICKENS

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**ABSTRACT:** An experiment was conducted to assess the influence of dietary benzoic acid supplementation on productive performance, egg hatchability, some blood parameters, intestinal Enterobacteriacea count, jejunum intestinal morphology and nitrogen retention for Sinai chickens. Sinai chickens as Egyptian breed were fed diets supplemented with different concentrations of benzoic acid consisting of 0 mg/kg diet (T1) and considered as control, 500 mg/kg diet (T2), 1000 mg/kg diet (T3), 1500 mg/kg diet (T4) and 2000mg/kg diet (T5). Egg number, egg mass and egg production percentage for chicken groups supplemented with 1500 and 2000 mg benzoic acid /kg diet, respectively were significantly improved compared to the rest of the experimental groups**.** Also, supplementing the diets with benzoic acid significantly improved feed conversion ratio for T5 group followed by those of T4 and T3 groups compared with the others. All doses of benzoic acid supplementation significantly improved eggshell strength and eggshell Ca concentration compared to those for control. Supplementing the diet with 2000mg/kg significantly improved the villus height and its surface area compared with control. Also experimental doses of 1500 and 2000mg benzoic acid/kg diet significantly reduced the total count of gram negative bacteria in the jejunum. Moreover, the birds fed diets supplemented with all levels of benzoic acid except that of 500 mg recorded the lowest ($P<0.0001$) value of N excretion (g/day). Supplementing the diet with concentration of 2000mg benzoic acid (T5) significantly improved hatchability of fertile eggs percentage and hatched chick weight. It is concluded that supplementing the chicken diet with doses of 1500 or 2000 mg/kg diet benzoic acid had beneficial influence on egg quality, egg production%, jejunum intestinal morphology, hatchability% and loading reduction of harmful bacteria in the intestine.

**Key words:** Benzoic acid, laying hens, production, reproduction, bacterial count

## INDRODUCTION

The effective utilization of organic acid and other alternatives have been used instead of antibiotics to establish appropriate production parameters and health of birds. Organic acids are defined as carboxylic acids including fatty acids with specific chemical characters (Shahidi et al.,2014). Benzoic acid is an organic acidifier, colorless crystalline solid and aromatic carboxylic acid (Sim et al., 1955). Organic acid has antibacterial properties and vital role in the improvement of intestinal digestibility and mineral utilization (Kim et al., 2005). Several studies have reported the potential ability of improving nutritional digestion improvement and retention, intestinal health and ultimate growth development of non-ruminant animals (Luise et al., 2017). The mode of action for organic acid may be attributed to different factors such as mineral chelation and stimulation on intermediary metabolism, inhibition the pathogenic microbes, and facilitation of proper digestion due to lowering intestine PH (De Lange et al., 2010). In addition, Rychen et al. (2018) reported that using benzoic acid with concentration of 500mg/Kg diet is safe for laying hens. Also, Gong et al. (2021) mentioned that there are few studies on the effect of addition benzoic acid to the laying hens diets especially with high levels

The present study aimed to determine the effect of dietary benzoic acid as a member of organic acid group on productive performance, egg hatchability, some blood parameters, intestinal Enterobacteriacea count, jejunum intestinal morphology and nitrogen retention for Sinai chickens.

## MATERIALS AND METHODS

### Experimental Design

This study conducted at EL- Serw poultry Research Station, Animal poultry Research Institute, Agriculture Research center, Ministry of Agriculture, Egypt. Four thousand and five hundred Sinai chickens aged 42- wk were randomly allocated into 5 experimental groups. The basal diet of these groups were supplemented with 0 mg/kg diet(T1) and considered as control, 500 mg/kg diet (T2), 1000 mg/kg diet (T3), 1500mg/kg diet (T4) and 2000mg/kg diet (T5) of benzoic acid. The basal diets were prepared according to feed composition of Animal and Poultry Feed stuffs in Egypt (2001) as shown in Table 1. The birds were fed a conventional layer mash. Feed and water were provided for chickens *ad-libitium.* throughout four months of the experimental period and the birds were subjected to 16-hr light:8-hr dark cycle. Each treatment group included ten birds (9 hens and 1 male) had been raised in floor pens and replicated nine times.

### Productive and Egg Quality Parameters

Daily egg production (%), egg weight (g) and egg mass (g egg/hen/day) were recorded for each group. Daily feed intake (g/hen/day) and feed conversion ratio (g feed/g egg) were detected. Three eggs from each replicate were randomly chosen to determine egg quality traits. Eggs were individually weighed to nearest 0.1g, egg shape index was determined as the percentage of the greatest width to the greatest length. Eggshell strength was done with Digital Force Gauge-FGC-50 at Agricultural Engineering Research Institute Egypt as described by Bennett et al. (1988). Also the shells of broken eggs were weighed without shell membranes to the nearest 0.1g then eggshell weight percentages were calculated. Eggshell thickness was measured without membranes by micrometer to the nearest 0.01mm. Egg yolk color was determined by Roche yolk color fan. Albumen height and yolk index were detected. The quantities of crude ash of eggshells were determined by burning for one night in ash oven and analyzed for calcium (Ca) % using atomic absorption.

**Total Enterobacteriacea Count**

At the end of the experimental period, three hens from each replicate were randomly selected and slaughtered to determine the total Enterobacteriacea count in the jejunum of the small intestine according to the method of Clench and Mathias (1995).

**Biochemical Parameters.**

Blood samples were collected from the same previous slaughtered hens at the end of experiment. Plasma was obtained by centrifugation of blood at 3000 rpm for 20 minutes, and subsequently stored at -20 Ċ for biochemical analyses. Plasma total protein concentration (g/dI) was measured using Biuret method as described by Armstrong and Carr (1964). Albumin concentration (g/dI) was estimated colorimetrically and globulin concentration (g/dI) was calculated. Plasma total cholesterol concentration (mg/dI) was determined according to the method of Bogin and Keller (1987). Plasma glucose concentration (mg/dI) was detected according to the method of Donaldson and Christensen 1991. Plasma uric acid and creatinine concentration (mg/dL) were determined according to the method of Kumar and Kumbhakar (2015)

**Electron Microscopy Scanning**

At the end of experiment, small pieces measured 2 cm from fresh specimens of the jejunum for the same slaughtered birds were removed and prepared for measuring villus height (V)(µm)and crypt depth (C)(µm). Scanning of small intestine by electron microscope was carried out at Faculty of Science, Alexandria University. Villus height to crypt depth ratio (V/C) was detected. Also, apparent surface area was determined according to the method of Ruttanavut and Yamauchi (2010). Average measures for six villi having a lamina were randomly selected for scanning. Villus height was measured from the tip of the villus to top of lamina propria, whereas, villus width was measured as the basal and apical ends excluding the crypt. Apparent villus surface area ($mm^2$) was calculated using the formula [(2π)×(villus width/2)×(villus height)] (Sakamoto et al., 2000).

**Nitrogen Retention**:

At 48 wk of age, 15 males were selected on the basis of the average body weight (one male per replicate). Birds were individually housed in metabolic cages (60cm long, 50 cm width and 60cm height) and fed their respective experimental diets for a period of two days for adaptation the birds in cages. Then the excreta were quantitatively collected for three days and feed intake was also recorded. Any feathers or foreign debris were removed out, then, the excreta were dried in a forced oven at 65Ċ for 24 hours. Finally, the excreta were ground well and stored in plastic bags. The proximate analysis of experimental diet and the excreta were carried out according to the official methods (A.O.A.C., 1990).

**Hatchability Performance**

One thousand hatching eggs produced from Sinai hens representing the five experimental groups were incubated in Egyptian made incubator at 37.5Ċ and 55%RH during setting phase of incubation and egg trays were randomly distributed in the incubator. At $18^{th}$ day of incubation, the eggs were candled and those with evidence of living embryos were transferred to hatcher and incubated at 37Ċ and 70%RH. Hatchability of fertile eggs% was determined. Eggs that failed to hatch at the end of incubation and having full opportunity for hatching were broken out and then examined with naked eye to estimate embryonic mortality percentage. Body weights (g) for all hatched chicks at the time of removal from the hatcher were recorded and termed as chick weight at pull out.

**Statistical Analysis**

Data were statistically analyzed according to one-way ANOVA implemented in SAS program (SAS, 2016) using GIM procedure and mean differences were tested by Tukey's test and used for comparisons at P≤0.5 significant level.

The following model was used:

$Y_{ij} = \mu + H_i + e_{ij}$

$Y_{ij}$ = observed traits, μ = the overall mean, $H_i$ = the effect of dietary benzoic acid and $e_{ij}$ = random error

## RESULTS AND DISCUSSIONS

**Productive Performance**

Table 2 represents the influence of dietary benzoic acid on some traits of productive performance for Sinai chickens. Egg number, egg mass and egg production percentage for groups supplemented with 1500 and 2000mg benzoic acid/kg diet namely T4 and T5, respectively were significantly improved compared to the rest experimental groups included control group which represented the worst results of the previous mentioned parameters. Also, egg weight was increased (P≤0.01) for T5 group compared with those for T1 and T2 groups with significant different with T3 and T4 groups. Moreover, birds of T3 and T4 groups consumed (P≤0.0001) less amounts of feed compared to those of the other groups. Also, supplementing the diets with benzoic acid significantly improved (P≤0.0001) feed conversion ratio for T5 group compared with the others followed by those of T4 and T3 groups compared with the others. Discrepancies were found in the published data regarding the influence of the organic acids on laying performance and little information was found on benzoic acid supplementation. Supporting to our results referring to the improvement of production traits such as egg number, egg mass, egg production and feed conversion rate, Grashorn et al. (2013) and Youssef et al. (2013) reported that supplementation of encapsulated organic acid in laying diet improved egg production and feed conversion compared to control. Gong et al. (2021) worked on laying diet with benzoic acid with 1000 mg and 2000mg found improvement of laying rate % and feed conversion ratio with non-statistical change with control, while feed intake did not represent any valuable change. Benzoic acid as an acidifier could activate the digestive enzymes through decreasing the PH value in gastrointestinal tract and promote the production performance (Mao et al., 2019). Most of the published researches for benzoic acid were performed on the broilers and they found improvement of body weight and feed conversion rate (Amaechi and Anueyiagu,2012).

**Egg Quality**

Some egg quality traits for Sinai chickens supplemented with benzoic acid are shown in Table 3. Highest egg shape index is observed for group T1 (control) compared to the other experimental groups with statistical increase compared to those forT5. Apparently, all doses of benzoic acid supplementation significantly improved eggshell strength compared to control. Also, the significant increase of shell thickness was detected for eggs of T5 group compared with T1 and T2 groups with numerical increase with T3 and T4 groups. Moreover, T4 and T5 groups represented significant increase of eggshell weight compared with T1. Supplementing the diet with 2000mg benzoic acid/kg diet significantly increased albumen height compared to control and with non-statistical change compared to T2, T3 and T4 groups. Also, yolk color for eggs produced from birds of T5 group was significantly increased compared to those for T1 and T2 groups. Additionally, yolk index was increased ($P\leq0.0001$) for T4 and T5 compared with T1 and T2. Moreover, all studied concentrations of benzoic acid significantly ($P\leq0.0001$) increased Ca concentration in eggshell compared to control group. Little information was published in this regard as Gong et al. (2021) mentioned that benzoic acid levels of 1000 and 2000 mg/kg diet significantly improved egg quality and they added that role of acting benzoic and organic acids in not clear. Also, Dhawale (2005) indicated that organic acid in Ca salt form may provide some extra improvement of mineral absorption for shell formation. In addition, Soltan (2008) indicted that the improvement in eggshell quality was connected with the increase of Ca concentration in serum which attributed to the beneficial effect of Ca absorption. Moreover, Klug et al. (2010) stated that benzoic acid improved the digestibility of Ca and P in swan and this mechanism is related to reduction of intestinal PH, which leads to an increase of digestive enzymes activity and solubility of minerals. It has also been proposed that organic acids improved Ca availability by chelating Ca and reducing the formation of insoluble Ca-phytate complexes (Boling et al.,2000). Finally, Khan and Iobal (2015) mentioned that organic acid had a beneficial effect on calcium digestibility and protein absorption in layers as the organic acids lower the diet acidity and PH thus increase the solubility of all minerals, thereby increase calcium retention and shell mineralization and the improvement in egg shell quality is coincided with shell weight and thickness. Also Zhang et al. (2022) revealed that benzoic acid might relieve the stress leading to egg quality improvement.

**Jejunum ntestinal Morphology**

Table 4 illustrates the effect of benzoic acid supplementation on jejunum morphology of Sinai chickens. Supplementation the diet of Sinai chickens with 2000mg benzoic acid /kg improved ($P\leq0.0001$) the villus height (V) compared with those for control and numerically lengthen than those for T2, T3 and T4 groups. Opposite trend was observed for crypt depth (C) as it significantly decreased for birds of T3, T4 and T5 groups compared with control. Accordingly, V/C ratio was significantly increased for T4 and T5 birds compared with control. Moreover, highest dose of benzoic acid (2000mg/kg) recorded highest surface area ($P\leq0.0001$) of the apparent villus with descending order with the other experimental doses compared to control.

The increase of jejunum villus height and decrease of crypt depth due to benzoic acid supplementations in the current results is in accordance with those previously reported by Giannenas and Papaneophytou (2014) who mentioned that benzoic acid had positive effect on gut mucosal architecture. Also, Samanta et al. (2010) reported that villus length linearly increased with the increase of dietary organic acid dose. Previously, Gong et al. (2021) reported that benzoic acid had the potential influence to improve growth and intestinal health. Moreover, Pham et al. (2022) reported that dietary benzoic acid supplementation improved the intestinal pathology score and crypt depth. The observed improvement of intestinal morphology due supplementing the diet with benzoic acid is supported by Senkoylu et al.

(2007) who detected the same effect of organic acids on broilers.

**Total Enterobacteriacea Count**

Data of Fig. 1 illustrate the effect of benzoic acid supplementation on total Enterobacteriaceae count in jejunum of Sinai chickens. Results revealed that experimental doses of 1500 and 2000mg benzoic acid significantly (P ≤0.05) reduced the total count of gram negative bacteria in the jejunum namely Klebsiella, Shigella, Salmonella, E.Coli, Citrobacter, Enterobacter, Edwarsiella, Proteus and Yersina composing Enterobacteriaceae count compared to those for the rest experimental doses and control. These results are coincided with those previously reported by Pelicano et al. (2005) who mentioned that organic salts reduced intestinal Enterobacteriacea and infectious process, thereby decreased inflammatory of intestinal mucosa, thus improved villus height and secretion function and consequently the digestion of nutrient absorption. Also, Gheisari et al. (2007) found that supplementation of organic acids to the diet might improve the proliferation of useful microflora and diminish the population of harmful bacteria in poultry gut. The principle key of the mode of action for benzoic as organic acid is its ability to penetrate the bacterial cell wall and disrupt the normal physiology of certain bacterial types due to the sensitivity towards the wide internal and external PH gradient as documented by Khan and Iobal (2015). Moreover, Van-Immerseel et al. (2006) reported that organic acids have the ability to change form un- dissociated to the dissociated form depending on the environmental PH, which enhance their antimicrobial effect. The results of laying rate improvement as shown in Table 2 and villus height increase with apparent surface area (Table 4) and diminishing the load of pathogenic bacteria could have related to the ability of benzoic acid supplementation for improving the physiological and health status of Sinai chickens.

**Biochemical Parameters.**

Data of Table 5 show the effect of benzoic acid concentration on some plasma biochemical traits for Sinai chickens. All concentrations of benzoic acid had no significant influence on plasma albumin, uric acid, creatinine, cholesterol and glucose. Experimental concentrations of benzoic acid for T3, T4 and T5 groups represented higher (P≤0.05) plasma total protein and globulin levels compared to T2 and control groups. These results are in harmony with those reported by Zhang et al. (2022) who found that benzoic acid had no effect on some biochemical traits. Also, Rathnayake et al. (2021) reported that birds fed diets supplemented with organic acid had higher level of plasma globulin and total protein besides they added that the improvement of birds immunity could be related to the inhibitory effect of organic acids on the pathogens in gut system. Moreover, Partanen and Mroz (1999) mentioned that organic acids have strong antimicrobial activities and serve as substrates in the intermediate metabolism and improve digestion, absorption and retention of numerous dietary nutrients.

**Nitrogen Retention**:

Results in Table 6 illustrated that no significant alternations were detected in nitrogen (N) intake (g/day) among control and the dietary treatments due to using any of dietary levels of benzoic acid, but N intake was numerically lowered in the diet supplemented with 2000 mg benzoic acid/kg diet compared to control group by about 10.72%. On the other hand, the birds fed diets supplemented with all levels of benzoic acid except that of 500 mg/kg diet recorded the lowest

($P<0.0001$) value of N excretion (g/day). In addition, the opposite trend was observed in respect of N retention % where, different levels of benzoic acid (1000, 1500 and 2000 mg/kg diet) resulted in a significant increase in N retention compared to the control diet. This effect in current study is apparently caused by a repartitioning of nitrogen excretion besides lowering N excretion and higher N retention from the birds fed diets supplemented with 1000, 1500 and 2000 mg benzoic acid/kg diet. This notion is expected due to lowering $NH_3$ emission as previously documented by Liang et al. (2005). Therefore, benzoic acid supplementation may be feasible option to mitigate $NH_3$ emission in the laying hens operation.

**Hatchability Performance**

Results of hatchability of fertile eggs%, total embryonic mortality % and hatched chick weight (g) as affected by dietary benzoic acid concentrations are shown in Table 7. Supplementing the diet with concentration of 2000mg benzoic acid (T5) significantly ($P\leq0.05$) improved hatchability of fertile eggs % and hatched chick weight (g) compared with those for T1 and T2 besides numerical improvement compared with T3 and T4 groups. Opposite trend was observed for embryonic mortality percentage as it decreased for T5 group compared to other groups. Published researches on the direct effect of benzoic acid on hatching traits of laying chickens are lacking but Yusuf et al. (2015) showed that hatchability% of fertile eggs and chick weight were higher for Japanese quail fed diet supplemented with mixture of benzoic and citric acid than the control. Furthermore, Toosi et al. (2016) indicated that including mixture of organic acid in broiler breeder diet increased hatchability and decreased embryonic mortality. Results herein for hatchability % and chick weight improvements besides embryonic mortality% diminish due to benzoic acid supplementation could be explained on the light of quality improvement (Table 3) and diminishing of bacteria loading (Fig. 1).

The conflicting results in the literature due to using organic acids in the diet could be related to the form and levels of these acids besides the chicken strains or environmental conditions and physiological response.

## CONCLUSION.

Supplementing of benzoic acid to chicken diet especially with doses of 1500 and 2000 mg/ Kg diet has beneficial effects on egg production, egg quality and hatchability %. In addition, benzoic acid plays an important role in improving the jejunum intestinal morphology and reducing harmful bacteria in the intestine and nitrogen excretion. Therefore, it is necessary to incorporate this promising supplement into chicken diet to obtain many positive impacts on performance and health status.

**Table (1):** Ingredients and calculated analysis of the layer diet

| **Ingredients** | **%** |
|---|---|
| Yellow corn | 64.00 |
| Soy bean meal (44 %) | 22.50 |
| Corn gluten (60%) | 1.58 |
| Wheat bran | 1.68 |
| Di-calcium phosphate | 1.40 |
| Limestone | 8.14 |
| premix [1] | 0.30 |
| Sodium chloride | 0.30 |
| DL- Methionine (99%) | 0.10 |
| Total | 100 |
| Calculated Analysis [2] | |
| Crude protein % | 16.10 |
| ME ( Kcal / kg ) | 2730 |
| Crude fiber % | 3.30 |
| Ether extract % | 2.87 |
| Calcium (%) | 3.43 |
| Av. Phosphorus (%) | 0.39 |
| Methionine % | 0.40 |
| Lysine | 0.84 |
| Methionine + Cystine % | 0.68 |

1-Each 3 kg of vitamins and Minerals premix contains 100 million IU vitamin A; 2 million IU Vit.$D_3$;10 g vitamin E; 1 g Vit.$K_3$; 1 g vitamin$B_1$; 5 g vitamin $B_2$ ;10 mg vitamin $B_{12}$; 1.5 g vitamin $B_6$; 30 g Niacin ;10 g Pantothenic acid ;1g Folic acid;50 mg Biotin; 300 g Choline chloride; 50 g Zinc; 4 g Copper; 0.3 g Iodine; 30 g Iron; 0.1 g Selenium; 60g Manganese ;0.1 g Cobalt; and carrier $CaCO_3$ to 3000 g. 2- According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

**Table (2): Effect** of dietary benzoic acid concentration on productive performance of Sinai chickens

| Items | Benzoic acid (mg/kg diet) | | | | | P- value |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | |
| Egg weight (g) | 48.18 ± 0.07$^{c}$ | 48.71 ± 0.15$^{bc}$ | 50.63 ± 0.31$^{ab}$ | 50.63 ± 0.31$^{ab}$ | 51.18 ± 1.07$^{a}$ | <0.0145 |
| Egg number | 75.40 ± 0.26$^{d}$ | 82.69 ± 0.42$^{cd}$ | 93.43 ± 0.99$^{c}$ | 106.54 ± 4.68$^{b}$ | 131.88 ± 4.30$^{a}$ | <0.000 |
| Egg mass (g/bird/ day) | 30.27± 0.19$^{d}$ | 35.23 ± 0.67$^{c}$ | 39.40 ± 0.63$^{c}$ | 44.90 ± 1.82$^{b}$ | 53.46 ± 1.59$^{a}$ | <0.000 |
| Egg production (%) | 51.30 ± 0.21$^{d}$ | 54.74 ± 0.28$^{c}$ | 59.50 ± 0.57$^{b}$ | 61.60 ± 0.44$^{a}$ | 61.99 ± 0.36$^{a}$ | <0.000 |
| Feed consumption (g/bird/day) | 113.04± 0.26$^{a}$ | 111.42 ± 0.44$^{b}$ | 110.09 ± 0.21$^{c}$ | 109.00 ± 0.23$^{c}$ | 113.19 ± 0.28$^{a}$ | <0.000 |
| Feed conversion ratio (g feed/ g egg mass) | 3.73 ± 0.07$^{a}$ | 3.17 ± 0.04$^{b}$ | 2.80 ± 0.04$^{c}$ | 2.46 ± 0.08$^{d}$ | 2.13 ± 0.04$^{e}$ | <0.000 |

a,b,c,d and e means having different letters in the same row are significantly different (P≤0.05)

**Table (3):** Effect of dietary benzoic acid concentration on some egg quality traits for Sinai chickens

| Items | Benzoic acid (mg/kg diet) | | | | | *P-value* |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | |
| Egg shape index | 82.18 ± 1.96$^{a}$ | 80.24 ± 0.22$^{ab}$ | 79.62 ± 1.24$^{ab}$ | 79.19 ± 0.33$^{ab}$ | 75.86 ± 0.99$^{b}$ | 0.006 |
| Egg shell strength (g/cm$^2$ ) | 3315.75 ± 10.09$^{b}$ | 4367.50 ± 5.83$^{a}$ | 4379.50 ± 8.11$^{a}$ | 4476.50 ± 13.47$^{a}$ | 4525.75 ± 1.75$^{a}$ | <0.000 |
| Egg shell thickness (mm) | 0.33 ± 0.04$^{b}$ | 0.34 ± 0.52$^{b}$ | 0.34 ± 0.29$^{ab}$ | 0.53 ± 1.30$^{ab}$ | 0.36 ± 1.67$^{a}$ | 0.002 |
| Egg shell weight (g) | 5.67 ± 0.18$^{b}$ | 6.17 ± 0.14$^{ab}$ | 6.30 ± 0.15$^{ab}$ | 6.40 ±0.11$^{a}$ | 6.79 ± 0.22$^{a}$ | 0.004 |
| Albumen height (mm) | 5.39 ±0.43$^{b}$ | 6.14 ± 0.45$^{ab}$ | 6.36 ± 0.51$^{ab}$ | 6.53 ± 0.33$^{ab}$ | 7.02 ± 0.54$^{a}$ | <0.000 |
| Yolk color | 6.50 ± 0.23$^{b}$ | 6.67 ± 0.28$^{b}$ | 7.50 ± 0.23$^{ab}$ | 7.67 ± 0.32$^{ab}$ | 8.00 ± 0.34$^{a}$ | 0.003 |
| Yolk index | 0.46 ± 0.007$^{c}$ | 0.48 ± 0.003$^{bc}$ | 0.49 ± 0.006$^{ab}$ | 0.50 ± 0.003$^{a}$ | 0.51 ± 0.010$^{a}$ | <0.000 |
| Calcium eggshell (mg/g) | 35.46±0.15$^{d}$ | 36.32±0.30$^{c}$ | 38.16±0.17$^{b}$ | 38.35±0.11$^{ab}$ | 38.72±0.3$^{a}$ | <0.000 |

a, b,c and d means having different letters in the same row are significantly different (P≤0.05)

**Table (4): Effect** of benzoic acid supplementation on jejunum morphology of Sinai chickens

| Items | Benzoic acid (mg/kg diet) | | | | | P-value |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | |
| Villus height (Mm)(V) | 1037.00 ± 6.70[b] | 1067.00 ± 13.05[ab] | 1075.33 ± 9.47[ab] | 1096 ± 27.71[ab] | 1109.00 ± 28.78[a] | <0.000 |
| Crypt depth (Mm)(C) | 146.80 ± 2.14[a] | 122.90 ± 6.45[ab] | 120.30 ± 5.03[b] | 116.64 ± 12.50[b] | 110.83 ± 3.93[b] | <0.000 |
| V/C ratio | 7.07 ± 0.08[b] | 8.87 ± 0.45[ab] | 9.09± 0.42[ab] | 10.25 ± 1.60[a] | 10.11 ± 0.06[a] | <0.000 |
| Apparent villus area $mm^2$ | 0.87 ± 0.03[c] | 0.97 ± 0.01[c] | 1.78 ± 0.02[b] | 1.88 ± 0.04[b] | 2.05 ± 0.03[a] | <0.000 |

a, b and c means having different letters in the same row are significantly different ($P \leq 0.05$)

**Table (5 ) :**Effect of benzoic acid concentration on some serum biochemical traits for Sinai chickens

| Items | Benzoic acid (mg/kg diet) | | | | | |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | ***P-value*** |
| Total protein (g/dl) | 5.83 ± 0.01[b] | 5.99 ± 0.11[b] | 6.45 ± 0.18[a] | 6.34 ± 0.09[a] | 6.51 ± 0.26[a] | 0.010 |
| Albumin (g/dl) | 2.76 ± 0.01 | 2.78 ± 0.09 | 2.75 ± 0.06 | 2.68 ± 0.05 | 2.68 ± 0.02 | 0.246 |
| Globulin (g/dl) | 3.03 ± 0.02[b] | 3.22 ± 0.02[b] | 3.64 ± 0.18[a] | 3.80 ± 0.16[a] | 3.85 ± 0.04[a] | <0.000 |
| Uric acid (mg/dl) | 2.96 ± 0.06 | 2.76 ± 0.29 | 2.81 ± 0.11 | 2.72 ± 0.02 | 2.87 ± 0.08 | 0.426 |
| Cholesterol (mg/dl) | 133.33± 0.59 | 135.78 ± 0.26 | 133.77 ± 0.84 | 135.82 ± 1.44 | 135.76 ± 0.27 | 0.406 |
| Glucose (mg/dl) | 168.36± 0.57 | 167.85 ± 0.81 | 166.90 ± 2.46 | 166.47 ± 1.67 | 167.42 ± 1.06 | 0.246 |
| Creatinine (mg/dl) | 8.26 ± 0.45 | 8.18 ± 0.61 | 8.77 ± 1.07 | 9.21 ± 0.46 | 8.77 ± 0.84 | 0.248 |

a and b means having different letters in the same row are significantly different ($P \leq 0.05$)

**Table (6):** Effect of benzoic acid concentration on nitrogen intake, nitrogen excretion and nitrogen retention

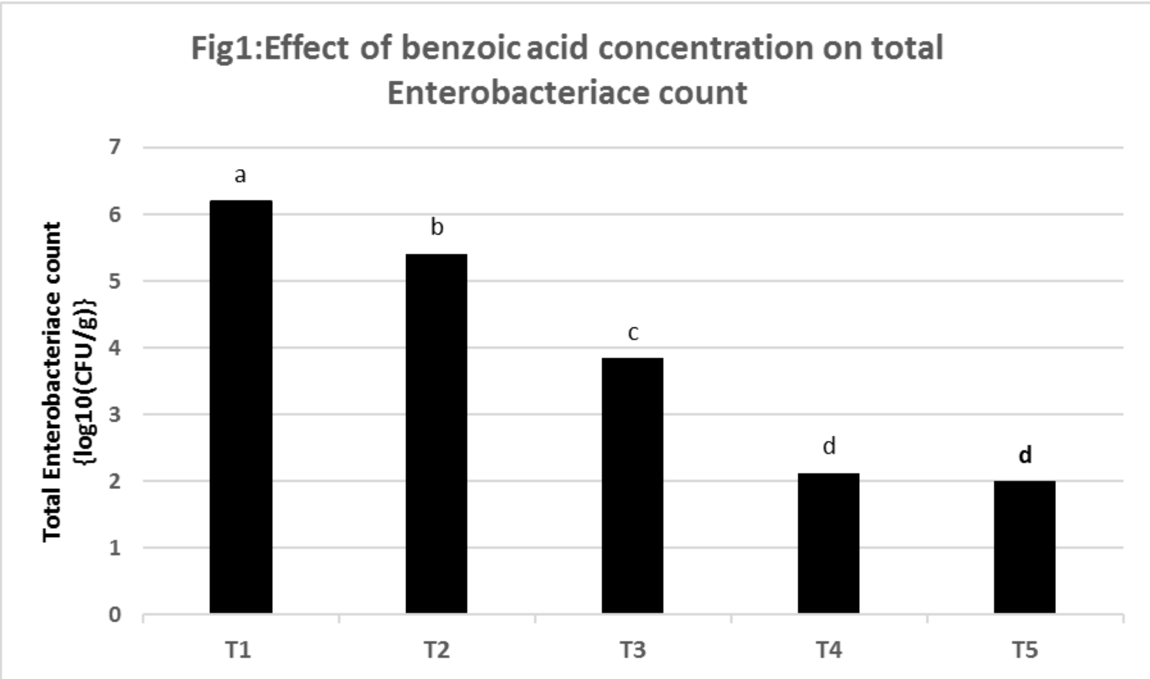
| **Items** | **Benzoic acid (mg/kg diet)** | | | | | ***P-value*** |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | |
| Nitrogen intake(g/day) | 4.29 ± 0.21 | 4.24± 0.24 | 4.29 ± 0.11 | 4.03 ± 0.20 | 3.83 ± 0.21 | 0.428 |
| Nitrogen excretion (g/day) | 1.11 ± 0.12$^{a}$ | 1.22 ± 0.08$^{a}$ | 0.72 ± 0.54$^{b}$ | 0.66 ± 0.04$^{b}$ | 0.61 ± 0.03$^{b}$ | <0.000 |
| Nitrogen retention % | 74.54 ± 1.45$^{b}$ | 71.27 ± 0.55$^{b}$ | 83.22 ± 1.21$^{a}$ | 83.16 ± 1.71$^{a}$ | 83.89 ± 0.72$^{a}$ | <0.000 |

a and b means having different letters in the same row are significantly different (P≤0.05)

**Table (7):** Effect of benzoic acid concentration on Fertility, hatchability of fertile eggs, total embryonic mortality percentage and hatched chick weight (g) for Sinai chickens

| **Items** | **Benzoic acid (mg/kg diet )** | | | | | **P-value** |
|---|---|---|---|---|---|---|
| | **T1 (control)** | **T2 (500mg/kg)** | **T3 (1000mg/kg)** | **T4 (1500mg/kg)** | **T5 (2000mg/kg)** | |
| Fertility% | 79.39±1.13$^{c}$ | 81.37±0.27$^{c}$ | 84.40±0.99$^{b}$ | 85.16±0.26$^{a}$ | 87.44±0.38$^{a}$ | <0.000 |
| Hatchability of fertile eggs% | 89.65±0.56$^{c}$ | 90.68±0.13$^{c}$ | 92.21±0.12$^{b}$ | 92.58±0.49$^{ab}$ | 93.27±0.19$^{a}$ | <0.000 |
| Total embryonic mortality% | 10.34±0.56$^{a}$ | 9.31±0.13$^{a}$ | 7.42±0.12$^{bc}$ | 7.78±0.49$^{b}$ | 6.28±0.19$^{c}$ | <0.000 |
| Hatched chick weight (g) | 34.42±0.44$^{b}$ | 34.68±0.48$^{b}$ | 34.90±0.14$^{ab}$ | 35.10±0.22$^{ab}$ | 36.32±0.37$^{a}$ | 0.010 |

a ,b and c means having different letters in the same row are significantly different (P≤0.05)




T1:control , T2:500mg/Kg diet, T3:1000mg/Kg diet, T4:1500mg/Kg die and T5:2000mg/Kg diet
CFU- colony forming unit

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## الملخص العربى
## كفاءه حمض البنزويك في تحسين الاداء الانتاجي والتناسلي لدجاج سيناء

**منى رفعت محمد أحمد ، مروه رمضان الدقن ،عزه رفعت فوزي ، ملاك منصور بشارة ،رءوف ادوارد رزق ، وسام اديب فارس**
معهد بحوث الإنتاج الحيواني-مركز البحوث الزراعية. مصر

اجريت هذه التجربه لتقييم تأثير إضافة حمض البنزويك للعليقه علي الأداء الانتاجي وفقس البيض وبعض صفات الدم والعدد البكتيري الامعاء Enterobacteriacea و الشكل الظاهري لصائم الامعاء الدقيقه والنيتروجين المحتجز لدجاج سيناء. وتم تغذية دجاج سيناء كسلاله مصريه علي علائق مضاف لها حمض البنزويك بتركيزات مختلفه وهي صفر ملجم / كجم عليقه واعتبرت المجموعه الاولي كمجموعه مقارنه (T1 ) ، الثانية 500 ملجم / كجم عليقه (T2 ) ، المجموعة الثالثه 1000 ملجم / كجم عليقه (T3 ) ، الرابعة 1500 ملجم / كجم عليقه (T4 )، الخامسة 2000 ملجم / كجم عليقه (T5 ). سجلت المجموعتين الرابعه والخامسة والمغذاه علي 1500 و2000 ملجم حمض بنزويك/ كجم عليقه زياده معنويه في كلا من عدد البيض ، حجم البيض ونسبة انتاج البيض مقارنة بالمجاميع المستخدمة الاخري وتحسنت الكفاءه الغذائيه لدجاج المجموعه الخامسه ( 2000 ملجم حمض بنزويك / كجم عليقه) يتلوها كلا من طيور المجموعه الرابعة ثم الثالثه مقارنة بباقي مجاميع التجربه وسجلت كل التركيزات المستخدمة لحمض البنزويك تحسنا معنويا في كلا من قوة القشرة ونسبة تركيز كالسيوم القشره مقارنة بالكنترول . وحدث تحسن معنوي في كلا من ارتفاع ومساحة خملات الامعاء نتيجة لاضافة 2000 ملجم حمض بنزويك / كجم عليقه مقارنة بالكنترول. ادي اضافة تركيزات مقدارها كلا من 1500 و2000 ملجم حمض بنزويك/ كجم عليقه تقليل معنوي للعدد الكلي للبكتريا السالبه لجرام في طائم الامعاء الدقيقة. واظهرت الطيور المغذاه علي كل التركيزات المستخدمه من حمض البنزويك ما عدا المغذاه علي 500 ملجم / كجم عليقه علي انخفاض معنوي للنيتروجين المحتجز ( جم/ يوم). سجلت اضافة تركيز 2000 ملجم حمض بنزويك / كجم عليقة حدوث تحسن معنوي في نسبة الفقس للبيض المخصب و كذا وزن الكتاكيت الفاقسة. ومن ذلك يمكن استنتاج ان اضافة تركيز 15000و 2000 ملجم حمض بنزويك / كجم عليقه له تاثير مفيد علي جودة البيض ونسبة انتاجه والشكل الظاهري لصائم الامعاء الدقيه ونسبة الفقس وتقليل الحمل للبكتريا الضاره في الامعاء.