Comparison of medium chain organic acids with zinc oxide and benzoic acid; effect on growth performance of pigs

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Abstract

Medium chain organic acids (MCOA) show strong and specific antimicrobial activity and may be useful as part of an antibiotic reduction strategy. Two trials were conducted to evaluate the effect of MCOA on growth performance of pigs in comparison with benzoic acid (BA) and ZnO (ZnO). Each used 240 pigs in a randomised complete block design with six treatments in eight replicate pens. Trial 1 tested a blend of C6:0, C8:0, C10:0 and C12:0 for 56 d post-weaning. Treatments were (1) control basal diet (BD), (2) BD+ZnO (3) BD+ZnO+BA, (4) BD+MCOA (5) BD+MCOA+ZnO, (6) BD+MCOA+BA. Pigs receiving MCOA+BA showed higher average daily liveweight gain in the grower period (849 g/d) than pigs receiving the control diet (773 g/d) or the ZnO treatment (779 g/d; $P=0.040$), grew 7% more efficiently ($P=0.008$) and were 3.02 kg heavier at 56 d than control pigs. Trial 2 tested the MCOA blend at two inclusion levels in comparison with C6:0/C12:0 (50/50) and C6:0/C12 monolaurin (C12M; 50/50), for 28 d post-weaning. Treatments were (1) control basal diet, (2) BD+ZnO (3) BD+1,500 mg/kg MCOA (4) BD+750 mg/kg MCOA (5) BD+ C6/C12 (total 3,000 mg/kg), (6) BD + C6/C12M (total 3,000 mg/kg). Pigs receiving 750 mg/kg MCOA grew more slowly than those receiving C6/C12M or ZnO ($P<0.05$) to d 21. Pigs fed ZnO were 1.61 kg heavier than these pigs ($P<0.05$), while those fed C6/C12M were 1.29 kg heavier ($P<0.05$) at d 21. During 0-14 d pigs fed ZnO showed the best feed conversion ratio (FCR), but at 15-28 d the improvement was no longer seen. Both the C6/C12 and the C6/C12M treatments appeared to improve FCR during this second phase ($P<0.01$). The results suggested that supplementation with a combination of MCOA and BA may be a promising strategy to improve growth performance while replacing ZnO and reducing antibiotic dependence.

Keywords: antimicrobial resistance, antibiotic reduction strategy

1. Introduction

There is continuing pressure to reduce the use of antibiotics in pig production. Despite progress towards reductions in many countries, antibiotics are still commonly used routinely, both prophylactically and to treat infection on farm. The highest priority critically important antimicrobials as classified by the World Health Organization are quinolones, third and higher generation cephalosporins, macrolides and ketolides, glycopeptides and polymyxins. Accurate assessment of use at farm level is difficult because of differences in reporting methods. Thus,

large variations in use exists between different countries (Lekagul *et al.*, 2019), whereby aminopenicillins accounted for 30-40% of total antibiotic use in studies from Sweden, Germany and Canada, while tetracyclines were the most commonly used in Denmark, Japan, Netherlands, Australia, Spain, France and Germany. The population correction unit (PCU), an index used by the European Medicines Agency, is calculated by dividing the active ingredient weight (in mg) of all antibiotic products sold in the country by the standardised average weight (in kg) of all animals at time of treatment multiplied by the number of animals (based on national statistics; Veterinary Medicines Directorate,

2016). In the United Kingdom, antibiotic use in 2019 was similar to use in 2018 at 110 mg/PCU, having fallen 60% in the three years prior (AHDB, 2019), while the use of critically important antibiotics decreased from 0.06 mg/PCU in 2018 to at 0.04 mg/PCU in 2019. A figure of 110 mg/PCU indicated that 110 mg of antibiotic active ingredient was used for every kg of bodyweight at time of treatment.

Regulation EU 2019/4, which will come into force on January 29th, 2022 (European Commission, 2019), will prohibit the prophylactic use of antibiotics in the European Union, except for very exceptional cases and to treat individual animals rather than groups. In parallel, there is an urgency to remove pharmacological levels of zinc oxide (ZnO) from piglet starter feeds to meet legislation in place due to environmental reasons and concerns of cross-resistance. Zinc oxide is used routinely to prevent intestinal infection of young animals at weaning, although its mode of action has not been clearly defined. However, it has been shown to alter the enterobacterial population in the ileum of piglets (Starke *et al.*, 2014; Vahjen *et al.*, 2011), and it has been proposed that the influence on gastrointestinal microbiota may be through suppression of Gram-positive commensal microorganisms rather than through suppression of Gram-negative organisms (Hojberg *et al.*, 2005) and may participate in the stabilisation of the epithelial cell membrane (Srivastava *et al.*, 1995). Zinc oxide was estimated to be used in 70–90% of weaned pig rations in 2017 (Driver, 2017) although it can be expected that this figure has since reduced in response to pending legislation. The proposed removal of ZnO leads to further concerns of a future rise in antibiotic use. Despite the progress towards reductions in the United Kingdom, antibiotics are still commonly used in the post-weaning period; both in medicated starter feeds and to treat infection on farm. The replacement of prophylactic antibiotics with safe, effective and reliable alternatives, therefore, remains a focus point.

Among candidate alternatives showing increasing promise are medium chain organic acids. These are unbranched and unsaturated monocarboxylic acids of caproic acid (C6:0, hexanoic acid), caprylic acid (C8:0, octanoic acid), capric acid (C10:0, decanoic acid) and lauric acid (C12:0, dodecanoic acid). Monoesters of free fatty acids are another type of single-chain lipid amphiphile, comprising esterified compounds consisting of one fatty acid molecule and one glycerol molecule. For example, monolaurin is the monoester formed from glycerol and lauric acid. The antimicrobial activity of medium chain organic acids (MCOA) has already been reviewed and described (Hanczakowska, 2017). The undissociated acid molecules penetrate the phospholipid bilayer membrane of bacteria and enter the cell where they dissociate due to the more alkaline pH. The pH of the cell cytoplasm is lowered, which suppresses cytoplasmic enzymes and nutrient transport systems, leading to cell death. While

some studies have shown stronger inhibitory effects with medium chain organic acid than with short or long chain organic acids (Hanczakowska *et al.*, 2013; Hassinen *et al.*, 1951; Nieman, 1954) and there are conflicting reports about the relative antimicrobial efficacy of the different medium chain acids. Only caprylic acid (C8:0), and, to a lesser extent, capric acid (C10:0) have shown significant antimicrobial activity against two strains of *Escherichia coli* in one study (Marounek *et al.*, 2003). Other studies showed that both lauric acid (C12:0) and caprylic acid (C8:0) had the ability to damage the cytoplasmic structure of bacteria (Skrivanová *et al.*, 2006). Lauric acid can disrupt *Clostridium* spp. cell membranes and cytoplasm (Shilling *et al.*, 2013) whereas monolaurin has been shown to be more active against Gram-positive bacteria than the free fatty acid, lauric acid (Batovska *et al.*, 2009). Previous *in vitro* studies (Neath *et al.*, in press), confirm the efficacy of monolaurin against the Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus suis*. Caproic acid has efficacy against Gram-negative bacteria including *Salmonella typhimurium* and *E. coli*, as well as against Gram-positive bacteria. Different MCOA may, therefore, be expected to show differing efficacy in pigs, and it may successfully replace prophylactic zinc oxide, to reduce antibiotic or benzoic acid use, without any reduction in pig performance. To test this, two trials were carried out to evaluate the effects of MCOA on the growth performance of weaner/grower pigs in comparison with standard ZnO or benzoic acid.

2. Materials and methods

Animals and trial design

The studies were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. Both trials used two hundred and forty (Large White × Landrace) × Danish Duroc pigs. Trial 1 pigs had an initial weight of 8.00±1.25 kg (mean ± SD) at 28.4±0.69 d of age. Trial 2 pigs had an initial weight of 7.88±1.31 kg (mean ± SD) at 27.8±0.52 d of age. Each trial consisted of 48 pens, each containing five mixed sex pigs. Start weight, sex and litter of origin were balanced across treatments within replicate. Pens within each replicate were randomly assigned to one of the six dietary treatments shown in Table 1 and 2. Pigs in Trial 1 remained on trial for 56 d after weaning (final weight 40.60±4.06 kg); Trial 2 pigs remained on trial for 28 d after weaning (final weight 19.40±2.88 kg). Feed and water were provided *ad libitum* throughout the experimental period.

Dietary treatments

A three-phase feeding programme was used: Diet 1 from weaning to d 14, diet 2 from d 15 to d 28 and diet 3 from d 29 to 56. Day 0 to 28 was classed as the weaner period and d 29 to 56 the grower period. Diet formulations were provided by a UK commercial feed producer, according to a commercial

Table 1. Dietary treatments used in Trial 1.¹

Diet	Treatment	Day 0-14 of trial Weaner phase	Day 15-28 of trial Weaner phase	Day 29-56 of trial Grower phase
1	Control	Basal diet	Basal diet	Basal diet
2	ZnO	ZnO	Basal diet	Basal diet
3	ZnO + BA	ZnO + BA	BA	BA
4	MCOA	MCOA 1000 mg/kg	MCOA 1000 mg/kg	MCOA 500 mg/kg
5	MCOA + ZnO	ZnO + MCOA 1000 mg/kg	MCOA 1000 mg/kg	MCOA 500 mg/kg
6	MCOA + BA	BA + MCOA 1000 mg/kg	BA + MCOA 1000 mg/kg	BA + MCOA 500 mg/kg

¹ BA = benzoic acid at 5,000 mg/kg benzoic acid; MCOA 1000 mg/kg = 2,000 mg/kg [medium chain organic acid/ silica blend] containing 1000 mg/kg MCOA (C6:C8:C10:C12 1:1:1:1); MCOA 500 mg/kg = 1000 mg/kg [medium chain organic acid/ silica blend] containing 500 mg/kg MCOA (C6:C8:C10:C12 1:1:1:1); ZnO = zinc oxide at 2,500 mg/kg zinc.

Table 2. Dietary treatments used in Trial 2.¹

Diet	Treatment	Day 0-14 of trial Weaner phase	Day 15-28 d of trial Weaner phase
1	Control	Basal diet	Basal diet
2	ZnO	ZnO	Basal diet
3	MCOA ₁₅₀₀	MCOA 1,500 mg/kg	MCOA 1,500 mg/kg
4	MCOA ₇₅₀	MCOA 750 mg/kg	MCOA 750 mg/kg
5	C6/ C12	C6 1,500 mg/kg + C12 1,500 mg/kg	C6 1,500 mg/kg + C12 1,500 mg/kg
6	C6/ C12M	C6 1,500 mg/kg + C12M 1,500 mg/kg	C6 1,500 mg/kg + C12M 1,500 mg/kg

¹ C6 1,500 mg/kg = 1,500 mg/kg C6:0 medium chain organic acid. C12 1,500 mg/kg = 1,500 mg/kg C12:0 medium chain organic acid; C12M 1,500 mg/kg = 1,500 mg/kg C12 monolaurin (monoglycerides of lauric acid); MCOA 1,500 mg/kg = 3,000 mg/kg [medium chain organic acid/ silica blend] containing 1,500 mg/kg MCOA (C6:C8:C10:C12 1:1:1:1); MCOA 750 mg/kg = 1,500 mg/kg [medium chain organic acid/ silica additive blend] containing 750 mg/kg MCOA (C6:C8:C10:C12 1:1:1:1); ZnO = zinc oxide at 2,500 mg/kg zinc.

specification based on AHDB recommendations (Table 3). In Trial 1, the MCOA blend used consisted of a blend of C6:0, C8:0, C10:0 and C12:0 fatty acids (1:1:1:1) on a silica carrier (50%). Feed-grade benzoic acid and ZnO were used. Trial 2 used the MCOA blend as in trial 1 in addition to a blend of purified C6:0 and C12:0 fatty acids and a blend of purified C6:0 fatty acid and C12 monolaurin. The additives were mixed into the commercial diets at the manufacturing stage and pelleted using a commercial pelleting process.

Measurements and analytical methods

Individual pig weights were recorded at start and on d 7, 14, 21, 28 (Trial 1 and 2), 35, 42, 49 and 56 (Trial 1). Feed troughs were emptied daily from weaning to d 21 and then on d 28 (Trial 1 and 2), 35, 42, 49 and 56 (Trial 1) for the determination of pen feed intake. Average pen feed conversion ratio was calculated from body weight and pen feed intake measurements. Individual use of antibiotics, incidence of scour or other health problems and death were recorded. Faecal scores were evaluated by the same individual once daily at the same time each day from Monday to Friday for the duration of the 56 d trial, using the following scoring system: 1 = firm, 2 = soft, spreads slightly, 3 = very soft, spreads readily, 4 = watery, liquid consistency. Health scores were designated based on the proportion of pigs within a pen displaying signs of poor

health, averaged over a particular period. For example, if a pen of five pigs had one pig that seemed unwell it would score 0.2 (1/5).

Statistical analysis

Trial 1 and 2 data were analysed according to a randomised complete block design using the general linear model function of SPSS (version 21; IBM, Armonk, NY, USA) with the pen serving as the experimental unit. In trial 1, data that violated that the assumptions of the GLM (normal distribution of residuals or homogeneity of variance) were reciprocally transformed prior to statistical analysis (d 0 to 7 feed conversion ratio (FCR), d 29 to 35 FCR and Diet 1 FCR). Significantly different means were separated using the Tukey's post-hoc test. Data are presented as least-square means along with the pooled SEM. Significance was recorded at $P < 0.05$ and P -values of 0.05 to 0.1 were noted as trends. Separate two-treatment analyses were performed between control vs ZnO, control vs MCOA/ benzoic acid (BA) and control vs MCOA. Faecal and health scores were analysed by Kruskal-Wallis using the non-parametric function of SPSS. Antibiotic usage was analysed by chi square analysis.

Trial 2 data were checked for normality of residuals using the Kolmogorov-Smirnov test and homogeneity of

Table 3. Basal diet specifications.¹

Ingredients (%)	0-14 d	15-28 d	29-56 d	Nutrients (% DM)	0-14 d	15-28 d	29-56 d
Barley	15.00	17.31	15.00	Crude protein	21.64	21.14	19.51
Oats	5.00	-	-	Oil A ⁴	7.30	4.27	-
Wheat	30.00	45.00	46.13	Oil B ⁵	7.98	4.94	3.80
SBM, extracted 48%	-	-	22.10	Crude fibre	2.10	2.43	3.11
SBM, extruded 48%	16.00	23.56	-	NDF	7.43	9.03	10.31
SBM, full fat	3.00	-	-	Ash	5.29	5.17	4.85
Confectionary by-product	-	-	8.00	Calcium	0.70	0.72	-
RSM, extracted	-	-	3.19	Phosphorus	0.70	0.65	0.45
Fish meal 68%	5.00	4.00	-	Sodium	0.18	0.19	0.20
Dry skimmed milk	3.00	-	-	SID lysine	1.37	1.23	1.20
Whey	14.44	4.65	-	SID methionine	0.53	0.44	0.42
Soya oil	5.00	2.70	0.47	SID threonine	0.89	0.80	0.78
Limestone	0.20	0.57	0.76				
Molasses	-	-	2.00				
Salt	0.01	0.27	0.44				
Lysine HCl	0.35	0.31	0.79				
Methionine	0.20	0.15	0.16				
Threonine	0.17	0.14	0.19				
Tryptophan	0.03	0.00	-				
Potato protein	1.25	-	-				
Monocalcium phosphate	0.77	0.81	-				
Monocalcium phosphate	-	-	0.47				
L-valine	0.05	-	-				
Phytase ²	0.04	0.04	-				
Premix ³	0.50	0.50	0.30				

¹ HCl = hydrochloride salt of L-lysine; NDF = neutral detergent fibre; RSM = rapeseed meal; SBM= soya bean meal; SID = standard ileal digestible.

² 6-phytase at 2,000 units/kg feed.

³ Premix additives (per kg feed): vitamin A (3a672a-retinyl acetate) 8,000 IU, vitamin D3 (3a671-cholecalciferol) 1,500 IU, vitamin E (all rac-alpha tocopherol acetate) (3a700) 125 IU, iodine (calcium iodate anhydrous 3b202) 1 mg, manganese (manganous oxide 3b502) 30 mg, copper (cupric sulphate pentahydrate E4-Copper) 80 mg, iron (ferrous sulphate monohydrate 3b103) 120 mg, selenium (sodium selenite E8-selenium) 0.25 mg, 1,220 U endo-1-4-beta-xylanase (EC 3.2.1.8), 152 U endo-1,3(4)-beta glucanase (EC3.2.1.6), 5 mg BHT (E321).

⁴ Oil A is the crude fat; ether extractable portion; contains additional non-polar (ether soluble) components of diet.

⁵ Oil B is the total fat; an acid hydrolysis step prior to the ether extraction releases any bound/ protected fats so that they can be extracted in the ether extract stage.

variance using the Levene's test prior to analysis. Diet 1 average daily feed intake (ADFI) data were heteroscedastic and therefore subject to a Kruskal-Wallis test analysis. Significantly different means were separated using the Tukey's *post-hoc* test. Data are presented as least-square means along with the pooled SEM. Significance was recorded at $P < 0.05$ and P -values of 0.05 to 0.10 were noted as trends. Faecal and health scores were analysed by Kruskal-Wallis using the non-parametric function of SPSS. For both trials, incidence of death or pigs taken off trial were not analysed statistically due to insufficient numbers. Data were analysed in terms of pen weight at the start and weekly thereafter, pen ADFI each week, for each diet and overall, pen average daily liveweight gain (ADLG) each week, for each diet and overall, pen average FCR each week, for each diet and overall, overall health and faecal scores and antibiotic usage.

3. Results

Trial 1

Low initial growth rates reflected high antibiotic usage and the removal of the poorly performing pigs during the early stages of the study. Three pens were removed

from the trial due to scouring and poor performance on d 7 (two from the MCOA + BA treatment and one from the ZnO + BA treatment). As these pens were removed from the trial early in the study, the observations were removed from the data set prior to statistical analysis. A further 13 pigs were removed in the weaner phase due to scouring or other signs of ill health, with an additional four losses due to mortality from undetermined causes (three during the 0-28 d phase and one in the 29-56 d phase). A total of 77 pigs (32.1%) required antibiotic treatment throughout the trial. There were notable (although non-significant) differences between treatment groups for piglets receiving antibiotics (Table 4), whereby eight pigs received antibiotics in the MCOA group compared to an average of 14 in all the other groups. For the remaining pigs, overall health (0.14 ± 0.153) and faecal (2.16 ± 0.059) scores were uniformly good throughout the trial, with no differences observed between the treatments.

The total feed intake of pigs was comparable between the dietary treatments throughout the study. In the grower period (29-56 d) pigs receiving MCOA + BA showed higher ADLG (849 g/d) compared to pigs receiving the control diet (773 g/d) or those which had received ZnO in the two-week post-weaning period (779 g/d; $P = 0.040$). Pigs

Table 4. Effect of replacing zinc oxide (ZnO) and benzoic acid (BA) with medium chain organic acids on pig performance.^{1,2}

	Con	ZnO	ZnO/BA	MCOA	MCOA/ZnO	MCOA/BA	SEM	P
Liveweight (kg)								
d 0	7.97	7.95	8.00	7.93	7.93	7.94	0.032	0.492
d 14	10.39	10.75	10.71	10.18	10.62	10.79	0.398	0.774
d 28	17.62	18.22	18.49	17.34	17.81	18.85	0.589	0.366
d 56	39.23	40.08	42.17	39.79	40.54	42.25	0.895	0.126
ADFI(g/d) ³								
0-14 d	213	215	199	196	200	229	19.0	0.759
15-28 d	700	678	677	654	641	680	41.5	0.877
29-56 d	1,221	1,250	1,318	1,241	1,305	1,287	30.2	0.163
Overall	834	860	861	826	840	856	24.2	0.855
ADLG (g/d) ⁴								
0-14 d	173	198	194	161	193	202	27.3	0.833
15-28 d	515	500	540	504	490	551	25.2	0.389
29-56 d	773 ^a	779 ^a	845 ^{ab}	803 ^{ab}	801 ^{ab}	849 ^b	19.2	0.040
Overall	560	575	609	570	581	613	15.8	0.151
ADLG using individual pig as experimental unit								
Overall	559 ^a	576 ^{ab}	605 ^{ab}	569 ^{ab}	580 ^{ab}	631 ^b	16.6	0.044
FCR ⁵								
0-14 d	1.17	1.08	1.10	1.26	1.04	1.06	0.062	0.158
15-28 d	1.36	1.35	1.26	1.30	1.32	1.24	0.054	0.623
29-56 d	1.58	1.60	1.55	1.55	1.63	1.52	0.025	0.071
Overall	1.50	1.46	1.41	1.45	1.44	1.39	0.025	0.065
Antibiotics ⁶	14	12	14	8	12	17		
Off trial	1	4	7	1	4	11		
Dead	0	1	1	0	0	2		

¹ Con = control; MCOA = medium chain organic acids (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial; MCOA/ZnO = ZnO at 2,500 mg/kg day 0-14 of trial, MCOA (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial; MCOA/BA = MCOA (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial, benzoic acid at 5,000 mg/kg throughout trial; ZnO = ZnO at 2,500 mg/kg day 0-14 of trial; ZnO/BA = ZnO at 2,500 mg/kg zinc day 0-14 of trial, benzoic acid at 5,000 mg/kg throughout trial.

² Data given are means of each treatment. Con, ZnO, ZnO/BA, MCOA, 8×5 pigs per treatment; MCOA/ZnO 7×5 pigs; MCOA/BA 6×5 pigs. Means within row that do not share a common superscript differ significantly ($P<0.05$).

³ Least square means of average daily feed intake.

⁴ Least square means of average daily liveweight gain.

⁵ Least square means of FCR.

⁶ No. of pigs requiring antibiotic treatment; standard treatment of amoxycillin 15 mg/kg/day for 5 d. Specific two-treatment comparisons Con vs MCOA/BA: ADLG 29-56 d $P=0.07$, 0-56 d $P=0.086$, FCR 0-56 d; $P=0.008$.

receiving MCOA showed an intermediate growth rate between the control and the MCOA + BA treatment in this period (803 g/d). When individual pig growth data were analysed, the difference between the MCOA + BA and the control group reached significance for the overall trial period (631 g/d vs 559 g/d; $P=0.044$). Differences were most pronounced in the period 43-49 d, where pigs receiving MCOA + BA gained 922 g/d compared to 765 g/d for pigs receiving ZnO ($P=0.002$). Consequently, pigs receiving MCOA + BA ended the trial 3.02 kg heavier than control pigs. There was a tendency for treatment to influence FCR ($P=0.065$) throughout the 56 d trial; two-way treatment comparisons showed the difference between the control pigs and the MCOA + BA treated pigs was highly significant ($P=0.008$) whereas neither ZnO or MCOA as treatments significantly influenced FCR. Pigs receiving ZnO did not show any increased ADLG compared to control pigs. However, when ZnO was removed from the diet after 14 d, there was a reduction in performance (Table 5) which appeared to be mitigated by the addition of benzoic acid (485 vs 383 g/d; $P=0.039$).

Trial 2

Pigs were in good health throughout the study with overall health scores of 0.04 ± 0.09 , irrespective of dietary treatment. Antibiotic treatment was relatively low at 12.9%, whereby eight pigs were removed from the trial and there were no mortalities. Mean faecal consistency scores were good (mean 2.23 ± 0.16 ; soft) throughout and there was little incidence of post-weaning diarrhoea. Piglets fed the diets containing ZnO had firmer faecal material than those fed the 750 mg/kg MCOA or the C6/C12M treatment ($P<0.05$). Growth performance data are presented in Table 6. Pigs receiving 750 mg/kg MCOA had the lowest feed intakes, which was reflected in growth rate as these pigs grew more slowly than those receiving ZnO or the C6/C12 M ($P<0.05$) until 21 d after weaning. At 21 d, pigs fed the ZnO were the heaviest and grew the fastest up until day 21, but, by d 28, there were no significant differences in growth rate or liveweight between the treatment groups. During the first feeding phase the pigs receiving ZnO showed the best FCR, whereas, in the second phase, this effect was no longer seen. Both C6/C12 and C6/C12M treatments appeared to improve FCR during the second period (d 15-28), for C6/C12 M ($P<0.01$).

Table 5. Trial 1: Effect of replacing zinc oxide (ZnO) and benzoic acid (BA) with medium chain organic acids on ADLG weekly average (g/day).^{1,2}

	Con	ZnO	ZnO/BA	MCOA	MCOA/ZnO	MCOA/BA	S.E.M	P
0-7 d	48	75	37	40	49	75	18.7	0.571
8-14 d	298	325	352	281	334	325	34.9	0.736
15-21 d	465 ^{ab}	363 ^a	485 ^b	409 ^{ab}	411 ^{ab}	443 ^{ab}	27.5	0.039
21-28 d	568	603	577	585	555	630	31.3	0.654
29-35 d	751	716	771	708	714	764	38.4	0.755
36-42 d	689	703	768	698	720	692	29.9	0.471
43-49 d	770 ^a	765 ^a	866 ^{ab}	865 ^{ab}	853 ^{ab}	922 ^b	27.0	0.002
50-56 d	879	941	981	940	920	986	33.8	0.265

¹ Con = control; MCOA = medium chain organic acids (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial; MCOA/ZnO = ZnO at 2,500 mg/kg day 0-14 of trial, MCOA (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial; MCOA/BA = MCOA (C6:C8:C10:C12 1:1:1:1) at 1000 mg/kg day 0-28 and 500 mg/kg day 29-56 of trial, benzoic acid at 5,000 mg/kg throughout trial; ZnO = ZnO at 2,500 mg/kg day 0-14 of trial; ZnO/BA = ZnO at 2,500 mg/kg zinc day 0-14 of trial, benzoic acid at 5,000 mg/kg throughout trial.

² Data given are means of each treatment. Con, ZnO, ZnO/BA, MCOA, 8×5 pigs per treatment; MCOA/ZnO 7×5 pigs; MCOA/BA 6×5 pigs. Means within row that do not share a common superscript differ significantly ($P<0.05$)

4. Discussion

In trial 1, the below-average growth rates and above-average antibiotic use throughout the weaner phase indicated a sub-clinical disease challenge. The reduction of antibiotic intervention in animals receiving MCOA compared to all other groups, although non-significant, was noteworthy. The positive health effect may be explained by a direct antimicrobial effect of MCOA and supports earlier findings that a blend of C6:0, C8:0 and C10:0 reduced the coliform count in the colon (Yen *et al.*, 2015). The absence of a notable effect of MCOA on growth performance in this

experiment may have been partly compounded by the removal of poorly performing animals from the other treatment groups. These results did not agree with those of Hanczakowska *et al.* (2011), who demonstrated that piglets receiving C8:0, C10:0, or a blend of these fed at 0.2% from 7-84 d of age showed improved growth performance (ADLG, FCR $P<0.05$) compared to control-fed animals. The magnitude of response was likely influenced by the type or chain length of medium chain acid (C6:0, C8:0, C10:0 and C12:0), inclusion level, and background pathogen challenge, although there are too few published studies to confirm this. The lack of growth improvement in response to ZnO in this

Table 6. Trial 2: Effect of replacing zinc oxide (ZnO) with different types and levels of medium chain organic acids on weaning pig performance.¹

	Con	ZnO	MCOA ₁₅₀₀	MCOA ₇₅₀	C6/C12	C6/C12M	S.E.M	P
Liveweight (kg)								
d 0	7.79	7.89	7.82	7.82	7.89	7.87	0.031	0.161
d 14	11.66 ^{ab}	12.17 ^a	11.24 ^{ab}	10.66 ^b	11.24 ^{ab}	11.19 ^{ab}	0.255	<0.010
d 21	14.56 ^{ab}	15.33 ^a	14.71 ^{ab}	13.72 ^b	14.61 ^{ab}	15.01 ^a	0.336	<0.050
d 28	19.25	19.97	19.26	18.24	19.44	19.46	0.485	0.244
ADFI (g/d) ²								
0-14 d	291 ^{ab}	306 ^a	278 ^{ab}	239 ^b	258 ^{ab}	256 ^{ab}	13.9	<0.050
15-28 d	729	736	738	659	715	717	30.7	0.469
Overall	511	521	505	448	486	484	20.7	0.171
ADLG (g/d) ³								
0-14 d	276 ^{ab}	308 ^a	244 ^{ab}	203 ^b	239 ^{ab}	237 ^{ab}	18.2	<0.010
15-28 d	543	556	568	539	586	597	26.0	0.564
Overall	409	433	408	371	413	413	17.0	0.242
FCR ⁴								
0-14 d	1.07 ^{ab}	1.01 ^a	1.16 ^{ab}	1.20 ^b	1.11 ^{ab}	1.09 ^{ab}	0.037	<0.010
15-28 d	1.36 ^a	1.32 ^{ab}	1.32 ^{ab}	1.21 ^b	1.22 ^{ab}	1.21 ^b	0.034	<0.010
Overall	1.25	1.20	1.24	1.20	1.18	1.17	0.021	0.058
Antibiotics ⁵	0	4	5	3	9	10		
Off-trial	0	0	1	1	1	5		
Dead	0	0	0	0	0	0		

¹ Data given are means of each treatment. 8×5 pig per treatment. Means within row that do not share a common superscript differ significantly ($P<0.05$).

² Least square means of average daily feed intake.

³ Least square means of average daily liveweight gain.

⁴ Least square means of feed conversion ratio.

⁵ No. of pigs requiring antibiotic treatment; standard treatment of amoxycillin 15 mg/kg/day for 5 d.

study was noteworthy in view of the ongoing widespread use of this medication. When ZnO was removed from the diet after 14 d, in line with standard practice and regulatory requirements, there was an immediate reduction in growth rate; whereby pigs grew 102 g/d less than control pigs (d 15-21; Table 5). Performance reductions in response to ZnO withdrawal has previously been reported (Portocarero-Khan and Jurado-Candaliga, 2006) and the current results suggested that the short-term benefits of this medication may be negated by post-withdrawal performance reduction.

In contrast to the lack of growth response seen when MCOA or ZnO were used individually, the 13% increase in growth rate (compared to control animals) observed when benzoic acid was used in addition to MCOA was highly notable. The difference between these pigs and the pigs receiving BA plus ZnO appeared to confirm that the combination, not solely BA, was responsible for the large increase. Benzoic acid in combination with ZnO (which is widely practised in the U.K.) resulted in an 8% increase in ADLG. When ZnO was omitted from the diet, and BA was instead used in combination with MCOA, the increase in ADLG reached 13%, and there was a 7% improvement in FCR. This is the first published study that reported a combination of BA and MCOA could have a complementary effect, which deserves further investigation.

Pigs in the second weaner study had fewer health problems and overall better performance than those in the first trial, and treatment effects on health were less pronounced. Addition of ZnO tended to have a positive impact on average daily liveweight gain in the first two weeks post-weaning while the effects of the MCOA were less pronounced. In this trial, combinations of C6 and C12 or C6 and C12M were chosen based on previous *in vitro* experiments (Neath *et al.*, in press) which showed efficacy of C6 against the Gram-negative microorganisms *S. typhimurium* and *E. coli*, and efficacy of C12M against the Gram-positive microorganisms *S. suis* and *S. aureus*. Although both C6/C12 and C6/C12M treatments appeared to improve FCR during the second period, overall, the MCOA did not match the growth response seen with ZnO. Possibly, the inclusion levels of the individual MCOA were too low to be effective. Given the inconsistency of the response to ZnO (in the first study, pigs receiving ZnO showed little measurable improvement in growth performance compared to control pigs, while in the second study the effect of ZnO was more pronounced) it could not be concluded whether MCOA could fully replace ZnO. The results suggested that this may depend on the background pathogen challenge. The type of MCOA and the inclusion level appeared to play a role in the growth performance response and requires further attention.

5. Conclusions

A blend of MCOA in combination with BA resulted in improved growth performance in pigs from weaning up to 40 kg, compared to a control group. Type of MCOA (chain length and monoester) influenced the response in weaner diets. Further studies would be useful to explore and refine the use of MCOA in combination with BA, as a strategy to further reduce antibiotic use in the pig sector.

Conflict of interest

The author declare no conflict of interest.

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