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The Effects of Different Levels of Citric Acid on Growth Performance, Nutrient Digestibility and Gastrointestinal pH of Weaned Piglets

Shi Wenying, Lu Chunlian, Li Shang, Song Jiachun, Cao Hongzhan

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ABSTRACT

Background: This study was conducted to evaluate the effects of different levels of citric acid (CA) on the growth performance, nutrient digestibility and gastrointestinal pH of weaned piglets.

Methods: A total of 120 weaned piglets with an initial BW of 7.15±0.01 kg, were randomly divided into four groups including control treatment each with three replicates of 10 piglets each. Each group was supplemented with 0.5% (control group), 1%, 1.5% and 2% CA in the basal diet, respectively.

Result: The results showed that the average daily feed intake (ADFI) and average daily gain (ADG) of added 1% CA group were significantly higher than that of 0.5% group (P<0.05). Dietary CA significantly improved the utilization rates of crude protein, calcium and phosphorus (P<0.05), while there was no significant difference between adding 1% and 1.5% CA (P>0.05). Compared with the control group, adding CA effectively reduced the pH of the stomach, duodenum jejunum and ileum contents and adding 2% CA were significantly lower than those in other groups (P<0.05). Therefore, the addition of 1.0% CA in the diets of weaned piglets is suitable.

Key words: Citric acid, Gastrointestinal pH, Growth performance, Nutrient digestibility, Weaned piglets.

INTRODUCTION

The immune system, digestive system and intestinal flora of weaned piglets are not completely developed, showing low immunity, insufficient gastric acid secretion and poor digestive function. The change of external environment, physical properties and dietary composition of diet after weaning may very easily cause weaning stress syndrome in piglets, which is mainly manifested as increasing diarrhea rate and decreasing growth performance of piglet (Giannenas *et al*., 2014; Salisbury *et al*., 2002; WU *et al*., 2015).

Studies have shown that CA is a type of high-quality natural acidifier which can improve feed flavor, growth performance and nutrient digestibility, decrease the pH value of gastrointestinal tract, reduce the number of harmful bacteria, increase the number of beneficial bacteria such as lactobacillus and improve the anti-stress ability and immunity of animals (Boling *et al.,* 2000; Luo *et al.,* 2019; Xu *et al.,* 2018; Zheng *et al.,* 2020; Zhou and Chen *et al.,* 2010). However, in the practice of pig breeding, there are different reports on the amount and effect of CA in the diet of piglets (Huang *et al.,* 2002; Krause *et al.,* 1994; Krishnakumar *et al.,* 2008; Li *et al.,* 2009; Tsiloyiannis *et al.,* 2001), this may be due to differences in basic diet composition, breeding environment and animal species. Therefore, the present study evaluated the effects of different levels of CA on the growth performance, apparent nutrient digestibility and gastrointestinal pH of weaned piglets in order to give a more theoretical basis for the rational use of CA in production practices.

College of Animal Science and Technology, Hebei Agricultural University, Baoding, 071001, China.

Corresponding Author: Cao Hongzhan, College of Animal Science and Technology, Hebei Agricultural University, Baoding, 071001, China. Email: chz516@126.com

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MATERIALS AND METHODS

This study was conducted in the Yunong livestock breeding farm located in Shijiazhuang and College of Animal Science and Technology, Hebei Agricultural University. The CA monohydrate, the main ingredient is CA, belongs to food additive, the content is 99%, manufactured by Weifang Yingxuan Industry Co., LTD.

A total of 120 piglets which were weaned at 21±2D days, with weight of approximately 7.15±0.01 kg, were supplied from the Yunong Livestock Breeding Farm located in Shijiazhuang and randomly divided into four groups including control treatment each with three replicates of 10 piglets. Each group was supplemented with 0.5%(control group), 1%, 1.5% and 2% CA in the basal diet, respectively. The experiment was carried out for 45 days, including 3 days of pre-feeding trial. The basal diet formula, a corn-soybean basal diet was prepared according to the guiding feeding

level of the NY/T 65-2004 of China (Xiong and Zhang, 2010) and NRC (2012), as detailed in Table 1.

The ambient temperature of the piggery was controlled at 25-27°C and the humidity is maintained at 55-65%.The piggery was cleaned and disinfected regularly. The piglets were fed three times at 7:00, 14:00 and 18:00 every day, with unrestricted intake of food and water. Daily feed consumption and body weight at the beginning and end of the trial were determined to calculate for the average daily feed intake per day (ADFI), average daily gain (ADG) and feed to gain ratio. The fecal samples from the pigs were collected every day from day 39 to 40 for the determination of digestibility. 10 ml 10% hydrochloric acid per 100 grams of feces was added to fix the nitrogen.The feeds and feces were oven-dried at 65°C to obtain a constant weight and then ground to pass a 1-mm screen and store at 4°C for chemical analysis according to the method of AOAC (2012). The CP were estimated by the Kjeldahl method, ether extract (EE) by the Soxhlet ether extraction apparatus, Calcium(Ca) by Potassium permanganate titration, phosphorus(P) by Molybdate yellow colorimetric method and acid insoluble ash(AIA) was described by the GB/T23742(2009).The digestibility of nutrient were calculated as follows:

$$\text{Apparent nutrient digestibility (\%)} = 100 \times \left[1 - \frac{\text{Amount of nutrient in feces}}{\text{Amount of a nutrient in feed}} \times \frac{\text{Amount of acid insoluble ash in feed}}{\text{Amount of acid insoluble ash in feces}}\right]$$

(Zhou and Chen, 2010)

At the end of the trial,3 weaned piglets with similar body weight in each group were killed under anesthesia. The stomach, duodenum, jejunum, ileum and cecum were separated with sterile cotton thread and the pH of gastrointestinal contents were measured with a portable pH meter. SPSS 22.0 software was used for processing and statistical analysis of the experiment of single factor test data (ANOVA) and Duncan's method was used for multiple comparisons. The test results were expressed as "Mean ± SE". Statements of statistical significance were tested at the level $P<0.05$ and extremely significant when $P<0.01$.

RESULTS AND DISCUSSION

Growth performances

The growth performances are presented in Table 2. The ADFI and ADG of test group 3 were significantly higher ($p<0.05$) in comparison with the control. However,there was no significantly difference in ADFI in group 1, group 2 and group 3 and no significantly difference in ADG in group 1 and group 3. There was no significant difference in feed/gain ratio among all groups ($p>0.05$).

A previous study has shown that 2% (Giesting and Easter, 1985), 2% ~3% (Cronmwell, 1987), 2.5% (Krause *et al*., 1994). CA diets increased the ADG of weaned piglets. However, the current study testified that 1% CA diet had the best performance. But there was no significant difference in ADFI 1%, 1.5%. 2% CA groups. Similar to these findings were the studies by Huang *et al.* (2002), Wang (2011) and Zheng *et al*. (2005). The reasons for the inconsistent results among studies are not known but might be the weaning age of piglets, the buffering capacity of the diets,dietary components and the feeding environment.

Apparent nutrient digestibility

The apparent digestibility values were shown in Table 3. The digestibility of CP increased with the increase in dietary CA levels and there were significant differences among all groups ($p<0.05$). The digestibility of EE was reduced with the increase in dietary CA level and there was significant lower ($p<0.05$) in the 2% CA group in comparison with control and 1% CA groups. The digestibility of Ca and P were significantly increased with the increase in dietary CA levels ($p<0.05$), but there were no significantly differences in Ca and P digestibility between the 1% CA and the 1.5% CA groups ($p>0.05$), P digestibility between the 1.5% CA and the 2% CA groups ($p>0.05$).

Similar to this result, Debi M *et al.* (2010) indicated that supplementation of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% CA in growing rabbit diet improved CP digestibility. Broz *et al.* (1987) found that adding 0.5%, 1.0% or 2.0% CA in the basal diet of weaned piglets could significantly improve feed utilization rate. Cao *et al*. (2015) reported that adding 0.5% CA and compound acid (0.25% benzoic acid and 0.25% CA) significantly increased Ca and P digestibility. Diao *et al.* (2013) determined that dietary 0.5% CA significantly increased the digestibility of crude protein, ether extract,

Table 1: Composition and nutrient levels of the basal diets of the weaned piglets (%).

Itemts	Content (%)	Nutrient levels	
Corn	65.00	DE/(kcal/kg)	3520
Wheat bran	1.40	CP	18.20
Soybean meal	11.00	CF	2.50
Expanded soybean	6.00	Ca	1.22
Fish meal	2.00	TP	0.27
Fermented soybean meal	4.00		
Soy protein concentrate	2.50		
Limestone	0.80		
Whey powder	2.50		
$CaHPO_4$	1.00		
NaCl	0.30		
Soybean oil	2.00		
Lys	0.50		
Premix[a]	1.00		
Total	100.00		

Note: [a]Premix provides the following per kg of the diet; VB_1 1 mg; VB_2 2 mg; VB_6 1 mg; VB_{12} 0.1mg; VA 1300 IU; VD_3 1600 IU; VE 18 IU; VK_3 0.5 mg; nicotinic acid 9 mg; Pantothenic acid 10 mg;Biotin 0.09 mg; Choline Chloride 360 mg; Cu 3 mg; Zn 50 mg; Fe 60 mg; I 0.12 mg; Mn 2 mg; Se 0.13 mg.

Table 2: Effects of the CA additions on the growth performances and diarrhea rates of the weaned piglets.

Items	Groups				SEM	*P*-value
	Control	Group 1	Group 2	Group 3		
	0	1.0%	1.5%	2.0%		
IBW/kg	7.14±0.01	7.13±0.01	7.16±0.01	7.16±0.03	0.028	0.690
FBW/kg	24.87±0.09^{c}	27.39±0.17^{a}	25.75±0.72^{bc}	26.43±0.20^{ab}	0.549	0.010
ADFI/(g/d)	788.28±38.58^{b}	897.19±36.76^{a}	861.04±4.89^{ab}	815.61±22.28^{ab}	14.511	0.111
ADG(g/d)	454.56±2.54^{c}	519.41±4.61^{a}	476.55±18.84^{bc}	494.08±6.19^{ab}	10.604	0.012
F/G	1.74±0.09	1.73±0.08	1.81±0.06	1.65±0.02	0.100	0.498

Note: Different letters within the same row represent significant difference.

Table 3: Effects of CA additions on the apparent nutrients digestibility of weaned piglets (%).

Items	Groups				SEM	*P*-value
	Control	Group 1	Group 2	Group 3		
	0	1.0%	1.5%	2.0%		
CP	75.44±0.34^{d}	78.87±0.30^{b}	77.69±0.08^{c}	80.50±0.26^{a}	0.374	0.000
EE	73.98±0.62^{a}	73.56±0.22^{a}	72.82±0.78^{ab}	71.73±0.19^{b}	0.734	0.065
Ca	63.19±0.77^{c}	66.06±0.52^{b}	65.60±0.12^{b}	68.68±0.29^{a}	0.694	0.000
P	50.07±1.09^{c}	54.90±0.58^{b}	56.49±1.13^{ab}	59.32±0.98^{a}	1.378	0.001

Note: Different letters within the same row represent significant difference.

Table 4: Effects of the CA addition on the pH levels of the contents of the gastrointestinal tracts of weaned piglets (%).

Items	Groups				SEM	*P*-value
	Control	Group 1	Group 2	Group 3		
	0	1.0%	1.5%	2.0%		
Stomach	3.64±0.05^{a}	3.26±0.05^{b}	3.36±0.04^{b}	2.99±0.06^{c}	0.073	0.001
Duodenum	5.92±0.07^{a}	5.82±0.03^{ab}	5.72±0.04^{b}	5.51±0.05^{c}	0.068	0.002
Jejunum	6.44±0.04^{b}	6.35±0.01^{b}	6.61±0.01^{a}	6.14±0.08^{c}	0.065	0.001
Lleum	7.01±0.07^{a}	6.33±0.05^{b}	6.91±0.10^{a}	5.99±0.08^{c}	0.111	0.001

Note: Different letters within the same row represent significant difference.

calcium and phosphorus of weaned piglets.But this study showed that 2% CA group had the highest digestibility of ether extract, which was inconsistent with the report by Diao *et al.*(2013).

Gastrointestinal pH

As shown in Table 4, The gastrointestinal pH were significantly reduced with the increase in dietary CA levels (p<0.05), but there were no significantly differences in pH of stomach contents between the 1% CA and the 1.5% CA groups (p>0.05), pH of duodenum contents between the 0.5% CA and the 1% CA groups and between the 1% CA and the 1.5% CA groups (p>0.05), PH of jejunum contents between the 0.5% CA and the 1% CA groups, pH of ileum contents between the 0.5% CA and the 1.5% CA groups.

Klieviiūt *et al.* (2016) showed that organic acids had no significant effect on pH in different parts of the stomach and intestines of rabbits. There were no significant differences in stomach and small intestine pH of weaned piglets which also supported by Piva *et al.* (2007) who reported that no effect on gastrointestinal pH after inclusion of sorbic acid in swine diets. However, Esmaeilipour *et al.* (2011) reported that adding CA to a low-phosphorus diet in broilers significantly reduced gastrointestinal pH Risley *et al.* (1992) found that adding 1.5% CA to corn soybean meal diet of weaned piglets reduced the pH of ileum contents. The results of this study showed that additions of CA could significantly reduce the pH levels of gastric, ileal and jejunal contents. The results of different effects of acidifiers on the pH of gastrointestinal tract contents may be related to the treatment technology and the inclusion level of acidifiers as well as the differences of animal species and individuals.

CONCLUSION

In the present study, it was found that under the experimental conditions, the addition of 1% CA in the diets of weaned piglets in accordance with various indicators and production benefits had effectively improved intestinal development, significantly reduced the pH level of the gastrointestinal contents and increased the apparent nutrient digestibility in the feed, thereby enhancing the performance of the examined weaned piglets.

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