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PAPER

Lauric acid saponified with calcium ameliorates indices of intestinal function and gut health in weaned piglets

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ABSTRACT

Reducing the use of antibiotics is one of the largest challenges for pig production. The scientific community has investigated numerous alternative substances to antibiotics, including medium-chain fatty acids, due to their antimicrobial and protective effects on the gut health of piglets. The present study investigated the effect of lauric acid saponified with calcium (C12-Ca) on the growth performance and gut health parameters in post-weaning piglets. A total of 192 24-day-old piglets were assigned to one of three dietary treatments: CTR (basal diet alone), ANT (amoxicillin, 400 mg/kg) as a positive control diet, or C12-Ca (1 g/kg) for 28 days. C12-Ca did not affect performance, except for feed efficiency (FE), which increased ($p < .05$) in the C12-Ca and ANT groups from 15 to 28 days. On days 0 to 28, FE was higher ($p < .001$) in the C12-Ca group than in the CTR group. In the C12-Ca and CTR groups, antibiotic treatments against diarrhoea were reduced. A greater concentration of lactic acid was found in the small intestine in the C12-Ca group and the acetic acid concentration in the caecum decreased under C12-Ca treatment ($p < .001$). No differences in IL-10, IL-6, IgA, and IgG were found in faecal samples. In the duodenum and ileum, C12-Ca administration provided a higher total antioxidant capacity and lower malondialdehyde level ($p < .001$). C12-Ca improved the ileal villus height and width ($p < .001$). Our findings suggest that C12-Ca administration ameliorates the indices of intestinal function and gut health in weaned piglets.

HIGHLIGHTS

- Reducing the use of antibiotics is one of the largest challenges for pig production.
- Medium-chain fatty acids are important alternative substances to antibiotics.
- Lauric acid saponified with calcium represents a promising nutritional strategy for improving piglet gut health.

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Lauric acid; saponification; piglets; gut health

Introduction

It has been widely reported that weaning stress entails changes in gut structure and function and may cause developmental delays in piglets (Pluske et al. 1997; Yin et al. 2014). Antibiotics have long been used as growth promoters and therapeutic medicines to address these issues. However, the scientific community has recognised that this practice poses a serious threat to both humans and livestock, due to increases in drug-resistant pathogens (Czaplewski et al. 2016). The Feed Additives Regulation was implemented as a measure, with a total ban on antibiotics as growth promoters, from January 2006 (Regulation 1831/2003/EC and subsequent amendments). On 13 September 2018, the European Parliament adopted a resolution on a European One Health Action Plan against Antimicrobial Resistance (AMR), stressing the correct and prudent use of antimicrobial agents, in order to limit the emergence of antimicrobial resistance in animal husbandry, and remarked that actions to prevent antimicrobial resistance should be coordinated and implemented in all regions of the European Union . The use of antimicrobial medicinal products through the medicated feed is regulated by the Regulation

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(EU) 2019/4 on the manufacture, placing on the market, and use of medicated feed. The development of new management and feeding strategies can be considered valuable tools with which to reduce the use of antibiotics through the improvement of gut health and function in weaning piglets. Numerous complex mechanisms are involved in regulating the health and functioning of the gastrointestinal (GI) tract. Among these, diet, functional integrity of the GI barrier, microbiota, and effective immune status represent the main components of gut health (Celi et al. 2019). Considering the wide range of proposed feeding strategies, the use of medium-chain fatty acids (MCFAs) has increased, due to their antimicrobial and protective effects on the intestinal microarchitecture of piglets (Decuypere and Dierick 2003; Hanczakowska 2017). MCFAs are a family of straight-chain saturated free fatty acids of 6–12 carbon atoms in length, including lauric acid (C12:0). MCFAs are naturally present in coconut oil (Dayrit 2015), cow's milk, and human breast milk (Jensen 2002). Previous studies have shown that MCFAs can improve the growth performance of piglets and prevent the negative effects associated with weaning (Dierick et al. 2002; Zentek et al. 2011, 2013; Sol et al., 2019). Furthermore, *in vitro* studies have shown that MCFAs have antibacterial activity, with great efficacy against Gram-positive bacteria (Lieberman et al. 2006; Skrivanova et al. 2012; Dayrit 2015; De Smet et al. 2016), supporting their use against pathogens relevant in pig farming, such as *Escherichia coli* and *Streptococcus suis*. Organic acids possess antimicrobial properties, due to their ability to cross the cell membrane—related to the lipophilic nature of their undissociated forms—and modifying the proton (and associated anion) concentrations in the cytoplasm (Gómez-García et al. 2019). MCFAs can also alter the vital processes of bacterial cells, including the electron transport chain and oxidative phosphorylation, which are essential for energy production (Van Immerseel et al. 2006; Yoon et al. 2018). However, when administered in a free form, MCFAs are rapidly absorbed by the cells of the intestinal mucosa, as they represent an immediate source of energy, thereby reducing or nullifying their effects as gut health promoters in different gastro-intestinal tracts (Liu 2015). One effective strategy involves the use of medium-chain triglycerides as a feed additive, as they allow for bypassing the unpleasant smell (Oprean et al. 2011) and protect the MCFAs from rapid absorption, while the active compounds can be gradually released by lipases *in vivo* in the stomach, along with the progressive release in the foregut (Jackman et al. 2020). Different delivery strategies have been explored to encapsulate them (Zentek et al. 2012), in order to slow down absorption and target it to other gastrointestinal tracts, thus potentially enhancing their functional performance. Another solution might be saponification, a process that involves the conversion of fat into soap by adding calcium hydroxide or other elements to obtain the salts of the fatty acids, which can be easily included in animal feed in their powdered form (Schumann and Siekmann 2005). Saponification is also an effective method for introducing higher concentrations of MCFAs into the final product (Esperón-Rojas et al. 2017). Only a few recent studies are available on the effect of lauric acid (LA)-based additives on the growth performance, gut health (immune and oxidative stress status and microbiome), and faecal microbiota of piglets (Duarte et al. 2019; López-Colom et al. 2019; Rolinec et al. 2020). The products used in the mentioned studies consisted mainly of lauric acid, either distilled from coconut oil or in microencapsulated form. In this study, we consider a form of lauric acid saponified with calcium (C12-Ca), composed of 75% lauric acid and 10% calcium. Our hypothesis is that this product may have a better effect on the growth performance and gut health of piglets, compared to available forms, due to its high content of lauric acid, low cost of production, and positive technological properties, such as its resistance to high temperature and high mixing properties in feed. The overall aim of this study was to evaluate the effect of C12-Ca on growth performance and on some important indices of intestinal function and gut health. Moreover, the effect of C12-Ca was compared to a major antimicrobial substance in pig medicine (Amoxicillin), in order to assess its possible efficacy as a dietary strategy for reducing prophylactic antibiotics.

Material and methods

Animals, housing, and experimental design

The experiment was performed at the Animal Production Research and Teaching Centre of the Polo Veterinario, Università Degli Studi di Milano (Lodi, Italy). The study was carried out on a total of 192 weaned crossbreed York/Large White x Landrace piglets (average weight = 9.33 ± 1.90 kg). The trial started at the weaning of piglets at 24 post-natal days and lasted 28 days in total. The weaning of the piglets was considered day 0 of the trial. Treatments were randomLy assigned to animals housed in two identical rooms with 24 pens each, which were balanced for

BW, a litter of origin, and sex (4 pigs/pen, 2 males and 2 females) in an environmentally regulated, isolated stable, as described in our previous study (Jiang et al. 2015). Each pen had plastic slatted floors and was fitted with an adjustable stainless-steel feeder and two nipple waterers. The rooms were lit by a combination of daylight (through skylights) and artificial light. The temperature, humidity, CO_2, and ammonium concentration of the air were automatically controlled. Ventilation was achieved by single, variable-speed fans linked to temperature sensors. The temperature inside the building was approximately 28 °C at the start of the trial and was adjusted weekly until a final temperature of 24–25 °C was obtained. The relative humidity was controlled at 60–70%. The pigs had water and feed, provided in meal form, which was available *ad libitum*. All piglets were fed a standard commercial diet, formulated to meet or exceed the nutrient requirements for post-weaning piglets (NRC 2012; Table 1). The basal diet was divided into pre-starter (administered from 0 to 14 days of the trial) and starter (administered from 15 to 28 days of the trial). Pens were allocated to 1 of 3 dietary treatments, with 16 replicate pens per treatment. The experimental unit was the pen. Dietary treatments were as follows: Basal diet (CTR), ANT diet (CTR + 400 mg/kg amoxicillin), and C12-Ca diet (CTR + 1 g/kg C12-Ca). A C12-Ca soap (DRAX S) product containing 30 mg/kg of zinc oxide (0.03 g/kg) by DC Practical Solution (Via Petrarca 4, 20123, Milan, Italy) was used. The C12-Ca soaps were created by saponification, combining 750 g/kg lauric acid and 100 g/kg calcium hydroxide.

Animal health and therapeutic treatments

The veterinary doctor responsible for animal welfare during the trial made the diagnoses of *Streptococcus suis*, based on early nervous signs (depression, incoordination, and adoption of unusual stances), variable degrees of inappetence, and diagnoses of diarrhoea using a standard system of scoring (Spiehs et al. 2008), and decided upon the antibiotic therapy. The carcasses of the dead animals were sent to the Pathological Anatomy Laboratory of the University of Milan for necropsy analyses. Piglets eventually treated with antibiotics were excluded from the sample collection.

Measurements and samples

Pigs and feeders were weighed at 0, 14, and 28 days of the trial, in order to determine their body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE). Mortality and pathologies were recorded daily. Faecal samples were collected directly from the rectum of one piglet per replicate for each treatment ($n = 16$) at weaning (0 day of the trial), at diet change (14 days of the trial), and at the end of the trial (28 days of the trial). Aliquots of faecal samples were stored at −20 °C for the assessment of their immunomodulatory and anti-inflammatory parameters. At the end of the trial (i.e. at 28 days), six piglets from different cages were randomLy chosen from each dietary treatment and

Table 1. Ingredients and chemical compositions of the pre-starter (administrated from 0 to 14 days) and starter (administrated from 15 to 28 days) basal diets fed to post-weaning piglets during the trial (as-fed basis, %).

	Basal diets	
	Pre-starter (0–14 days)	Starter (15–28 days)
Ingredients, %		
Barley meal	20.71	20.00
Wheat meal	16.08	17.42
Sweet whey	10.00	5.00
Maize meal	8.32	22.00
Soycomil R	6.50	4.50
Flacked wheat	6.00	3.00
Dextrose	5.00	2.50
Flacked maize	5.00	3.50
Flacked barley	4.00	–
Soybean meal 48% CP	4.00	9.00
Wheat middlings	3.00	5.00
Herring meal	2.50	1.50
Plasma AP 820	2.50	0.50
Soybean oil	3.00	1.40
Cellulose	1.00	0.75
L-Lysine	0.55	0.45
Dicalcium phosphate	0.50	0.60
Calcium carbonate	0.40	0.60
L-Threonine	0.25	0.15
Vitamins + trace elementsa	0.25	0.25
DL-Methionine	0.23	0.10
Salt	0.15	0.25
L-Tryptophan	0.06	0.03
Animal fat	–	1.50
Analysed composition		
DM, %	90.29	89.34
CP, %	18.27	16.63
EE, %	4.95	5.04
CF, %	2.94	3.17
Ash, %	4.47	4.34
Calcium, %	0.57	0.61
Phosphorus, %	0.55	0.53
Calculated composition		
DE, kcal/kg	3500	3420
NE, kcal/kg	2480	2440
Lysine, %	1.37	1.13
Methionine + Cystine, %	0.87	0.70
Threonine, %	0.96	0.78
Tryptophan, %	0.27	0.22

CP: crude protein; DL-methionine: D (dextrogyre) and L (levogyre) optical isomers- methionine; DM: dry matter; EE: ether extract; CF: crude fibre; DE: digestible energy; NE: net energy.

[a]Vitamin–mineral premix supplies per kg, as fed: vitamin A, 10 000 IU; vitamin D_3, 1.000 IU; vitamin E, 50 mg; vitamin B_1, 1.0 mg; vitamin B_2, 3.0 mg; vitamin B_{12}, 0.02 mg; vitamin B_6, 3.0 mg; pantothenic acid, 10 mg; nicotinic acid, 15 mg; biotin, 0.06 mg; vitamin PP, 0.35 mg; folic acid, 0.99 mg; vitamin K_3, 2 mg; choline, 300 mg; Fe, 100 mg; Cu, 20 mg; Co, 0.75 mg; Zn, 100 mg; Mn, 10 mg; I, 0.75 mg; Se, 0.4 mg; ethoxyquin, 150 mg.

slaughtered, in accordance with current regulations, at a local commercial slaughterhouse, in order to collect the intestinal tissues and digesta content samples. The entire intestinal tracts were removed, and different sections of the duodenum, jejunum, ileum, and caecum were obtained from each animal. Approximately 1 cm segments each of the duodenum, jejunum, and ileum were promptly fixed in 10% paraformaldehyde in 0.01 M phosphate-buffered saline (pH 7.4) for 24 hours at 4 °C for histological measurements ($n = 6$). Segments 1 cm in length were also obtained from each tract of the small intestine, in order to analyse the antioxidant parameters ($n = 6$). Other sections from the duodenum, ileum and caecum were divested of their intestinal contents by squeezing them directly into 50 mL Falcon tubes, immediately frozen, and stored at −80 °C to determine their fermentative (5 mL of intestinal content) parameters ($n = 6$). The final set of sections from the duodenum, ileum and caecum were refrigerated (4 °C) and transported to the laboratory, in order to analyse their microbial populations (1 g of intestinal content) ($n = 6$).

Morphological analysis of the small intestine

Following 24 hours of fixation in 4% paraformaldehyde, the duodenum, jejunum, and ileum segments were dehydrated in a series of ethanol solutions with increasing alcohol content, immersed in a solution of xylene, and finally encased in paraffin. The paraffinic blocks were cut into 5 μm sections using a rotating microtome (Microm HM335E). The sections were collected, placed on slides, and then stained using haematoxylin-eosin for analysis. The slides were evaluated with a Nikon Eclipse E600 microscope equipped with a DS-Fi2 camera. The images of the sections were observed at 200× magnification and processed using the NIS-Elements software. The morphometry was quantified by measurements of the villi and crypts. A minimum of twenty well-oriented and intact villi and crypts were selected per image. The villus length was measured from the tip of the villus to the villus–crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villi. The measurements were made using the ImageJ software.

Count of the microbial population in the intestinal contents

Bacterial counts (viable counts; log10 CFU/g) in the contents of the duodenum, ileum, and caecum were performed using the ring-plate technique (Michiels et al. 2010). Seven serial 10-fold dilutions were made from fresh digesta and faeces in a sterilised peptone solution (peptone, 1 g/L; agar, 0.4 g/L; NaCl, 8.5 g/L; and cysteine, 0.7 g/L) and plated onto selective media (Oxoid) to count the following bacterial groups: *Escherichia coli* (Tryptone bile x-glucuronide agar, followed by incubation for 72 hours at 30 °C under 90% N_2 and 10% CO_2), coliform bacteria (violet red bile lactose agar, followed by incubation for 24 hours at 37 °C under 90% N_2 and 10% CO_2), anaerobic sulphite reducer (iron sulphite agar, followed by incubation for 24–48 hours at 37 °C under 90% N_2 and 10% CO_2), Lactobacillus (De Man, Rogosa, and Sharp agar, followed by incubation for 72 hours at 30 °C under 90% N_2 and 10% CO_2), Enterococcus and Streptococcus (Slanetz and Bartley agar, followed by incubation for 24 hours at 44 °C under 90% N_2 and 10% CO_2), and Enterobacteriaceae (violet red bile glucose agar, followed by incubation for 24 hours at 37 °C under 90% N_2 and 10% CO_2).

Fermentative parameters in intestinal contents

Volatile fatty acids (VFAs) were determined using a gas chromatograph equipped with a capillary column, according to the methods described by Erwin et al. (1961). Lactic acid concentrations were determined by the method described by Raeth-Knight et al. (2007). Briefly, 1 mL of 25% metaphosphoric acid was mixed with 5 mL of fresh digesta in a 15 mL centrifuge tube, and the mixture was frozen overnight. Samples were then thawed, neutralised with 0.4 mL of 25% NaOH, and vortexed. Thereafter, to condition the column, 0.64 mL of 0.3 M oxalic acid was added to the VFA and lactic acid standards and samples. After vortexing, the samples were centrifuged for 20 min at 3,000 × g and 4 °C. Then, 2 mL of the supernatant was transferred into gas chromatography vials for analysis.

Pro- and anti-inflammatory cytokines and immunoglobulins in faeces

Pro- and anti-inflammatory cytokines—namely, interleukin 6 (IL-6) and interleukin 10 (IL-10)—were evaluated in the faecal samples collected directly from the rectum of one piglet per pen for each treatment ($n = 16$), at 0, 14, and 28 days of the trial. The analyses were performed by an enzyme immunoassay (Swine IL-6 ELISA Kit, Thermo Fisher; Swine IL-10 ELISA Kit, Thermo Fisher), following the manufacturer's instructions. immunoglobulins IgG (specific for *Streptococcus suis*) and IgA were also analysed by ELISA kits (Porcine *Streptococcus suis* Antibody ELISA Test Kit, Krisghen Biosystems; Immunoglobulin A ELISA Kit, -R-Biopharm

Table 2. Growth performance parameters of post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[d].

	Dietary treatment				*p*-value		
Parameter	CTR	ANT	C12-Ca	SEM	Tr.	Tm.	Tr*Tm
Pigs/treat	64	64	64				
BW, kg							
Day 0	9.300	9.300	9.300	0.340	0.007	<0.001	0.198
Day 14	13.900	14.900	14.200				
Day 28	22.000	23.600	22.700				
ADG, g/d							
Days 0–14	335	398	344	173.070	0.005	<0.001	0.456
Days 15–28	572	626	614				
Days 0–28	453	512	479	14.770	0.059		
ADFI, g/d							
Days 0–14	510	542	535	309.120	0.474	<0.001	0.988
Days 15–28	964	1006	994				
Days 0–28	737	774	765	39.470	0.628		
FE							
Days 0–14	0.640[b]	0.730[a]	0.650[b]	0.010	0.001	<0.001	0.023
Days 15–28	0.570[a]	0.620[b]	0.620[b]				
Days 0–28	0.600[c]	0.660[a]	0.630[b]	0.010	0.001		

BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FE: feed efficiency; G:F: gain to feed; Tr: treatment; Tm: time; Tr*Tm: treatment*time; CTR: basal diet; ANT: amoxicillin; SEM: standard error of the mean.
[a,b,c]Within rows, different superscript letters indicate a significant difference.
[d]Pigs 24 post-natal days of age at the beginning of the trial.

AG), according to the manufacturer's instructions. Only faeces were used to analyse the interleukins and immunoglobulins, as blood sampling was considered a stressful procedure for the animals. Faecal sampling is a non-invasive technique that involves less stressful handling of the piglets.

Total antioxidant capacity (TAOC) and malondialdehyde (MDA) in intestinal tissue

Frozen intestinal tissue samples (0.1 g) of the duodenum, ileum, and caecum were homogenised using an ultra-turrax homogeniser with cold physiological saline solution, maintained in ice, and centrifuged (3,000 × g for 15 min at 4 °C). Supernatants were then collected for analyses. Malondialdehyde (MDA) is one of the most extensively studied end-products of polyunsaturated lipid peroxidation under radical-induced oxidative stress conditions. The MDA concentration in the intestinal tissue was calculated by the acid extraction of homogenised tissue for 2 h at room temperature and protected from light. Subsequently, the derivatization of the extract to facilitate liquid chromatographic separation was carried out with 2.4-dinitrophenylhydrazine. After derivatization, MDA was measured by LC-MS/MS using an internal method (Laboratorio Vailati srl, Via S. Rocco, 25020 San Paolo, Brescia, Italy). The total antioxidant capacity (TAOC) was determined using a commercial kit (ab65329, Abcam), following the manufacturer's instructions.

Statistical analysis

A completely randomised design was used. Growth performance was analysed using the Statistical Analysis System software (SAS version 9.4; SAS Institute Inc., Cary, NC, USA), applying a MIXED procedure for repeated measurements and accounting for the effects of treatment, time, and the treatment × time interaction. Total average daily gain (0–28 study days), total average daily feed intake (0–28 study days), total feed efficiency ratio (0–28 study days), fermentative parameters, IgA, IL-6, TAOC, and MDA were analysed using a one-way analysis of variance (ANOVA), in order to compare the means of the three groups using the GLM procedure in SAS. For the statistical analysis of mortality and antibiotic treatment, Fisher's exact test was used. Intestinal morphology was analysed using a one-way analysis of variance (ANOVA) followed by a *post-hoc* Tukey's test in SAS. The pen represented the experimental unit for the growth performance parameters, while each pig represented the experimental unit for mortality and antibiotic treatment, fermentative parameters, IgA, IL-6, TAOC, MDA, and intestinal morphology. All the numerical data in the tables are presented as the least-square means (LSMeans), accompanied by a standard error of the mean (SEM) values. Differences between groups were considered statistically significant at $p < .05$.

Ethics statement

The experimental protocol was reviewed and approved by the Animal Welfare Committee of the Università Degli Studi di Milano (OPBA_147_2017).

Table 3. Mortality and antibiotic treatment of post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[c].

	Dietary treatment			*p*-value		
Parameter	CTR	ANT	C12-Ca	CTR vs. ANT	CTR vs. C12-Ca	ANT vs. C12-Ca
Pigs/treat.	64	64	64			
Dead	7a	0b	3ab	0.013	ns[d]	ns
Treated with antibiotics:						
Streptococcus suis	5	0	3	ns	ns	ns
Diarrhoea	1[b]	9[a]	0[b]	0.017	ns	0.003

Data are presented as Fisher test and chi-squared test results.CTR: basal diet; ANT: amoxicillin
[a,b]Within rows, different superscript letters indicate a significant difference.
[c]Pigs 24 post-natal days of age at the beginning of the trial.
[d]ns: not significant.

Results

Animal performance and health

Table 2 summarises the piglet growth performance data. No differences in BW, ADG, or ADFI were observed among the groups. Conversely, the FE from 15 to 28 days was significantly ($p < .05$) higher in the C12-Ca soap and ANT groups than in the CTR group. Considering the 0–14 and 0–28-day study periods, the ANT group showed a significantly higher FE, compared to the CTR and C12-Ca groups ($p < .001$).

Mortality and antibiotic treatment

As shown in Table 3, during the trial, ten piglets died: 7 in the CTR group and 3 in the C12-Ca group. The ANT group suffered no mortality, with a significant difference compared to the CTR group ($p < .05$). Eight piglets were treated against *Streptococcus suis*—5 in the CTR group and 3 in the C12-Ca group—with individual injections of Amoxicillin, but there were no significant differences between groups. Notably, the three treated piglets in the C12-Ca group completely recovered. One piglet in the CTR group and nine in the ANT group were treated for diarrhoea with individual injections of Enrofloxacin, whereas no piglets in the C12-Ca group were affected by diarrhoea. Therefore, the diarrhoea cases were significantly ($p < .05$) higher in the ANT group than in the C12-Ca and CTR groups.

Fermentative parameters

Table 4 shows the data on acetic and lactic acid in the intestinal contents of the duodenum, ileum, and caecum. The C12-Ca group displayed the significantly ($p < .001$) highest concentration of lactic acid in the small intestine and caecum. The acetic acid concentration in the C12-Ca and ANT groups was significantly ($p < .001$) decreased, compared to the CTR group, in the duodenum, ileum, and caecum. The propionic, butyric, and valeric acid results were under 9 mmol/kg, without significant differences.

Table 4. Acetic and lactic acid concentration in the duodenum, ileum and caecum content in post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[d].

	Dietary treatment				
Parameter	CTR	ANT	C12-Ca	SEM	*p*-value
Pigs/treat.	6	6	6		
Acetic acid, mmol/kg					
Duodenum	25.644[a]	21.648[b]	20.149[b]	0.933	<0.001
Ileum	42.963[a]	38.633[b]	36.968[b]	1.016	<0.001
Caecum	33.471[a]	29.474[b]	26.810[c]	1.216	<0.001
Lactic acid, mmol/kg					
Duodenum	12.211[c]	14.099[b]	20.981[a]	0.666	<0.001
Ileum	22.869[c]	26.643[b]	33.415[a]	0.999	<0.001
Caecum	11.434[c]	12.544[b]	20.870[a]	0.777	<0.001

[a,b,c]Within rows, different superscript letters indicate a significant difference.
[d]Pigs 24 post-natal days of age at the beginning of the trial.

Intestinal content microbial populations

As shown in Table 5, no significant differences in microbial populations were found between groups in the duodenum, ileum, and caecum content.

Antioxidant and anti-inflammatory status

Regarding the IgA, IL-6, IL-10 and *Streptococcus suis* IgG results, no significant differences were found. IgA and IL-6 results are reported in Table 6. The IL-10 values were below the limit of detection (<7.80 pg/g) in the considered analysis. The quantitative analysis of IgG specific for *Streptococcus suis* in faeces was negative for all samples analysed. In contrast, the results of the antioxidant parameters in the duodenum, ileum, and caecum tissue (Table 7) showed that the addition of C12-Ca soaps led to an improvement ($p < .001$) in the total antioxidant capacity and a reduction in malondialdehyde levels ($p < .001$) in the duodenum and ileum, compared to the CTR and ANT groups.

Table 5. Intestinal microbial populations (Log10 CFU/g) of the post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[a].

	Dietary treatment				
Parameter	CTR	ANT	C12-Ca	SEM	*p*-value
Duodenum content					
Escherichia coli, CFU/g	2.00	2.04	2.00	1.00	ns
Coliforms, CFU/g	2.00	2.00	2.00	0.00	ns
Anaerobic sulphite reducers, CFU/g	3.42	3.30	3.16	2.96	ns
Lactobacillus, CFU/g	2.86	2.76	2.63	2.14	ns
Enterococcus/Streptococcus, CFU/g	2.00	2.15	2.00	1.60	ns
Enterobacteriaceae, CFU/g	2.00	2.00	2.00	0.00	ns
Ileum content					
Escherichia coli, CFU/g	5.08	5.54	4.98	5.44	ns
Coliforms, CFU/g	5.34	6.18	5.04	5.80	ns
Anaerobic sulphite reducers, CFU/g	4.50	2.28	3.36	4.47	ns
Lactobacillus, CFU/g	6.14	6.54	6.80	6.36	ns
Enterococcus/Streptococcus, CFU/g	4.98	2.70	5.16	5.11	ns
Enterobacteriaceae, CFU/g	6.05	6.70	5.64	6.50	ns
Caecum content					
Escherichia coli, CFU/g					
Coliforms, CFU/g	2.11	2.91	2.8	2.68	ns
Anaerobic sulphite reducers, CFU/g	2.95	2.72	2.49	2.35	ns
Lactobacillus, CFU/g	6.06	6.10	6.22	5.76	ns
Enterococcus/Streptococcus, CFU/g	3.03	2.72	3.00	2.63	ns
Enterobacteriaceae, CFU/g	2.81	3.19	2.81	2.85	ns

[a]Pigs 24 post-natal days of age at the beginning of the trial.
ns: not significant; CTR: basal diet; ANT: amoxicillin; CFU: colony forming unit; SEM: standard error of the mean.

Table 6. Concentrations of TAOC and MDA in the duodenum, ileum, and caecum tissue in post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[d].

	Dietary treatment				
Parameter	CTR	ANT	C12-Ca	SEM	*p*-value
Pigs/treat.	6	6	6		
MDA, nmol/mg					
Duodenum	2.530^{a}	2.080^{b}	1.860^{c}	0.100	<0.001
Ileum	2.510^{a}	2.130^{b}	1.870^{c}	0.100	<0.001
Caecum	2.510^{a}	2.100^{b}	1.900^{b}	0.100	<0.001
TAOC, nmol/µl TROLOX eq.					
Duodenum	4.270^{c}	4.580^{b}	5.130^{a}	0.140	<0.001
Ileum	5.980^{b}	6.210^{b}	6.930^{a}	0.134	<0.001
Caecum	6.230^{b}	$6.590^{a,b}$	7.150^{a}	0.111	<0.001

MDA: malondialdehyde; TAOC: total antioxidant capacity; CTR: basal diet; ANT: amoxicillin; TROLOX: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; SEM: standard error of the mean.
[a,b,c]Within rows, different superscript letters indicate a significant difference.
[d]Pigs 24 post-natal days of age at the beginning of the trial.

MDA was also reduced in the caecum in pigs fed C12-Ca and ANT, compared to CTR ($p < .001$).

Duodenum, jejunum, and ileum morphology

Table 8 shows the data for the morphology of the small intestine. No statistical differences were found in the duodenum for villus height (VH), villus width (VW), or crypt depth (CD). Only the villus height to crypt depth ratio (VH:CD) was significantly ($p < .01$) higher in the CTR than the C12-Ca treatment group in the duodenum. In the jejunum, VH was significantly ($p < .001$) increased in the ANT group, compared to the CTR and C12-Ca groups. VW was significantly ($p < .05$) higher in the ANT group, compared to the CTR group, whereas CD was decreased ($p < .001$) in the C12-Ca group, compared to the CTR and ANT groups. In contrast, VH:CD increased ($p < .05$) in pigs supplemented with C12-Ca soap, compared to the control group, in the jejunum. However, C12-Ca soap ameliorated the villus morphology in the ileum, where VH and VW were significantly ($p < .001$) higher in the animals fed C12-Ca soap, compared to the CTR and

Table 7. Effects of C12-Ca soaps on IgA and IL-6 in faecal samples of post-weaning piglets fed a basal diet alone (CTR) or supplemented with amoxicillin (ANT, 400 mg/kg) or lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[a].

	Dietary treatment				
Parameter	CTR	ANT	C12-Ca	SEM	*p*-value
IgA, µg/g					
Study days 0	41.24	34.89	32.77	4.10	ns
Study days 14	43.71	32.50	32.20	7.09	ns
Study days 28	34.82	30.77	35.80	3.19	ns
IL-6, pg/g					
Study days 0	791.88	634.98	640.15	86.58	ns
Study days 14	449.70	419.41	396.31	67.63	ns
Study days 28	278.23	274.62	332.03	65.04	ns

[a]Pigs 24 post-natal days of age at the beginning of the trial.
ns: not significant; CTR: basal diet; ANT: amoxicillin; IgA: immunoglobulin A; IL: interleukin; SEM: standard error of the mean.

Table 8. Intestinal morphology parameters of the duodenum, jejunum, and ileum for post-weaning piglets fed a basal diet alone (CTR), supplemented with amoxicillin (ANT, 400 mg/kg), or supplemented with lauric acid saponified with calcium (C12-Ca soaps, 1 g/kg) for 28 days[c].

	Dietary treatment				
Parameter	CTR	ANT	C12-Ca	SEM	*p*-value
Pigs/treat.	6	6	6		
Villus height, µm					
Duodenum	233.880	226.370	211.110	10.390	ns
Jejunum	192.270^{b}	225.770^{a}	172.270^{b}	7.860	<0.001
ileum	172.580^{b}	173.180^{b}	211.530^{a}	6.740	<0.001
Villus width, µm					
Duodenum	125.240	128.660	104.960	7.350	ns
Jejunum	91.990^{b}	117.020^{a}	$113.340^{a,b}$	5.360	<0.050
Ileum	96.800^{b}	100.540^{b}	128.880^{a}	5.040	<0.001
Crypt depth, µm					
Duodenum	111.350	134.190	134.220	7.790	ns
Jejunum	147.850^{a}	127.330^{a}	99.250^{b}	7.610	<0.001
Ileum	151.260^{a}	78.340^{b}	146.680^{a}	6.190	<0.001
VH:CD ratio					
Duodenum	2.270^{a}	1.950^{ab}	1.740^{b}	0.990	<0.010
Jejunum	1.550^{b}	$1.930^{a,b}$	2.020^{a}	0.130	<0.005
Ileum	1.270^{b}	2.580^{a}	1.500^{b}	0.130	<0.001

VH: CD ratio: Villus height to crypt depth ratio; ns: not significant; CTR: basal diet; ANT: amoxicillin; SEM: standard error of the mean.
[a,b]Within rows, different superscript letters indicate a significant difference.
[c]Pigs 24 post-natal days of age at the beginning of the trial.

ANT diets. Crypt depth was increased in the CTR and C12-Ca groups, compared to ANT, while the VH:CD ratio was greater in the ANT treatment group ($p < .001$).

Discussion

In recent years, lauric acid has been shown to have great potential to ameliorate the indices of intestinal function and gut health in weaned piglets. Moreover, interest in it as a supplement to diets for post-weaning piglets has increased, due to its possible antimicrobial activity against Gram-positive bacteria, which could represent a solution to reduce the use of antibiotics (Jackman et al. 2020). In this study, the addition of C12-Ca soap in the diet of piglets was considered, as a strategy to reduce the use of antibiotics to improve growth performance and ameliorate the indices of intestinal function and gut health in weaned piglets. When the 15- to 28-day period of the trial is considered, the FE in pigs supplemented with C12-Ca soaps was comparable to that of pigs receiving antibiotics and higher, when compared to the control. The finding that dietary supplementation with C12-Ca soaps at 0.1% did not result in significant differences in BW, ADG, and ADFI was comparable to the findings of other studies (Duarte et al. 2019; López-Colom et al. 2019). A recent review (López-Gálvez et al. 2021) has indicated that doses of organic acids at or above 1 g/kg in the diet can positively affect the growth and gut health of the piglets, thus confirming the possibility of using these substances as alternatives to antibiotics, due to their positive effects in preventing post-weaning diarrhoea (PWD) or restoring animal physiology following a PWD event. Moreover, the study of Jackman et al. (2020) concluded that MCFAs and monoglycerides, in the dose range of 0.2–1%, can be useful in supporting pig growth performance and gut health and, in the range of 1–2%, for feed pathogen mitigation. Comparing our results with those in the literature and considering the high content of lauric acid in C12-Ca, the dose of 0.1% can be considered as the minimal effective dose to influence growth performance, with the most appropriate economic criteria for pig farming, representing a valid alternative to antibiotic use.

Lauric acid has a remarkable bactericidal action against *Streptococci* (Bergsson et al. 2001). Streptococcosis is a disease that causes significant economic losses in the pig industry (Fittipaldi et al. 2012). It is generally accepted that the main route of infection is through the respiratory system. *Streptococcus suis* is transferred from the vaginal secretions of sows into the oral–nasal cavities of piglets during parturition and colonises the tonsils immediately after birth (Amass et al. 1996). According to Segura et al. (2017), the gastrointestinal tract cannot be excluded as a secondary site of infection in piglets. It has been found that, after weaning, the relative intestinal concentrations of *Streptococcus suis* increased or remained constant (Su et al. 2008; Wijtten et al. 2011). In our study, dietary supplementation of lauric acid saponified with calcium in post-weaning piglets did not significantly reduce the therapeutic treatments against *Streptococcus suis*. Moreover, our results did not show a significant reduction in mortality due to *Streptococcus suis* in all the dietary treatments; however, the pigs of the C12-Ca group that showed disease, once adequately treated, quickly recovered, unlike the control group pigs, which died. It is well-known that diarrhoea is another major challenge for post-weaning piglets, which results in increased morbidity and mortality and causes large economic losses in the global swine industry (Vondruskova et al. 2010; Yang et al. 2014). In our study, the antibiotic treatments against diarrhoea were decreased in CTR piglets and those supplemented with C12-Ca soap, compared to the ANT group ($p < .05$). This could be related to the difference in mechanism of action between MCFA and antibiotics. The bactericidal effect of MCFA is mainly hydrogen ion ($H+$)-related, but this is not the case for antibiotics (Yen et al. 2015).

Microbial metabolites were measured, in addition to the determination of the intestinal microbial composition as indicators of microbial activities. Our results displayed a significantly higher concentration of lactic acid in the duodenum, ileum, and caecum of treated piglets. On the other hand, the acetic acid concentration significantly decreased in the duodenum and caecum in the C12-Ca soap group and ANT, compared to the CTR group. The acetic acid concentration in the caecum was the lowest in the piglets fed a diet supplemented with C12-Ca soaps. In this regard, the literature contains numerous studies on the effect of organic acids on fermentation patterns in pigs, but it is difficult to compare these various experimental studies, due to the use of different substances (pure acids or salts; individually or in combination), as well as other factors, such as the intestinal segments chosen for analysis and the composition of the diet, which affected the microbial composition (Zentek et al. 2012; Lai et al. 2014; Tugnoli et al. 2020). Dierick et al. (2004) reported a reduction in the lactate

concentration of the proximal and distal jejunum of piglets after using a combined administration of triglycerides with medium-chain fatty acids and lipase, whereby the proportion of acetate was increased. These results were not confirmed in this study and Zentek et al. 2012. The different modulation of MCFA on the intestinal concentrations of short-chain fatty acids could be explained by the different diet compositions. The composition of the experimental diet considered in our study was characterised by a high content of lactose, which could have affected the lactate content in the intestine. By contrast, in our study, a reduction in acetic acid concentration was found in the caecum of piglets. However, unlike what was reported in Świątkiewicz et al. (2020), with our data we cannot conclude that this reduction is due to the higher digestibility of the fibre and the greater efficiency of nutrient use following MCFA supplementation. On the other hand, the diet we used for our trial was characterised by a higher digestibility, which may have made the effects of MCFAs on the production of volatile fatty acids in the large intestine less evident. The modulation of VFA production reported in our study demonstrated the ability of C12-Ca to reach the intestine in a concentration sufficient to modulate the microbial metabolites. Whether this was due to a higher bacterial metabolic activity of the selected bacterial groups, or a change in the microbial composition needs to be further explored (e.g. considering microbiota analysis measured by qPCR), as the microbial populations in the intestinal contents of the piglets were not affected. Furthermore, the antibacterial effects of medium-chain fatty acids have been studied mostly using *in vitro* techniques at comparatively high concentrations, and more studies are needed to evaluate the amount of lauric acid which is able to reach the intestinal tract (Kabara et al. 1972; Petschow et al. 1996; Sprong et al. 1999; Bergsson et al. 2001).

Weaning stress causes growth reduction and gut dysfunction in piglets and may lead to intestinal inflammation and minor immune responses (Bomba et al. 2014; Bhat et al. 2015). However, the results of the anti-inflammatory parameters in faeces showed that supplementation with C12-Ca soaps did not result in significant differences, compared to the other groups, demonstrating the absence of intestinal inflammation in all animals.

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and their elimination through antioxidative mechanisms (Castellani et al. 2014; Yin et al. 2014). Weaning pigs with such an imbalance and immature antioxidant systems in their intestines are susceptible to oxidative stress (Cao et al. 2018). In the present study, TAOC levels in the intestine, which reflect the total antioxidant defense system, were significantly higher in the animals treated with C12-Ca soaps. Furthermore, the levels of MDA, one of the most-used markers of lipid peroxidation, were lower. This result can also be considered as an indirect indicator of intestinal inflammatory status. Indeed, several human studies have shown that inflammation and oxidation are related (Mittal et al. 2014; Biswas 2016) and that both participate in the pathogenesis of many chronic diseases (Dierick et al. 2002; Cao et al. 2018).

Intestinal villi represent the main site of nutrient absorption and their improved development has been associated with greater nutrient utilisation, resulting in better piglet growth (Mekbungwan et al. 2002; Ray et al. 2002). In this study, some favourable results were found with the use of C12-Ca soaps in pig diets, particularly in the ileum, where the C12-Ca diet modulated the intestinal absorption surface. The stressful events related to weaning cause changes in the gut morphology, such that the villus length is reduced and the crypt depths are increased (Pluske et al. 1997). Dierick et al. (2004) reported that feeding MCFAs to weaning pigs positively influenced their intestinal morphology, resulting in a significant increase in villus length and a decrease in crypt depth in the small intestine. However, other authors have found no evidence of differences in intestinal morphology after the dietary administration of MCFAs (Hanczakowska et al. 2013; Ferrara et al. 2017). The comparison of our results with other studies demonstrates that the concentration of MCFA able to bypass the stomach or the upper digestive tract is critical in observing an evident positive effect at the intestinal level (Tugnoli et al. 2020).

Considering the results obtained, we hypothesised that C12-Ca may not completely dissociate in the stomach, meaning that lauric acid, in the form of Ca-laurate, cannot be completely absorbed in the proximal regions of the small intestine and can reach the distal regions of the small intestine (i.e. the ileum) and perhaps even further (i.e. the caecum). This assumption was supported by the modulation by C12-Ca of intestinal VFAs, antioxidant status, lipid peroxidation, and favourable changes in the structure of the ileum epithelium.

Conclusion

Our results suggest that supplementing a basal diet with C12-Ca soaps (1 g/kg) may ameliorate the gut

health and indices of intestinal function in piglets. The present contributions of C12-Ca to growth performance, intestinal microbial populations, and anti-inflammatory and immunomodulatory properties are not conclusive and justify further investigation, in order to determine the content and the absorption of C12-Ca along the gastrointestinal tract to retard the absorption, thereby achieving higher efficiency of the product in the distal parts of the porcine GIT. The treatment demonstrated a modulation in intestinal VFA production, as well as beneficial effects on intestinal antioxidant status and some intestinal morphology parameters in the ileum. Considering these properties, C12-Ca soaps can be considered as a valuable additive to weaned piglet feed, which deserves further investigation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [Prof. Valentino Bontempo; valentino.bontempo@unimi.it], upon reasonable request.

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