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Effect of Organic Acids in Crossbred Weaned Piglets

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ABSTRACT

The present study was aimed to evaluate the effect of dietary organic acids on growth performance, nutrient digestibility and fecal microflora of crossbred weaned piglets. A total of thirty weaned crossbred piglets (75% LWY × 25% Desi) with average body weight of 7.5 ± 0.5 kg (2 months old) were randomly divided (CRD) into five homogenous treatments with six piglets in each. The dietary treatments were T1 (control) - Basal diet as per NRC (2012), T2 - Basal diet + 1.5% Citric acid, T3 - Basal diet + 1.5% Fumaric acid, T4 - Basal diet + 1.5% Benzoic acid, T5 - Basal diet + 1.5% blend of citric, Fumaric acid and Benzoic acids (each at 0.5%). Results revealed that piglets received the diet containing blend of organic acids improved ($P < 0.05$) the ADG, feed efficiency, nutrient digestibility (CP and EE) and reduced fecal *E. coli* counts as compared with piglets received individual organic acids and control. The highest ($P < 0.05$) weight gain was observed in piglets fed T5 or T4 diets, followed by T2 or T3 and lowest in control. There was no effect ($P > 0.05$) of organic acids on average daily feed intake of piglets of different treatments. The digestibility of CF (%) was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared to control. The *Salmonella* count was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared with control. The cost per Kg weight gain (Rs.) was found to be significantly ($P < 0.05$) higher in organic acid supplemented groups as compared to control. In conclusion, dietary supplementation of blend of organic acids at 1.5% indicated beneficial effects on growth performance and nutrient digestibility coexisting with reduced fecal microbiota.

KEYWORDS: Fecal microbiota, Growth performance, Nutrient digestibility, Organic acids and Weaned piglets

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INTRODUCTION

Weaning is one of the most stressful events for pigs, which can cause dysfunction of the gastrointestinal tract (GIT) and immune system, leading to reduced growth performance and feed intake in the first week after weaning (Tang et al., 2017). Use of subtherapeutic doses of antibiotic growth promoters (AGPs) was in practice to alleviate gastrointestinal disturbances and improve microbial balance in the GIT of weaned piglets (Rathnayake et al., 2021). The use of antibiotic growth promoters in swine production contributes to the emergence of antibiotic resistant strains both in livestock and human posing a significant public health threat (Ferronato and Prandini, 2020 and Gois et al., 2016). Thus, exploring the alternatives that can promote growth, improve feed efficiency and reduce enteric diseases in swine has become a trending topic for researchers reduce/ eliminate the use of antibiotic growth promoters.

Among different compounds exploited for the replacement of antibiotics, organic acids (OAs) are potential alternatives as they are known to reduce the load of pathogenic bacteria and modulate the microbial composition in the GIT and enhance the growth performance of pigs (Long et al., 2018; Xu et al., 2018). In recent years, multiple reports have described the beneficial effects of dietary organic acids such as formic, fumaric, citric, butyric, benzoic, lactic and propionic acids by enhancing growth performance (Oh et al., 2018; Xu et al., 2018; Ngoc et al., 2020; Long et al., 2018 and Xiang et al., 2021), nutrient digestibility (Dutta et al., 2015; Yang et al., 2018; Oh et al., 2018 and Bujnak et al., 2021) and modulating the intestinal microflora (Long et al., 2018; Dutta et al., 2015; Bharathidhasan et al., 2022 and Ahmed et al., 2014).

However, the effectiveness of organic acids varies with several factors such as type and dose of their inclusion, dietary composition, age, health status and physiological condition of the animal (Mroz et al., 2006). Hence, the aim of the present study is to evaluate the response of weaned crossbred (LWY×Desi) piglets upon incorporation of single and blend of organic acids.

MATERIALS AND METHODS

Experiment location and ethical statement

The experimental work of the current study was carried out at AICRP on pigs, Sri Venkateswara Veterinary University, Tirupati with the approval of Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Reg No 281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/026 /Animal Nutrition /2022 dated 22.06.2022).

Experimental design and animals

In a completely randomized design, thirty crossbred (75% LWY × 25% Desi) weaned piglets with average body weight of 7.5 ± 0.5 kg (2 months old) were divided into five homogenous treatments with six piglets in each.

Housing and feeding of experimental animals

The piglets were housed in individual pens in a well-ventilated semi-opened animal shed having provision for individual feeding and watering. Five iso-nitrogenous and iso-caloric experimental diets were formulated as per NRC (2012) requirements (Table 1). The dietary treatments were T1 (control) - Basal diet as per NRC (2012), T2 - Basal diet + 1.5% Citric acid, T3 - Basal diet + 1.5% Fumaric acid, T4 - Basal diet + 1.5% Benzoic acid, T5 - Basal diet + 1.5% Citric, Fumaric acid and Benzoic acids (each at 0.5%).

Table 1. Ingredient and nutrient composition (%) of experimental diets during weaner phase

Ingredient	T1	T2	T3	T4	T5
Maize	56.9	55.0	55.0	55.0	55.0
Soybean meal	35.6	36.0	36.0	36.0	36.0
DORB	5.0	5.0	5.0	5.0	5.0
Salt	0.5	0.5	0.5	0.5	0.5
Mineral mixture #	1.5	1.5	1.5	1.5	1.5
Lysine	0.5	0.5	0.5	0.5	0.5
Citric acid	-	1.5	-	-	0.5
Fumaric acid	-	-	1.5	-	0.5
Benzoic acid	-	-	-	1.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Proximate composition (%)*					
Dry matter	89.4	88.9	88.8	90.6	90.01
Organic matter	88.2	86.5	87.9	90.1	89.7
Crude protein	18.9	18.9	18.8	18.8	18.9
Ether extract	0.78	1.24	1.01	1.36	1.32
Crude fiber	5.81	7.01	5.02	6.52	6.53
Total ash	8.75	8.43	8.07	7.86	8.28
Acid insoluble ash	2.21	2.95	3.02	2.12	3.06
Nitrogen free extract	65.7	65.4	67.1	65.4	64.9
ME (Kcal/Kg)**	3189.4	3173.5	3179.7	3231.3	3194.8

*On dry matter basis ** Calculated value # Each Kg contained Calcium - 25.5%; Phosphorous - 12.75%; Sulphur - 0.72%; Zinc - 9600 mg; Manganese - 1500 mg; Sodium - 5.9 mg; Magnesium - 6000 mg; Potassium - 100 mg; Iron - 1500 mg; Iodine - 325 mg; Copper - 1200 mg and Cobalt - 150 mg.

Growth trial

Piglets were offered respective experimental rations daily at 9:00 AM and 3:00 PM. Fresh water was offered *ad libitum* daily to all the animals. The growth trial last for 83days (83 days were required for the piglets to reach 25 Kg body weight). The piglets were weighed individually at fortnightly intervals in the morning before feeding and the body weights were recorded to assess the changes in body weight, average daily gain.

Digestion trial

At the end of the growth trial, the piglets were allowed for a period of two days for acclimatization to new system of housing and management followed by a collection period of 7 days. During digestion trial, piglets were offered experimental rations daily at 9:00 AM and 3:00 PM. Fresh water was offered *ad libitum* daily to all the animals. The total quantity of faeces voided during preceding 24 h was weighed, mixed and representative samples (5%) from each piglet was taken separately in a polythene zip lock covers and stored in a deep freezer at -20^{0}C. The faecal sample was dried and ground to pass through 2 mm screen and preserved in air tight bottles for further analysis. The proximate composition of feed and fecal sample was determined as per AOAC (2019).

Fecal microbiota

For enumeration of Faecal microbiota, fresh faecal sample was obtained directly by massaging rectum, collected in an aseptic container and analyzed for faecal pathogens (*E. coli, Salmonella and Staphylococcus spp*). About 1.0 g of composite faecal sample was weighed aseptically and transferred to a sterile test tube and diluted with 9 ml of sterile nutrient broth and was homogenized. Viable count of bacteria in the faecal samples were conducted by plating 10 folds dilution in the nutrient broth on to MacConkey agar, XLD agar and Mannitol salt agar for growth of *E. coli*, *Salmonella and Staphylococcus,* respectively. The plates after incubation at 37^0 C for 24 hours (*E. coli* and *Salmonella*) and 24 – 48 hours (*Staphylococcus*) were counted immediately for the developed colonies. Each colony will account for one viable microbial cell or one colony-forming unit. Therefore, the number of CFU in the sample was determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor.

Cost economics

Economics of feeding was calculated from the records of daily feed consumption and by considering the prevailing market price of feed ingredients and organic acids used for feeding of experimental piglets.

Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) as per the methods of Snedecor and Cochran (1995) and the means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Feed intake of piglets

It revealed that supplementation of different organic acids (either individually or in combination) at 1.5% did not affect ($P > 0.05$) the feed intake of crossbred piglets (Table 2). Similar results by Namkung et al. (2004); Gerritsen et al. (2010) and Zhai et al. (2017) also indicated that the ADFI was not affected by supplementation of organic acids. Contrary to the present study, reduced ADFI upon formic acid supplementation (Ma et al*.,* 2021) and increased ADFI (Diao et al., 2016 and Kludge et al*.,* 2006) upon supplementation of benzoic acid was reported. The variation in these studies might be due to strong pungent odours of some organic acids such as formic acid and propionic acid which may reduce diet palatability, thus decreasing feed intake and thereby negatively impact the growth rate (Kil et al*.,* 2005). In the current study, the organic acids (citric, fumaric and benzoic acids) offered a good acceptability without any considerable difference in the palatability and feed intake of diets in all the experimental groups.

Growth performance and FCR

It was observed that the ADG (g) and feed efficiency during the experimental period of 83 days was highest ($P < 0.05$) in T5 followed T2 or T3 or T4 and lowest in T1 (Table 2). The highest ($P < 0.05$) weight gain was observed in piglets fed T5 or T4 diets, followed by T2 or T3 and lowest in control. Similar results of improved average daily gain and feed efficiency was observed by several researchers (Grilli et al*.,* 2010; Li et al*.,* 2008; Long et al*.,* 2018 and Xiang et al*.,* 2021) in weaner piglets. However, contrary to the present study, Gerritsen et al. (2010)

and Lee et al. (2021) observed no effect of organic acids on growth performance in weaned piglets. These inconsistent results might be attributed to the various factors such as composition of diet, type of acid, purity of the acid, inclusion level of acid, age, breed and health status of the piglets.

Addition of organic acid blend in the current study resulted a positive effect on the growth performance in the weaned piglets. Among several factors which might have involved in improved growth performance in piglets, lowering of gastro-intestinal pH and subsequent modification of intestinal microflora is one of the main reasons for enhanced growth performance in the current study (Kil et al., 2005 and Long et al., 2018). Another possible explanation for improved growth performance was attributed to enhanced digestibility of nutrients. In the current study, increased apparent digestibility of nutrients (CP, EE and CF) was observed in the piglets fed diets containing organic acid/s (Table. 3). As a result of antimicrobial activity of organic acids, there was a reduction in pathogenic microflora such as *E. coli*, *Salmonella* and *Staphylococcus* as evinced by reduction in their count in the fecal matter (Table. 4). This in turn reduces the metabolic needs of microbes, thus enhancing the availability of dietary nutrients to piglets, thereby improved the growth performance. In the current study, blend of organic acids found to be effective as compared to individual organic acid supplementation due to the synergistic activity and dissociation properties (Partanen et al., 2007).

Nutrient digestibility

In the present study, no significant (P > 0.05) difference in digestibility of DM, OM and NFE was observed among different treatments (Table. 3). While there was significant (P< 0.05) difference in digestibility of CP (%) among different treatments and was in the order of T5 > T2 > T3 > T4 > T1. The results agree with several authors who reported improved CP digestibility with supplementation of either single (Diao et al., 2016; Guggenbuhl et al., 2007 and Kiarie et al., 2018) or blend of organic acids (Dutta et al., 2015; Yang et al., 2018 and Bujnak et al., 2021). Contrary to present results, several researchers reported that the digestibility of CP was not affected due to supplementation of either single (Kludge et al., 2006) or blend of organic acids (Kil et al., 2005 and Omogbenigum et al., 2003).

Table 2. Effect of dietary organic acids on growth performance of crossbred weaned piglets.

Particulars	Treatment Groups					P-Value
	T1	T2	T3	T4	T5	
Initial BW (Kg)	7.60±0.53	7.60±0.96	7.50±0.75	7.50±0.54	7.40±0.73	0.863
Final BW (Kg)*	22.6±0.48[c]	25.4±0.81[b]	25.1±1.22[b]	25.7±1.17[a]	26.2±0.53[a]	0.031
Weight gain (Kg)*	15.0±0.47[b]	17.8±0.84[ab]	17.6±0.67[ab]	18.2±0.73[a]	18.8±1.03[a]	0.032
Feed intake (g)	634.00±2.01	634.30±2.47	636.60±2.29	635.40±2.01	636.60±2.65	0.483
ADG (g/day)*	180.72±7.76[c]	214.45±10.18[b]	212.04±8.17[b]	219.27±8.85[b]	226.50±12.49[a]	0.043
FCR*	3.52±0.10[a]	2.98±0.14[b]	3.02±0.12[b]	2.91±0.11[b]	2.84±0.17[c]	0.037

[abc] Values bearing different superscripts in a row differ significantly (P < 0.05) *

Weaned piglets have an unfavorable pH in their stomach because of the limited HCl secretion. Nutrient digestibility is closely related to secretion and functionality of digestive enzymes which is more pronounced at lower pH. Improved CP digestibility associated with organic acid supplementation in the current study might be attributed to the lower pH in the gastrointestinal tract, resulting in increased activity of digestive enzymes (Kim et al., 2005). Low pH is the favorable condition for activation of several digestive enzymes (Mroz, 2005) especially pepsin and trypsin which are the prime enzymes responsible for the digestion of proteins, as evinced in the current study.

Similar trend (T5 > T2 > T3 > T4 > T1) of improved ($P < 0.05$) digestibility of EE was observed in the current research is comparable with the reports of Diao et al. (2016) supplemented with benzoic acid. Contrary to the present study several authors (Bujnak et al., 2021; Dutta et al., 2015; Gerritsen et al., 2010 and Kil et al., 2005) did not indicate any improvement in the digestibility of EE. The improved digestibility of EE observed in the piglets received blend of organic acids could be due to reduced passage rate of digesta into the small intestine and stimulation of the enzyme secretion of the pancreas (lipase, amylase) leading to an increased digestibilities of nutrients (Ma et al., 2021).

The digestibility of CF (%) was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared with control. Like the present study, Dutta et al. (2015) and Gerritsen et al. (2010) also observed an improvement in CF digestibility. The improved fiber digestion could be attributed to low pH in GIT, inhibiting the activity of enterobacteria (*E. coli* and *Salmonella*), thus creating a better environment for bacteria that ferments crude fibre (Hansen et al., 2007), resulted in better digestibility of CF.

Table 3. Effect of dietary organic acids on nutrient digestibility (%) of crossbred weaned piglets.

Particulars	Treatment Groups					P-Value
	T1	T2	T3	T4	T5	
Dry matter (%)	82.62±1.13	84.37±2.41	84.58±0.91	85.41±0.98	84.67±1.05	0.561
Crude protein (%)*	76.70±4.32[c]	78.54±5.15[b]	79.63±4.35[b]	77.41±3.61[b]	81.95±2.11[a]	0.024
Ether extract (%)*	63.76±3.02[c]	67.93±4.26[b]	65.45±2.91[b]	65.23±2.06[b]	68.78±1.62[a]	0.043
Crude fiber (%)*	43.14±2.17[b]	47.54±4.54[a]	45.78±3.40[a]	47.58±1.49[a]	48.19±3.11[a]	0.048
Organic matter (%)	82.34±1.21	84.34±1.54	85.43±2.43	83.55±2.10	84.94±0.95	0.932
NFE (%)	87.03±1.23	89.35±2.41	87.64±2.42	88.67±3.12	89.47±0.43	0.562

[abc] Values bearing different superscripts in a row differ significantly ($P < 0.01$)**

Fecal microbiota

In accordance to the results of present study (Table. 4), several authors observed a reduction in fecal microbial count by supplementing single (Bharathidhasan et al., 2022) or blend of organic acids (Yang et al., 2019; Long et al., 2018; Dutta et al., 2015; Ahmed et al., 2014). Contrary to the present study, non-significant difference in fecal microbial count was observed due to supplementation of single (Canibe et al., 2001) or blend of organic acids (Lee et al., 2021; Oh et al., 2018; Li et al., 2008; Namkung et al., 2004).

The reduced fecal microbiota in the current study might be attributed to reduced pH in the GIT due to supplementation of organic acid/s. This agrees with Richards et al. (2005) who reported that lower pH in the GIT of weaned piglets resulted in reduced proliferation of pathogenic bacteria (*E. coli*) in the GIT. Supplementation of organic acids influences the intestinal microbiota by changing intestinal environment to a uncongenial conditions for pathogenic bacterial growth (Long et al., 2018) resulting in reduced pathogens as evinced in the current study. Another possible explanation for reduced pathogenic microflora can be attributed to direct anti-microbial activity of organic acids. Organic acids in their undissociated from, passively diffuse through the bacterial cell wall and interfere with bacterial biochemical processes leading to cell death (Kluge et al., 2006 and Graber et al., 2012). Therefore, results of current study demonstrated the favorable impact of supplementation of organic acid/s by reducing pathogenic micro-organisms, thereby contributed to improved growth performance in weaned piglets.

Table 4. Effect of dietary organic acids on fecal microbiota (CFU/ml) of crossbred weaned piglets

Species	Treatment Groups T1	T2	T3	T4	T5	P-Value
E. coli ($\times10^5$)**	5.09±0.24^a	4.93±0.23^b	4.44±0.21^b	4.71±0.22^b	4.15±0.31^c	0.006
Salmonella ($\times10^5$)**	2.18±0.07^a	1.34±0.05^b	1.29±0.12^b	1.38±0.16^b	1.08±0.17^b	0.001
Staphylococcus ($\times10^5$)**	1.54±0.08^a	1.27±0.04^b	0.96±0.04^c	1.26±0.05^b	1.12±0.05^{bc}	0.001

^{abc} Values bearing different superscripts in a row differ significantly (P < 0.01)**

Cost economics

There was increased feed cost (Rs./Kg) due to the incorporation of organic acids in the diets as compared to control (Table. 5). The cost (Rs./Kg) was increased by 3.59, 4.04, 2.84 and 3.49 in T2, T3, T4 and T5, respectively as compared to control (T1). The cost variation among the treatments was due to the inclusion of organic acids. The cost of organic acids were in the order of fumaric acid > citric acid > benzoic acid. Hence the diets containing fumaric acid (T3) resulted in the maximum cost and diets containing benzoic acid (T4) resulted in the minimum cost. The cost per Kg weight gain was lowest (P < 0.05) in T4 or T5 groups followed by T2 or T3 and highest in T1 group. Though there was an increase in feed cost due to organic acid supplementation, the cost per Kg weight gain (Rs.) was found to be significantly (P < 0.05) lower in organic acid supplemented groups as compared to control.

CONCUSION

The present study suggested that dietary inclusion of blend of organic acids at 1.5% improved overall growth performance, nutrient digestibility and reduced fecal pathogenic microbial load. Therefore, the blend of organic acids (citric, fumaric and benzoic acid) can serve as an alternative to antibiotic growth promoters in weaned piglets to promote growth and improve the health status. Further research is warranted to determine if the gut microbiota was altered by organic acids during early stage would lead to long-lasting beneficial effects on swine performance in their later stages.

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Table 5. Effect of dietary organic acids on cost economics of pork production in crossbred weaned piglets

Particulars	Treatment Groups					P-Value
	T1	T2	T3	T4	T5	
Cost of weaners feed (Rs. /Kg)	37.25	40.84	41.29	40.09	40.74	
Total feed intake (Kg /animal)	52.62±1.12	52.64±0.80	52.83±0.63	52.73±1.40	52.83±2.20	0.378
Total feed cost (/Rs./animal)	1960±40^{d}	2149±54^{b}	2189±79^{a}	2114±33^{c}	2152±82^{b}	0.041
Weight gain (Kg)*	15.00±0.47^{b}	17.80±0.84^{ab}	17.60±0.67^{ab}	18.20±0.73^{a}	18.80±1.03^{a}	0.032
Cost / Kg weight gain (Rs.)*	130.69±2.65^{c}	120.78±1.13^{b}	123.94±1.89^{b}	116.15±2.07^{a}	114.49±1.27^{a}	0.032

^{abc} Values bearing different superscripts in a row differ significantly ($P < 0.05$) *

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