546

Arch. Anim. Nutr., 2003, Vol. 57, pp. 49–63

Taylor & Francis
Taylor & Francis Group

THE COMBINED USE OF WHOLE *CUPHEA* SEEDS CONTAINING MEDIUM CHAIN FATTY ACIDS AND AN EXOGENOUS LIPASE IN PIGLET NUTRITION[1]

N.A. DIERICK*, J.A. DECUYPERE and I. DEGEYTER

Ghent University, Faculty of Agricultural and Applied Biological Sciences, Department of Animal Production, Melle, Belgium

(*Received 25 July 2002, accepted 8 October 2002*)

In search for an alternative for nutritional antimicrobials in piglet feeding, the effects of adding whole *Cuphea* seeds, as a natural source of medium chain fatty acids (MCFA), with known antimicrobial effects, and an exogenous lipase to a weaner diet were studied. The foregut flora, the gut morphology, some digestive parameters and the zootechnical performance of weaned piglets were investigated. Thirty newly weaned piglets, initial weight 7.0 ± 0.4 kg, were divided according to litter, sex and weight in two groups (control diet; *Cuphea* + lipase diet). The *Cuphea* seeds (*lanceolata* and *ignea*) (50 g kg^{-1}) were substituted for soybean oil (15 g kg^{-1}), Alphacell (25 g kg^{-1}) and soy protein isolate (10 g kg^{-1}) in the control diet. Also 500 mg kg^{-1} microbial lipase was added to the *Cuphea* diet. The piglets were weighted individually on days 0, 3, 7, 14 and 16. Feed intake was recorded per pen during days 0 to 3, 3 to 7, 7 to 14 and 14 to 16. On day 7 five piglets of each experimental group were euthanized for counting the gastric and small intestinal gut flora and for gut morphology at two sites of the small intestine (proximal, distal). The results indicate a trend towards improved performances parameters by feeding *Cuphea* + lipase. The enzymic released MCFA (1.7 g kg^{-1} fresh gastric contents) tended to decrease the number of *Coliforms* in the proximal small intestine, but increased the number in the stomach and distal small intestine. With *Cuphea*, the number of *Streptococci* was significantly lower in small intestine, but not in the stomach, while the number of *Lactobacilli* was significantly lower in the distal small intestine and tended to be lower in the stomach and proximal small intestine. No differences between the diets were noted for the total anaerobic microbial load in the stomach or in the gut. Feeding *Cuphea* + lipase resulted in a significantly greater villus height (distal small intestine) and a lesser crypt depth (proximal and distal small intestine) and greater villus/crypt ratio depth (proximal and distal small intestine). The intra-epithelial lymphocyte (IEL) counts per 100 enterocytes were significantly decreased in the proximal small intestine and tended to decrease in the distal small intestine by feeding the *Cuphea* + lipase diet. Both phenomena are indicative for a more healthy and better functional state of the mucosa. Present results are in line with foregoing research, showing that manipulation of the gut ecosystem by the enzymic *in situ* released MCFA in the stomach and foregut can result in improved performances of the piglets, which makes the concept a potential alternative for in-feed nutritional antibiotics.

Keywords: Cuphea; Seeds; Weaning; Piglets; Medium chain fatty acids; Antimicrobial properties; Intestinal microorganisms; Intestine; Morphology

*Corresponding author: Dr N.A. Dierick, Ghent University, Faculty of Agricultural and Applied Biological Sciences, Department of Animal Production, Proefhoevestraat, 10, B-9090, MELLE, Belgium. Tel: + 32 9 264 90 10; Fax: + 32 9 264 90 99; E-mail: Noel.Dierick@rug.ac.be

[1]Part of this research was presented as a poster at the 3rd Joint RRI-INRA 2002 'Beyond antimicrobials – the future of gut microbiology 'symposium organized by the Institut National de la Recherche Agronomique, Clermond-Ferrand Theix (France) and the Rowett Research Institute, Aberdeen (UK), 12–15 June 2002 (Aberdeen, UK).

ISSN 0003-942X print; ISSN 1477-2817 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0003942031000086626

1. INTRODUCTION

Due to concerns about the problem of selection of antibiotic-resistent micro-organisms and drug residues, the use of most antibiotics, as growth promotors, was banned in the EU. A number of alternative additives has been proposed: enzymes, probiotics, oligosaccharides (prebiotics), Zn, dietary acidifiers (for review see: Piva, 1998; Roth and Kirchgessner, 1998; Thomke and Elwinger, 1998; Partanen and Mroz, 1999; Verstegen and Schaafsma, 1999). The most promising alternatives in pig rearing for the moment seem to be short-chain organic acids (Partanen and Mroz, 1999). However, the zootechnical improvements with organic acids are not always consistent.

The antimicrobial properties of various free fatty acids, especially medium chain fatty acids (MCFA: C4 : 0 to C12 : 0) and their fatty acid glycerol esters have been known for many years (Kabara *et al.*, 1972, 1977; Kabara, 1984; Meeus, 1994; Decuypere and Meeus, 1995). Moreover medium chain triacylglycerols (MCTAG) have nutritional, physiological and metabolic properties in mammals unique from those of long chain triacylglycerols (LCTAG) (Timmermann, 1993; Velasquez *et al.*, 1996; Odle, 1998; Willis and Marangoni, 1999; Traul *et al.*, 2000).

Earlier research at our department revealed that the *in situ* enzymic release of MCFA from commercial MCTAG sources in the stomach and fore-gut of piglets, result in persistent and significant effects on the gut luminal flora combined with improved performances, making this strategy a valuable alternative for in feed nutritional antibiotics. A patent has been applied for by the authors and funding partners for the protection of this concept (Decuypere and Dierick, 2000).

Present use of MCTAG is severely restricted however by the cost of synthesizing them from C8 : 0 and C10 : 0 fractions of coconut and palm kernel oil. In search of natural, unrefined and cheaper MCTAG oils, it was found that *Cuphea* has potential as a very rich source of MCFA (Litchfield *et al.*, 1967). *Cuphea* is a genus of low-growing annual plants, originating from central regions of America belonging to the *Lythraceae* family, which is known as a rich source of medium chain fatty acids (Knapp, 1993). However, the plant is not commercially utilized in large-scale agriculture due to its low frost-tolerance, sequential maturation and the release of seeds from the seed pods, which precludes temperate cultivation and mechanized harvesting. Nevertheless, interest in *Cuphea* hybrids is very great as a third crop in the corn/soybean rotation in the USA. Recently a shatter-resistent transgenic *Cuphea* was created with the potential for practical and economical exploitation. First successes have also been made transferring genes that regulate the fatty acid synthesis in *Cuphea* to rapeseed plants (Dehesh *et al.*, 1996; Voelker *et al.*, 1997a,b; Zarhloul *et al.*, 2000).

In present work, the effects of adding whole *Cuphea* seeds and an exogenous lipase to a weaner diet on the foregut flora, the gut morphology and histology, some digestive parameters and on the zootechnical performance of weaned piglets are investigated.

The available *Cuphea* cultivars, *Cuphea lanceolata* and *Cuphea ignea (polycentra)* are species from central USA, containing 25–30% oil in the seed. Over 80% of the fatty acids in the oil is capric acid (C10 : 0).

2. MATERIALS AND METHODS

In order to test possible negative effects (e.g. feed refusals, presence of anti-nutritional factors (ANF)), of feeding whole *Cuphea* seeds to weaned piglets, a preliminary feeding

test was carried out. Five newly weaned piglets (mean live weigh 7.6 ± 0.9 kg) were fed a weaner diet supplemented with 50 g kg^{-1} *Cuphea* seeds for 1 week. Animal behaviour, feed intake and health status were followed.

For the actual experiment, 30 newly weaned piglets (3 weeks of age, Seghers Hybrid F1, Belgium), initial weight 7.0 ± 0.4 kg, were divided according to litter, sex and weight in two groups (control diet; *Cuphea* + lipase diet) of 15 piglets (five per pen). The experiment was carried out in commercial settings in temperature controlled facilities. For the formulation of the diets, two batches of *Cuphea* seeds were available and were thoroughly mixed (3 kg, *Cuphea lanceolata*, Oregon State University, USA; 350 g *Cuphea ignea*, Aveve N.V., Merksem, Belgium). The used *Cuphea* seeds, the ingredients and calculated composition of the two diets are presented in Tables I and II, respectively. The *Cuphea* seeds (50 g kg^{-1}) were substituted for soybean oil (15 g kg^{-1}), Alphacell (25 g kg^{-1}, ICN Biomedicals, USA) and soy protein isolate (10 g kg^{-1}, Ralston Purina, Brussels, Belgium) in order to mimic the composition of the control diet, based on the analysis of the seeds. The weaner feeds were formulated to contain 11 g kg^{-1} apparent ileal digestible lysine, 6.9 g kg^{-1} apparent ileal digestible methionine + cystine, 6.3 g kg^{-1} apparent ileal digestible threonine, 2.1 g kg^{-1} apparent ileal digestible tryptophane and a net energy content (NE) of 9.8 mJ kg^{-1} according to Dutch Feeding Standards (CVB, 1998). For the determination of digestibilities, Celite 545 (Fluka, Switzerland) was added to the diets as inert marker. Before inclusion in the diet, the seed mix was mixed with ground barley (50/50, w/w) and carefully milled in a Brabender mill (sieve: 1 mm; Duisburg, Germany) to avoid any evaporation or loss of the oil, which has a very low boiling point. Lipase L5 (500 mg/kg) (microbial source, 6563 U/g, Kemin Europa N.V., Belgium) was added to the *Cuphea* diet. Lipolytic activity is expressed as the amount of enzyme (1 U) producing 1 μmol free acid per minute and per gram from tricaprylin at pH 5.5 and 40°C. There was no third treatment either without lipase but supplemented with *Cuphea* or a control diet with only lipase supplemented as preliminary research revealed that MCFA from native fat in *Cuphea* seeds could not be liberated by pig endogenous gastric lipase alone, without extra added lipases, and that long fatty acids, possibly liberated in the stomach by pig gastric lipase from normal fat sources (e.g. soy oil) do not have any antimicrobial effect.

TABLE I Proximate analysis of the seeds of the *Cuphea* cultivars

Cultivar *Origin*	Cuphea lanceolata *Oregon State University, USA*	Cuphea ignea (platycentra) *Aveve N.V., Merksem, Belgium*
DM [g·kg^{-1}]	937.0	954.0
Crude protein [g·kg^{-1}]	187.0	146.0
TDF[1] [g·kg^{-1}]	429.0	471.0
Crude fat [g·kg^{-1}]	267.0	362.0
Fatty acids [as % of total fatty acids]		
C6:0	0.0	0.0
C8:0	0.9	3.0
C10:0	75.7	86.9

[1]Total dietary fibre.

TABLE II Ingredients and chemical composition of the diets (as fed basis)

Ingredients	*Control diet*	*Cuphea + lipase diet*
Basal diet [g·kg^{-1}][1]		
Barley	350.0	350.0
Wheat	150.0	150.0
Corn, pressure cooked	150.0	150.0
Wheat feedflour	62.7	62.7
Soybean meal	80.0	80.0
Soya Danex[2]	75.0	75.0
Whey permeate	55.0	55.0
Herring meal	50.0	50.0
Fat	3.0	3.0
Methionine	2.0	2.0
Lysine-HCl	4.5	4.5
Threonine	2.0	2.0
Tryptophane	0.5	0.5
Salt	4.0	4.0
Vitamin E (50%)	0.1	0.1
Choline (60%)	0.4	0.4
Copper sulphate (25%)	0.6	0.6
Vitamin-mineral premix	2.2	2.2
Monocalcium phosphate	8.0	8.0
Supplements [g kg^{-1} basal diet]		
Cuphea seeds[3]	0.0	50.0
Soybean oil	15.0	0.0
Alphacell[4]	25.0	0.0
Soybean isolate[5]	10.0	0.0
Lipase L5[6]	0.0	0.5
Celite[7]	10.0	10.0
Dicalcium phosphate	5.0	5.0
Limestone	5.0	5.0
Chemical composition of the complete diet [g kg^{-1}]		
DM	889.8	887.4
Crude protein	150.1	151.1
Crude ash	65.8	62.3
TDF[8]	163.6	151.9
Crude fat	66.0	64.4
Total fatty acids	57.3	52.7
C6:0	0.0	0.0
C8:0	0.1	0.2
C10:0	0.2	7.9
C12:0	0.8	1.1
[4N HCl] insoluble ash	11.5	12.0

[1]DSP diet; Vitamex N.V. (Belgium)
[2]Expanded full fat soybeans; Danis, N.V. (Belgium)
[3]Mixture of 3 kg of *Cuphea lanceolata* and 350 g of *Cuphea ignea* whole seeds
[4]ICN (USA)
[5]Purina Protein (Belgium)
[6]Kemin Europa N.V. (Belgium)
[7]Celite 545, Fluka (Switzerland)
[8]Total dietary fibre.

The feed was offered dry, *ad libitum*, while water was continuously available via nipples. The experiment lasted only 16 days, as the availability of *Cuphea* seeds was strictly limited. The piglets were weighted individually, at weaning and on days 3, 7, 14 and 16. Feed intake was recorded per pen during the following periods: days 0–3, 3–7,

7–14 and 14–16. The visual 'faeces + health' conditions of the piglets was checked daily and coded per pen on a scale ranging from 0 (extremely bad condition, long skin hair, diarrhoea and mortality) to 10 (normal faeces, normal skin hair, excellent condition).

On day 7, five piglets taken at random from each experimental group, without fasting, were euthanized. The experimental protocols used were approved by the Ghent University Animal Ethics Committee.

The gastro-intestinal tract (GIT) was immediately removed and dissected; samples of the contents were taken from the stomach, the upper small intestine (first 3 m) and the lower small intestine (last 3 m), for bacteriological and chemical analyses. Gut tissue samples for histomorphometry were taken from two sites of the small intestine: 3 m distal from the pylorus and 3 m proximal of the caecum. The samples for light microscopy were fixed in a phosphate-buffered 10% formalin solution, dehydrated in 70% alcohol, embedded in paraffin, sectioned (5 μ) and stained with haematoxylin and eosin (HE), using standard procedures (Kik, 1991). No more than 5 min elapsed between the dissection of the intestine and the fixation of the samples in the formalin solution. Measurements of villus height and crypt depth were conducted with a light microscope (Nikon, Eclipse E400, Analis, N.V., Belgium) connected with a laboratory imaging system (Sony SSC-DC58AP Camera and Lucia 4.51 Image Archiving and Measurement System, Analis N.V., Belgium). From each sample at least 10 well-oriented vertically cut villi with their adjacent crypts were selected at random and measured from the tip of the villus to the base of an adjacent crypt and from crypt mouth to base, respectively. Quantitation of IELs and enterocytes was done on each sample on at least 10 well-oriented vertically cut and HE stained villi, per site, and data are expressed as IELs counts per 100 enterocytes. The dietary treatments were unknown to the person performing the cell morphometry and histology.

Feeds and digesta (stomach and small intestinal contents, faeces) were analysed for dry matter (DM), crude protein (Kjeldahl method), crude fat (ether extraction after acid hydrolysis, Soxhlet method) and ash (550°C, 2 h) according to EU standard methods (Anonymous, 1971, 1972). The other analysis (total fatty acids, TFA, and free fatty acids, FFA) were analysed as described before (Dierick *et al.*, 2002a), while total dietary fibre (TDF) and (4 N HCl) unsoluble ash, including Celite, were carried out as described by Dierick *et al.* (2002b). The bacteriological enumerations were carried out on freshly serial 10-fold diluted samples. The selective media and culture conditions used were Eosin Methylene Blue agar (24 h, 37°C, aerobically, greenish to brown colonies), Rogosa agar (24 h, 37°C, anaerobically, white colonies), Slanetz-Bartley (48 h, 37°C, aerobically, pink to brown colonies) and Reinforced Clostridial agar + 0.001% hemin (48 h, 37°C, anaerobically, total count of colonies) (all from Oxoid, Basingstoke, UK), for *Coliform, Lactobacilli, Streptococci* and total counts respectively (Dierick *et al.*, 2002a).

Before preparing the final feeds, the endogenous *Cuphea* lipase activity as well as the activity of the added lipase L5 (500 mg kg^{-1}) to *Cuphea* seeds, milled in corn starch (Sigma S-4126, St Louis, USA) (50/50, w/w), were followed during 28 days of storage, at room temperature. Therefore, the release of individual FFA, as % of total fatty acids present, at days 0, 14 and 28, was followed.

3. CALCULATIONS AND STATISTICAL ANALYSIS

The mean values per treatments (weight, growth, histomorphology, digestibility) were compared using analysis of variance (one-way ANOVA) followed by a least significance difference test (LSD). The computation was done using the SPSS 7.5 program for Windows (SPSS Inc., Chicago, ILL, USA). Means of bacterial counts were calculated as decimal values and presented as ($\log_{10}$ CFU (colony forming units) per g fresh contents), while SD values were calculated on ($\log_{10}$) values. A non-parametric test Mann–Whitney–Wilcoxon was used to compare the flora components.

4. RESULTS AND DISCUSSION

The concept of the use of a refined MCTAG oil combined with an exogenous lipase for the *in situ* release of antimicrobial FFA as a potential alternative for in-feed antibiotics was thorougly validated in previous experiments *in vitro* (Dierick *et al.*, 2002a) and *in vivo* (Dierick *et al.*, 2000b). The aim of this study was to use whole *Cuphea* seeds, as a non refined and cheaper MCTAG source.

In order to test possible negative effects of feeding whole *Cuphea* seeds to weaned piglets, as only very limited nutritional information about the occurence of ANF in the seeds was available, a preliminary feeding test was carried out. *Cuphea* has a C_3 physiology and seems to contain no saponins or cyanogens while alkaloids are mostly absent (Watson and Dallwitz, 1994). To our knowledge, feed experiments with pigs have never been carried out with whole *Cuphea* seed. Studying only the oil fraction, Hendrich *et al.* (1993) reported no any specific toxic effect in mice during long-term and multi-generational feeding of crude *Cuphea* oil (8.5% in the diet).

During the preliminary experiment, the piglets had a normal feed intake (318 g/d), growth (259 g/d) and feed/gain ratio (1.23 kg/kg). The rectal temperature ranged between 39.2°C and 39.7°C and the piglets had a normal health condition. From this, it can be concluded that most probably no deleterious compounds were present in the seeds used, as no negative clinical symptoms were noted with the piglets.

Earlier research (Dierick and Decuypere, 2002) revealed that in raw materials, after grinding, endogenous lipase activity may increase considerably and cause levels of FFA of more than 50% of the total lipid fraction, in 2 weeks of storage. Supplementation of feeds with exogenous lipases should thefore always be done with care by appropriate choice of exogenous lipases in order to prevent any excessive but inevitable endogenous lipolysis in the prepared feeds. Therefore, before preparing the final feeds, the endogenous lipase activity in the milled *Cuphea* seeds was followed and the results presented Table III. From Table III it can be seen that in *Cuphea lanceolata*, FFA, as% of total fatty acids present, amounted to about 16% without any effect of storage time or addition of exogenous lipase. In *Cuphea ignea*, lipolysis amounted to about 8%, again, without any effect of storage time or addition of exogenous lipase. In both milled cultivars, endogenous lipase activity remain very low in comparison with lipase activity in grinded cereals stored at normal temperature (Dierick and Decuypere, 2002); also the addition of lipase L5 did not influence the release of FFA during storage. Also Hellyer *et al.* (1999) found a very low degree of endogenous lypolysis in *Cuphea lanceolata* seeds (0.82%, 30°C, 65 h of incubation).

TABLE III Release of free fatty acids from milled *Cuphea* seeds during storage at room temperature as influenced by endogenous and added lipases (Mean values)

	Mixture without addition of exogenous lipase L5					*Mixture with addition of lipase L5*[1]				
Storage time [d]	0 FFA[2]	14 FFA	28 FFA	28 TFA[3]	28 FFA/TFA[4]	0 FFA	14 FFA	28 FFA	28 TFA	28 FFA/TFA
Cuphea lanceolata										
MCFA[5]										
C6:0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0
C8:0	0.5	0.4	0.8	3.0	27.6	0.4	0.5	0.9	3.0	29.6
C10:0	27.1	23.6	25.5	188.5	13.5	26.9	23.4	25.3	189.7	13.3
C12:0	1.0	0.5	0.6	5.4	10.6	0.9	0.5	0.6	5.5	10.4
LCFA[6]										
C14:0	1.6	1.0	0.9	4.6	20.5	1.5	0.9	1.1	5.2	20.6
C16:0	4.7	1.8	1.9	9.7	19.4	2.8	3.0	2.9	7.9	36.7
C16:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C18:0	2.4	0.0	0.0	4.1	0.0	0.0	1.9	1.1	1.7	61.1
C18:1	2.4	2.0	1.1	12.4	9.0	3.1	2.9	2.7	9.0	30.0
C18:2	5.4	3.7	1.8	13.6	13.5	5.0	3.6	2.6	11.8	21.7
C18:3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum	45.1	33.0	32.6	243.3	13.4	40.7	36.6	37.1	233.9	15.9
Cuphea ignea										
MCFA										
C6:0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	2.1	0.0
C8:0	0.9	1.1	1.6	12.0	13.1	1.1	1.1	1.7	12.3	14.1
C10:0	16.5	21.4	20.0	267.9	7.5	19.6	20.7	19.9	266.7	7.5
C12:0	0.3	0.1	0.1	2.4	6.2	0.7	0.1	0.2	2.3	8.4
LCFA										
C14:0	0.0	0.0	0.1	0.9	7.9	0.0	0.0	0.2	0.8	19.4
C16:0	1.3	0.9	1.1	5.8	18.6	1.3	0.8	1.1	6.0	18.1
C16:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C18:0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
C18:1	0.0	1.0	0.0	4.9	0.0	0.8	1.5	0.0	4.8	0.0
C18:2	2.5	2.5	0.0	10.6	0.0	1.4	0.0	0.0	10.6	0.0
C18:3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum	21.5	27.0	22.9	306.6	7.5	24.8	25.2	23.1	305.6	7.5

[1] Lipase L5: Kemin Europa N.V. (B), 500 ppm in the mixture or 1000 ppm on *Cuphea* seeds
[2] FFA: free fatty acids, expressed as [g kg^{-1}] *Cuphea* seeds
[3] TFA: total fatty acids, expressed as [g kg^{-1}] *Cuphea* seeds
[4] FFA/TFA: free fatty acids as [% of total fatty acids]
[5] MCFA: Medium chain fatty acids
[6] LCFA: Long chain fatty acids.

This can explain the fact that in the preliminary feed trial no lower feed intake was observed, when feeding 50 g kg^{-1} whole *Cuphea* seeds in the diet. Otherwise the release of high levels of free MCFA with a known strong 'rancid (C4 : 0–C6 : 0), goat-like (C8 : 0), goat-cheesy (C8 : 0) and unclean and soapy (C10 : 0–C12 : 0)' odour (Hamilton, 1983; Molimard *et al.*, 1997) and averse taste most probably should have resulted in a lower feed intake (Cera *et al.*, 1989).

In Table IV the performance data are presented. During the first week after weaning, the piglets on the *Cuphea* diet ate less, had the same growth rate combined with a better feed conversion ratio (FCR) than those on the control diet. During the second week, the piglets on the *Cuphea* diet had a 10% higher feed intake, a 23% better growth rate and a 20% better FCR than those on the control diet. Overall, the piglets on the *Cuphea* diet showed a 8% higher feed intake, a 25% faster growth and a 14% better FCR. However, the differences never reached significancy due to the low number of animals, especially after week 1, when five piglets, taken at random, of each group, were euthanized for the study of some digestive parameters. Therefore, the results need to be interpreted with caution. However, present results are in line with previous experiments where piglets performed better (daily weight gain, + 10%; feed conversion, − 3%) on diets containing a commercial MCTAG oil (25 g kg^{-1}) + lipase (1000 mg/kg on feed) compared with control or acid supplemented diets (Dierick *et al.*, 2002b).

In Table V the fatty acid composition is given for the gastric contents of the slaughtered piglets. In the stomach of the piglets on the control diet, FFA concentration amounted to 3.6 g kg^{-1} contents, mainly of C18 : 2 and without MCFA. In the stomach of the piglets on the *Cuphea* diet however, FFA concentration amounted to 4.8 kg^{-1} contents, the main MCFA being C10 : 0 (1.4 g kg^{-1}). The concentration of FFA, as% of total fatty acids, amounted to 22.8% and 33.4% in the stomach of the piglets on the control and *Cuphea* + lipase diet, respectively. About 50% of the total amount of C10 : 0 present in *Cuphea* was released in the stomach. In previous experiments with diets containing purified commercial MCTAG oils + exogenous lipase (1 g kg^{-1} in diet), gastric lipolysis of MCTAG oil increased from 20% to about 65% under the influence of the exogenous lipase added (Dierick *et al.*, 2002b). As a result of the endogenous gastric lipase activity, lipolysis in the stomach of piglets normally may reach approximately 25%, as found in earlier research. The reason for the rather small

TABLE IV Performance of the piglets as influenced by the diets (Mean values)

		Days			
	Diet	0–7	7–14	14–16	0–16
Number of piglets per treatment		15 (10)[1]	10	10	10
Feed intake [g/d; per pen]	Control	212	360	489	311
	Cuphea	194	396	607	336
Growth [g/d; individual]	Control	135 (128)[1]	242	311	205
	Cuphea	135 (128)[1]	298	389	256
P value		1.00 (1.00)[1]	0.19	0.25	0.19
FCR (per pen)	Control	1.57	1.49	1.57	1.51
	Cuphea	1.43	1.18	1.54	1.30

[1]Ten piglets per treatment performed the whole trial, indicated as ( ); five piglets at random taken from each group of 15 were slaughtered at day 7.

TABLE V Fatty acid composition of the diets and content of total (TFA) and free fatty acids (FFA) in gastric and upper small intestinal contents of slaughtered piglets (Means ± SD, $n = 5$)

	Diet		*Gastric contents*		*Contents of upper small intestine*[1]	
	Control	Cuphea + *lipase*	*Control*	Cuphea + *lipase*	*Control*	Cuphea + *lipase*
FFA [g·kg^{-1} fresh basis]						
C6:0			0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
C8:0			0.0 ± 0.0	0.1 ± 0.0	0.0	0.0
C10:0			0.0 ± 0.0	1.4 ± 0.7	0.0	0.1
C12:0			0.1 ± 0.1	0.2 ± 0.1	0.0	0.0
C14:0			0.1 ± 0.0	0.1 ± 0.1	0.0	0.0
C16:0			0.9 ± 0.3	1.0 ± 0.5	0.3	0.3
C16:1			0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
C18:0			0.2 ± 0.1	0.2 ± 0.1	0.2	0.0
C18:1			0.5 ± 0.3	0.4 ± 0.3	0.2	0.0
C18:2			1.5 ± 1.3	1.2 ± 1.2	0.2	0.0
C18:3			0.2 ± 0.2	0.2 ± 0.2	0.0	0.0
Sum			3.6 ± 2.3	4.8 ± 3.0	1.0	0.5
TFA [g·kg^{-1} fresh basis]						
C6:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
C8:0	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0	0.0
C10:0	0.2 ± 0.2	7.9 ± 0.1	0.0 ± 0.1	2.6 ± 1.1	0.0	0.3
C12:0	0.8 ± 0.0	1.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.2	0.1	0.0
C14:0	0.4 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.0	0.0
C16:0	6.6 ± 0.1	5.7 ± 0.3	1.6 ± 0.6	1.4 ± 0.6	0.4	0.3
C16:1	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0	0.0
C18:0	11.0 ± 0.0	8.2 ± 0.4	0.5 ± 0.2	0.4 ± 0.1	0.2	0.2
C18:1	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.8	1.7 ± 0.7	0.7	0.5
C18:2	33.3 ± 0.3	25.4 ± 0.6	8.2 ± 3.0	5.7 ± 2.6	2.0	0.9
C18:3	4.6 ± 0.3	3.3 ± 0.1	1.1 ± 0.4	0.8 ± 0.3	0.2	0.0
Sum	57.3 ± 0.4	52.7 ± 1.5	14.4 ± 5.2	13.2 ± 5.7	3.6	2.2
FFA [as % of TFA]						
C6:0			0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
C8:0			0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
C10:0			0.0 ± 0.0	52.0 ± 5.3	0.0	52.2
C12:0			41.1 ± 4.4	50.8 ± 6.9	48.5	79.6
C14:0			60.5 ± 5.0	73.2 ± 4.2	81.3	93.6
C16:0			54.2 ± 3.0	70.9 ± 7.6	82.0	82.9
C16:1			13.8 ± 15.1	0.0 ± 0.0	0.0	0.0
C18:0			47.0 ± 9.8	48.4 ± 27.2	100.0	0.0
C18:1			16.3 ± 8.6	20.5 ± 11.3	25.7	0.0
C18:2			15.9 ± 9.5	17.1 ± 13.3	7.8	0.0
C18:3			18.1 ± 16.2	19.0 ± 14.1	0.0	0.0
Sum			22.8 ± 7.9	33.4 ± 9.5	27.2	20.9

[1]Pooled samples per treatment; contents of first 3 meters.

increase in degree of lipolysis from 23% to 33% may be due to the fact that the exogenous added lipase activity (0.5 g kg^{-1} in diet) may not have been sufficient for acting on the fat, incorporated in the seed matrix.

The influence of feeding *Cuphea* seeds and lipase on the luminal flora in the foregut of the piglets is given in Table VI. The enzymic *in situ* released antimicrobial MCFA (1.7 g kg^{-1} fresh gastric contents) tended to decrease the number of coliforms in the proximal small intestine, but not in the stomach or distal small

TABLE VI Influence of diet on luminal microbial ecology in stomach and small intestine [CFU $\log_{10}$ /g fresh material] (Means ± SD, $n = 5$)

	Diet	*Stomach*	*SI 1*[1]	*SI 2*[2]
Coliforms	Control	$3.6^{b} \pm 0.1$	$5.4^{a} \pm 0.9$	$5.9^{a} \pm 0.8$
	Cuphea	$4.4^{a} \pm 0.3$	$4.6^{a} \pm 0.3$	$6.6^{a} \pm 1.2$
Streptococci	Control	$7.6^{a} \pm 0.1$	$7.2^{a} \pm 0.1$	$7.9^{b} \pm 0.4$
	Cuphea	$7.5^{a} \pm 0.4$	$6.9^{a} \pm 0.2$	$6.8^{a} \pm 0.9$
Lactobacilli	Control	$7.6^{a} \pm 0.1$	$7.4^{a} \pm 0.8$	$7.7^{b} \pm 0.5$
	Cuphea	$7.3^{a} \pm 0.4$	$6.8^{a} \pm 0.7$	$6.4^{a} \pm 0.7$
Total count	Control	$7.1^{a} \pm 0.7$	$6.6^{a} \pm 0.1$	$7.1^{a} \pm 0.0$
	Cuphea	$7.1^{a} \pm 0.1$	$6.4^{a} \pm 0.4$	$7.2^{a} \pm 0.6$

[1] SI 1: Upper small intestine: 0–3 m distal from the pylorus
[2] SI 2: Distal small intestine: 0–3 m proximal of the caecum
Means with different superscript letters indicate significant differences ($P < 0.05$).

intestine. With *Cuphea,* the number of *Streptococci* was significantly lower in small intestine, but not in the stomach, while the number of *Lactobacilli* was significantly lower in the distal small intestine and tended to be lower in the stomach and proximal small intestine. No differences between the diets were noted for the total anaerobic microbial load in the the stomach or the gut. The reason for the less pronounced effects of the *Cuphea seeds* + lipase on the gut flora in comparison with earlier research with commercial MCTAG fat sources and lipases (where a 100-fold decrease in microbial load was noted) (Dierick *et al.*, 2002a,b), may be related to the smaller release of MCFA in the present experiment, probably due to the smaller amount of MCTAG fat used (50 g kg^{-1} *Cuphea* seeds representing 15 g kg^{-1} *Cuphea* oil in the diet vs. 25–50 g kg^{-1} commercial MCTAG oils in the diet), the smaller amount of lipase used (0.5 g kg^{-1} vs. 1 g kg^{-1} in the diet) and to the fact that the *Cuphea* fat was still incorporated in the whole seed and not extracted before use. Also non optimal enzymic conditions in the stomach (pH 3.8), differing from the pH-optimum of 5.5 of the used lipase, should be mentioned. Earlier research (Dierick *et al.*, 2002a,b) revealed that a minimal concentration of 3.5 g kg^{-1} MCFA or 0.025 M (with a C8:0/C10:0 ratio of 0.65/0.35) in the medium (stomach, proximal gut) was necessary for obtaining a significant (> 10-fold) suppression of the luminal flora. In the present research the concentration of free MCFA in gastric contents was only ± 1.7 g kg^{-1} with a C8:0/C10:0 ratio of 0.05/0.95. Because the antimicrobial activity is dependent on the pKa of the acid, on their molecular weight (MW) and on their lipophilic/hydrophilic character, MCFA with lower MW and lesser lipophylic character (C8:0) will be more effective in penetrating into the microbial cells and more bactericidal than C10:0, as was found in earlier studies (Dierick *et al.*, 2002a,b). So, the used *Cuphea* cultivars had a less favourable FFA profile than the commercial MCTAG sources used earlier.

The mode of action of organic acids including medium chain fatty acids on bacteria is well described and is based on the ratio dissociated(A-)/non-dissociated (HA) acid (Dierick *et al.*, 2002a). Briefly, the non-dissociated acid is responsible for the bactericidal effects, because it can cross the cell wall of the bacteria, while the dissociated form cannot. In the cell of the bacteria, where the internal pH is around 6.5–7.0, the acid will dissociate and the internal pH will drop. Enzymatic processes will stop and the proton motive force will collapse. These effects result in cellular death.

In Table VII, the pH and dry matter content in the stomach and foregut of the piglets are presented. Because the piglets were slaughtered with no preliminary fasting period, a gastric pH of 3.8 is rather normal (Cranwell *et al.*, 1976; Decuypere *et al.*, 1978). No significant differences were noted in pH in the stomach or along the foregut between the treatments. DM content in the lower small intestine was significantly lower with the *Cuphea* diet (66 g kg^{-1}) compared to that with the control diet (32 g kg^{-1}). *Cuphea* seeds are characterized by a hard seed coat with invaginated spiral mucilaginous hairs in the outermost cell layers. One case of emaciation and death in quail has been reported after feeding whole *C. carthegenensis* seeds. Moisture in the crop caused release of the mucilaginous seed hairs that subsequently formed an impaction that blocked any further passage of food and ultimately led to death of the birds (Hurst, 1978). Indeed the inverted spiral hairs containing primarily arabinose, become everted and highly mucilaginous upon soaking in water for 10–20 min. This could probably explain the higher water content in the proximal gut contents of the piglets, fed *Cuphea* seeds, in our experiments.

In Table VIII, the influence of the diet on the gastric, ileal and faecal apparent digestibility of some nutrients, calculated with 4 N HCl unsoluble ash as an inert marker, are given. No significant differences were noted here for any of the nutrients. The rather high apparent ileal digestibility coefficient (DC) for TDF (75%), compared to the faecal DC (45%) is probably caused by the high amount of endogenous material

TABLE VII pH and dry matter content [g kg^{-1} fresh basis] of gastric and small intestinal contents (Means ± SD, $n = 5$)

	Diet	*Stomach*	*SI 1*[1]	*SI 2*[2]
pH	Control	$3.8^a \pm 0.5$	$5.6^a \pm 0.4$	$6.8^a \pm 0.4$
	Cuphea	$3.8^a \pm 0.4$	$5.4^a \pm 0.5$	$7.1^a \pm 0.1$
DM	Control	$234^a \pm 16$	44[3]	$66^a \pm 27$
	Cuphea	$223^a \pm 33$	33[3]	$32^b \pm 7$

[1] SI1: upper small intestine: 0 - 3 m distal from the pylorus
[2] SI 2: 0 - 3 m proximal of the caecum
[3] Pooled samples
Means with different superscript letters indicate significant differences ($P < 0.05$).

TABLE VIII Influence of diet on gastric, ileal and faecal apparent digestibility coefficients (DC) of some nutrients (Means ± SD, $n = 5$)[1]

		Gastric DC	*Ileal DC*	*Faecal DC*
DM	Control	14.0 ± 7.8	66.5 ± 14.8	76.9 ± 3.2
	Cuphea	20.1 ± 13.3	66.7 ± 14.5	77.3 ± 1.8
CP	Control	N.D.[2]	52.9 ± 16.6	69.9 ± 9.1
	Cuphea	N.D.	56.5 ± 14.4	68.9 ± 4.9
Crude fat	Control	N.D.	50.3 ± 22.2	46.5 ± 18.3
	Cuphea	N.D.	43.3 ± 22.1	47.9 ± 7.9
TDF[3]	Control	N.D.	76.7 ± 6.9	45.4 ± 4.1
	Cuphea	N.D.	74.8 ± 28.3	43.4 ± 5.1

[1] No significant differences
[2] N.D.: not determined
[3] Total dietary fibre.

and the very low ileal DM content ($\pm$ 50 g kg^{-1}). The gastric DC value for the sum of the fatty acids amounted to 24%, without difference between the diets, while the DC value for C10:0 and C12:0, being the most important MCFA in the *Cuphea* diet, amounted to only 2% and 6% respectively (data not shown). In earlier experiments, gastric DC values for total and MCFA ranged between 20–40% and 5–20%, respectively (Dierick *et al.*, 2002b). Evidence of direct absorption of MCFA across the stomach wall is also given by Herting *et al.* (1956), Clark *et al.* (1969) and Aw and Grigor (1980). From these data it is clear that almost all C10:0 from *Cuphea* will be delivered to the duodenum for further antimicrobial action, after lipolytic release from the fat under the influence of exogenous and pancreatic lipases.

In Table IX the effect of diet on the mean villus height and crypt depth at two sites of the small intestine is given. Feeding *Cuphea* + lipase resulted in a significant lesser crypt depth and greater villus/crypt ratio in the proximal small intestine and in a significant increase in villus height and decrease in crypt depth and increase in villus/crypt ratio in the distal small intestine. In Table X, the effect of diet on the intra-epithelial lymphocyte counts is presented. With the *Cuphea* + lipase diet, the number of IEL per 100 enterocytes in the small intestine is significantly lower at proximal and tended to be lower at the distal site. Intra-epithelial lymphocytes are T-lymphocytes, with the majority of the CD8 phenotype, of which the functions are not entirely elucidated. However, IEL play an important role in the defense against invasive pathogens, the induction of apoptosis and the conservation of the mucosal integrity. High villus/crypt ratios and low IEL counts are generally accepted for a more healthy and more functional capacity of the mucosa because shortening of villi, lengthening of crypts (indicative for an increased mitotic activity) and high IEL counts are characteristic for pathologic changes and damages (Kik, 1991). Hampson and Kidder (1986) and Pluske

TABLE IX Effect of diet on the villus height and crypt depth (Means $\pm$ SD, n = 5; 10 measurements per pig and per site) at the proximal and distal small intestine[1]

Diet	*Villus height SI 1*	*Crypt depth SI 1*	*V/C SI 1*[3]	*Villus height SI 2*	*Crypt depth SI 2*	*V/C SI 2*
Control diet	381.9 $\pm$ 56.2	244.2 $\pm$ 39.0	1.61 $\pm$ 0.34	447.7 $\pm$ 56.0	246.6 $\pm$ 38.2	1.85 $\pm$ 0.37
Cuphea + lipase diet	365.7 $\pm$ 64.6	201.0 $\pm$ 40.1	1.88 $\pm$ 0.46	507.1 $\pm$ 85.5	235.2 $\pm$ 40.7	2.23 $\pm$ 0.54
Significance or P value[2]	0.14	***	***	***	**	***

[1] Measuring sites: SI1: 3 m distal from the pylorus and SI 2: 3 m proximal of the caecum
[2] Significant from control: * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)
[3] Villus height/crypt depth ratio.

TABLE X Effect of diet on the number of intra-epithelial villous lymphocytes (IELs) in the proximal and distal small intestine (mean value of five piglets $\pm$ SD; at least 10 measurements per pig and per site)[1]

Diet	*SI 1*[2]	*SI 2*[2]
Control diet	33.9 $\pm$ 3.9	30.3 $\pm$ 6.8
Cuphea + lipase diet	26.7 $\pm$ 2.3	26.0 $\pm$ 1.8
Significance or P value[3]	**	0.21

[1]Expressed as IELs per 100 enterocytes
[2]Measuring sites: SI 1: 3 m distal from the pylorus and SI 2: 3 m proximal of the caecum
[3]Significant from control: ** ($P < 0.01$)

et al. (1996) stated that an increase in crypt depth is compatible with an increase in crypt-cell production rate and an overall stimulation of cell turn-over in the small intestine. Both phenomena are associated with reduced digestive and absorptive capacity. Minimal villus height and maximal crypt depth were normally found at about 8 days post-weaning, being the slaughter time of the piglets in present experiment (Kenworthy, 1976; Deprez *et al.*, 1987; Pluske *et al.*, 1997; Van Beers-Schreurs *et al.*, 1998). Therefore the observation that MCFA in the gut lumen might prevent this post-weaning villus height reduction may be of particular interest in the prevention of post-weaning enteric disease. This is in line with the results of Galluser *et al.* (1993) reporting that rats who were given a MCTAG/LCTAG diet showed a higher mucosal mass, protein content and increased villus length and crypt depth in the proximal part of the small intestine compared with the LCTAG and control diet groups, suggesting that MCTAG represent a rapidly available and high energy fuel for the tissue. Also Czernichow *et al.* (1996) found that enteral infused MCTAG enhanced mucosal mass and favoured epithelial cell renewal in the proximal intestine in rats. Possible interferences arising from the mucilaginous arabinose containing hairs of the *Cuphea* seeds with the gut flora and/or the intestinal surfaces may not be excluded however and need further research.

The present results, although less pronounced for some parameters, are in line with foregoing research, showing that manipulation of the gut ecosystem by the enzymic *in situ* released MCFA in the stomach and fore-gut may result in improved performances of the piglets (Dierick, *et al.*, 2002a,b), which makes the concept a potential alternative for in feed nutritional antibiotics, which show established similar effects (Vervaeke *et al.*, 1979; Thomke and Elwinger, 1998; Anderson *et al.*, 1999). The present obtained growth promotion could be improved however by increasing the level of *Cuphea* seeds and/or lipase in the diet and/or by choosing *Cuphea* cultivars with a higher C8 : 0/C10 : 0 ratio e.g. *Cuphea painteri* or *hookeriana* (0.70/0.30) (Kleinman, 1990). More and long-lasting experiments are needed however, before *Cuphea* can be used in practical pig feeding, in the supposition that the seeds become commercially available in the future, as at present, most *Cuphea* seeds are rather harvested by hand and on a small scale.

Finally, attention must be drawn to the impact of feeding MCFA to pigs on product quality. Although the conversion of MCFA into long-chain fatty acids by hepatic *de novo* lipogenesis is likely to be a less important metabolic pathway, higher levels of MCFA (4% or more in the diet) selectively increase the firmness of the carcass fat by MCFA chain elongation (Bergner and Sommer, 1994). In order to avoid these increases in degree of saturation of the carcass fat in slaughter pigs and because the major changes in fatty acid composition due to diet will occur within 4–5 weeks (Wiseman and Agunbiade, 1998), high levels of MCTAG are not recommended in the finishing phase of pigs.

Acknowledgements

The authors are indebted to Dr. Jim M. Crane, Department of Crop and Soil Science, Oregon State University, USA and to Dr. E. Vanderbeke, R and D, Aveve NV, Merksem, Belgium for providing the Cuphea seed samples and to Dr. L. Van Campenhout, R and D, Kemin Europa N.V., Herentals, Belgium, the lipase. The authors are also grateful to Dr. K. Molly, Vitamex N.V., Belgium for piglets and basal

feed supply, to Dr. C. Van Nevel for aid by performing the morphometric and histologic measurements and to A. Ovijn, A. Maes and A. Turtelboom for skillful technical assistance. This work was part of a thesis submitted by I. De Geyter to obtain the degree of 'Bio-ingenieur' at Ghent University, Belgium.

References

Anderson, D., McCracken, V., Aminov, R., Simpson, J., Mackie, R., Verstegen, M. and Gaskins, H. (1999) Gut microbiology and growth-promoting antibiotics in swine. *Pig News and Information*, **20,** 115N–122N.

Anonymous (1971) *Publicatieblad Europese Gemeenschappen.* **14** (L279), 8.

Anonymous (1972) *Publicatieblad Europese Gemeenschappen.* **15** (L123), 9.

Aw, T. and Grigor, M. (1980) Digestion and absorption of milk triacylglycerols in 14-day-old suckling rats. *J. Nutr.*, **110,** 2133–2140.

Bergner, H. and Sommer, A. (1994) Einsatz von freien Fettsäuren in der Tierernährung. *Arch. Anim. Nutr.*, **46,** 217–236.

Cera, K., Mahan, D. and Reinhart, G. (1989) Postweaning swine performance and serum profile responses to supplemental medium-chain free fatty acids and tallow. *J. Anim. Sci.*, **67,** 2048–2055.

Clark, S., Brause, B. and Holt, P. (1969) Lipolysis and absorption of fat in the rat stomach. *Gastroenterol.*, **56,** 214–222.

Cranwell, P., Noakes, D. and Hill, K. (1976) Gastric secretion and fermentation in the suckling pig. *Brit. J. Nutr.*, **36,** 71–86.

CVB (1998) *Voedernormen landbouwhuisdieren en voederwaarde veevoeders, verkorte tabel, cvb-reeks nr 24,* Centraal Veevoederbureau, Lelystad, NL, 116 pp.

Czernichow, B., Galluser, M., Cui, S., Gosse, F. and Raul, F. (1996) Comparison of enteral or parenteral administration of medium chain triglycerides on intestinal mucosa in adult rats. *Nutr. Res.*, **16,** 797–804.

Decuypere, J., Bossuyt, R. and Henderickx, H. (1978) Gastric secretion in suckling pigs and early-weaned pigs given a dry cow's milk formula ad. lib. *Brit. J. Nutr.*, **40,** 91–102.

Decuypere, J. and Dierick, N. (2000) The combined use of triglycerides containing medium chain fatty acids and selected lipases as feed supplements as an alternative for in feed antibiotics in early weaned pigs. *European Patent Publication EP 1059041* (13 December 2000); *PCT Publication WO 00/74497* (14 December 2000).

Decuypere, J. and Meeus, J. (1995) Aspects nouveaux de la mode d'action des acides dans l'alimentation porcine. Journée d'étude KEMIN EUROPE, Rennes, 27 Juin, 1995, 22 pp.

Dehesh, K., Jones, A., Knutzon, D. and Voelker, T. (1996) Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of *Ch FatB2*, a thioesterase cDNA from *Cuphea hookeriana*. *The Plant Journal*, **9,** 167–172.

Deprez, P., Deroose, P., Van den Hende, C., Muylle, E. and Oyaert, W. (1987) Liquid versus dry feeding in weaned piglets: the influence on small intestinal morphology. *J. Vet. Med., B*, **34,** 254–259.

Dierick, N. and Decuypere, J. (2002) Endogenous lipolysis in feedstuffs and compound feeds for pigs: effects of storage time and conditions and lipase and/or emulsifier addition. *Anim. Feed Sci. Technol.*, **102,** 53–70.

Dierick, N., Decuypere, J., Molly, K., Van Beek, E. and Vanderbeke, E. (2002a) The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition. I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets. *Livest. Prod. Sci.*, **75,** 129–142.

Dierick, N., Decuypere, J., Molly, K., Van Beek, E. and Vanderbeke, E. (2002b) The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative to nutritional antibiotics in piglet nutrition. II. In vivo release of MCFAs in gastric cannulated and slaughtered piglets by endogenous and exogenous lipases; effects on the luminal gut flora and growth performance. *Livest. Prod. Sci.*, **76,** 1–16.

Galluser, M., Czernichow, B., Dreyfus, H., Gossé, F., Guérols, B., Kachelhoffer, J., Doffoel, M. and Raul, F. (1993) Comparison of different lipid substrates on intestinal adaptation in the rat. *Gut*, **34,** 1069–1074.

Hamilton, R. (1983) The chemistry of rancidity in foods. In: Allen, J. and Hamilton, R. (Eds.) *Rancidity in Foods*, Applied Science Publishers, London, pp. 1–20.

Hampson, D. and Kidder, D. (1986) Influence of creep feeding and weaning on brush border enzyme activities in the piglet. *Res. Vet. Sci.*, **40,** 24–31.

Hellyer, S., Chandler, I. and Bosley, J. (1999) Can the fatty acid selectivety of plant lipases be predicted from the composition of the seed triglyceride? *Biochim. Biophys. Acta*, **1440,** 215–224.

Hendrich, S., Lii, C., Myers, R. and Dupont, J. (1993) Effect of feeding *Cuphea* oil to three generations of CBA/2 and C57B1/6 mice. *J.A.O.C.S.*, **70,** 797–801.

Herting, D., Embree, N. and Harris, P. (1956) Absorption of acetic acid and glycerol from the rat stomach. *Am. J. Physiol.*, **186,** 224–226.
Hurst, G. (1978) Crop impactions in bobwhite quail in Louisiana. *J. Wildl. Dis.*, **124,** 355.
Kabara, J. (1984) Medium-chain fatty acids and esters as antimicrobial agens. In: Kabara, J. (Ed.), *Cosmetic and Drug Preservation, Principles and Practice,* Marcel Dekker, N.Y., pp. 275–304.
Kabara, J., Vrable, R. and Liekenjie, M. (1977) Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. *Lipids,* **12,** 753–759.
Kabara, J., Swieczkowski, D., Conley, A. and Truant, J. (1972) Fatty acids and derivates as antimicrobial agents. *Antimicrobial Agents and Chemotherapy,* **2,** 23–28.
Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. *Res. Vet. Sci.*, **21,** 69–75.
Kik, M. (1991) Effects of lectins in legume seeds on the structure and functions of the small intestinal mucosa. *Ph.D. Thesis, Rijksuniversteit Utrecht,* The Netherlands, 130 pp.
Kleiman, R. (1990) Chemistry of new industrial oilseed crops. In: Janek, J. and Simon, J. (Eds.) *Advances in new crops,* Timber Press, Portland, OR, pp. 196–203.
Knapp, S. (1993) Breakthroughs towards the domestication of *Cuphea.* II, In Janick J. and Simon J. (Ed.), *New Crops,* Wiley, New York, pp. 372–379.
Litchfield, C., Miller, E., Harlow, R. and Reiser, R. (1967) The triglyceride composition of 17 seed fats rich in octanoic, decanoic or lauric acid. *Lipids,* **2,** 345–350.
Meeus, J. (1994) *De antimicrobiële activiteit van organische zuren en pH op de darmflora bij het varken..* Thesis Landbouwkundig Ir., University of Ghent, Faculty Agricultural and Applied Biological Sciences, 110 pp.
Molimard, P., Le Quéré, J. and Spinner, H. (1997) Les lipides et la flaveur des produits laitiers. *Journal français des oléagineux, corps gras, lipides (OCL),* **4,** 301–312.
Odle, J. (1998) Medium-chain triglycerides: A unique energy source for neonatal pigs. *Pig News and Information,* **20,** 25N–32N.
Partanen, K. and Mroz, Z. (1999) Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.*, **12,** 117–145.
Piva, A. (1998) Non-conventional feed additives. *J. Anim. Feed Sci.*, **7,** 143–154.
Pluske, J., Williams, I. and Aherne, F. (1996) Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Anim. Sci.*, **62,** 131–144.
Pluske, J., Hampson, D. and Williams, I. (1997) Factors influencing the structure and function of the small intestine in the weaned pig: A review. *Livest. Prod. Sci.*, **51,** 215–236.
Roth, F. and Kirchgessner, M. (1998) Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. *J. Anim. Feed Sci.*, **7,** 25–33.
Thomke, S. and Elwinger, K. (1998) Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. *Ann. Zootechn.*, **47,** 153–167.
Timmermann, F. (1993) Medium chain triglycerides, the unconventional oil. *Int. Food Ingredients,* **3,** 11–18.
Traul, K., Driedger, A., Ingle, D. and Nakhasi, D. (2000) Review of the toxicologic properties of medium-chain triglycerides. *Food Chem. Toxicol.*, **38,** 79–98.
Van Beers-Schreurs, H., Nabuurs, M., Vellenga, L., Kalsbeek-van der Valk, H., Wensing, T. and Breukink, H. (1998) Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentration of short-chain fatty acids in the large intestine and blood. *J. Nutr.*, **128,** 847–953.
Velasquez, O., Seto, R. and Rombeau, J. (1996) The scientific rationale and clinical application of short-chain fatty acids and medium-chain fatty acids. *Proc. Nutr. Soc.*, **55,** 49–78.
Verstegen, M. and Schaafsma, G. (1999) Some developments for antibiotics as growth promotor. In: Jansman, A. and Huisman, J. (Eds.) *Nutrition and gastrointestinal physiology – today and tomorrow,* TNO, Wageningen (NL), pp. 65–73.
Vervaeke, I., Decuypere, J., Dierick, N. and Henderickx, H. (1979) Quantitative in vitro evaluation of the energy metabolism influenced by Virginiamycin and Spiramycin used as growth promoters in pig nutrition. *J. Anim. Sci.*, **49,** 846–856.
Voelker, T. and Davies, H. (1997a) Plant medium-chain thioesterases. United States Patent 5.639.790, June 17, 1997.
Voelker, T., Davies, H. and Knutzon, D. (1997b) C8 and C10 medium-chain thioesterases in plants. United States Patent 5.667.997, September 16, 1997.
Watson, L. and Dallwitz, M.J. (1994) *The Families of Flowering Plants.* CD-ROM, Version 1.0 for MS-DOS, CSIRO Publications: Melbourne.
Willis, W. and Marangoni, A. (1999) Biotechnological strategies for the modification of food lipids. *Biotechnol. Gen. Rev.*, **16,** 141–175.
Wiseman, J. and Agunbiade, J. (1998) The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. *Livest. Prod. Sci.*, **54,** 217–227.
Zarhloul, M., Lühs, W., Syring-Ehemann, A., Hausmann, L., Friedt, W. and Töpfer, R. (2000) Molecular efforts to the biosynthesis of medium-chain triglycerides in rapeseed (*Brassica napus*). *Proceedings Deutsche Gesellschaft für Fettwissenschaft – Association Française pour l'Etude des Corps Gras Joint Congress,* Würzbrug, 8.–10.10.2000.