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Response of Weanling Rabbits to Diet Contained Citric Acid During Growing Period

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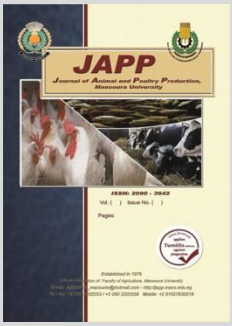

ABSTRACT

This study aimed to investigate the effect of dietary CA as feed additive on growth performance and nutrient digestibility as well as economic efficiency of rabbits during fattening period. Sixty unsexed NZW aged 35 days were randomly distributed into equal five groups. The control group was fed on the basal diet without CA addition. The groups from 2 to 5 were fed on diets contained 0.5, 1.0, 1.5 and 2.0% CA, respectively at 6-11 wks. of age. Rabbits fed diet contained 1.5 and 2.0% CA had heavier (P>0.05) live body weight at 11 wks of age, and higher daily weight gain during the whole experimental period as compared with the control group. Moreover, rabbits fed diet contained 1.5% CA recorded a significant (P≤0.05) improvement in FCR than the control group at 6-11 wks. of age. Crude fiber digestibility was significantly enhanced for rabbits fed different CA diets than the control, while both DM, OM and CP digestibility coefficients were improved by feeding diets contained CA at 1.5 and 2.0% than the control without significant effects. Feeding economic efficiency revealed the best value for rabbits fed 1.5 % CA diet than other groups at 11 wks. of age. Therefore, CA at 1.5% in the diet may be an alternative feed additive to maximize productivity and profitability without adverse effects for fattening rabbits.

Keywords: rabbits, citric acid, performance, nutrients utilization.

INTRODUCTION

Deficiency of arable land is the main restriction for large animal breeding to produce animal protein available for human due to over population in Egypt. Therefore, producing animal protein from small animal could be an necessary alternate option to mitigate the protein requirement for the nation. In view of this point, rabbit could use as a good alternative source of animal protein for humans because it high reproductive output and can digested high fibers diet such as forages and agriculture by-product to produce high quality meat (McNitt *et al.*, 2013) that contained high protein and low fat level (Dalle, 2002).

The mucosa of the small intestine has a great role in the digestion and absorption of nutrients and acts an important area of defense against antigenic aggressiveness in weanling rabbits (Gallois *et al.*, 2005). Due to stopping the use of antibiotics as growth promoters, the interest in natural antimicrobial feed additives as alternatives growth promoters was increased because its improve the economic efficiency of animal output through mounting growth rate, lowering feeding cost and detraction the risk of diseases (Uddin *et al.*, 2014). Organic acids can be used as replacement for antibiotics in rabbits' diet, so, using it appears interesting, although the available data on their effect on microflora population, mucosal immunity and growth performance are few and contradictory in rabbits (Falcao *et al.*, 2007). Moreover, the mode of action of organic acids on caecal microflora is not clearly understood, although it's played a direct action on the bacterial cell integrity (Maertens *et al.*, 2006). There are many benefits of using organic acids as a feed additives such as antifungal property, antibacterial activity against anaerobic pathogens, reduced mortality caused by gastrointestinal tract disease and stimulate gastrointestinal mucosal growth (Cardinali et al 2008; Kishawy *et al.*, 2018).

Citric acid (CA) exert anti-microbial activity in rabbits (*Skřivanová and Marounek 2007*). It is can improve nutrients utilization and consequently rabbit performance (*Debi et al. 2010; Romero et al. 2011*), and it improve minerals deposition in the bone, especially calcium, then improving bone metabolism. Citric acid classified as a growth simulator, acidifier, bacterial inhibitor, antioxidant and antitoxin (Salgado-Tránsito *et al.*, 2011), furthermore, it diminishing gut pH level, thus reducing harmful microbiota and modifying the distribution of bacterial species in them and improving the animal health (Chowdhury *et al.*, 2009). In addition, CA increase live weight gain, feed conversion efficiency and availability of nutrients (Nezhad *et al.*, 2007). Some investigations stated that dietary CA had a positive effect on pig and broiler chicks production (Atapattu *et al.*, 2005*;* Moghadam *et al., 2006 and Shen-Hui et al. 2005*). Because the scare of CA information on rabbit productive. Therefore, the current study carried out to get some knowledge about citric acid (CA) effect as feed additive on productive performance and nutrients digestibility of weanling rabbits during growing period.

MATERIALS AND METHODS

Unsexed sixty weanling healthy NZW rabbits aged 35 days used in this work. All treatment groups of rabbits had nearly similar average of initial live weight. Citric acid (CA) a weak organic acid, crystalline white solid, odorless compound used as a feed additive. All rabbits individually housed in galvanized wire cages within well-ventilated pen under similar hygienic and husbandry conditions. Every

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cage contained a metal feeder and a stainless steel nipple for drinking. Diet and water were offered ad libitum all the time. Rabbits divided into five groups, 12 animals each. The 1st group consumed the complete pelleted basal diet in the absence of CA (control, T1). The 2nd , 3rd , 4th and 5th groups consumed diets included 0.5, 1.0, 1.5 and 2.0 % CA, respectively. Diets composition and chemical analysis are shown in Table 1.

Table 1. Diets composition and chemical analysis (%)

Ingredients, %	T1	T2	T3	T4	T5
Alfalfa (dehydrated.)	40.0	39.5	39.0	38.5	38.0
Wheat bran	23.0	23.0	23.0	23.0	23.0
Barley	11.0	11.0	11.0	11.0	11.0
SBM (44%)	12.0	12.0	12.0	12.0	12.0
Maize	09.0	09.0	09.0	09.0	09.0
Citric acid	0.00	0.50	1.00	1.50	2.00
Molasses	03.0	03.0	03.0	03.0	03.0
Limestone	00.9	00.9	00.9	00.9	00.9
Salt	00.6	00.6	00.6	00.6	00.6
Vit.&min. premix*	00.5	00.5	00.5	00.5	00.5
Total	100.0	100.0	100.0	100.0	100.0
Price of kg, LE	4.29	4.32	4.35	4.38	4.40
Calculated chemical Analysis**					
Dry matter	88.10	88.10	88.10	88.10	88.10
Crude protein	18.30	18.21	18.13	18.03	17.94
Ether extract	3.12	3.10	3.09	3.07	3.06
Crude fiber	14.50	14.37	14.24	14.11	13.98
NFE	44.25	44.53	44.82	44.91	45.2
Ash	7.93	7.87	7.82	7.81	7.75
DE Kcal/kg***	2656	2662	2668	2675	2680

LE, Egyptian pound, the feed price was calculated according to ingredients price at the experiment time

*** Each 3 kg Premix of Vit. & Min. contains: vit. A, 12, million IU; Vit.D3, 2, 5 million IU; Vit. E, 10 g; Vit. K, 2.5g; Vit. B2, 5 g; Vit. B6, 1.5 g; Vit. B12, 10 mg; Biot, 50 mg; Folic, 1.0 g; Nicotc, 30 mg; Pantoth., 10 g; Antioxidant, 10 g; Mn, 60 g; Cu, 10 g; Zn, 55 g; Fe, 35 g; I, 1.0 g; Co, 250 mg and Se, 150 mg. **calculated according to NRC(1977). *** Calculated according to Perez *et al.* (1998).**

At the end of the 11th week of age, 3 males of each treatment randomly taken for digestion trial. Feed intake and excreted feces were daily weighed and recorded for five days. Chemical analysis for feed and feces were estimated according to AOAC (2002). Apparent digestibility coefficient was calculated as the percentage of digested nutrient/ total nutrient intake.

Live body weight for each rabbit recorded weekly, while feed intake recorded weekly then expressed per day for each rabbit. Daily weight gain and feed conversion were calculated weekly. Protein conversion ratio (PCR) as weight gain (g)/crude protein consumed (g) at 6-11 weeks was determined. Similarly, energy utilization efficiency (EEU, as consumed digestible energy; Kcal/weight gain, g) at 6-11 weeks was also calculated. Feeding economic efficiency (FEE %) as the percentage of A-B/B, where A= price of 1 kg gain (LE) and B= feed cost (LE)/kg gain was determined at 6-11 weeks.

Statistically analysis: The data obtained analyzed according to one-way ANOVA of GLM Procedure of SAS® (*SAS Institute, 2004*). Significant differences between means were detected using *Duncan (1955*).

RESULTS AND DISCUSSION

Growth performance:

Live weight (LW) of NZW rabbits not significantly affected among the experimental groups due to dietary citric acid (CA) at different ages (Table 2). Rabbits fed diet contained 1.5 and 2.0% CA had heavier LW compared to the control at the end of the 11th week of age. Also, daily weight gain (WG) significantly affected among the experimental groups of growing NZW at the 7, 8 and 10th wks of age only, while it not affected at other studied ages and the entire period (6-11 weeks). Generally, daily BWG recoded the higher value by all CA diets except of 0.5% CA diet when compared with the control through the entire experimental period (6-11 weeks). Rabbits consumed 1.5 and 2.0% CA diet recorded the best daily BWG than other groups at the end of the experimental period The improvement in LBW and BWG for rabbits fed 1.5 or 2.0% CA in their diets may be due to citric acid can plays a growth stimulator, bacterial inhibitor, antioxidant and antitoxin (*Salgado-Tránsitoet al., 2011*), which prevent the pathogenic bacteria growth and elevated the nutrients availability for the animal, which may improve the animal output (*Abdel-Fattah et al. 2008*).

Table 2. Effect of dietary CA on live body weight and daily weight gain of weanling NZW rabbits during growing period

Age, wks	Control	Citric acid (CA) level, %			
		0.5	1.0	1.5	2.0
		Live body weight, g			
6	1122.5±52.7	1020.8±48.6	1069.2±61.3	1081.7±59.4	1074.2±48.9
7	1329.2±54.9	1204.2±50.0	1255.0±61.8	1284.2±55.9	1300.8±51.6
8	1527.5±52.2	1391.7±56.3	1458.3±65.2	1498.3±52.7	1535.0±53.8
9	1755.8±65.3	1615.0±65.1	1669.2±60.4	1724.2±50.4	1765.8±55.8
10	1974.2±67.7	1836.7±73.7	1919.2±60.0	1956.7±59.0	1986.7±61.1
11	2186.7±70.1	2044.2±79.2	2146.7±71.0	2211.7±54.7	2204.2±62.9
		Daily body weight gain, g			
7th	29.51±1.85^{ab}	26.49±1.62^{b}	27.29±1.72^{ab}	29.41±1.71^{ab}	32.41±1.39^{a}
8th	28.28±1.92^{ab}	26.81±1.83^{b}	29.07±1.65^{ab}	30.62±1.53^{ab}	33.45±2.11^{a}
9th	32.65±2.42	31.93±1.71	30.17±2.07	31.69±2.00	32.98±2.18
10th	31.19±1.83	31.69±1.98	35.72±1.43	33.48±2.23	31.57±1.44
11th	30.38±1.63^{ab}	29.67±1.84^{b}	32.53±2.15^{ab}	36.22±1.72^{a}	31.10±2.10^{ab}
6-11	30.35±1.36	29.23±1.29	30.78±1.12	32.28±1.26	32.28±1.22

a,b: means in the same row within each item with different superscript are significantly different at P ≤ 0.05 .

Also, citric acid could depressed pH level in the gut, thus diminution harmful microbiota and modifying the distribution of bacterial species in the gut and improving the health status of the animal (*Chowdhury et al., 2009*). These observations are similar with *Shen-Hui et al. (2005), Moghadam et al. (2006), Nezhad et al. (2007), Abdel-*

Fattah et al. (2008) who reported dietary citric acid increased broilers live weight and weight gain. *Debi et al. (2010)* found that CA addition with 2% resulted a significant (P<0.05) improvement of rabbits body weight. *Uddin et al. (2014)* concluded that adding CA up to 1.50% to the diet had positive effect on growth traits of growing rabbit. Also, *Kliševičiūtė et al. (2016)* found that adding organic acid such as citric acid to rabbit's diet increased body weight and weight gain. However, *Biggs and Parsons (2008)* stated that inhibition in growth rate at 21 days of chicks due to dietary citric acid addition. Also, *Khalil and El-Zarie (2012)* found that rabbits weight gain not affected by fed citric acid at g/kg diet. Moreover, the reduction in rabbits LBW by feeding 0.5% CA diet compared with the control group could be the decrease in daily feed intake which reached 8.72% during the entire period (6-11 weeks).

Non-significant differences were observed among CA groups of daily feed intake (FI) comparing with the control group at different experimental ages (Table 3), while FI was significantly enhanced for rabbits fed 2.0%CA diet than those fed 1.5 %CA at the 9th wk of age as well as those fed 0.5% CA diet at the entire experimental period (6-11 weeks) only. This observation agree with *Nezhad et al. (2007)* who demonstrated that feed intake not affected for broilers fed on CA diet as compared with the control, however, *Moghadam et al. (2006)* found a significant positive effect. A significant differences were observed in feed conversion ratio (FCR) among the experimental groups at the lasted weeks of fattening rabbit's period as well as the whole experimental period (Table 3). A better FCR (P≤0.05) was found for rabbits fed 1.5 % CA diet, while the other CA groups had preferable FCR values without significant effects when compared with the control during the entire period (6-11 weeks).

The FCR improvement may be due to citric acid could increased villus height/crypt depth ratio and increment beneficial bacteria while diminution pathogenic organisms in the feed and gut (*Yesilbag and Çolpan, 2006*), which may increase the digestibility of crud protein, fat, crud fiber as well as well-developed intestinal glands and an elevate in the number of goblet cells as well as minimize the most anti nutritional factors (*Sharma et al., 2013*). This result is agree with *Afsharmanesh and Pourreza (2005), Atapattu and Nelligaswatta (2005), Abdel-Azeem et al. (2000)* in poultry and rabbit. Similarly, *Mvan and Suresh (2013)* concluded that citric acid supplementation at level 0.9% in the diet improved the efficiency of feed utilization of crossbred pigs. *Uddin et al. (2014)* found that an improvement of FCR was occurred by CA addition with 0.5 up to 1.5% in rabbit's diet. *Kliševičiūtė et al. (2016)* reported that dietary butyric, citric acid and propionate improved FCR of rabbits. However, *Debi et al. (2010)* stated that FCR not significantly affected due to CA addition up to 2% in growing rabbit's diet. Also, feed conversion of broilers not (P≥0.05) affected by CA (*Atapattu and Nelligaswatta , 2005, Gong et al., 2006 and Moghadam et al.,2006)* .

Table 3. Effect of dietary CA on daily feed intake and feed conversion ratio of weanling NZW rabbits during growing period

Age, wks	Control	Citric acid (CA) level, %			
		0.5	1.0	1.5	2.0
		Daily feed intake, g			
7th	77.2±4.48	64.3±4.79	77.8±4.33	77.36±8.10	83.3±7.65
8th	97.0±4.70	84.9±4.68	94.7±4.75	100.3±6.44	99.1±4.44
9th	106.4±3.40^{ab}	103.1±5.97^{ab}	103.3±6.08^{ab}	90.2±6.90^{b}	117.4±5.08^{a}
10th	114.4±8.31	107.4±7.89	116.4±8.25	110.2±8.50	122.4±5.13
11th	127.9±5.43	114.6±6.03	127.1±6.51	125.9±5.53	118.5±5.04
6-11	103.2±3.47^{ab}	94.2±4.40^{b}	103.1±4.53^{ab}	98.9±3.61^{ab}	107.5±3.74^{a}
		Feed conversion			
7th	2.72±0.20	2.52±0.22	2.99±0.25	2.71±0.30	2.60±0.25
8th	3.54±0.20	3.20±0.96	3.33±0.19	3.33±0.27	3.11±0.27
9th	3.43±0.24^{ab}	3.24±0.11^{ab}	3.60±0.22^{b}	2.93±0.27^{a}	3.63±0.18^{b}
10th	3.56±0.24^{ab}	3.43±0.23^{ab}	3.25±0.17^{a}	3.33±0.23^{ab}	3.94±0.20^{b}
11th	4.25±0.13^{b}	3.93±0.18^{ab}	4.00±0.21^{ab}	3.51±0.12^{a}	3.93±0.20^{ab}
6-11	3.42±0.06^{b}	3.25±0.09^{ab}	3.35±0.09^{ab}	3.09±0.11^{a}	3.36±0.10^{ab}

a,b: means in the same row within each item with different superscript are significantly different (P ≤ 0.05).

No significant differences were observed in all digestibility coefficients of diet nutrients due to dietary CA except for crud fiber among the experimental groups (Table 4). Crud fiber significantly improved by increasing CA level in the diet comparing to the control. Rabbits fed both 1.0 and 2.0 % CA diets had the highest CF digestibility comparing with both the control groups. Both dry and organic matter (DM& OM) digestibility coefficient (%) were insignificantly improved by feeding diet contained varying CA levels comparing to the control groups. While, crude protein digestibility coefficient recorded the superior value by feeding 0.5 and 2.0% CA diet followed 1.5 % CA diet than the control. However, EE digestibility coefficient was approximately similar by feeding different CA diets, but 0.5 % CA diet recoded the superior EE digestibility value than the control group.

The higher values of nutrients digestibility coefficients in rabbits fed CA diets may be due to an increase in these nutrients utilization in the intestinal tract. Improvement in nutrients digestibility coefficients in rabbits fed CA diets may be due to CA can decrease PH and increase acidic environment in the intestinal tract in the gut, thus reducing harmful microbiota (*Chowdhury et al., 2009*). Also, dietary organic acids could be suppressed bacteria pathogenic growth and improving digestion, absorption and mucosal immunity (*Zdzislaw, 2005*). Moreover, improving nutrient digestibility may be due to dietary CA addition increases the peptides, amino acids, and glucose transporters (*Gilbert et al., 2008*). This result is agree with *Debi et al. (2010)* who found that CA supplementation to rabbit diet improved DM, CP, NFE and EE digestibility's However, *Debi et al. (2010)* found that dietary CA by 0.5 up to 2.5%in rabbits diet not affect CF

digestibility. Improving of crud fiber digestibility for rabbits may could be CA acts a weaken of crude fiber structure, thus making them more susceptible for enzymatic digestion and facilitate the nutrient digestibility *(Uddin et al., 2014)*

Results of Table 5 shows that, protein efficiency ratio (PER), energy utilization efficiency (EEU) for NZW rabbits during growing period. Rabbits fed 1.5%CA diet had a significant improvement in PCR than the control, while both 0.5 and 2.0% CA groups recorded non-significant (P>0.05) improvement. Also, efficiency of energy utilization (EEU) recorded the best value by feeding 1.5 % CA diet than those fed 2.0%CA and the control diet. Feeding economic efficiency (%) for rabbits not significantly affected although it elevated by feeding 0.5 and 1.5%CA diet than other groups during the entire experimental period.

The PCR improvement of rabbits fed 1.5 %CA diet may be due to the improvement in BWG and the decrease in crude protein consumed when compared with rabbits fed both 1.0%CA or the control diet at the entire experimental period. The elevation of EEU by feeding 1.5%CA diet than the control may be due to improve rabbits performance especially rabbits BWG and stop harmful bacteria effects as well as improve diet nutrients digestibility

Table 4. Effect of dietary CA on nutrients digestibility's of weanling NZW rabbits during growing period

Parameters	Control	Citric acid (CA) level, %			
		0.5	1.0	1.5	2.0
Dry matter (DM)	70.2±2.8	74.1±1.2	71.5±0.6	71.0±1.4	72.7±3.0
Organic matter (OM)	71.3±2.8	74.9±1.0	72.6±0.6	72.3±1.3	74.1±2.9
Crude protein (CP)	62.6±4.0	70.9±2.1	61.2±1.7	64.5±2.6	70.8±3.9
Ether extract (EE)	81.4±1.7	84.1±1.6	80.7±0.7	81.9±1.8	81.0±0.6
Crude fiber (CF)	10.4±6.1^{b}	29.2±3.1^{a}	33.0±3.0^{a}	29.1±0.9^{a}	36.1±7.7^{a}
Nitrogen free extract (NFE)	83.4±2.1	84.3±1.6	82.3±0.5	82.2±1.1	81.9±1.9

a,b: means in the same row within each item with different superscript are significantly different (P ≤ 0.05).

Table 5. Effect of dietary CA on protein conversion ratio (PER), efficiency of energy utilization (EEU) and feeding economic efficiency (FEE, %) of weanling NZW rabbits during growing period

Parameters	Control	Citric acid (CA) level, %			
		0.5	1.0	1.5	2.0
PCR	1.56±0.03^{b}	1.68±0.04^{ab}	1.60±0.05^{b}	1.78±0.07^{a}	1.64±0.06^{ab}
EEU	9.04±0.17^{a}	8.57±0.23^{ab}	8.88±0.24^{ab}	8.15±0.33^{b}	8.94±0.28^{a}
FEE, %	173.0±5.1	195.8±8.1	176.0±7.8	200.1±11.8	173.8±9.7

a,b: means in the same row within each item with different superscript are significantly different (P ≤ 0.05).

CONCLUSION

It could be advised that the presence of 1.50 % citric acid in the diet of weanling rabbits could be an alternative method to maximize their productivity and profitability and nutrients digestibility's as well as economic efficiency without adverse effects during growing period.

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تأثير حمض الستريك فى عليقة الأرانب المفطومة على أداء النمو والتمثيل الغذائي خلال فترة النمو

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تجري الدراسة لمعرفة تأثير وجود حمض الستريك فى علائق الأرانب المفطومة على أداء النمو وهضم العناصر الغذائية وكذلك الكفاءة الاقتصادية خلال فترة النمو. حيث استخدم عدد ٦٠ أرنب نيوزيلاندى أبيض عمر ٣٥ يوم ، تم وزن الأرانب وتوزيعهم الى خمس مجموعات متساوية عشوائيا. تم تغذية المجموعة الأولى (الضابطة) على العليقة الأساسية بدون حمض الستريك بينما تغذت المجموعات من الثانية حتى الخامسة على العليقة المحتوية على ٠,٥ ، ١,٠ ، ١,٥ ، ٢,٠ % حمض الستريك على التوالى خلال الفترة التجريبية الكلية (٦- ١١ أسبوعًا من العمر).أظهرت النتائج زيادة غير معنوية فى وزن الجسم الحى للأرانب التي تغذت على العلائق المحتوية على ١,٥ و ٢,٠ % حمض الستريك عند عمر ١١ أسبوعًا وكذلك أفضل معدل للزيادة اليومية فى الوزن خلال الفترة الكلية للتجربة (٦-١١ أسبوع من العمر) بالمقارنة بتلك التى تغذت على العليقة الضابطة (الكنترول). كما تحسن معامل التحويل الغذائي معنويا بالتغذية على العليقة المحتوية ١,٥ % حمض الستريك مقارنة بالكنترول خلال الفترة الكلية للتجربة (٦ - ١١ أسبوعًا من العمر). كما تحسن معامل هضم الألياف الخام معنويا بالتغذية عل جميع علائق حمض الستريك بينما تحسنت بدون معنوية معاملات هضم المادة الجافة والعضوية فضلا عن البروتين الخام بالتغذية على ١,٥ ، ٢,٠% حمض الستريك بالمقارنة مع الكنترول. أظهرت الكفاءة الاقتصادية للتغذية قيما أفضل للأرانب المغذاة على ١,٥% حمض الستريك فى العليقة بالمقارنة بالمجموعات الأخرى عند عمر ١١ أسبوع. لذلك تشير النتائج الى امكانية وجود حمض الستريك بمستوى ١,٥% فى عليقة الأرانب المفطومة كاضافة غذائية لتحسين أدائها الانتاجى وتمثيل العناصر الغذائية فضلا عن الكفاءة الإقتصادية دون أى آثار ضارة لها خلال فترة التسمين.