FACULTY OF BIOSCIENCE ENGINEERING

DEPARTMENT APPLIED BIOSCIENCES

HET GEBRUIK VAN ESSENTIËLE OLIËN EN ORGANISCHE ZUREN IN DE VARKENSVOEDING OM DE MICROBIOTA TE STUREN

Prof. Dr. Ir. JORIS MICHIELS

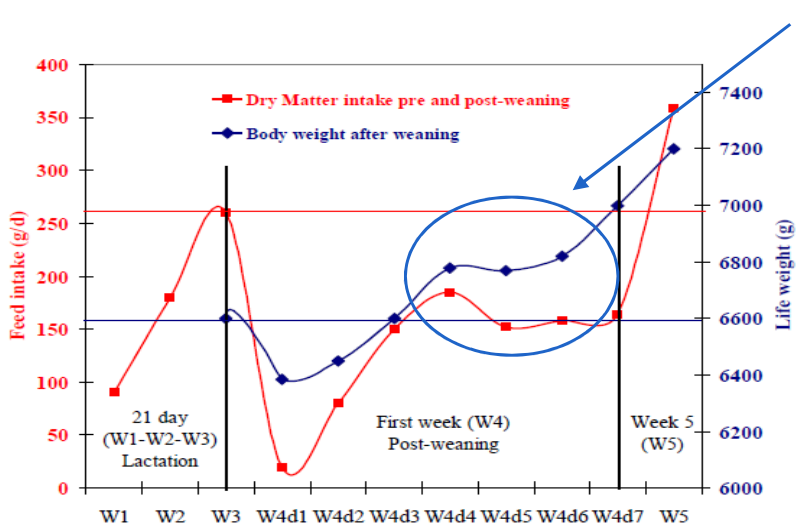
200 YEARS GHENT UNIVERSITY

AOAC Lowlands Symposium 2017, 21 September 2017

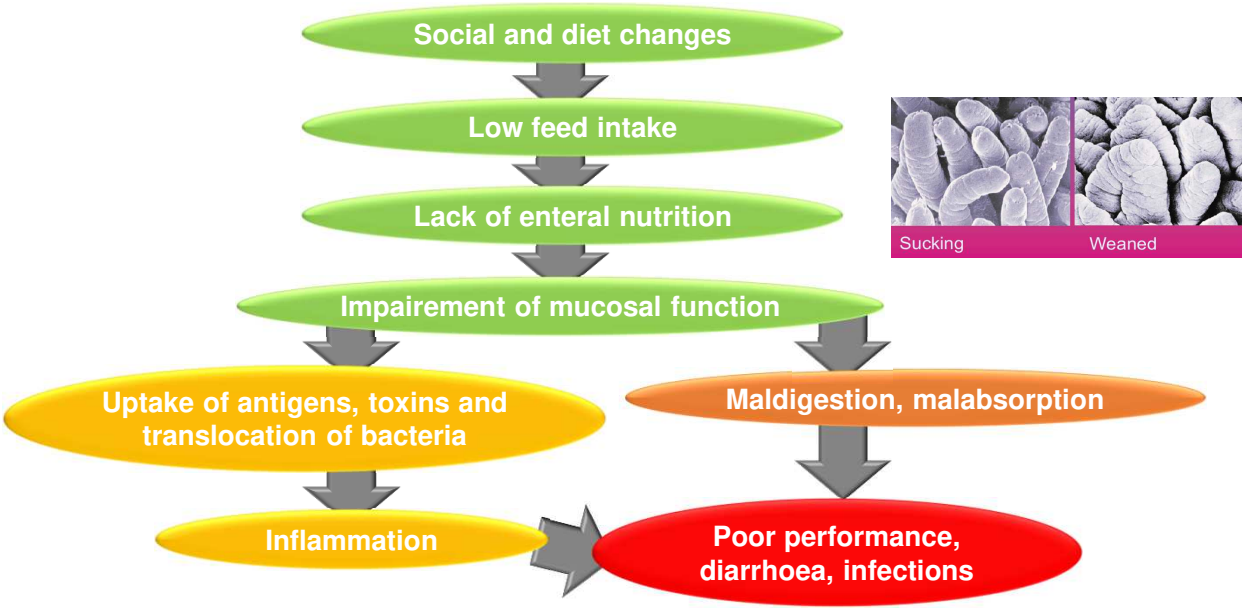
ABRUPT WEANING OF PIGLETS CAUSES STRESS AND REDUCES FEED INTAKE

- Weaning: gradual process
- Starts a week after birth, stops at 8-20 weeks

- Weaning: abrupt event at 3 - 4 weeks

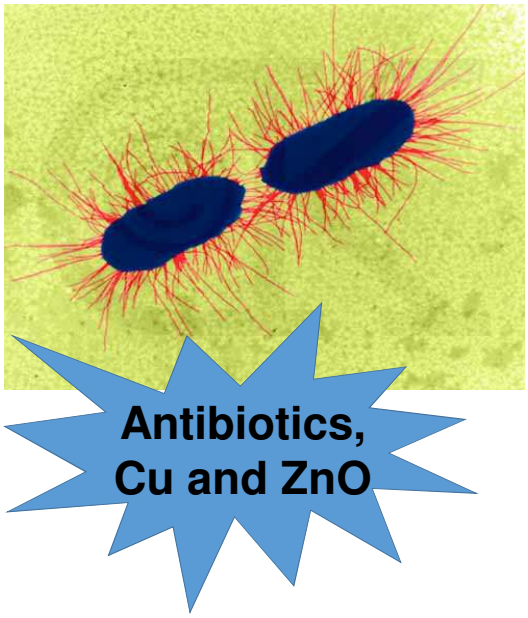


(Brooks, 2003)



(Vente-Spreeuwenberg and Beynen, 2003)

ENTEROTOXIGENIC *E.COLI* (ETEC)



Fimbriae bind to specific complex carbohydrate receptors on epithelial surface

↓

Colonisation of GIT

↓

Production enterotoxins

(heat labile or stable)

↓ ↓

increased secretion | decreased absorption

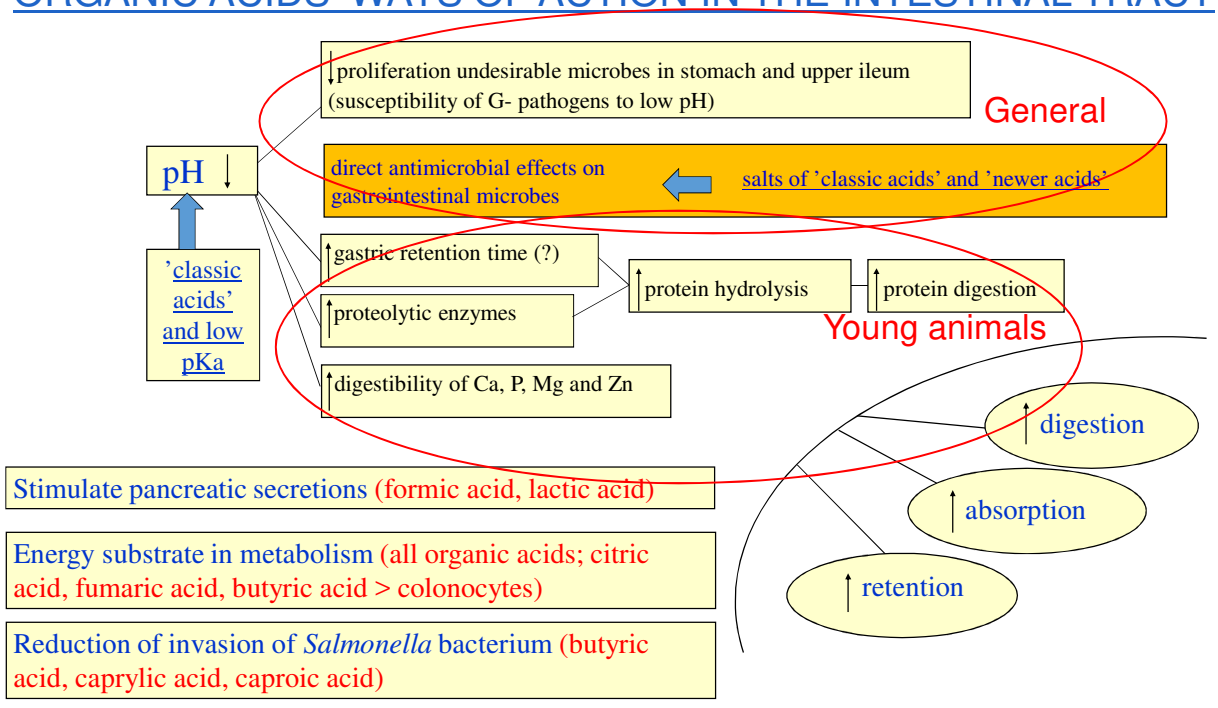
↓

Hypersecretion that exceeds absorption in colon

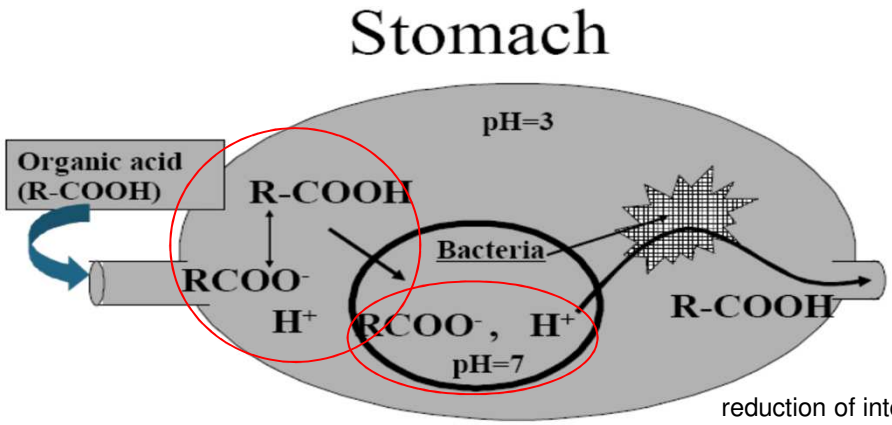
↓

Diarrhoea, dehydration, reduced feed intake digestibility and growth, morbitity and mortality

ORGANIC ACIDS' WAYS OF ACTION IN THE INTESTINAL TRACT



DIRECT ANTIMICROBIAL EFFECTS OF ORGANIC ACIDS ON MICROBIOTA



reduction of internal pH, preventing enzyme activity, loss of proton motive force, malfunction of bacterial cell …

Organic acid	Dose g/kg	Changes in microbial counts, log10 CFU						References
		Stomach			Small intestine			
		Total	LAC	COLI	Total	LAC	COLI	
Formic acid	6					↑	↑	Gedek et al. 1992a
	12					↓	↓	Gedek et al. 1992a
	18					↓	↑	Gedek et al. 1992a
	24					↓	↓	Gedek et al. 1992a
	7					↓	↓	Maribo et al. 2000
	14		-	↓		↓	↓	Maribo et al. 2000
Ca_ formate	18					↑	↑	Torralardona et al. 2007
Na_diformate	18					↓	↓	Gedek et al. 1992b
K_diformiate	18	↓	↓	-	↓	↓	-	Canibe et al. 2001
	5							Liu et al. 2007
	18			↓		-	↓	Fevrier et al. 2001
Formic + Lactic acid	10+10	↓	↓	↓				Hansen et al. 2007
Blend_1	4				↓	↓		Metzler-Zebeli et al. 2009
Blend_2	11					-	↑	Namkung et al. 2004
Blend_3	21					-	↑	Namkung et al. 2004

Blend_2: 23.1% formic acid + 13.3% lactic acid + 12.4% acetic acid + 0.76 phosphoric acid + 0.76 citric acid
Blend_3: 51.7% lactic acid + 29.0% formic acid + 17.0% acetic acid + 16.0% phosphoric acid + 0.85% citric acid

Contents lists available at ScienceDirect

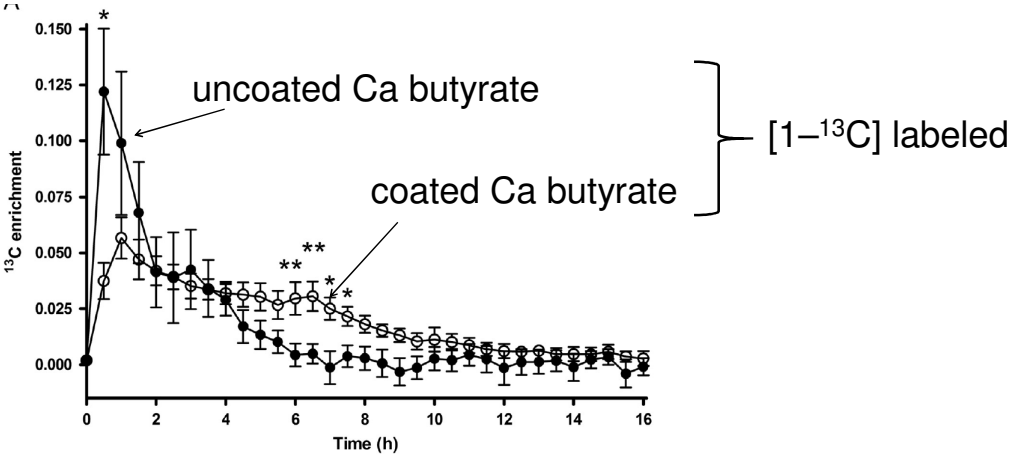
Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Fat coating of Ca butyrate results in extended butyrate release in the gastrointestinal tract of broilers

J.J.G.C. van den Borne [a], M.J.W. Heetkamp [b], J. Buyse [c], T.A. Niewold [d,*]

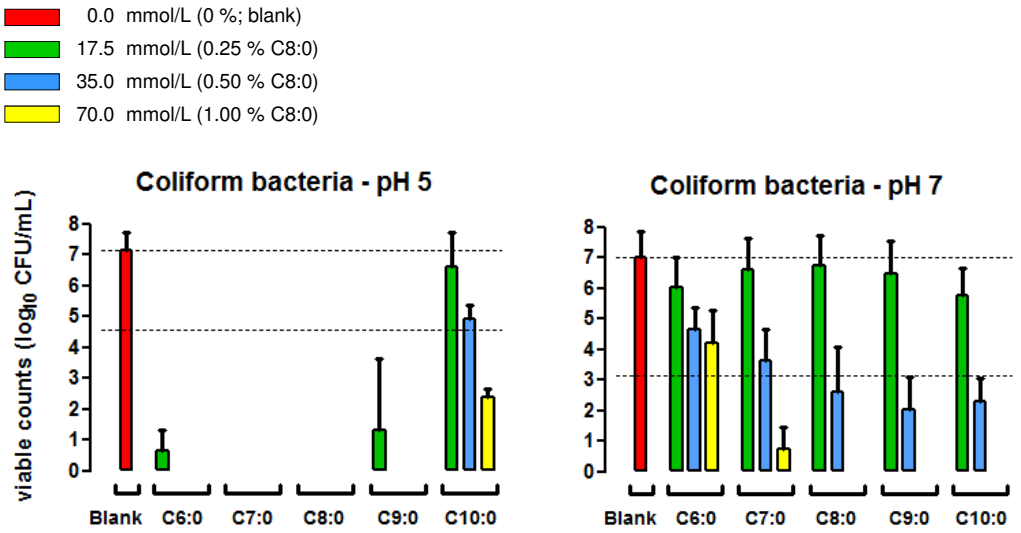


MEDIUM-CHAIN FATTY ACIDS (MCFA)

Common name	Systematic name		nC	MW	MP	pKa
Caproic acid	**hexanoic acid**	C6:0	**6**	**116 .2**	**-3.4**	**4.88**
Enanthic acid	**heptanoic acid**	C7:0	**7**	**130.2**	**-7.5**	**4.89**
Caprylic acid	**octanoic acid**	C8:0	**8**	**144.2**	**16.7**	**4.89**
Pelargonic acid	**nonanoic acid**	C9:0	**9**	**158.2**	**12.2**	**4.96**
Capric acid	**decanoic acid**	C10:0	**10**	**172.3**	**31.9**	**4.89**
Undecylic acid	**undecanoic acid**	C11:0	**11**	**186.3**	**28.6**	**4.96**
Lauric acid	**dodecanoic acid**	C12:0	**12**	**200.3**	**44.2**	**4.96**

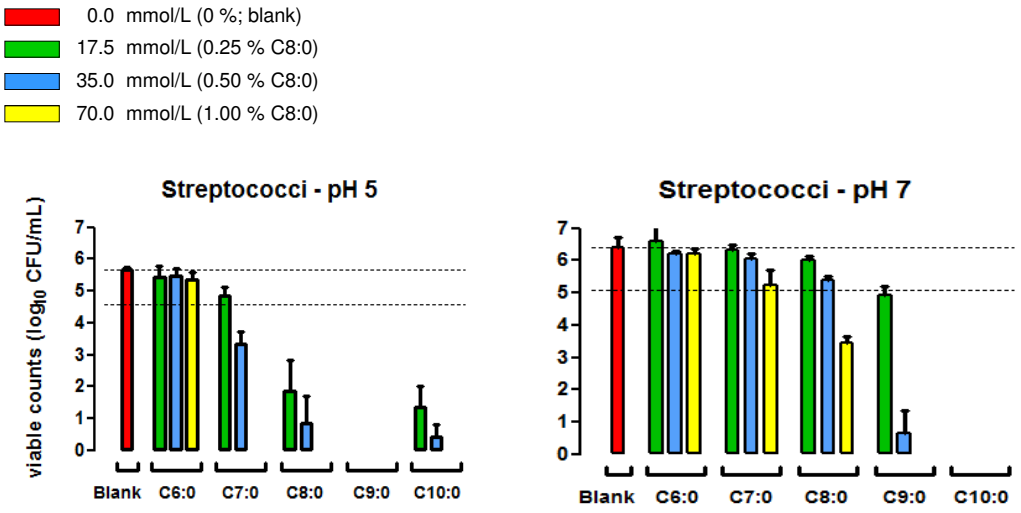
- Main sources :
  - **Oils from Coconut, Palm kernel, Tucum, Cohune, Babassu**
  - **Milk** from several mammalian species
- Others :
  - **Lythraceae** (> 25 % oil in DM) (***Cuphea*** genus) wide range of MCFAs according to cultivar :
    - high C8:0/C10:0 ratio e.g. *Cuphea painteri or hookeriana* (0.70/0.30)
  - Laurel family (*Umbellaria californica*) etc.
  - Transgenic Canola : gene (acyl-ACP thioesterase, *Ch FatB2*) that regulate (terminates) the fatty acid synthesis in *Cuphea,* transferred to canola seeds

IN VITRO ANTIMICROBIAL ACTIVITY AGAINST COLIFORMS OF MEDIUM-CHAIN FATTY ACIDS (MCFA)



(De Smet et al 2017)

IN VITRO ANTIMICROBIAL ACTIVITY AGAINST STREPTOCOCCI BACTERIA OF MEDIUM-CHAIN FATTY ACIDS (MCFA)



(De Smet et al 2017)

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Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets

E. Hanczakowska [a,*], M. Świątkiewicz [b], M. Natonek-Wiśniewska [c], K. Okoń [d]

	Experimental group (G)		
	C	C8	C10
Jejunum			
E. coli	3.12^{b}	2.05^{a}	2.77^{ab}
Clostridium perfringens	3.31	2.84	3.13
Cecum			
E. coli	2.87^{b}	2.18^{a}	2.35^{ab}
Clostridium perfringens	3.41^{b}	2.44^{a}	2.85^{ab}

Free MCFA at 0.3% in feed

	Experimental group (G)		
	C	C8	C10
No of born piglets in treatment	90	89	92
Dead and culled piglets (%)	6.7	4.5	2.2
Body weight (kg) on days of age			
1st day of age	1.78	1.80	1.79
7th day of age	2.99	3.21	3.18
28th day of age	7.65	7.70	7.57
56th day of age	14.32	14.59	15.12
70th day of age	22.37^{Aa}	22.58^{ABa}	24.08^{Bb}
Average body weight gains of piglets, g			
1–28th day of age	218	219	214
28–56th day of age	238^{Aa}	246^{ABa}	270^{Bb}
56–70th day of age	575^{ABa}	570^{Aa}	640^{Bb}
1–70th day of age	298^{Aa}	301^{ABa}	323^{Bb}

MCFA CONTENT OF VARIOUS PLANT SOURCES AND MILK FROM DIFFERENT SPECIES (G/100G FA)

Source	MCTAG	Coconut	Palm-kernel	Man	Horse	Cow	Rabbit	Sow
C4:0						4,8	0,0	0,1
C6:0	**1-2**	**0,5**	**0,3**	**0,1**		**2,2**	**0,0**	**0,1**
C8:0	**65-75**	**8,0**	**3,9**	**0,2**	**8,0**	**1,3**	**34,3**	**0,0**
C10:0	**25-35**	**6,4**	**4,0**	**1,0**	**17,1**	**2,9**	**21,4**	**0,0**
C12:0	**2**	**48,5**	**49,6**	**4,9**	**14,3**	**3,3**	**1,2**	**0,0**
C14:0		17,6	16,0	5,6	8,7	10,8	1,0	4,0
C16:0		8,4	8,0	20,3	15,3	26,2	11,7	36,6
C16:1				3,4	4,0		1,2	10,5
C18:0		2,5	2,4	7,5	1,2	10,8	2,1	5,9
C18:1		6,5	13,7	33,6	8,3	24,1	12,6	32,3
C18:2		1,5	2,0	12,6	6,1	2,4	13,8	8,4
C18:3				1,0	4,3	1,1	0,7	1,0

- Steam distillation (fractionation) of free MCFA after hydrolysis
- Enzymatic/chemical re-esterification to Medium-chain mono/tri-acylglycerides (MCTAG)

(Dierick, 2009)

OCCURENCE OF PREDUODENAL LIPASES IN MAN AND MAMMALS

Location	*rat*	*mouse*	*rabbit*	*dog*	*horse*	*pig*	*calf*	*man*
Tongue	xxx	xx	x	x	x	x		(x)
Pharynx							xxx	
Stomach -cardia						xx		
Stomach -fundus	x	x	xxxx	xxx	xx	x	x	(xxx)

1 LU = quantity of enzyme releasing 1 µmol FA / min. (pH 6.5; 30°C) form an olive oil emulsion in a pH-stat (Food Chemicals Codex, 1981)

X = < 10 U/g tissue
XX = 10-100 U/g
XXX = 100-200 U/g
XXXX = > 200 U/g
(XX) = no exact data available

Concept: "Combined use of MCTAGs + acid resistant microbial lipase"

IN VIVO ANTIMICROBIAL ACTIVITY AGAINST THE GUT MICROBIOTA OF MEDIUM-CHAIN TRI-ACYLGLYCERIDES (MCTAG)

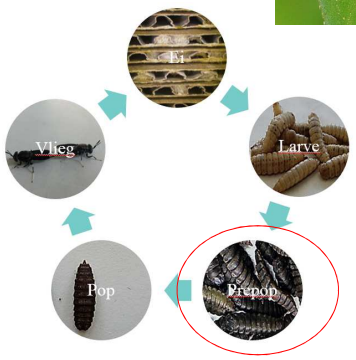
	2.5% soybean oil	2.5% MCTAG	2.5% MCTAG + 0.1 % lipase	0.25% citric acid + 0.75% fumaric acid + 0.50% calcium formate
Degree of fat hydrolysis in stomach (%)	26.7^{a}	35.2^{a}	70.4^{b}	28.9^{a}
Free MCFAs (g/100g FM) in stomach	0.02^{a}	0.22^{b}	0.45^{c}	0.01^{a}
Gastric bacteria; $\log_{10}$ CFU/g				
Lactobacilli	7.2^{ac}	7.6^{a}	6.6^{bc}	7.3^{a}
E. coli	4.6^{a}	0.6^{bc}	2.0^{b}	0.0^{c}
Proximal small intestinal bacteria; $\log_{10}$ CFU/g				
Lactobacilli	6.9	6.8	5.9	6.4
E. coli	4.9^{a}	4.8^{a}	1.8^{b}	1.8^{b}

(Dierick et al. 2002)

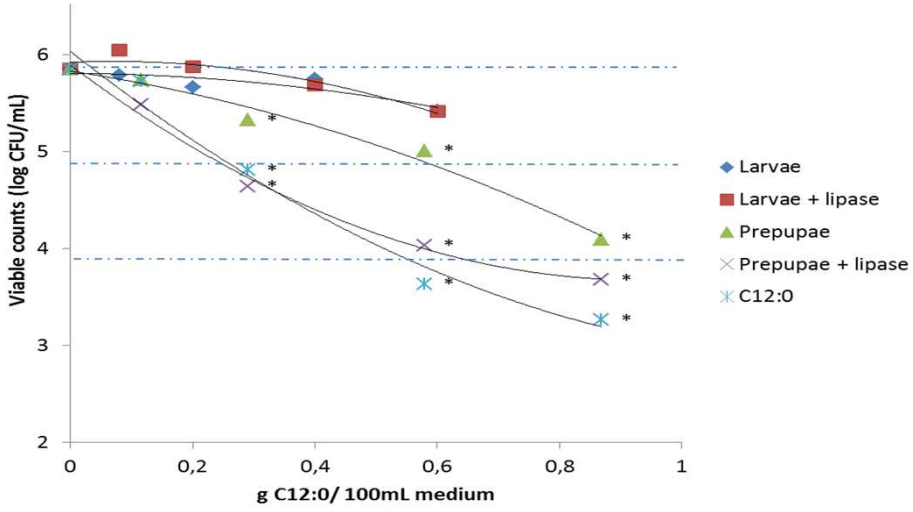
BLACK SOLDER FLY (*HERMETIA ILLUCENS* L.)

- Fast development and rapid reproduction
- Larvae are varocious feeders of organic waste
- Adults don't eat and are no vectors of diseases
- Favorable nutritional composition
  - 40-44 % protein on dry matter
  - High in essential amino-acids (6-8 % lysine on crude protein)
  - 32-40 % fat on dry matter
  - For prepupae: C12:0 = 58% of crude fat, of which app. half as free fatty acid

Antimicrobial effect?

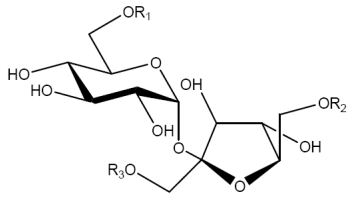


IN VITRO ANTIMICROBIAL ACTIVITY OF BLACK SOLDER FLY LARVAE AND PREPUPAE AGAINST STREPTOCOCCI



(Spranghers et al., 2017)

SUGAR FATTY ACID ESTERS OF MEDIUM-CHAIN FATTY ACIDS



EC10: R1 is capric acid
EC12: R1 is lauric acid

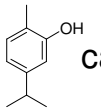
c (mg/ml)	EC_{10}	EC_{12}
***E. coli* CCM 3954**		
0	9.76 (0.25)	9.76 (0.25)
0.1	7.91 (0.54)*[a]	9.79 (0.28)[b]
0.2	7.03 (0.75)*[a]	9.50 (0.45)[b]
0.5	6.30 (0.40)*[a]	9.13 (0.11)[b]
1	6.43 (0.45)*[a]	9.32 (0.68)[b]
2	5.89 (0.26)*[b]	9.04 (0.17)[b]
3	5.64 (0.23)*[b]	9.27 (0.24)[b]
5	5.13 (0.44)*[b]	8.84 (0.14)[b]

c (mg/ml)	EC_{10}	EC_{12}
***C. perfringens* CNCTC 5459**		
0	9.30 (0.30)	9.30 (0.30)
0.1	6.74 (0.22)*[a]	< 2*[b]
0.2	6.79 (0.28)*[a]	< 2*[b]
0.5	6.22 (0.35)*[a]	< 2*[b]
1	4.08 (0.51)*[a]	< 2*[b]
2	< 2*	< 2*
3	< 2*	< 2*
5	< 2*	< 2*

(Skrivanova et al., 2014)

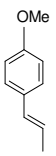
ESSENTIAL OILS WITH ANTIMICROBIAL PROPERTIES

Origanum vulgare (oregano)
50-80% in EO



carvacrol

anethole

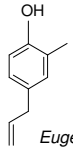


Pimpenella anisum (anis)
80-90% in EO

phenolic structure

synthetically prepared

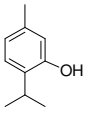
'nature identical'



eugenol

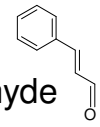
Eugenia caryophyllata (clove)
>80% in EO

Thymus vulgaris (thyme)
20-55% in EO



thymol

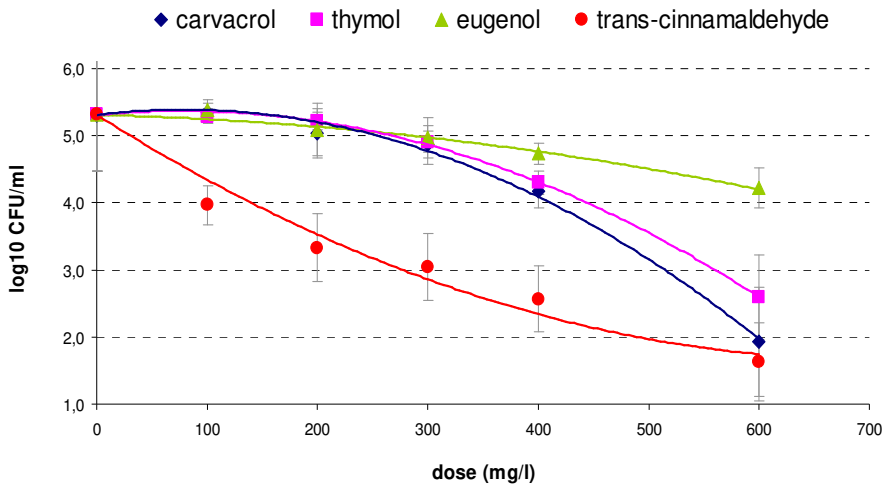
α,β-unsaturated aldehyde



cinnamaldehyde

Cinnamomum zeylanicum (cinnamon)
60-95% in EO

EFFECT ON *E. COLI* IN *IN VITRO* SIMULATIONS OF THE SMALL INTESTINAL FERMENTATION



(Michiels et al. 2009)

Feed additive	Dose, mg/kg	Measured responses	References
Herbal extracts	7500	Reduced coliform bacteria counts in fecal; less diverse of microbiota in ileal digesta base on PCR-DGGE	Namkung et al. 2004
EO blend	50-150	Increased Lactobacillus and decreased *E. coli* counts in feces	Li et al. 2012
EO blend	1000	Increased Lactobacillus counts	Zhang et al. 2012
Chinese medicinal herbs	1000/3000	Increased Lactobacilli counts in ileum and decreased Coliform counts in colon	Huang et al. 2012
EO blend	100	Reduced *E. coli* and total aerobic bacteria in the rectum; increased Lactobacilli to E. coli ratio in colon	Li et al. 2012
Phytogenic additive	50-150	Microbial counts in feces (aerobes, gram negatives, anaerobes and lactobacilli) didn't change	Muhl and Liebert 2007
Thymol, carvacrol	500-2000	No effect on different bacterial groups in stomach and small intestine	Michiels et al. 2010
Cinnamaldehyde	100-400	No effect on different bacterial groups in stomach and small intestine	Michiels et al. Unpublished
Carvacrol (5%), cinnamaldehyde (3%), capsicum oleoresin (2%)	300	Decreased ileum total microbial mass and increased the lactobacilli:enterobacteria ratio	Manzanilla et al. 2006

FACULTY OF BIOSCIENCE ENGINEERING

1.WEANING OF THE PIG IN COMMERCIAL CONDITIONS IS A STRESSFUL EVENT THAT RESULTS IN GUT MALFUNCTION , ULTIMATELY LEADING TO HIGH USE OF ANTIBIOTICS, AND SUPRANUTRITIONAL LEVELS OF CU AND ZNO

2. USING ORGANIC ACIDS AND ESSENTIAL OILS ARE A KEY STRATEGY TO IMPROVE GUT HEALTH AND PERFORMANCE OF WEANED PIGLETS, THOUGH COMMERCIAL APPLICATIONS VARY WIDELY IN MOLECULES, DOSE, AND PHYSICO-CHEMICAL FORMULATION

3. INNOVATIONS WILL ELUCIDATE NEW MODES OF ACTION AND NEW CHEMICAL FORMULATIONS, BOOSTING THEIR FUTURE USE IN ANIMAL FEEDS

200 YEARS GHENT UNIVERSITY

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