

## **EFFECT OF BENZOIC ACID ADDITION TO DIET ON PRODUCTIVE PERFORMANCE OF GROWING RABBITS**

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### **SUMMARY**

A total of 40 males of New Zealand White rabbits (NZW) at six weeks of age were randomly divided into five treatment groups, eight individuals each. The first group was fed the basal diet and served as a control, while the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were fed the basal diet with the addition of 0.5, 1.0 and 1.5 % benzoic acid (BA) while the 5<sup>th</sup> group was fed the basal diet with 150 ppm zinc bacitracin (ZB). The experiment duration was six weeks (6 to 12 weeks of age). Results showed that both BA addition and ZB had no significant effect on live body weight and daily feed intake, while daily weight gain was significantly increased in rabbits fed 1.0 % BA and ZB diets than the other groups with the lowest daily gain being recorded for the control group of rabbits. Feed conversion ratio was significantly better for rabbit groups fed the ZB diet than the other groups during 8 - 10 and 6 - 12 weeks of age compared to the control group. Total edible parts (%) was significantly higher in rabbits fed ZB (T5), BA (T3) and control (T1) – diets compared with rabbits that fed BA (T2 and T4) diets. Compared to rabbits of the control, all feed supplement levels had significant effects on plasma total protein, AST and ALT activity without any apparent effects on the other parameters. MDA level, as a good indicator of oxidative status, was insignificantly decreased by BA inclusion to diet which implies that the BA did not produce any oxidative stress on rabbits. It is concluded that benzoic acid supplementation to rabbits diet up to 1.0% level could be used to enhance the productive performance of growing rabbits without negative impacts on their physiological status.

**Keywords:** *growing New Zealand White rabbits, benzoic acid, zinc bacitracin, productive performance, physiological status.*

### **INTRODUCTION**

The use of organic acids appears interesting even though scientific data concerning their effect on microflora population, mucosal immunity and growth performance are scarce and often contradictory in rabbits (Falcao–e–Cunha *et al.*, 2007). The effects on digestibility and productive performance due to inclusion of organic acids in rabbits nutrition are not completely clear. However, improvements in daily gain have been reported in many studies, but no effects were reported by others (Hollister *et al.*, 1990; Scapinello *et al.*, 2001). Organic acids and their salts are used in monogastric animals nutrition as alternatives to antibiotic growth promoters (Hansen, *et al.*, 2007). The number of studies on the effects of organic acids on growth performance of rabbits and the results obtained were not consistent (Falcao – e – Cunha *et al.*, 2007). Similarly, Hamza (2008) showed that the addition of acetic acid and ascorbic acid to drinking water of growing NZW rabbits had some positive effects on productive performance but the fumaric acid addition almost had negative effects. To our knowledge, benzoic acid has not studied in rabbit feeding, however a preliminary trail was designed by Papadomichelakis *et al.* (2011) to study the effect of its inclusion in diets on nutrients digestibility and growth performance of rabbits. Therefore, the objective of the present study was to reevaluate the effect of benzoic acid addition to the diet of growing NZW rabbits on their growth performance, feed utilization, carcass traits and some blood plasma constituents.

### **MATERIALS AND METHODS**

The present study was carried out at a private rabbitary farm near Mansoura Governorate, Egypt. A total of 40 NZW male rabbits, at six weeks of age were divided into five groups, eight individuals each. The first group was fed the basal diet and served as a control (C). The 2<sup>nd</sup>, 3<sup>rd</sup> and the 4<sup>th</sup> groups were fed the basal diet with the addition of 0.5; 1.0 and 1.5 % benzoic acid (BA), respectively, while the 5<sup>th</sup> group was fed the

basal diet with the addition of 150 ppm zinc bacitracin (ZB). Rabbits were individually housed in galvanized wire cages fitted with feeders and automatic nipple drinkers. All cages were located in well-ventilated rabbitary.

The experimental diets were formulated and processed as pellets to meet the nutrient requirements of growing rabbits as recommended by NRC (1977). Composition and calculated analysis of the basal diet are shown in Table (1).

All the experimental groups of rabbits were fed their respective diet and subjected to the same environmental, managerial and hygienic conditions and had free access to feed and water throughout the experimental period from 6 - 12 weeks of age. The growth performance of rabbits was evaluated in terms of feed intake, live body weight, body weight gain and feed conversion ratio. At 10 weeks of age, three rabbits from each treatment were housed in metabolic cages, which enable us to calculate the individual feed intake and collect the excreted feces. Feces were collected daily for five consecutive days. The excreted feces were pooled, mixed and sampled for chemical analysis according to (AOAC, 1995).

At 12 weeks of age, three rabbits from each group were slaughtered after 12 hours fasting. Blood samples were collected at slaughtering in heparinized tubes, centrifuged at 3000 rpm for 15 minutes, and the obtained plasma were stored at -20 °C until biochemical analysis using commercial kits. Total protein (g/dl) according to Henry *et al.* (1974), albumin (g/dl) according to Doumas (1971), and globulin concentration was calculated. Moreover, plasma levels of creatinine and urea (mg/dl) were also determined using the method of Bartles *et al.* (1972), triglycerides (mg/dl) according to Fossati and Prencipe (1982), and total cholesterol (mg/dl) according to Stein (1986). Besides, the activity of plasma aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were estimated U/L according to Reitman and Frankel (1957) using commercial kits. Malondialdehyde level (nmol/dl) was determined by the method of Mihara and Uchiyama (1978).

Data obtained were statistically analysed using SAS (2002). Significant differences between means were detected according to Duncan's multiple range test (Duncan, 1955). The following statistical Model was used:

$$Y_{ij} = U + A_i + e_{ij}$$

Where,  $Y_{ij}$  = an observation,  $U$  = overall mean,  $A_i$  = effect of treatments ( $i= 1, 2, \dots, 5$ ),  $e_{ij}$  = standard error.

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

| Ingredient                      | %     |
|---------------------------------|-------|
| Yellow corn                     | 32.00 |
| Wheat bran                      | 26.80 |
| Clover hay                      | 30.00 |
| Soybean meal (44%)              | 7.47  |
| Molasses                        | 2.13  |
| Limestone                       | 1.00  |
| Salt (NaCl)                     | 0.30  |
| Vit&Min Premix <sup>1</sup>     | 0.30  |
| Calculated analysis (NRC, 1977) |       |
| CP (%)                          | 15.42 |
| CF (%)                          | 12.58 |
| EE (%)                          | 3.81  |
| NFE (%)                         | 48.29 |
| DE (Kcal/Kg)                    | 2790  |

<sup>1</sup>Vit+Min mix. Provided per kilogram of the diet Vit. A: 6000 IU, vit. E (dl- $\alpha$ -tocopherylacetate): 10 IU, menadione: 2.5 mg, Vit. D3: 2000 ICU, riboflavin: 2.5 mg, calcium pantothenate: 10 mg, nicotinic acid: 12 mg, Choline chloride: 300 mg, vit. B<sub>12</sub>: 4  $\mu$ g, vit. B<sub>6</sub>: 5 mg, thiamine: 3 mg, folic acid: 0.50 mg, and biotin: 0.02 mg. Trace mineral (mg/kg of diet: Mn: 80 mg, Zn: 60 mg, Fe: 35 mg, Cu: 8 mg and Se: 0.1 mg).

## RESULTS AD DISCUSSION

The effect of dietary inclusion of benzoic acid (BA) or zinc bacitracin (ZB) on growth performance of growing NZW rabbits is presented in Table (2).

### **Live body weight (LBW):**

It is clear from the results that both BA and ZB inclusion in the diets did not significantly affect LBW of rabbits at all ages (i.e., 8, 10 and 12 weeks of age). The initial LBW of rabbits (at 6 wk) was nearly similar for all treatments and ranged between 802.1 and 833.6 with no significant differences among treatments. However, at the other growing periods LBW of rabbits were heavier for treatments than the control. In this concern, the final LBW of rabbits - as influenced by dietary inclusion of BA or ZB - surpassed that of the control group by about 2.62; 7.14; 4.02 and 6.96 % for rabbits fed BA at 0.5, 1.0, or 1.5% levels and ZB, respectively.

This result may indicate that dietary inclusion of BA at 1.0% level or ZB addition are capable to improve LBW of growing rabbits in approximately similar manner, although the differences between treatments lacked the significant level due, in part to, the low number of rabbits / treatment group .

Our results are in close agreement with those reported by Papadomichelakis *et al.* (2011) who indicated that BA did not affect growth performance of rabbits. On the other hand, Carraro *et al.* (2005) fed growing rabbits on diets supplemented with sodium butyrate (0.0, 0.5, 1.0 and 2.0 g/Kg) from weaning to 70 d of age, and found no effect of treatments on LBW. Similarly used coated sodium butyrate in diets of rabbits from 23 to 63 d of age and found insignificant effect on LBW of rabbits from all treatments. Also, Cesari *et al.* (2008) studied the role of organic acids (a blend of formic and lactic acids) on growth performance of rabbits from 30 to 84 d of age, and observed that dietary inclusion of organic acids did not affect LBW of rabbits.

In contrast, Kamal *et al.* (2008) fed male NZW rabbits on diets supplemented with organic acids (0.5% of citric, formic and malic acids, either singly or in combination) and found that dietary inclusion of organic acids significantly increased LBW compared by those fed the un-supplemented control diet. A similar result was observed by Debi *et al.* (2010) who evaluated the effect of graded levels of citric acid (0.5 to 2.5 %) and reported that LBW of rabbits were positively affected by dietary citric acid inclusion up to 2%.

### **Daily weight gain (DWG):**

Daily weight gains of rabbits as affected by dietary inclusion of different BA levels during the experimental intervals are illustrated in Table (2).

Results clearly showed that BA supplementation to diets had significantly ( $p \leq 0.05$ ) increased DWG of rabbits during all periods compared by the controls. This effect was also observed when ZB was added to the diet, where ZB - fed rabbits gained more DWG than those of the control group. During the entire period (6 - 12wks), rabbits of the control group gained less weight than the other treatments including the ZB one. Interestingly, the best DWG for the whole period was recorded for rabbits fed both BA (1.0%) and ZB treatments, which were significantly better than the other BA (0.5 and 1.5%) groups, all of them differ significantly ( $p \leq 0.05$ ) than the control treatment. It appears from these results that BA gives comparable results as ZB which is widely used in rabbit diets. Since the effect of ZB in enhancing the most growth performance traits including DWG may be due to its role as a growth promoter. This was also observed Pinheiro *et al.* (2004) who used ZB in rabbits and Papatsiros *et al.* (2011) who used BA in pig diets and mentioned that both ZB and BA could be used as growth promoters and / or to prevent digestive tract disorders by their influence on pH of the intestine and its microflora population. Since it is well known that, ZB is the most used antibiotic for rabbits, especially post weaning, and also for promoting growth.

**Table (2): Growth performance traits as affected by Benzoic acid supplementation to growing rabbit's diet.**

| Variable                     | T1     | T2      | T3      | T4      | T5      | SEM  |
|------------------------------|--------|---------|---------|---------|---------|------|
| Live body weight ( LBW, g):  |        |         |         |         |         |      |
| 6 wk                         | 817.1  | 806.3   | 802.1   | 833.6   | 825.8   | 22.7 |
| 8 wk                         | 1238.1 | 1271.3  | 1265.3  | 1286.9  | 1303.8  | 35.9 |
| 10 wk                        | 1689.6 | 1755.0  | 1780.6  | 1745.5  | 1795.6  | 51.2 |
| 12 wk                        | 2145.6 | 2201.9  | 2298.8  | 22.31.9 | 2295.0  | 62.6 |
| Daily weight gain (DWG, g):  |        |         |         |         |         |      |
| 6-8 wk                       | 30.07c | 33.21ab | 33.08ab | 32.38b  | 34.14a  | 0.56 |
| 8-10 wk                      | 32.25b | 34.55ab | 36.81a  | 32.79b  | 35.14ab | 1.25 |
| 10-12 wk                     | 32.37b | 32.93b  | 37.01a  | 3474ab  | 35.67ab | 1.18 |
| 6-12 wk                      | 31.63c | 33.44b  | 35.62a  | 33.29b  | 3498b   | 0.33 |
| Daily feed intake (DFI, g):  |        |         |         |         |         |      |
| 6-8 wk                       | 75.25  | 78.75   | 79.75   | 78.63   | 77.00   | 2.69 |
| 8-10 wk                      | 116.88 | 108.63  | 108.63  | 116.75  | 99.00   | 6.56 |
| 10-12 wk                     | 123.75 | 121.13  | 125.13  | 118.38  | 115.38  | 6.28 |
| 6-12 wk                      |        |         |         |         |         | 5.04 |
| Feed conversion ratio (FCR): |        |         |         |         |         |      |
| 6-8 wk                       | 2.51   | 2.38    | 2.43    | 2.43    | 226     | 0.09 |
| 8-10 wk                      | 2.68a  | 3.24ab  | 3.21ab  | 3.20ab  | 2.84b   | 0.26 |
| 10-12 wk                     | 3.77   | 3.78    | 3.39    | 3.46    | 3.25    | 0.21 |
| 6-12 wk                      | 3.34a  | 3.08ab  | 3.01ab  | 3.02ab  | 2.78b   | 0.15 |

<sup>a,b,c,d</sup> Means in the same row followed by different superscripts are significantly different at ( $p \leq 0.05$ ); SEM= Standard error of means.

Our results are in agreement with the findings by Scapnello *et al.*, 2001 (used Fumaric acid); Kluge *et al.*, 2006 (used benzoic acid); Kamal *et al.*, 2008 (used citric, fumaic, malic acids); Debi *et al.*, 2010 (used citric acid) and Romero *et al.*, 2012 (used formic and citric acids) who reported that organic acids have a positive effect on DWG of rabbits. On the other hand, some other studies reported insignificant effects of different organic acids on DWG of rabbits (Skrivanova and Marounek, 2000; Carraro *et al.*, 2005; Papadomichelakis *et al.*, 2011; Ribeiro *et al.*, 2012 and Romero *et al.*, 2012).

#### **Daily feed intake (DFI) and feed conversion ratio (FCR):**

Daily feed intake of rabbits as affected by BA supplementation to diets is presented in Table (2). Results clearly showed that dietary supplementation with BA or ZB had no significant effect on DFI of rabbits during all studied intervals and the entire experimental period. However, DFI of rabbits fed ZB or BA (1.5%) diets were insignificantly lower than the control and other treatment groups of rabbits. This was not the case for FCR, where it was significantly improved during the period 8 - 10 wks of age, for rabbits in the T5 group (ZB) followed by those of the other treatment groups compared to the control group of rabbits which have the worst FCR. This trend was also observed for the whole experimental period, where rabbits that fed the basal diet with ZB achieved better FCR than the other treatments.

It is of interest to observe that the differences in FI and FCR among BA treatments, regardless the level of BA, were not significant. In harmony with the present results, Carraro *et al.* (2005) fed growing rabbits on diets supplemented with different levels of an organic acid salt (sodium butyrate) from 28 to 70 days of age and found that both feed intake and FCR were not significantly affected by treatments. This was also reported by Cesari *et al.* (2008) who used a blend of formic and lactic acids and found that that FI was not affected, but FCR was positively enhanced by organic acids.

However, Kamal *et al.* (2008) who fed male NZW rabbits on diets supplemented with organic acids (0.5% of citric, formic and malic acids, either singly or in combination) found significant decrease in FI and better FCR for treatment group of rabbits compared with those of the control group. Similarly, Ribeiro *et al.* (2012) used coated sodium butyrate in rabbit's diet and found similar results.

**Carcass characteristics**

The effect of benzoic acid inclusion to diets on carcass traits of growing rabbits is presented in Table (3). It is clear from the results that most of the carcass traits, expressed as relative weights of LBW at slaughter did not significantly influenced by dietary supplementation with BA or ZB. Only, liver and edible parts % were significantly ( $p \leq 0.05$ ) affected by treatments, where liver % was significantly decreased in rabbits that fed ZB (T5) and the higher levels of BA (T4 and T3) compared with those of the control and T2 treatments. On the other hand, total edible parts (%) were significantly higher in rabbits fed ZB (T5), BA (T3) and control (T1) - diets compared with rabbits that fed BA (T2 and T4) diets. It appears from the previous results that the percentages of carcass traits increases or decreases in the studied traits, either significant or not, were not great enough to suggest a clear - cut trend for the effect BA on general performance of rabbits. Although the previous BA studies are very scarce (Suiryarayna and Ramana, 2015) our results showed positive effects of its inclusion to rabbit diets as a safe acidifier with promising health and growth promoting effects.

**Table (3): Carcass traits as affected by benzoic acid or Zink bacitracin supplementation to growing rabbits diet.**

| Variable         | T1                  | T2                 | T3                  | T4                 | T5                 | SEM  |
|------------------|---------------------|--------------------|---------------------|--------------------|--------------------|------|
| LBW-fasted (g)   | 2021.7              | 2048.3             | 2116.3              | 2080.0             | 2104.0             | 73.3 |
| Blood %          | 4.20                | 4.73               | 3.61                | 3.58               | 4.89               | 0.78 |
| Fur %            | 13.97               | 13.01              | 13.98               | 13.42              | 13.40              | 0.69 |
| Head %           | 5.99                | 6.33               | 5.78                | 5.74               | 6.16               | 0.19 |
| Liver %          | 3.02 <sup>a</sup>   | 3.07 <sup>a</sup>  | 2.78 <sup>ab</sup>  | 2.53 <sup>b</sup>  | 2.52 <sup>b</sup>  | 0.13 |
| Kidneys %        | 0.63                | 0.68               | 0.62                | 0.64               | 0.68               | 0.03 |
| Heart %          | 0.33                | 0.37               | 0.32                | 0.32               | 0.29               | 0.03 |
| Edible parts (%) | 61.83 <sup>ab</sup> | 59.57 <sup>b</sup> | 61.10 <sup>ab</sup> | 59.88 <sup>b</sup> | 63.20 <sup>a</sup> | 7.70 |

<sup>a,b,c,d</sup> Means in the same row followed by different superscripts are significantly different at ( $p \leq 0.05$ ); SEM= Standard error of means.

Our results are in close agreement with many studies which benefits different organic sources or their salts in rabbits diets as digestive tract stimulators, antibacterial, antibiotics alternatives and reported that carcass traits did not affect by organic acids supplementation (Abd El-Rahim *et al.*, 1994; Carraro *et al.* (2005); Cesari *et al.*, 2008; Papadomichelakis *et al.* 2011; Ribeiro *et al.* 2012 and Romero *et al.* 2012).

**Blood constituents**

The biochemical blood constituents of rabbits as influenced by BA or ZB inclusion to diets are shown in Table (4). All feed supplement levels, used herein, had significant ( $p \leq 0.05$ ) effects on plasma total protein, AST (GOT) and ALT (GPT) activity without any apparent effects on the other parameters. It is likely that BA has no negative impacts on the physiological status of rabbits as indicated by the normal values of the plasma biochemical parameters, with no mortality, observed in our results, since all measurements fell within the normal blood constituents reference values for rabbits as documented by Kaneko *et al.* (2008). However the observed increases in ALT and AST with the increase of BA level (T3 and T4) and with ZB addition (T5) may reflect slight liver malfunction but without negative effects on rabbits performance. Of interest the plasma level of MDA as a good indicator of oxidative status, where our results revealed insignificant effects of BA on MDA level which implies that the BA did not produce any oxidative stress on rabbits. This may be due to that BA is excreted predominantly through the urine as hippuric acid and benzoyl glucuronide, which were reported to be safe and had no deleterious effects on liver and kidney functions. This confirms the earlier results by Bridges *et al.* (1970) who explained the fate of BA in living organisms.

It is concluded that benzoic acid supplementation to rabbits diet up to 1.0% level could be used to enhance the productive performance of growing rabbits without negative impacts on their physiological status.

**Table (4): Effect of benzoic acid or Zink bacitracin supplementation to diet on some blood parameters of rabbits.**

| Variable | T1                  | T2                 | T3                  | T4                  | T5                 | SEM  |
|----------|---------------------|--------------------|---------------------|---------------------|--------------------|------|
| PTP      | 6.48 <sup>a</sup>   | 6.07 <sup>ab</sup> | 6.53 <sup>a</sup>   | 6.22 <sup>ab</sup>  | 5.74 <sup>b</sup>  | 0.21 |
| Alb.     | 3.93                | 3.73               | 4.00                | 3.90                | 3.62               | 0.13 |
| Glob.    | 2.55                | 2.34               | 2.53                | 2.32                | 2.12               | 0.12 |
| T.lipids | 198.4               | 193.6              | 203.9               | 198.0               | 201.2              | 6.24 |
| Tri-g.   | 61.3                | 58.37              | 63.03               | 62.57               | 64.47              | 2.94 |
| Cholest. | 74.57               | 77.30              | 80.47               | 80.43               | 79.77              | 2.95 |
| Creat.   | 0.97                | 1.04               | 0.93                | 1.06                | 1.08               | 0.05 |
| Urea     | 18.84               | 22.54              | 25.46               | 23.62               | 26.31              | 2.33 |
| AST      | 32.05 <sup>ab</sup> | 29.91 <sup>b</sup> | 33.94 <sup>ab</sup> | 35.61 <sup>ab</sup> | 37.90 <sup>a</sup> | 1.78 |
| ALT      | 12.78 <sup>ab</sup> | 11.88 <sup>b</sup> | 14.15 <sup>ab</sup> | 14.51 <sup>ab</sup> | 15.11 <sup>a</sup> | 0.82 |
| MDA      | 43.99               | 37.39              | 38.43               | 37.46               | 42.16              | 2.85 |

<sup>a,b,c,d</sup> Means in the same row followed by different superscripts are significantly different at ( $p \leq 0.05$ ); SEM= Standard error of means.

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## تأثير إضافة حمض البنزويك إلى العليقة على الأداء الإنتاجي للأرانب النامية

نها تاج الدين حسن تاج الدين

قسم الإنتاج الحيواني - كلية الزراعة - جامعة دمياط - دمياط - مصر

أجريت الدراسة علي عدد 40 ذكر من سلالة النيوزيلاندي الأبيض في عمر ستة أسابيع حيث قسمت إلى خمسة مجموعات بكل مجموعة ثمانية أرانب . غذيت أرانب المجموعة الأولى علي العليقة الأساسية دون إضافات بينما غذيت أرانب المجموعات 2 , 3 , 4 علي نفس العليقة مضافا إليها حمض البنزويك بمعدل 0.5 , 1 , 1.5 % علي التوالي وفي المجموعة الخامسة تم إضافة مركب " زنك باسيتراسين " للعليقة بمعدل 150 جزء في المليون . وقد استمرت التجربة لمدة ستة أسابيع . وتوضح النتائج أن هذه الإضافات كلها لم تؤثر معنويا علي وزن الجسم واستهلاك الغذاء مع زيادة معنوية في معدل الزيادة الوزنية مصحوبة بتحسين في كفاءة تحويل الغذاء نتيجة إضافة 1% من حمض البنزويك او الزنك باسيتراسين . وجدت زيادة معنوية في وزن الأجزاء المأكولة من الذبيحة وخاصة في المجموعة التي غذيت علي علائق مضافا إليها مركب الزنك أو حمض البنزويك بمستوي 1% مقارنة بالمجموعات الأخرى . كان للمعاملات تأثيرات معنوية علي البروتينات في الدم , والإنزيمات الناقلة لمجموعات الأمين كما تحسنت الحالة التأكسدية نتيجة إضعف حمض البنزويك للعليقة . وقد خلصت نتائج الدراسة إلي أن إضافة حمض البنزويك لعلائق الأرانب النامية بمستوي 1% يؤدي إلي تحسن معنوي في الأداء الإنتاجي دون أي تأثيرات سلبية علي الحالة الفسيولوجية للأرانب النامية .