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Research Article
Effect of Tributyrin Supplementation in Diet on Production Performance and Gastrointestinal Tract of Healthy Nursery Pigs

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Abstract

Background and Objective: The period after weaning is a critical period in swine production due to several factors affecting the function of the small intestine. Since, tributyrin is a potential alternative for overcoming these problems, this study was conducted to evaluate the effects of supplementing tributyrin in diet on productive performance and gastrointestinal tract of nursery pigs. **Materials and Methods:** The 64 castrated commercial piglets were studied for 56 days (from 21-77 days of age) and kept under evaporative cooling system. The piglets were arranged by completely randomized design; a t-test was used to compare mean different of the treatments. The pigs were randomly divided into 2 treatments and each treatment consisted of 8 replications, 4 pigs each. **Results:** Feeding tributyrin had no significant effects on the growth performance ($p>0.05$), while ileum villous height tended to increase ($p = 0.07$). Gastrointestinal pH, populations of *E. coli* and *Lactobacillus* spp., in the caecum and caecal short-chain fatty acids concentration were not influenced by tributyrin supplementation ($p>0.05$). **Conclusion:** Although, tributyrin did not have significant effect on productive performance and gut ecology of healthy piglets, it may positively support intestinal morphology. In healthy piglets, the effect of various levels of tributyrin supplementation in diet on intestinal morphology is interesting and should be more investigated.

Key words: Tributyrin, butyric acid, organic acids, production performance, gastrointestinal pH, intestinal morphology, villi, short-chain fatty acids, intestinal microflora, piglets

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The weaning of pigs is generally considered as a critical point since there are many negative factors affecting the anatomy and functions of the small intestine. Weaning piglet was challenged with physiological, environmental and social change that can contribute to intestinal and immune system dysfunctions[1], decrease active small intestinal absorption[2], increase enteric infections and diarrhea and depression of growth performance in piglets[3].

Now a days, consumers are focusing greater attention on the quality and safety of food. The ban on antibiotics as a growth promoter in animal feed in the European Union (EU) has motivated research on alternative ways to optimize the digestive process, increase nutrient availability, improving growth performance and minimizing the use of antibiotics such as the supplementation of pro-prebiotics, organic acids or phytogenics substance in the diets[4-8]. It is accepted that organic acid is an alternative to protect animals from harmful bacteria and to improve growth performance due to its beneficial effect on antimicrobial function, digestibility and nutrient resorption[9].

Butyric acid is a volatile fatty acid that serves as a primary source of energy for colonocytes, is a strong mitosis promoter and a differentiation agent in the gastrointestinal tract[10], while n-butyrate is an effective anti-proliferation and anti-differentiation agent in various cancer cell lines[11]. Tributyrin is a precursor of butyric acid that can improve the trophic status of the epithelial mucosa in the gut of nursery piglets[12]. Butyrate can be released from tributyrin by intestinal lipase, releasing three molecules of butyrate and then is absorbed by the small intestine[13]. The supplementation of tributyrin in the diet may improve the production performance of piglets and act as a mitosis promoter agent in the gastrointestinal tract to stimulate the proliferation of villi in the small intestine of piglets post-weaning.

Therefore, this study was conducted to study the effect of supplementing tributyrin in the diet of weaning pigs on the productive performance, intestinal morphology, gastrointestinal pH, large intestine selected microflora and ceacal short-chain fatty acid concentrations.

MATERIALS AND METHODS

Animals and management: Total 64 castrated commercial crossbred piglets (21 days old) were tested in a completely randomized design. The pigs were randomly divided into two groups and each group consisted of 8 replications (pens) of 4 pigs each. The average body weight of each replication was homogenized and balanced (7.53±0.29 kg). Comparison of treatments was calculated using a t-test.

The piglets were kept, maintained and treated in adherence to accepted standard for human treatment of animal for 8 weeks under an evaporative cooling system to control air ventilation and temperature. Feed and water were offered *ad libitum*. During the feeding trial, the house was cleaned weekly and the feces were removed every day.

Experimental diets: The nutrients composition of the experimental diets were according to the recommendation of National Research Council[14] without any antimicrobial agents. The diet was divided into three phases consisting of pre-starter (after weaning-14 days), starter I (15-42 days) and starter II (43-56 days). Two experimental diets were supplemented with tributyrin at the level of 0.00% (control diet) and 0.40% of the diet according to the recommendation of Perstorp Waspik B.V (Table 1, 2). Feed was in pellet form (3 mm diameter) and prepared at 3-weekly intervals.

Measurements

Production performance: The body weight of each pig was measured at the end of each feeding phase (0-14, 15-42 and 43-56 days after weaning) and the body weight gain was calculated. The feed intake of each pen was measured weekly. The Feed Conversion Ratio (FCR) was calculated from the body weight gain and feed intake data.

Morphology of small intestine: At the end of the trial, one pig from each replication was exsanguinated. Tissue samples were collected from the duodenum, jejunum and ileum and were immediately fixed in 10% neutral buffer formalin. Then, the tissues were carefully embedded in paraffin. For each specimen, at least 10 sections of 7 μm thickness were prepared. Tissues were then stained with haematoxylin-eosin for histological evaluation. Histology of the duodenum, jejunum and ileum tissue was assessed under a light microscope in accordance with Nunez *et al.*[15]. The morphology of the small intestines in this study included the villous height, crypt depth and the villous height to crypt depth ratio which were conducted using a computer-assisted image-analysis system (Biowizard, Thaitec, Thailand). Measurements of the villous height from the tip of the villous to the villous-crypt junction and the crypt depth from the villous-crypt junction to the lower limit of the crypt were recorded as the mean of 10 fields for each specimen.

Table 1: Compositions of experimental diets

	Pre-starter		Starter I		Starter II	
Ingredient name	Control	Tributyrin	Control	Tributyrin	Control	Tributyrin
Corn	5.000	5.000	10.000	10.000	43.302	43.302
Broken rice	31.558	31.558	37.384	37.384	15.000	15.000
Soybean oil	1.651	1.651	3.694	3.694	3.822	3.822
Raw rice bran	-	-	5.000	5.000	8.000	8.000
SBM (46.5% CP)	5.000	5.000	22.183	22.183	17.909	17.909
Full-fat soybeans	40.858	40.858	10.000	10.000	5.000	5.000
Fish meal (58% CP)	2.000	2.000	3.000	3.000	3.000	3.000
L-lysine HCL 78%	0.349	0.349	0.296	0.296	0.248	0.248
DL-methionine	0.149	0.149	0.128	0.128	0.059	0.059
L-threonine	0.077	0.077	0.066	0.066	0.044	0.044
Whey	10.000	10.000	5.000	5.000	-	-
Monodicalciumphosphate	1.264	1.264	0.806	0.806	0.720	0.720
Calcium carbonate	0.654	0.654	0.616	0.616	0.712	0.712
Salt	0.463	0.463	0.365	0.365	0.488	0.488
Vitamin and mineral premix[A]	0.500	0.500	0.500	0.500	0.500	0.500
Tributyrin	-	0.400	-	0.400	-	0.400
Inert filler (corn starch)	0.400	-	0.400	-	0.400	-
Choline choride 75%	0.077	0.077	0.062	0.062	0.046	0.046
Sodium bicarbonate	-	-	0.500	0.500	0.750	0.750
Total (%)	100.000	100.000	100.000	100.000	100.000	100.000

[A]Premix content; vitamin A (retinyl acetate) 4 MIU, vitamin D (cholecalciferal) 0.64 MIU, vitamin E (DL-α-tocopheryl acetate) 24,000 IU, vitamin K3 (menadione) 1.4 g, vitamin B_1 (thiamine) 0.6 g, vitamin B_2 (riboflavin) 0.3 g, vitamin B_6 (pyridoxine) 0.75 g, vitamin B_{12} (cyanocobalamin) 14 mg, nicotinic acid 20 g, pantothenic acid 10 g, folic acid 0.44 g, d-biotin 0.04 g, choline chloride 60 g, Fe ($FeSO_4.H_2O$) 45 g, Cu ($CuSO_4.5H_2O$) 40 g, Mn (MnO) 15 g, Zn (ZnO) 40 g, Co ($CoCO_3$) 0.2 g, [$Ca(IO_3)_2$] 0.4 g, Se (Na_2SeO_3) 0.06 g, carrier (ground corn cobb) added to 1 kg

Table 2: Calculated nutrient content of experimental diet

Nutrient name	Pre-starter	Starter I	Starter II
Metabolizable energy for swine (cal kg^{-1})	3,400.00	3,350.00	3,300.00
Protein (%)	23.00	21.00	18.00
Fat (%)	9.96	7.75	8.23
Fiber (%)	2.77	2.85	3.28
Calcium (%)	0.80	0.70	0.66
Total phosphorus (%)	0.74	0.64	0.61
Available phosphorus (%)	0.40	0.33	0.31
Salt (%)	0.80	0.60	0.60
Choline (%)	0.05	0.04	0.03
Linoleic acid (%)	4.25	3.07	3.37
Lactose (%)	7.00	3.50	0.00
Arginine, digestible (%)	1.37	1.22	1.00
so-leucine, digestible (%)	0.95	0.82	0.65
Lysine, digestible (%)	1.35	1.23	0.98
Methionine+cysteine, digestible (%)	0.74	0.68	0.55
Methionine, digestible (%)	0.47	0.44	0.34
Threonine, digestiblele (%)	0.79	0.73	0.59
Tryptophan, digestible (%)	0.32	0.31	0.20
Valine, digestible (%)	0.86	0.82	0.72
Potassium (%)	1.02	0.84	0.69
Sodium (%)	0.32	0.37	0.43
Chloride (%)	0.51	0.40	0.39
Dietary electrolyte balance (Na^++K^++Cl^-) (meq kg^{-1})	254.00	263.00	255.00

Determination of gastrointestinal pH: At the end of the trial, one pig from each replication was exsanguinated. Immediately thereafter, the pH of contents in each part of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, caecum, colon and rectum) were directly measured using a pH meter (IQ Scientific Instruments, Carlsbad, CA, USA).

Large intestine selected bacterial counts: The contents in the caecum were collected immediately from one pig from each replication after exsanguination into sterile centrifuge tubes, placed on ice and transported (within 1 h after collection) to the laboratory for selected bacterial enumeration. Five grams of the caecum digesta were sampled and diluted with 45 mL of 1% peptone solution (Oxoid Laboratories, Basingstoke, UK) and homogenized using a Stomacher (Stomacher Laboratory Blender 400, Seward Medical, West Sussex, UK). Ten-fold serial dilution was used to reduce to 1:10 of the concentration and 0.1 mL was applied onto duplicate agar plates for each dilution. The spread plate technique was used to determine bacterial numbers. The populations of *E. coli* were grown on McConkey agar according to the methods of Biagi *et al.*[16]. All agar plates were incubated for 24 h at 37°C under aerobic conditions. The populations of *Lactobacillus* spp., were grown on de Man, Rogosa and Sharpe agar. All agar plates were incubated for 24 h at 39°C under anaerobic conditions according to the methods described by Franklin *et al.*[17].

Concentration of short-chain fatty acids (SCFAs) in caecum: The contents in the caecum were immediately collected after the pigs were exsanguinated. The samples were prepared according to Krutthai *et al.*[18]. About 1.5 mL aliquot of each sample was centrifuged (model MX-301; TOMY Kogyo, Tokyo, Japan) in microcentrifuge tubes at 14,000×g and 4°C for

10 min. The 900 µL supernatant of each sample was acidified using 100 µL 50 mM H_2SO_4, mixed by vortex for 30 sec and then left to stand at room temperature. The samples were centrifuged at 14,000×g at 4°C for 10 min and the supernatant was analyzed for SCFAs using High Performance Liquids Chromatography (HPLC).

The HPLC system consisted of an Agilent 1100 series (Agilent Technologies), Photodiode Array Detector (Agilent Technologies), Razex ROA-Organic Acids H+ (8%) Column (300×7.8 mm) and Razex ROA-Organic Acids H+ (8%) Guard Column (50×7.8 mm). The supernatants were filtered with a 0.22 µm nylon syringe filter (13 mm diameter; No. 2166) (Alltech Associated Inc., Deerfield, IL, USA) according to the method of Fernandes *et al.*[19]. An amount of 20 µL of each sample was injected into the HPLC with autosampling and 0.005 N H_2SO_4 as the mobile phase. The running conditions provided for a column heat of 60°C, a flow rate of 0.5 mL min^{-1} and the absorbance detector was operated at a wavelength of 210 nm. A mixture of succinic acid, lactic acid, formic acid, acetic acid, propionic acid, butyric acid and valeric acid was included as a standard in all analyses. Qualitative acids analysis was determined by the retention time of the acid peaks, while quantitative analysis was carried out using a standard curve composed of the various acid concentrations.

Statistical analysis: A t-test was used to compare the measurement values obtained from the two independent groups for productive performance, intestinal morphology, gastrointestinal pH, population of selected ceacal bacteria and the concentration of short-chain fatty acids. Statements of statistical significance were based on $p<0.05$.

RESULTS AND DISCUSSION

The effects of supplemental tributyrin in the diet on the production performance are presented in Table 3. There were no significant effects of tributyrin on the growth, feed intake and feed conversion ratio of piglets throughout the experimental period ($p>0.05$).

Tributyrin supplementation at 0.4% did not positively promote the productive performance of piglets. Araujo *et al.*[20] also reported no apparent advantages of supplementing milk replacer with sodium butyrate or tributyrin (0.3%) on the performance and glucose metabolism in calves. In accordance with Fang *et al.*[21], supplementing 0.1% sodium butyrate did not affect the average daily feed intake, average daily gain and feed to gain of piglets post-weaning and Hou *et al.*[22] also stated that dietary supplementation with 0·1% tributyrin did not significantly affect the growth performance of weaning piglets. Although, Hou *et al.*[23] found that 0.5% tributyrin in the diet improved the growth rate and FCR of piglets and Hanczakowska *et al.*[24] also reported that body weight gains of piglets fed diets supplemented with 0.3% sodium butyrate were higher than controls diet. In agreement with Chiofalo *et al.*[25] who stated that sodium butyrate supplemented diet (0.15%) improved the growth performance of weaning piglet. Moreover, He *et al.*[26] reported that tributyrin could effectively alleviate the inflammation of

Table 3: Effect of tributyrin in diet on production performance of piglets

Item	Control	Tributyrin 0.4%	p-value	SEM
Initial weight (kg)	7.57	7.51	0.73	0.08
Pre-starter (0-14 days after weaning)				
Body weight (kg)	12.17	12.22	0.93	0.25
Average daily gain (kg day^{-1} pig^{-1})	0.33	0.34	0.79	0.01
Average daily feed intake (kg day^{-1} pig^{-1})	0.36	0.35	0.75	0.01
Feed conversion ratio	1.09	1.05	0.23	0.02
Starter I (15-42 days after weaning)				
Body weight (kg)	31.96	31.60	0.74	0.53
Average daily gain (kg day^{-1} pig^{-1})	0.71	0.69	0.59	0.01
Average daily feed intake (kg day^{-1} pig^{-1})	0.96	0.96	0.98	0.02
Feed conversion ratio	1.35	1.38	0.31	0.01
Starter II (43-56 days after weaning)				
Body weight (kg)	43.57	43.11	0.72	0.60
Average daily gain (kg day^{-1} pig^{-1})	0.83	0.82	0.72	0.01
Average daily feed intake (kg day^{-1} pig^{-1})	1.33	1.29	0.83	0.03
Feed conversion ratio	1.60	1.58	0.64	0.03
Total (0–56 days after weaning)				
Initial weight (kg)	7.57	7.51	0.73	0.08
Body weight (kg)	43.57	43.11	0.72	0.60
Average daily gain (kg day^{-1} pig^{-1})	0.64	0.64	0.73	0.01
Average daily feed intake (kg day^{-1} pig^{-1})	0.90	0.90	0.95	0.02
Feed conversion ratio	1.40	1.41	0.53	0.01

the liver and enhance the immune function of intrauterine growth-restricted piglet's liver. Conversely, too high a level of tributyrin (1.0%) in the diet adversely affected performance[12]. The inconsistent effects of tributyrin on the growth performance of piglets may have resulted from several factors such age, breeding, performance, environmental factors, dosage and health status. In the present study, with good management and good health conditions, supplemental tributyrin (0.4) resulted in no advantage with regard to growth performance since the maximal productive performance had already been achieved.

There were no significant effects of tributyrin on the pH in each part of the gastrointestinal tract of piglets (p>0.05) as shown in Table 4. There were no significant effects of tributyrin on the pH in each part of the gastrointestinal tract of piglets (p>0.05).

Post weaning is a critical period in swine production because of limitations in the digestive and absorptive capacity due to several factors such as insufficient production of HCl, pancreatic enzymes and sudden changes in feed consistency and intake[27]. Lowering dietary pH by supplemental organic acid or their salt reduces gastric pH, resulting in the increased activity of proteolytic enzymes and gastric retention time and improved protein digestion[28]. Diao *et al.*[29] reported that benzoic acid supplementation in the diet of weaning piglets decreased the pH values of the digesta in the colon on 14th day and in the ileum and cecum on 42nd day after weaning. According to Radcliffe *et al.*[30], supplementing citric acid (1.5 and 3.0%) in diet caused a 1.51 and 2.16 U decrease in diet pH, which resulted in a linear decrease in the pH of the stomach digesta in weaning piglet. However, in the current study, tributyrin supplementation in the diet did not affect the acid-base status in the gastrointestinal tract of piglets. In accordance with Hanczakowska *et al.*[24] and Manzanilla *et al.*[31] there were no significant differences in acidity of digesta in each part of the digestive tract of piglets fed diets supplemented with 0.3% sodium butyrate. In addition, Zentek *et al.*[6] also reported that organic acid supplementation (0.416% fumaric and 0.328% lactic acid) in diet of weaning piglet did not influence the pH of the digesta in each part of the gastrointestinal tract and also agree with another study which showed that supplementing organic acids in diet of weaning pig did not affect the pH of the gastrointestinal tract[32]. Although, butyric acid is acidic (pKa 4.82) and can be used as an acidifier[33], tributyrin is a neutral, short-chain, fatty acid triglyceride[34]. It is hydrolyzed by intestinal lipases, yielding glycerol and three butyrate molecules[13], the liberated butyrate molecules can exit the proximal intestinal lumen by passive diffusion and be actively transported across the apical membrane via monocarboxylate transporters[35]. This indicates that tributyrin does not induce any acidity throughout the gastrointestinal tract.

Table 4: Effect of tributyrin on the pH of gastrointestinal tract of piglets

Item	Control	Tributyrin 0.4%	p-value	SEM
Stomach	3.69	3.54	0.59	0.27
Duodenum	5.06	5.11	0.52	0.13
Jejunum	6.14	6.20	0.33	0.09
Ileum	6.47	6.44	0.49	0.09
Caecum	5.80	5.83	0.98	0.07
Colon	6.08	6.03	0.90	0.07
Rectum	6.53	6.52	0.83	0.05

Table 5: Effect of tributyrin in the diet on intestinal morphology of piglets

Item	Control	Tributyrin 0.4%	p-value	SEM
Villous height (μm)				
Duodenum	571.46	609.89	0.51	27.37
Jejunum	501.51	556.66	0.43	33.27
Ileum	383.44	443.69	0.07	16.92
Crypt depth (μm)				
Duodenum	233.10	232.87	0.99	6.24
Jejunum	210.66	222.30	0.41	6.69
Ileum	177.97	186.14	0.62	7.77
Villous height: crypt depth (μm)				
Duodenum	2.46	2.63	0.50	0.12
Jejunum	2.41	2.53	0.71	0.16
Ileum	2.20	2.42	0.46	0.14

The effects of supplemental tributyrin in the diet on the intestinal morphology of piglets are shown in Table 5. There were no significant effects of tributyrin on the villous height and crypt depth of duodenum, jejunum and ileum (p>0.05) and the ratio of the villous height to crypt depth in each segment of the small intestine (p>0.05). However, the tributyrin supplemented group tended to have an increased ileum villous height compare to the control group (p = 0.07).

In the post-weaning period, a reduction in the villous height and an increase in the crypt depth is often observed[36]. This abnormal intestinal morphology is usually associated with diarrhea and retarded growth in weanling piglets[3]. However, there is evidence that supplementing tributyrin in the diet reduced the adverse effect after weaning associated with gastrointestinal dysfunction, villous atrophy, crypt hyperplasia and a decline in enzyme activity in the small intestine[12,23].

The study of Hou *et al.*[23] showed that supplementing 0.5% tributyrin in the diet resulted in a decrease in the crypt depth, an increase in the ratio of the villous height to the crypt depth in the duodenum and ileum, a decrease in the villous width in the duodenum and an increase in duodenal lactase and ileal maltase activity. Moreover, supplementing tributyrin at 0.1% showed a positive effect on the caecal crypt depth in piglets[12], improved intestinal villus morphology and increased intestinal villus surface areas[37]. In addition, Chiofalo *et al.*[25] also reported that supplementing 0.15% sodium butyrate

increased villouslength and crypt depth of weaned piglets. Conversely, the study of Hou *et al.*[22] showed that there were no significant effect of supplementing tributyrin (0.10%) in the diet of piglets on villus height, crypt depth and the ratio of villus height:crypt depth in the ileum. In accordance with Hanczakowska *et al.*[24], supplementing 0.3 % sodium butyrate in the diet of piglets did not affect villus height, crypt depth and the ratio of villus height: crypt depth in duodenum and jejunum. In addition, the study of De Santana *et al.*[32] showed that the histology of piglet intestinal epithelial was not affected by the supplementation of a combination of sodium butyrate with plant extracts and nucleotides in the diet of weaning pigs. However, the present study showed that tributyrin supplementation (0.4) in the diet tended to improve the intestinal morphology in the ileum section of healthy piglets ($p = 0.07$). It is assumed that tributyrin which is a source of butyric acid has a beneficial effect on the small intestinal morphology of healthy weaning pigs.

The effects of supplemental tributyrin in the diet on bacterial flora in the caecum of piglets are shown in Table 6. There were no significant effects of tributyrin on the populations of *E. coli* and *Lactobacillus* spp., in the caecum ($p>0.05$).

Organic acid is one alternative feed additive for the replacement of antibiotics as growth promoters. Organic acids associated with specific antimicrobial activity are short-chain fatty acids (SCFAs: C1-C7) and either simple monocarboxylic acids such as formic, acetic, propionic and butyric acid or carboxylic acids bearing a hydroxyl group such as lactic acid[33]. The undissociated form of organic acids can easily penetrate the lipid membrane of the bacterial cell. In the cell which the pH of cell cytoplasm is higher than the pKa of organic acids, the organic acids are dissociated into anions and protons. The generation of anions and protons potentially presents problems for bacteria that must maintain a near-neutral pH in their cytoplasm to sustain functional macromolecules[38]. For this reason, the bacteria cells have to use energy to export of excess proton in order to maintain a cellular pH[39] that may result to inhibit the growth of bacterial cells. There is evidence that supplementing 0.5% benzoic acid in the diet of weaned piglet can decrease the number of *Escherichia coli* in ileum and caecum and the number of *Enterococci* in ileum and can increase the number of *Bifidobacterium* in ileum and *Bacillus* in the caecum of piglets[29]. Moreover, previous studies have shown that dietary supplementation of citric acid (0.5%) and acidifiers blend (17.2% formic acid, 4.1% propionic acid, 10.2% lactic acid, 9.5% phosphoric acid, SiO_2 34.0%) increased fecal *Bacillus* spp., counts and reduced fecal counts of *Salmonella* spp. and *E. coli*[40].

The study of Walia *et al.*[41] reported that feeding sodium butyrate (0.3%) to finishing pigs in the late finishing period was effective in reducing *Salmonella* shedding and seroprevalence. In addition, Chiofalo *et al.*[25] stated that supplementing 0.15% of fat-protected sodium butyrate increase lactic acid bacteria on intestinal mucosa of weaning pigs. Nevertheless, in the present study, as tributyrin did not influence the acid-base status of the gastrointestinal tract (Table 4), the populations of *E. coli* and *Lactobacillus* spp., in the caecum were also not affected by the supplementation. According to Walsh *et al.*[42] organic acid supplementation (predominantly propionic, acetic and benzoic acid; at 2.58 mL L^{-1}) in drinking water of weanling pig challenged with *Salmonella typhimurium* had no effect on the prevalence of the pathogen in organs or digesta. Calveyra *et al.*[43] also reported that there was no significantly different in quantification of *Salmonella typhimurium* in fecal samples from *Salmonella typhimurium* challenged pigs fed an encapsulated organic acid supplemented diet (fumaric acid 20%, citric acid 10%, malic acid 10%, phosphoric acid 10%) or a blend of short chain free organic acids supplemented diet (formic acid 26%, propionic acid 10%, plant fatty acids 18%). In addition, Acikgoz *et al.*[44] observed that formic acid administration to the drinking water did not affect the intestinal population of *E. coli*, total organism and *Salmonella* spp., in the caecum of broiler chick. In the present study, tributyrin may have crossed the membrane and been absorbed in the small intestine, so very little may have reached the lower part of the gastrointestinal tract, which is consistent with Van Immerseel *et al.*[45] who reported that acids from feed and water were not effective further down the intestinal tract. Furthermore, there is evidence that organic acids which are used in diets or water are metabolized in the upper part of the gastrointestinal tract in poultry[46]. Thus, the effectiveness of tributyrin on modifying host microflora populations in the lower part of the gastrointestinal tract is limited in healthy piglets.

The effects of supplemental tributyrin in the diet on the concentration of SCFAs are shown in Table 7. There were no significant effects of tributyrin on the concentration of succinic acid, lactic acid, formic acid, acetic acid, propionic acid, butyric acid and valeric acid in the caecal digesta of nursery pigs ($p>0.05$).

According to Hanczakowska *et al.*[24], there were no significant differences in short-chain fatty acids content of the digesta in caecum of piglets fed sodium butyrate supplemented diet (0.3%) and control diet. Hanczakowska *et al.*[47] also reported that supplementing acidifiers containing the short-chain fatty acids (0.5% of 1:1

Table 6: Effect of tributyrin on bacterial flora in caecum of piglets

Item	Control	Tributyrin 0.4%	p-value	SEM
E. coli (log_{10} CFU mL^{-1})	6.65	6.87	0.46	0.14
Lactobacillus spp. (log_{10} CFU mL^{-1})	9.18	9.23	0.79	0.09

Table 7: Effect of tributyrin on the concentration of short-chain fatty acid in caecum of piglets

Item (mmol L^{-1})	Control	Tributyrin 0.4%	p-value	SEM
Succinic acid	8.70	8.38	0.88	1.01
Lactic acid	47.36	43.60	0.75	5.60
Formic acid)	29.19	28.71	0.91	1.95
Acetic acid	104.69	109.70	0.53	3.84
Propionic acid	40.50	41.09	0.86	1.66
Butyric acid	24.07	26.95	0.59	2.52
Valeric acid	279.84	264.40	0.71	19.41

w/w ratio mixture of propionic and formic) in the diet of piglets did not have any effect on volatile fatty acid content of caecum digesta. In accordance with Lampromsuk *et al.*[48], dietary supplementation of organic acid base acidifier (sodium benzoate, formic acid) did not significantly affect the concentration of acetic, propionic, butyric, valeric acid and total volatile fatty acid in caecum of weaning pigs. In contrast with Diao *et al.*[29], supplementing benzoic acid in diet of weaning pig significantly increased the content of propionic acid and total volatile fatty acids in the cecum on14th day. The study of Poeikhampha and Bunchasak[49] showed that supplementation of 0.8% potassium diformate in the diet of nursery pig tended to increase butyric acid and total short-chain fatty acids in caecal digeta. Conversely, Zentex *et al.*[6] reported that the concentration of short-chain fatty acids in the colon digesta of piglets fed organic acid supplemented diets (0.416% fumaric and 0.328% lactic acid) was reduced when compared with those fed unsupplemented diets. It has also been documented that acidic conditions favor the growth of *Lactobacillus* spp., in the stomach which possibly inhibits the colonization and proliferation of *E. coli* by blocking the sites of adhesion or by producing lactic acid and other metabolites which lower the pH and inhibit *E. coli*[50]. In the present study, the gastrointestinal pH, bacterial population and SCFAs concentrations in the ceacum were not significantly affected by tributyrin supplementation. It is assumed that tributyrin may be rapidly absorbed in the small intestine to be used as an effective energy source for enterocytes and this was not effective further down the intestinal tract.

CONCLUSION

In conjunction with the good management of weaning pigs, tributyrin supplementation in the diet has no significant effects on the productive performance, gastrointestinal acid-base, ceacal bacteria and short-chain fatty acids. However, it seems that tributyrin tends to improve the intestinal morphology of piglets.

SIGNIFICANT STATEMENTS

In this study, the study was conducted to evaluate the effects of supplementing tributyrin in the diet as an alternative to antibiotic growth promoter in post-weaning piglets. This study reported the effects of supplementing tributyrin in the diet on the productive performance, intestinal morphology, gastrointestinal pH, large intestine selected microflora and short-chain fatty acids concentrations of nursery pigs. Although tributyrin supplementation in the diet did not have any benefit on the productive performance and gut ecology of healthy piglets, tributyrin tends to improve the intestinal morphology of piglet. Supplementing tributyrin in appropriated level may be a tool to reduce the negative impact on the intestinal mucosal morphology of piglets post-weaning.

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