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RESPONSE OF GROWING RABBITS TO DIFFERENT LEVELS OF DIETARY CITRIC ACID

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Abstract

An experiment was conducted for a period of 56 days with 36 healthy New Zealand White (NZW) rabbits aged about one months having weight from 370 to 390g to evaluate the effects of dietary citric acid on growth performance, feed consumption and digestibility of nutrients as well as immune status. The experiment was designed with 6 dietary treatments having 6 rabbits per treatment. Rabbits of control treatment (T_1) were given the diet without citric acid (CA) but the dietary treatments T_2, T_3, T_4, T_5 and T_6 contained 0.5, 1.0, 1.5, 2.0 and 2.5% CA respectively. Green grass was supplied on *ad libitum* basis. The total body weight gain was 734, 776, 812, 862, 911 and 740g for the rabbits fed 0, 0.5, 1.0, 1.5, 2.0 and 2.5% CA containing diets respectively. Addition of CA at the level of 2% enhanced body weight significantly ($P<0.05$). Total DM intake also increased with increasing the percentage of CA up to 2% level ($P<0.05$). Incase of feed conversion ratio, there was no significant difference in addition to different levels of CA. Supplementation of CA improved dry matter, crude protein and ether extract digestibility ($P<0.05$) but incase of crude fiber and nitrogen free extract, there was no significant difference. Non significant difference was also found incase of acidity of feed and feces. The highest lymphocyte (73%) was counted in dietary treatment T_6 having 2.5% CA, which was 17% higher than the control diet. From the result of this experiment, it may be concluded that the addition of CA up to 2% level in diet improves performance, digestibility of nutrients and immune status of growing rabbit.

Key words: Rabbit, Citric acid, Performance, Immunity

Introduction

An adult people require at least 250g milk and 120g meat every day and 104 eggs per year. But per capita availability in Bangladesh is about 41g of milk, 21g meat per day and 41 eggs in a year (MOFL, 2006). These products obtained from 22.87 million cattle, 1.21 million buffalo, 20.75 million goat, 2.68 million sheep and 245.97 million poultry that are insufficient in quantity and also very low to meet the national demand (DLS, 2007). Scarcity of land is the main constraints for the production of large animal to make animal protein available for human due to over population in Bangladesh (1067 persons per square kilometer; UNDP Report, 2009). Therefore, production of animal protein from small animal

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should be emphasized as an alternate option to mitigate the protein requirement for the nation. Considering this point, rabbit would be an important micro livestock (Vietmeyer, 1985). Numerous attempts have been made to improve the productive performance of small animal by using growth promoters (Ashour *et al*., 2004 and El-Kholy *et al.,* 2008a) or enzyme supplementation (Sarhan, 2001 and El-Mahdy *et al*., 2002). But, considering health hazard the feed manufacturers and the animal growers have been actively looking for an efficacious alternative. Several alternatives such as organic acids, probiotics, prebiotics, herbs, enzymes and essential oils have recently been used to serve the purpose. Among those the organic acids are important alternatives to antibiotics exclusively used as a growth promoter and for improvement of the feed conversion efficiency in farm animals (Esteive *et al.,* 2001). Among other organic acids citric acid is cheap, available non-corrosive and has greater growth promotion property. Its addition significantly increase live weight gain, feed conversion efficiency and availability of nutrients (Shen *et al.,* 2005; Atapattu *et al*., 2005; Moghadam *et al*., 2006 and Nezhad *et al*., 2007). It also increases the immunity of animals (Abdel-Fattah *et al.,* 2008 and Rahmani and Speer, 2005). The experiment was conducted to investigate the effect of different levels of dietary citric acid on growth performance, feed consumption, digestibility of nutrients and immune status of growing rabbit.

Materials and Methods

Rabbit and test substance

A total of 36 weaned apparently healthy crossbred New Zealand White (NZW) rabbits aged about 30 to 45 days were collected from Madhupur, Tangail. The initial live weight of rabbits ranged from 370 to 390g. Citric acid ($C_6H_8O_7$), a weak organic acid with molecular weight 192.123 g/mol, crystalline white solid, odorless compound used as a test substance.

Trial house and adaptation of rabbit

The cages of rabbit were steel, Quonset style (Harris, 1983) measuring 1.9m ×1.8m × 1.3m in dimension where two rabbits were placed. J-shaped screened metal feeders and 250 ml plastic mug as waterers were provided in cages. The cages were properly washed and cleaned with forced water and then kept for drying properly. The feeders, waterers and trays for feces collection were cleaned properly and disinfected by using phenyl solution and were left for few days. Before the commencement of the study, the animals were kept for two weeks to adapt with the experimental feeds and environment.

Experimental design

The experiment was conducted in a Completely Randomized Design (CRD) having 6 dietary treatments with 3 replicate cages each of 2 rabbits. The rabbits were given green grass and concentrate mixture, where concentrate mixture were further fortified with 0, 0.5, 1.0, 1.5,

2.0 and 2.5% citric acid for the dietary treatment T_1 T_2, T_3, T_4, T_5 and T_6 respectively (Table 1).

Table 1. Formulation of diet

Ingredients (kg/100kg)	Dietary treatments (% CA)					
	T_1	T_2	T_3	T_4	T_5	T_6
Maize	58.5	58.0	57.5	57.0	56.5	56.0
Soybean meal	25	25	25	25	25	25
Rice polish	14.1	14.1	14.1	14.1	14.1	14.1
Citric acid	0.0	0.5	1.0	1.5	2.0	2.5
DCP	1.5	1.5	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5	0.5	0.5
Premix	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100

Feeding of the experimental rabbit

Concentrate mixture and green grass were supplied on *ad libitum* basis. Locally available road side grass was collected every morning and evening followed by cleaning, chopping, weighing and supplied to the animals. The green grass and concentrate mixture was offered twice daily, once at 7.00 am and other at 4.00 pm. Clean and safe drinking water was made available to the animals at all times.

Measurement

The amount of concentrate and grass offered were recorded twice a day. The refusal of both roughage and concentrate of the subsequent days were collected, weighed and recorded. Digestibility trial was conducted for last 7 days of the trial. pH value of feed and feces were measured using pH meter. For determination of the immunity of the experimental rabbit, differential leukocyte count (DLC) was performed as per technique described by Shastry (1983).

Feed, feces and grass sample were analyzed for proximate composition following the method described by AOAC (1990). Data collected for different parameters were analyzed statistically (Steel and Torrie, 1980).

Results and Discussion

Growth performance

Addition of different levels of citric acid significantly ($P<0.05$) increased live weight of the experimental rabbit. The final weight was 1085, 1147, 1178, 1254, 1297 and 1082g for the rabbit fed diet containing 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5% CA respectively and corresponding

total weight gain was 734, 776, 812, 862, 911 and 739g in different dietary groups. The trend of increase of body weight followed with the increasing level of CA up to 2% but declined at 2.5% level (739g) which was nearly similar to control. This result agreed with Cesari *et al.* (2008), who found that addition of organic acid (formic and lactic acid) increased the live weight gain of rabbit. The other researchers (Abdel-Fattah *et al.* 2008 and Nezhad *et al.* 2007) found that addition of dietary citric acid, acetic acid and lactic acid in broiler ration improved the live weight of broiler chicks as compared to those of un-supplemented diet. Moghadam *et al.* (2006) and Maiorka *et al.* (2004) reported that citric acid had positive effect on live weight of broiler. Other researchers reported that inclusion of citric acid in broiler diet improved weight gain which was consistent with the present finding (Afsharmanesh and Pourreza, 2005; Shen *et al.*, 2005; Ivanov 2005 and Snow *et al.* 2004). This result disagreed with the result of Zaghini *et al.* (1986) who mentioned that addition of 1.5% of either citric or formic acid, replacing an equivalent amount of maize meal had no significant effects on daily body weight gain in comparison with the control diet.

Feed intake

Significant ($P<0.05$) responses were observed with increasing levels of citric acid in case of feed (Concentrate and roughage) intake. Total concentrate and roughage intake were 3616, 3776, 3840, 4435, 4562, 3588g and 2234, 2371, 2424, 2573, 2837, 2240g for dietary treatments T_1, T_2, T_3, T_4, T_5 and T_6 respectively. Concentrate and roughage intake increased as the level of citric acid was increased up to 2% but decreased at 2.5% level which was similar to the control. In case of rabbit, little information is available about the effect of citric acid on performance. Atapathu *et al*. (2005) found that the effects of citric acid on feed intake of broiler chicks were significant, similar to the result as observed by Moghadam *et al.* (2006). However, it is not consistent with the observation of Nezhad *et al.* (2007) who found no significant effect of citric acid on feed intake in broiler. From different citations it is clear that, effect of citric acid on feed consumption has a positive effect.

Dry matter (DM) intake

Total DM intake was 3286, 3365, 3400, 3605, 3693 and 3282g for the group of rabbit fed 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5% citric acid containing diet respectively. Clear indication was found that citric acid increased the DM intake of the experimental rabbit. In case of 2% citric acid level, DM intake was highest and it was 12% more than the control. In this experiment, it was found that DM intake increased as the level of citric acid increased up to 2% level.

Feed conversion ratio (FCR)

A linear trend of improvement of feed conversion ratio observed up to the inclusion of citric acid at 2% level, but subsequent increment (2.5%) of citric acid level reduced the FCR which was similar to the control (0.0% CA). Castrovilli (1991) found that feed conversion efficiency of rabbits was improved with the addition of 0.15 or 0.3% mixture of organic acids including citric acid. This result agreed with the result of Radcliffe *et al.* (1998). Who found that addition of citric acid and phytase enzyme in the pig diet increased the feed conversion efficiency. Addition of citric acid increased the feed conversion efficiency of

broiler (Denil *et al*., 2003). Nezhad *et al.* (2007) and Shen *et al.* (2005) found significant effect of CA on FCR but the result disagreed with the result of other researchers who mentioned that the addition of CA in broiler diet had no effect on FCR (Gong-YiFeng *et al.*, 2006; Atapattu *et al.*, 2005 and Moghadam *et al.*, 2006).

Table 2. Growth performance, feed intake, dry matter intake and feed conversion ratio of the experimental rabbit under different levels of dietary citric acid

Parameters	Dietary treatments (% CA)							
	T_1 (0.0)	T_2 (0.5)	T_3 (1.0)	T_4 (1.5)	T_5 (2.0)	T_6 (2.5)	SEM	Level of Sig.
Initial weight (g)	351 ±34.0#	372 ±2.0	367 ±4.4	393 ±5.2	386 ±18.2	342 ±59.2	7.7	NS
Final weight (g)	1085[a] ±16.4	1147[b] ±5.0	1178[b] ±31.8	1254[c] ±13.3	1297[c] ±14.6	1082[a] ±65.7	21.3	*
Total live weight gain (g)	734[a] ±52.0	776[ab] ±7.5	812[bc] ±6.6	862[cd] ±12.1	911[d] ±12.0	739[a] ±21.0	20.5	*
Daily live weight gain (g)	13.1[a] ±1.2	13.9[ab] ±1.0	14.5[bc] ±3.1	15.4[cd] ±2.0	16.3[d] ±1.4	13.2[a] ±1.0	3.70	*
Intake (g)								
Concentrate	3616[a] ±15.2	3776[ab] ±51.3	3840[ab] ±50.4	4435[ab] ±17.5	4562[b] ±75.4	3588[a] ±41.2	14.0	*
Roughage	2234[a] ±44.4	2371[b] ±26.4	2424[bc] ±87.8	2573[c] ±29.9	2837[d] ±18.5	2240[a] ±34.2	12.5	*
Dry matter	3286[a] ±12.8	3365[b] ±7.9	3400[b] ±5.7	3605[c] ±19.1	3693[d] ±11.1	3282[a] ±12.3	41.3	*
FCR (kg FI/kgLWG)	4.5 ±0.36	4.4 ±0.21	4.2 ±0.15	4.2 ±0.20	4.0 ±0.17	4.4 ±0.19	0.10	NS

[a,b,c,d] Mean values within the same row with uncommon superscripts differed significantly (P<0.05), # Mean ± SE (Standard error)
SEM\ = Standard error of mean
NS = Non-significant, * = Significant at 5% level

pH of feed and feces

Addition of citric acid decreased the level of p^H in feed and feces linearly. In both the cases, the lowest p^H value was found in case of dietary treatment T_6 containing 2.5 % citric acid. This result is similar to the result of Radcliffe *et al*. (1998) and Scipioni *et al.* (1979). They found that addition of citric acid in ration reduced dietary P^H. Similar result was reported by Sammel and Claus (2003).

Digestibility of different nutrients

Digestibility of different nutrients indicated that citric acid increased digestibility of nutrients. In most of the cases, diet with 2% CA (T_5) fed to the growing rabbits tended to

improve the digestibility (P<0.05). Significant difference (P<0.05) was found on DM, CP and EE digestibility. These findings reflected that the level of 2% CA may be the most suitable concentration compared to other doses. The present results agree with those of Scipioni *et al.* (1979) who mentioned that addition of citric acid in the diet increased digestibility.

Table 3. Effect of citric acid on pH of feed and feces

pH	Dietary treatments (% CA)						SEM	Level of Sig.
	T_1 (0.0)	T_2 (0.5)	T_3 (1.0)	T_4 (1.5)	T_5 (2.0)	T_6 (2.5)		
Feed	6.4±.015[#]	6.1±0.17	5.9±0.20	5.7±0.25	5.5±0.28	5.3±0.31	0.12	NS
Feces	7.5±0.21	7.4±0.21	7.2±0.17	7.2±0.22	6.9±0.15	6.7±0.19	0.09	NS

[#] Mean ± SE (Standard error)
SEM = Standard error of mean; NS = Non-significant

Table 4. Effect of citric acid on digestibility (%) of different nutrients

Digestibility (%)	Dietary treatments (% CA)						SEM	Level of Sig.
	T_1 (0.0)	T_2 (0.5)	T_3 (1.0)	T_4 (1.5)	T_5 (2.0)	T_6 (2.5)		
DM	62.1^{a} ±1.6[#]	63.1^{ab} ±0.5	63.4^{ab} ±0.6	64.3^{b} ±0.7	64.8^{b} ±0.8	62.0^{a} ±1.9	0.58	*
CP	60.3^{ab} ±0.5	59.4^{a} ±0.7	60.4^{ab} ±0.6	62.1^{c} ±0.9	61.7^{b} ±0.3	60.1^{ab} ±1.6	0.47	*
CF	41.1 ±0.5	41.3 ±0.7	43.2 ±0.2	42.5 ±1.7	43.5 ±1.3	41.9 ±2.1	0.38	NS
EE	44.4^{b} ±2.6	38.1^{a} ±0.6	41.1^{ab} ±0.7	44.7^{b} ±0.9	42.1^{ab} ±0.5	43.7^{b} ±1.5	0.81	*
NFE	70.7 ±1.8	70.0 ±0.6	72.8 ±0.9	71.1 ±0.6	73.0 ±1.9	71.3 ±1.8	0.60	NS

[abc] Mean values within the same row with uncommon superscripts differed significantly (P<0.05), [#] Mean ± SE (Standard error)
SEM = Standard error of mean; NS = Non-significant, * = Significant at 5% level

Leukocytic count

Different leukocyte cells (lymphocyte, neutrophil, monocyte, eosinophil and basophil) were counted in the last week of the experimental period. Among the different cells, the highest cell was observed in case of lymphocyte and it was increased linearly with different level of CA (highest value 73 at 2.5% level of CA) and significant difference (P<0.05) was observed. Neutrophil content decreased when the percentage of citric acid was increased. In case of other cells, no significant difference (P<0.05) was found. Leukocytes are responsible for protecting the body against foreign invaders, such as bacteria, viruses, fungi and parasites and lymphocyte increases the immunity of the animal (Macer, 2003). He also concluded that

lymphocytes are the second most common leukocyte in small animals and horses and the most common leukocyte of ruminants. Among the leukocytes, mainly lymphocyte maintains the immune status of the animal. Lymphocyte content increased with increasing dietary level of citric acid, and therefore, citric acid has got positive effect on immunity of rabbit. Their general function is to provide adaptive immunity, by creating a specific defense against specific pathogens.

Table 5. Effect of citric acid on leukocyte content of rabbit blood

Cells	Dietary treatments (% CA)						SEM	Level of Sig.
	T_1 (0.0)	T_2 (0.5)	T_3 (1.0)	T_4 (1.5)	T_5 (2.0)	T_6 (2.5)		
Lymphocyte	62[a] ±3.8[#]	66.[b] ±1.8	66[b] ±1.2	69[b] ±2.7	71[b] ±1.0	73[c] ±1.4	1.55	*
Neutriphil	32[c] ±3.5	27[b] ±2.0	24[b] ±1.5	25[b] ±1.5	23[b] ±0.7	16[a] ±0.5	1.64	*
Monocyte	3± 0.0	0.3 ±0.3	4.0 ±2.7	1.0 ±1.2	2.0 ±1.2	4 ±2.1	0.59	NS
Eosinophil	2± 1.0	6.0 ±2.3	5.0 ±2.0	4 ±0.9	4.0 ±1.5	6 ±1.2	0.71	NS
Basophil	1 ±0.9	0.3 ±0.33	1 ±0.57	0.3 ±0.33	0 ±0.0	0.7 ±0.9	0..24	NS

[abc] Mean values within the same row with uncommon superscripts differed significantly (P<0.05), [#] Mean ± SE (Standard error)
SEM= Standard error of mean; NS = Non-significant, * = Significant at 5% level

Conclusion

The result concluded that the addition of citric acid up to 2.0% level in concentrate mixture enhances performance (body weight and feed intake) of rabbit. The use of CA up to 2.5% level improves innate immunity of rabbit by increasing defense mechanism against non-specific pathogens. At the level of 2.0% CA both performances and innate immunity are improved and this level may be recommended for growing rabbit.

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