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FINAL REPORT

Using dietary medium-chain triglycerides to improve post-weaning performance of pigs

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Abstract

Eighty-four weaned piglets were used to determine the effects of medium-chain triglycerides (MCT; predominately C_8 to C_{10}) and coconut oil in diet of weaner pigs on the incidence of post-weaning diarrhoea and production indices. To this effect, seven treatments were used: (i) Negative control diet (no antimicrobials), (ii) Positive control diet (antibiotic + ZnO), (iii) 0.625% MCT diet, (iv) 1.25% MCT diet, (v) 2.5% MCT diet, (vi) 5% MCT diet and (vii) a diet with coconut oil at 5%, according to a randomised complete block arrangement of treatments. There were 12 replicates per treatment. Pigs were housed individually in wire-mesh floored cages, in an environmentally controlled room and fed the experimental diets for 14 days, and then a follow-on (Phase 2) weaner diet for the following 14 days after which time the experiment ceased. Pigs had *ad libitum* access to feed and water. Daily weight gain, feed intake and feed conversion ratio were calculated on weekly basis. A blood sample was collected from 6 'focus' pigs on days 7 and 14 of the experiment and plasma analysed for triglycerides, glycerol, NEFA and glucose levels and neutrophil and lymphocyte count, and for growth hormone activity on samples collected on day 14 only. There was a complete lack of diarrhoea in this experiment, so the proposition that MCT/coconut oil would have antibacterial effects could not be tested. Results showed that there was no difference between experimental diets on pig performance up to 4 weeks post-weaning. Blood metabolites measured were similar between pigs from different treatments except GH which was significantly higher (P=0.019) in pigs fed 1.25% MCT compared to the positive control pigs. Neutrophil counts were significantly lower in the positive control pigs than pigs fed 0.125% or 2.5% MCT, and lymphocytes count significantly higher in the positive control compared to negative control pigs or those fed 0.125% or 2.5% MCT.

Introduction

Preliminary data from a previous CRC–funded activity (Investigating the interaction between ghrelin, medium-chain triglycerides and weaning transition in young pigs) suggests that the use of medium-chain triglycerides (MCT) in a liquid milk diet for young pigs: (i) increased circulating concentrations of the hormone ghrelin that is implicated in gastrointestinal tract development, and (ii) decreased the incidence of scouring (diarrhoea) of piglets consuming the liquid milk replacer. The weaning transition in pigs is commonly accompanied by diarrhoea (1984), and is associated with adverse changes in intestinal morphology and function (Hampson, 1986). Inadequate nutrient intake in the immediate post-weaning period contributes to intestinal inflammation and can compromise gut morphology and function (McCracken et al., 1999). From the nutritional perspective, avoiding the weaning transition growth stasis and controlling intestinal insults (eg, inflammation) that could predispose to post-weaning diarrhoea is a challenge to the pig industry.

MCT are used directly for the acyl-modification (activation) of ghrelin within the gut of rats (Nishi et al., 2005). Ghrelin is primarily produced by endocrine cells of the gastric mucosa for secretion into the circulation. Studies have identified multiple physiological functions for ghrelin, including growth hormone (GH) release, appetite stimulation, cellular proliferation, apoptosis inhibition, and regulation of lipid metabolism and tissue fat distribution in muscle (Nishi et al., 2005). Ghrelin is also reported to be involved in the inhibition of pro-inflammatory cytokine production

and gastroprotection against stress-induced gastric damage in rats (Konturek et al., 2004). Furthermore, exogenous ghrelin administration for 5 days to 18 day-old weaned pigs increased their weight gain (Salfen et al., 2004). If MCT are being utilized for the activation of stomach-derived ghrelin in young pigs, this could assist in overcoming the problems associated with the weaning transition period. Unfortunately our last study was of short duration (5 d) and we did not see a growth enhancement effect, but a longer growth period may allow for expression of growth benefits.

Antibiotics and/or mineral compounds such as zinc oxide (ZnO) are commonly used in post-weaning diets to control diarrhoea and improve growth performance, however their sustained use in the pig industry is questionable and hence alternatives to existing compounds require investigation. MCT have been shown to have antibacterial activity (Isaacs et al., 1995; Skrivanova et al., 2006) including effects in pigs (Dierick et al., 2002), and hence show promise as an alternative to traditional antimicrobial compounds. To date though there is a general lack of information pertaining to the optimum dose of MCT needed in a standard post-weaning diet to cause an antibacterial effect. Additionally, the most efficient and/or economical way to provide MCT in the diet also requires investigation. To this effect, an additional treatment in this experiment will examine the influence of coconut oil. Coconut oil is comprised of slightly longer-chain (but still saturated) fatty acids than MCT but when digested in the small intestine, can cause similar physiological effects. Moreover, coconut oil is most likely cheaper than supplying MCT in a diet.

Materials and Methods

This study was approved by the WA Department of Agriculture and Food Animal Ethics Committee (AEC No. 5-06-40) to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Animals, experimental design, housing, diets and feeding

The experiment used 84 newly-weaned Large White x Landrace piglets, initial age of approximately 21 days and 6.2 ± 1.09 kg (mean ± SD) live weight. The experiment was a randomised complete block arrangement with seven treatments: (i) Negative control diet (no antimicrobials), (ii) Positive control diet (antibiotic + ZnO), (iii) 0.625% MCT diet, (iv) 1.25% MCT diet, (v) 2.5% MCT diet, (vi) 5% MCT diet and (vii) a diet with coconut oil at 5%.

Upon arrival, the pigs were allocated at random to one of the seven treatments after stratification on their live weight and pen location. There were 12 replicates per treatment. Pigs were housed individually in wire-mesh floored cages, in an environmentally controlled room where temperature was maintained at 29 ± 1ºC, and was decreased by approximately 1½ºC per week thereafter. The pigs were fed *ad libitum* in a round feeder and water was freely accessible through a nipple drinker set in each crate.

Pigs were fed the experimental diets for 14 days and a follow-on (Phase 2) weaner diet was then offered until the experiment ceased at 28 days after weaning. Experimental diets were isonitrogenous and isoenergetic and formulated to meet or

exceed the nutrient and energy requirements of pigs of this age and weight. Its composition is shown in Table 1. The Negative and Positive control diets contained tallow as the dietary supplemental fat source because this is the fat source most commonly used in practice. The MCT source used was derived from coconut oil and consists of food grade caprylic (C8) 65-75% and capric (C10) 25-35% acids with less than 0.5% C6 and less than 1% C12 esterified to pure glycerine (Melrose laboratories P/L, Mitcham, Victoria, Australia). The coconut oil source used was "Fiji Gold" a commercial brand.

Table 1. Composition of experimental diets (g/kg as-fed)

	Stage I							Stage II
	Controls		MCT inclusion (%)				Coconut	
Ingredient	Positive	Negative	0.625	1.25	2.5	5	oil	
Wheat	631	638	638	636	636	616	641	680
Soybean meal	155	154	154	154	154	162	153	201
Fish meal	100	100	100	100	100	100	100	50
Whey	50	50	50	50	50	50	50	25
Tallow	41	39	33	28	16	-	-	18
MCT	-	-	6.6	11.9	25.1	50.3	-	-
Coconut oil	-	-	-	-	-	-	35.7	-
Dicalcium phosphate	7.16	7.14	7.14	7.14	7.14	7.16	7.13	13.6
Limestone	5.4	5.4	5.4	5.4	5.4	7.4	5.42	6.25
L-Lysine	2.63	2.64	2.64	2.64	2.64	2.62	2.66	3
Salt	1	1	1	1	1	1	1	1
DL-Methionine	0.75	0.74	0.74	0.74	0.74	0.81	0.74	0.77
L-Threonine	0.81	0.81	0.81	0.81	0.81	0.82	0.82	0.9
Mineral/Vitamin supplement[1]	1	1	1	1	1	1	1	1
Zinc oxide	2.5	-	-	-	-	-	-	-
Olaquindox	1	-	-	-	-	-	-	-
Calculated diet composition:								
DE (MJ/kg)	15.0	15.0	15.0	15.0	15.0	15.2	15.0	14.5
Available Lys (g/MJ DE)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.8
Protein (%)	22.2	22.3	22.3	22.3	22.2	22.4	22.3	20.9

[1]Supplied per kg of diet: 60.0 mg Fe ($FeSO_4$); 10.0 mg Cu ($CuSO_4$); 40.0 mg Mn (MnO); 100.0 mg Zn (ZnO); 0.30 mg Se (Na_2SeO_3); 0.50 mg I (KI); 0.20 Co ($CoSO_4$); vitamin A, 7,000 IU; vitamin D_3, 1,400 IU; vitamin E, 20.0 mg; vitamin K_3, 1.0 mg; thiamin, 1.0 mg; riboflavin, 3.0 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.015 mg; pantothenic acid, 10.0 mg; Folic acid, 0.2 mg; niacin, 12.0 mg and biotin 0.03 mg.

Sampling and measurements

Pigs were weighed upon entry and then on days 7, 14 and 28 of the experiment. Feed refusals and/or waste were weighed daily and the daily weight gain, feed intake and feed conversion ratio calculated on a weekly basis.

All pigs were swabbed to determine the shedding of β-haemolytic *E.coli* upon arrival, and then on days 3, 5, 7, 10 and 14. Swabs were cultured on sheep blood agar plates and plates were assessed for β-haemolytic colonies displaying morphology characteristic of *E. coli*, after overnight incubation. The presence of haemolytic *E. coli* was scored (0 = no growth, 1 = haemolytic *E. coli* in 1st section, 2 = haemolytic *E. coli* in 2nd section, 3 = haemolytic *E. coli* in 3rd section, 4 = haemolytic *E. coli* in 4th section, 5 = haemolytic *E. coli* in 5th section).

A blood sample (10 mL) was collected from 6 'focus' pigs per treatment on days 7 and 14 via jugular vein puncture into vacutainer tubes coated with lithium heparin and with no coating for glucose analysis. Blood samples were immediately centrifuged at 2,000 × g for 10 minutes at 5 ºC to recover plasma which was immediately stored at -20ºC until analysed. Pig plasmas were analysed for glycerol, triglycerides, glucose and free fatty acids (NEFA) levels by an enzymatic colorimetric method using Glycerol kinase (Roche Diagnostics, Indianapolis, IN, USA), Lipase and Glycerol kinase (Roche Diagnostics, Indianapolis, IN, USA), Hexokinase (Roche Diagnostics, Indianapolis, IN, USA) and acyl-CoA synthetase (WAKO NEFA-C Kit, Novachem Pty Ltd, Collingwood, Vic, Australia), respectively. Neutrophil and lymphocyte counts were performed using the Bayer ADVIA 120 Hematology Analyser (Bayer Diagnostics Division (Siemens), Tarrytown, NY, USA). Growth hormone (GH) activity was also measured in plasma samples by an enzyme method using a porcine/canine growth hormone enzyme-linked imunosorbent (ELISA) kit (DSL-10-73100, Diagnostic Systems Laboratories, Inc, Webster, Texas, USA).

Statistical analyses

Data were analysed using the ANOVA procedures of SPSS (SPSS for Mac OS X version 11.0.4 by Software Mackiev, SPSS Inc., 1989-2005). One-way ANOVA was performed to compare the response of pigs between all seven treatments. Six pre-planned orthogonal comparisons between the positive treatment and each of the other treatments were also made. The experimental unit was each pig for all measurements. Statistical significance was accepted at $P < 0.05$ and LSD comparisons were used (at the 5% significance level) to examine differences between the mean values of the different variables.

Results

Table 2 shows that performance between pigs fed different experimental diets was overall similar in this experiment. During week 2, ADG in pigs fed 5% MCT was significantly lower than in the positive control pigs and FCR was better in positive than in negative control pigs. The FCR data in Week 1 are clearly distorted due to variable growth rates and feed intakes, however data up to 4 weeks after weaning shows that there was no benefit in adding olaquinox and ZnO to weaner diets, at least in the current experiment. It should be noted however that there was **NO** diarrhoea whatsoever in this experiment.

There was no significant difference between treatments in the plasma levels of triglycerides, glycerol, NEFA or glucose (Table 3). Neutrophil counts in the positive control pigs were significantly lower than in the negative control pigs or in those fed 0.125 or 2.5% MCT ($P<0.05$). Lymphocyte counts were significantly higher in the

positive control pigs compared to pigs fed the negative control, 1.25 or 2.5% MCT diets ($P<0.05$). However the N/L ratio was similar between pigs fed different experimental diets (Table 3). Growth hormone levels were significantly higher in the 1.25% MCT-pigs than in the positive control pigs ($P=0.019$), but it was not reflected in higher ADG in those pigs.

Table 2. Performance of pigs fed different levels of medium-chain triglycerides and coconut oil.

Treatment	1	2	3	4	5	6	7	
	Controls		MCT inclusion (%)				Coconut	SEM
	Positive	Negative	0.625	1.25	2.5	5	oil	
n=	11	11	10	9	10	10	8	
Live weight (kg)								
Start (21 days of age)	6.34	6.18	6.31	5.82	6.15	6.28	5.88	0.131
7 days of the experiment	6.51	6.49	6.36	6.03	6.29	6.53	6.14	0.143
14 days of the experiment	8.36	8.16	7.97	7.79	7.92	7.82	7.79	0.176
28 days of the experiment	14.00	14.62	14.77	13.96	14.50	13.62	14.31	0.325
Week 1								
ADG (g/pig/day)	23	44	7	30	20	35	37	7.1
VFI (g/pig/day)	108	135	88	109	103	112	117	5.3
FCR (g/pig/day)	0.47	2.92	2.05	4.03	4.76	2.82	1.57	0.989
Week 2								
ADG (g/pig/day)	264	239	229	251	233	185	235	9.3
VFI (g/pig/day)	320	362	325	328	316	281	312	10.2
FCR (g/pig/day)	1.22	1.59	1.45	1.39	1.39	1.61	1.34	0.376
Total (weeks 1 and 2)								
ADG (g/pig/day)	144	141	118	140	126	110	136	6.6
VFI (g/pig/day)	214	249	207	219	209	196	215	7.1
FCR (g/pig/day)	1.73	1.87	1.83	1.79	1.73	1.91	1.61	0.530
Weeks 3 and 4								
ADG (g/pig/day)	403	461	486	441	470	414	466	13.8
Total (weeks 1 to 4)								
ADG (g/pig/day)	274	302	302	291	298	262	301	8.9

	P – value						
	Treatment	Positive vs Negative	Positive vs 0.625% MCT	Positive vs 1.25% MCT	Positive vs 2.5% MCT	Positive vs 5% MCT	Positive vs Coconut oil
Live weight (kg)							
Start (21 days of age)	0.927	0.737	0.955	0.308	0.698	0.897	0.383
7 days of the experiment	0.963	0.975	0.785	0.389	0.681	0.972	0.520
14 days of the experiment	0.973	0.762	0.554	0.407	0.507	0.422	0.419
28 days of the experiment	0.970	0.604	0.533	0.974	0.686	0.756	0.815
Week 1							
ADG	0.846	0.418	0.522	0.825	0.881	0.651	0.642
VFI	0.366	0.141	0.318	0.941	0.818	0.825	0.631
FCR	0.934	0.498	0.669	0.350	0.248	0.537	0.779
Week 2							
ADG	0.409	0.437	0.299	0.708	0.356	**0.022**	0.424
VFI	0.553	0.259	0.891	0.843	0.905	0.292	0.829
FCR	**0.053**	**0.006**	**0.091**	0.213	0.213	**0.004**	0.416
Total (weeks 1 and 2)							
ADG	0.773	0.913	0.289	0.883	0.465	0.174	0.748
VFI	0.564	0.172	0.788	0.856	0.862	0.499	0.976
FCR	0.841	0.473	0.624	0.784	0.984	0.360	0.579
Weeks 3 and 4							
ADG	0.634	0.240	0.106	0.471	0.190	0.827	0.248
Total (weeks 1 to 4)							
ADG	0.848	0.389	0.396	0.616	0.460	0.733	0.439

Table 3. Blood indices of pigs fed different levels of medium-chain triglycerides and coconut oil.

Treatment	1	2	3	4	5	6	7	
	Controls		MCT inclusion (%)				Coconut	SEM
	Positive	Negative	0.625	1.25	2.5	5	oil	
n=	6	6	6	4	6	6	6	
First blood collection (Day 7 of the experiment)								
Triglycerides (mmol/L)	0.38	0.39	0.38	0.38	0.54	0.53	0.42	0.272
Glycerol (µmol/L)	31.9	37.7	28.4	42.7	35.6	32.1	32.9	2.04
NEFA (mmol/L)	0.20	0.29	0.24	0.36	0.32	0.19	0.19	0.041
Glucose (mmol/L)	5.5	5.0	5.5	5.2	5.4	5.3	5.2	0.08
Neutrophils (%)	36.3	41.0	41.9	44.7	38.2	35.6	42.2	1.47
Lymphocytes (%)	56.0	52.4	50.0	47.1	54.3	55.7	49.2	1.42
N/L ratio	0.68	0.79	0.88	1.00	0.75	0.68	0.92	0.05
n=	6	6	6	6	6	5	6	
Second blood collection (Day 14 of the experiment)								
Triglycerides (mmol/L)	0.45	0.48	0.42	0.53	0.34	0.40	0.51	0.021
Glycerol (µmol/L)	52.8	50.4	37.4	40.7	31.9	21.8	38.5	3.39
NEFA (mmol/L)	0.21	0.37	0.23	0.30	0.18	0.12	0.20	0.025
Glucose (mmol/L)	5.3	5.6	5.6	5.3	5.9	5.8	5.6	0.09
Neutrophils (%)	35.0	48.2	43.1	48.6	44.7	44.5	45.4	1.36
Lymphocytes (%)	57.7	44.1	49.3	44.2	47.1	48.0	46.0	1.34
N/L ratio	0.62	1.17	0.89	1.13	0.96	0.97	0.99	0.05
GH (ng/ml)	11.4	6.6	10.1	24.4	11.3	9.8	7.1	1.55

	P-value						
	Treatment	Positive vs Negative	Positive vs 0.625% MCT	Positive vs 1.25% MCT	Positive vs 2.5% MCT	Positive vs 5% MCT	Positive vs Coconut oil
First blood collection (Day 7 of experiment)							
Triglycerides	0.425	0.960	0.960	0.976	0.131	0.139	0.689
Glycerol	0.679	0.457	0.653	0.193	0.630	0.976	0.900
NEFA	0.728	0.600	0.760	0.224	0.320	0.911	0.903
Glucose	0.649	0.097	0.910	0.305	0.747	0.535	0.399
Neutrophils (%)	0.608	0.441	0.359	0.143	0.733	0.902	0.279
Lymphocytes (%)	0.541	0.549	0.319	0.116	0.746	0.955	0.206
N/L ratio	0.573	0.621	0.359	0.129	0.735	0.980	0.235
Second blood collection (Day 14 of the experiment)							
Triglycerides	0.223	0.708	0.758	0.284	0.164	0.560	0.442
Glycerol	0.246	0.843	0.214	0.328	**0.096**	**0.021**	0.249
NEFA	0.139	**0.073**	0.764	0.313	0.817	0.346	0.983
Glucose	0.712	0.537	0.477	0.962	0.147	0.269	0.484
Neutrophils (%)	0.110	**0.017**	0.151	**0.020**	**0.040**	**0.066**	**0.093**
Lymphocytes (%)	**0.074**	**0.013**	0.130	**0.019**	**0.021**	**0.057**	**0.056**
N/L ratio	0.124	**0.077**	0.404	0.117	**0.073**	0.234	0.293
GH	**0.072**	0.339	0.800	**0.019**	0.998	0.757	0.463

Discussion

The potential antibacterial effects of MCT could not be evaluated in this study, as there were absolutely **NO** clinical signs of diarrhoea in any of the pigs at any time during the study and *E. coli* shedding was extremely low. A follow up study where pigs are given a mild challenge of an enterotoxigenic *E. coli* (to mimic conditions of PWD) under diet conditions is more likely to provide answers to this issue.

Results from this study showed no differences between pigs fed MCT oil at different levels of inclusions, coconut oil or tallow on pig performance. This was an unexpected result, since the MCT oil used, which contained approximately 70% caprilyc acid (C8) and 30% capric acid (C10) and the coconut oil, are more digestible to animals than tallow (included in the control treatments) (1988). Higher digestibility of MCT has been attributed to the content of shorter chain fatty acids and more unsaturated fatty acids than those present in tallow (Cera et al., 1988). Supporting this hypothesis, a linear improvement in ADG and feed efficiency in piglets fed MCT at 20-60 g/kg (2-6%) during the first 2 weeks post-weaning, compared to tallow or milk fat, was observed by Rodas and Maxwell (1992). However in a study by Cera et al. (1988) corn oil did not increase post-weaning performance in pigs weaned at 21 days of age, even though corn oil showed a higher fat and dry matter apparent digestibility than lard or tallow. Some research has also shown reduced ADG in weaned pigs fed MCT compared to soybean oil at 8% (Fakler et al., 1993) or coconut at 10% (Chi and Lepine, 1993), findings attributable to toxicity of the MCT.

The lack of any differences in performance in the present study might have been due to reduced feed intake, particularly in the first week post-weaning and adverse morphological changes in the pig intestine after weaning. Also, there is evidence of low luminal lipase levels initially post-weaning which limit fat digestion and therefore growth responses (Cera et al., 1990). After 7 days post-weaning lipase activity increases reaching a peak after 2-3 weeks post weaning (in pigs weaned at 21 days of age) and coincides with an increase in fat digestibility (Cera et al., 1990), which also plateau at 3 weeks post-weaning (Cera et al., 1988). Therefore after 2-3 weeks post-weaning pigs are more sensitive to dietary fat manipulation.

None of the metabolites measured in plasma indicated a difference between fat sources in improving the energy status of the pigs. Neutrophil and lymphocyte counts on the other hand, were lower and higher, respectively, in pigs fed the positive control than in pigs fed negative control diet or MCT oil at 0.125 and 2.5%, which might be a reflection of the antibiotics included in the positive control diet. However, the ratio N/L was not significantly different between experimental groups.

The fact that GH was higher in pigs fed 1.25% MCT compared to positive control pigs and that it was not reflected in ADG in those pigs can most likely be explained by the fact that GH is pulsatile in its nature of secretion, and repeated blood samples are needed to be collected to be able to have a representative sample. Since GH was measured in a single plasma sample, the results might have been affected by time of sampling and variation between animals.

In conclusion, results from this experiment did not show differences between MCT, coconut oil and tallow on pig performance and blood metabolites. The absence of diarrhoea during the experiment did not allow evaluating the antibacterial potential of MCT included in a commercial weaner diet. Therefore a follow up experiment where pigs are given a mild challenge of an enterotoxigenic *E. coli* under diet conditions might help providing answers to this issue.

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