Alternatives to Antibiotics

USDA

With the support of

OIE WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

3rd International Symposium
on Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Health and Production

The Berkeley Hotel, Bangkok, Thailand
16-18 December 2019

Organic Acids as Antibiotic Alternatives in Monogastric Animals

Dr. Chengbo Yang, Assistant Professor

Department of Animal Science
Faculty of Agricultural and Food Sciences
University of Manitoba

University of Manitoba

Growing demand for animal protein

7 billion people consuming on average 37 kg of meat and 83 kg of dairy per year

9 billion people consuming on average 50 kg of meat and 99 kg of dairy per year

Production increase needed by 2050:

+ 53% + 75%

University of Manitoba

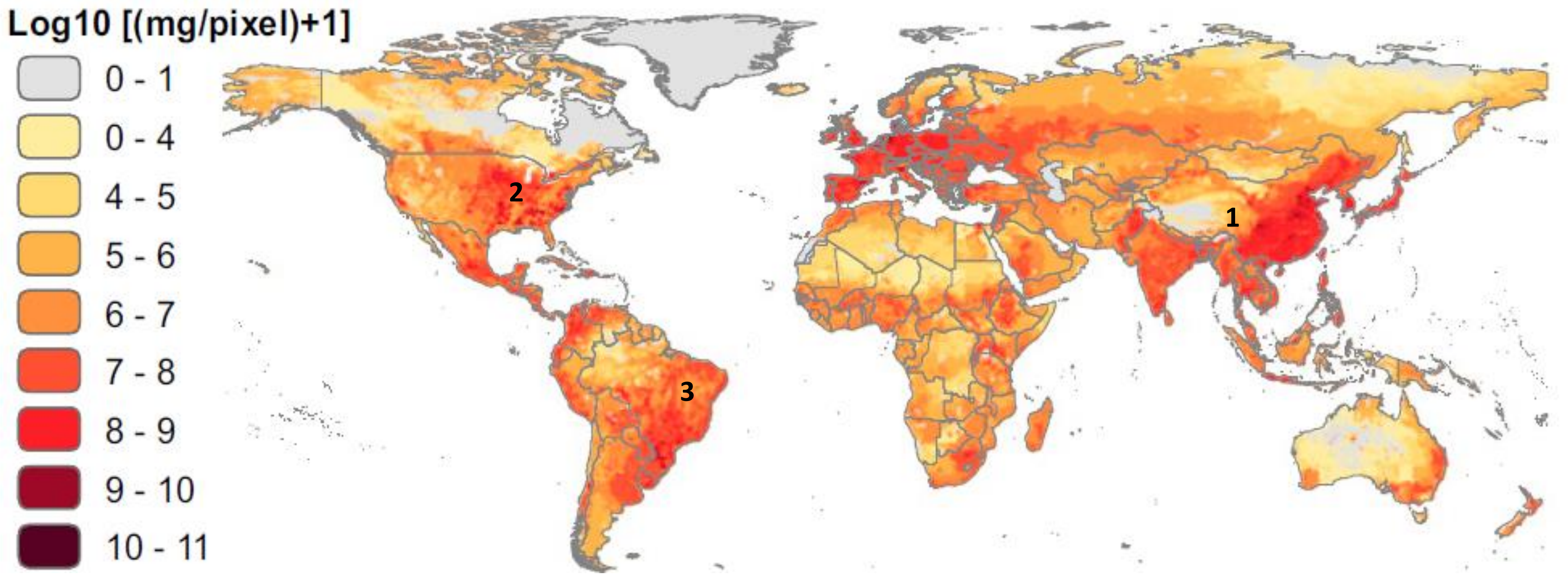
Effect of AGP on Growth Rates in Swine (>32,000 pigs)

Class of pigs	% increase in ADG
Weanling (7-25 kg)	16.4
Growing pigs (17-49 kg)	10.6
Growing-finishing (24-84 kg)	4.2

(Source: NRC, 1998)

University of Manitoba

Global trends in antimicrobial use in food animals



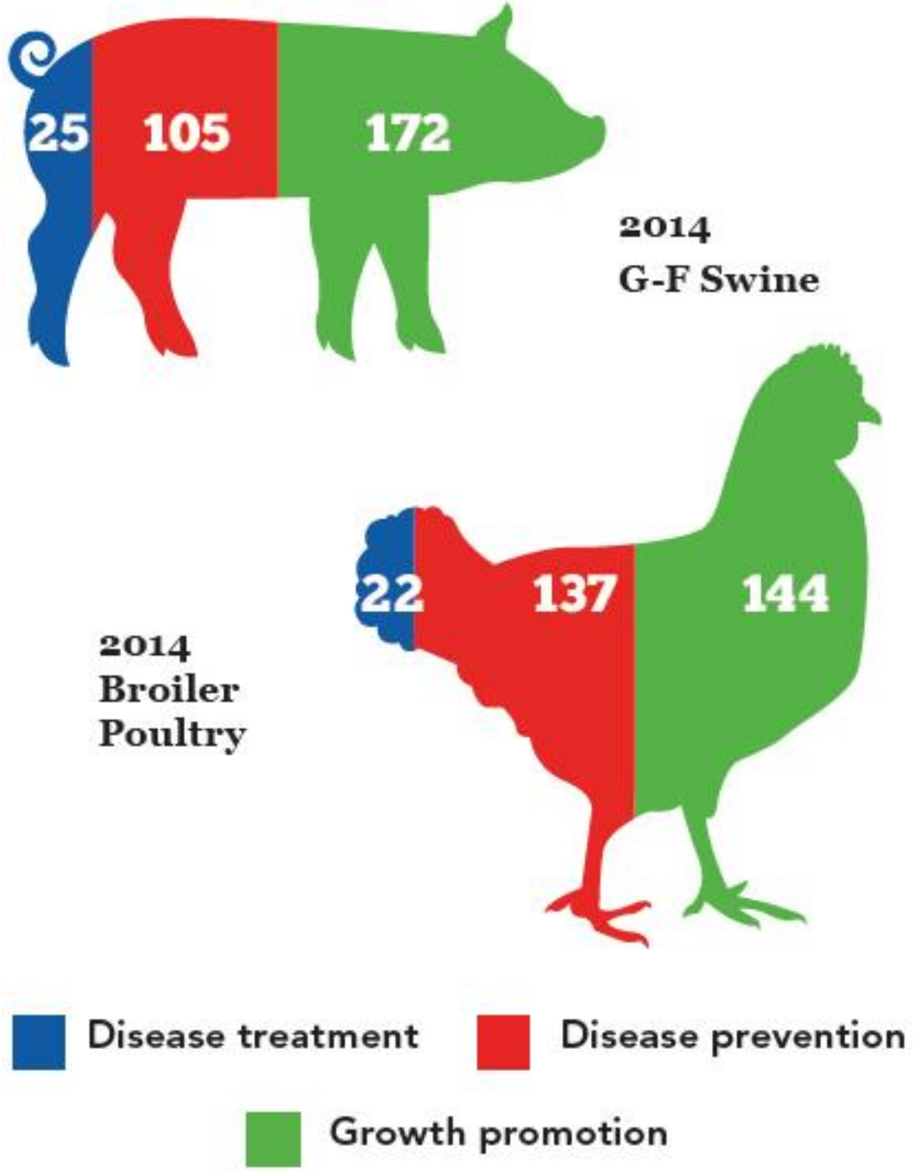
1. **China**
2. **United States**
3. **Brazil**

63,151 tons in 2010.
Predicted to rise dramatically by 67% by 2030.

(Van Boeckel et al., 2015, PANS)

University of Manitoba

Antimicrobial use in feed adjusted for populations and weights



Source: Canadian Antimicrobial Resistance Surveillance System (CARSS) – Report 2016

University of Manitoba

Consumers want a food system that assures food is safe, affordable, healthful and sustainable

Tyson
Tyson Foods, Inc.

CHIPOTLE MEXICAN GRILL

Pizza Hut

Cargill™

McDonald's®

Panera BREAD®

COSTCO® WHOLESALE

Wendy's

SUBWAY®

Chick-fil-A®

Applebee's

University of Manitoba

The Opportunities

- Development of better solutions to improve gut development and health in livestock; and

- Development of antibiotic alternatives for sustainable livestock production.

University of Manitoba

Alternatives to antibiotics

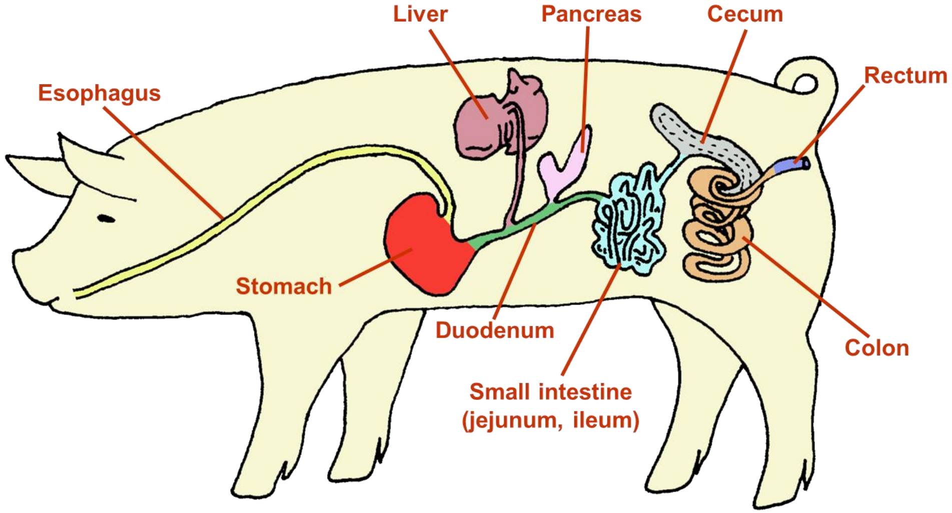
- Probiotics
- Prebiotics
- Synbiotics
- Feed enzymes
- Phytochemicals
- **Organic acids**
- Antimicrobial peptides
- Bacteriophage
- Bacteriophage gene products
- Antibacterial lytic enzymes
- Small interfering RNAs
- Therapeutic antibodies
- Vaccines

University of Manitoba

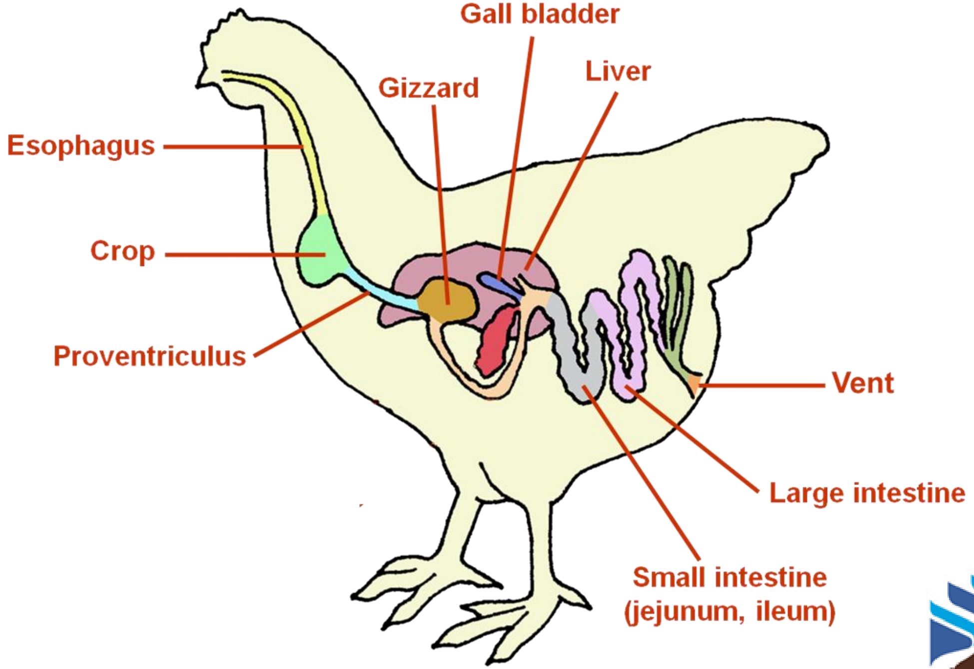
Potential antibiotic alternatives and their estimated relative effectiveness compared to antibiotic growth prompters

Compound	Relative Effectiveness	Comments
Antibiotic growth promotants	**+++++**	The standard for comparison purposes
Zinc oxide	**++++**	Fed at 2000 to 3000 ppm for the first two weeks post weaning. Decrease in scours and improved performance.
Copper sulfate	**+++**	Improved performance at 200 to 250 ppm, similar (but independent) to antibiotics. Largest effect in the nursery.
Plasma protein	**+++**	Increased feed intake and improved growth performance. Effects appear to be greater under unsanitary conditions.
Specific antibodies (egg yolk)	**++**	Limited data, but potentially promising. Results will likely depend on disease condition.
Organic acids	**+++**	**Likely most effective in newly weaned pigs. Inconsistent results. Formic acid may be most effective, but is not approved in the U.S.**
Direct-fed microbials	**++**	Suggested to promote beneficial bacteria in the gut. Inconsistent results. May depend on strain selection.
Prebiotics	**++**	Suggested to promote beneficial bacteria in the gut. Research with oligosaccharides have shown beneficial results.
Enzymes	**++**	Potential benefit through improved digestibility of feed ingredients and subsequent improved gut health.
Bioactive peptides	**++**	Limited research. Some peptides have antibiotic properties and could have potential benefits.
Botanicals (herbs and spices)	**+**	More research is necessary, There are many potential products.
Essential oils	**+**	More research is necessary.
Fermented liquid feeds	**+**	Fermentation will produce acids that can help in the maintenance of gut pH.

Non-ruminant (monogastrics)



Segment	pH
Stomach	2.6 to 4.2
Duodenum	5 to 6
Jejunum	6.0 to 7.0
Ileum	6.0 to 7.4
Cecum/colon	6.4 to 7.5



Segment	pH
Crop	5.5
Proventriculus/gizzard	2.5 to 3.5
Duodenum	5 to 6
Jejunum	6.5 to 7.0
Ileum	7.0 to 7.5
Cecum/colon	8

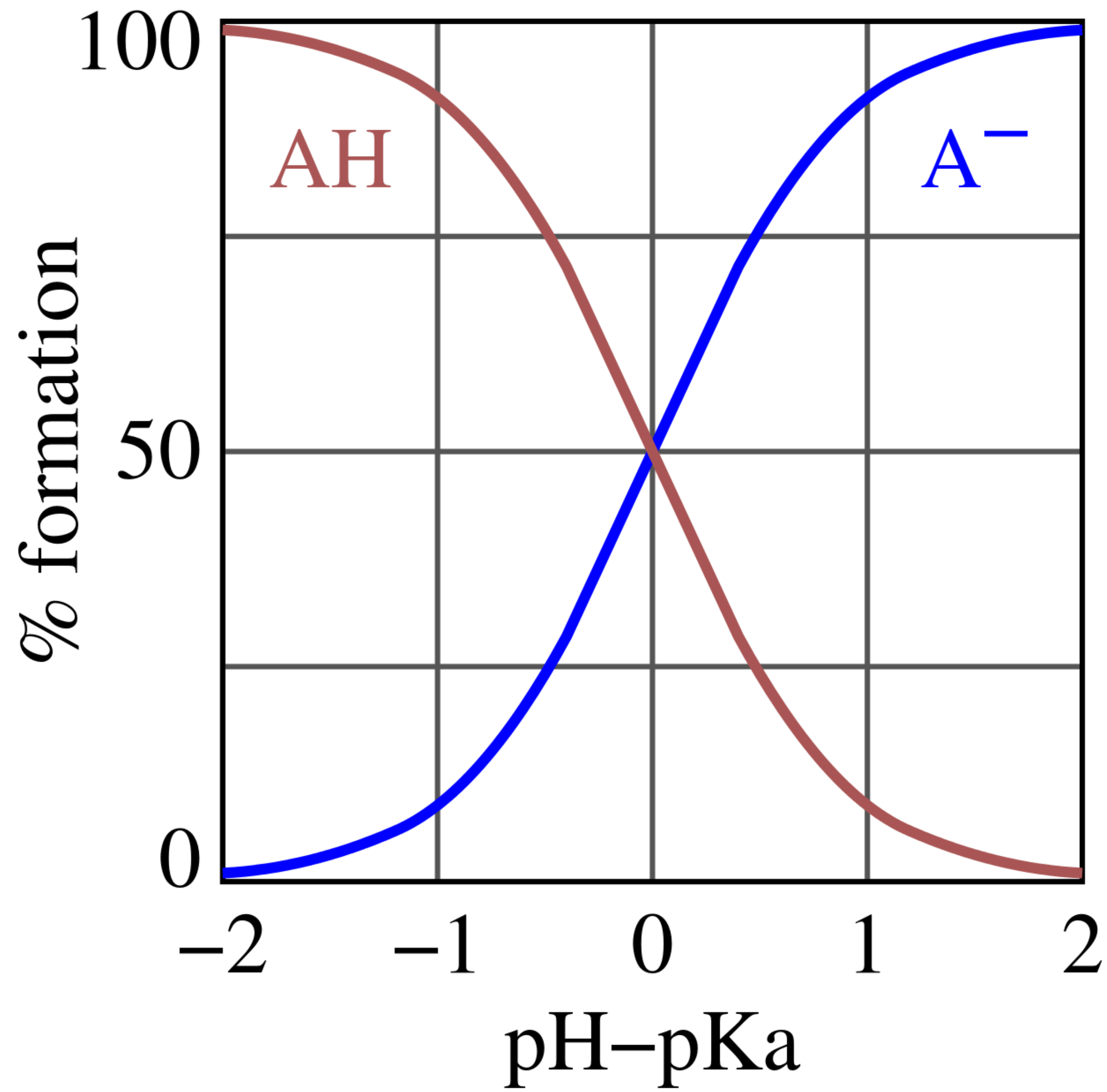
University of Manitoba

Organic acids

- **Organic acids**: formic acid, citric acid, propionic acid, fumaric acid, humic acid, medium chain fatty acids (e.g. **lauric acid**).
- **Salt of organic acids**: sodium formate, sodium butyrate
- **Organic acid derivative**: Tributyrin
- **Combination of organic acids and inorganic acids** has lower inclusion levels.
- **Protected organic acids or slowly release organic acids** may have a better performance with lower inclusion levels.

University of Manitoba

Association and dissociation equilibrium of organic acids



$$\mathbf{pH = p\mathit{K}a + \log\left(\frac{[A^-]}{[HA]}\right)}$$

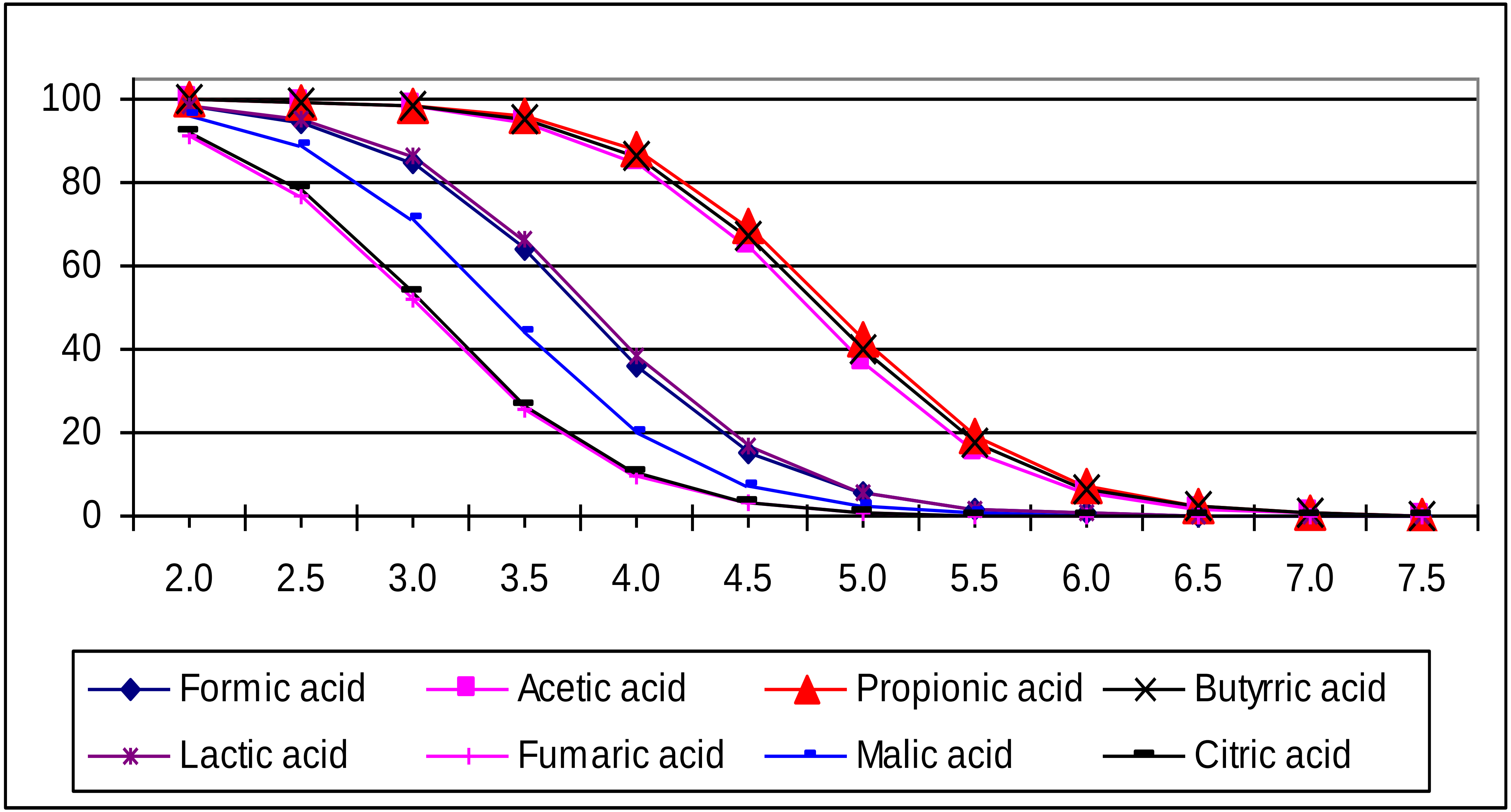
(https://en.wikipedia.org/wiki/Acid_dissociation_constant)

University of Manitoba

The pKa of organic acids and HCl

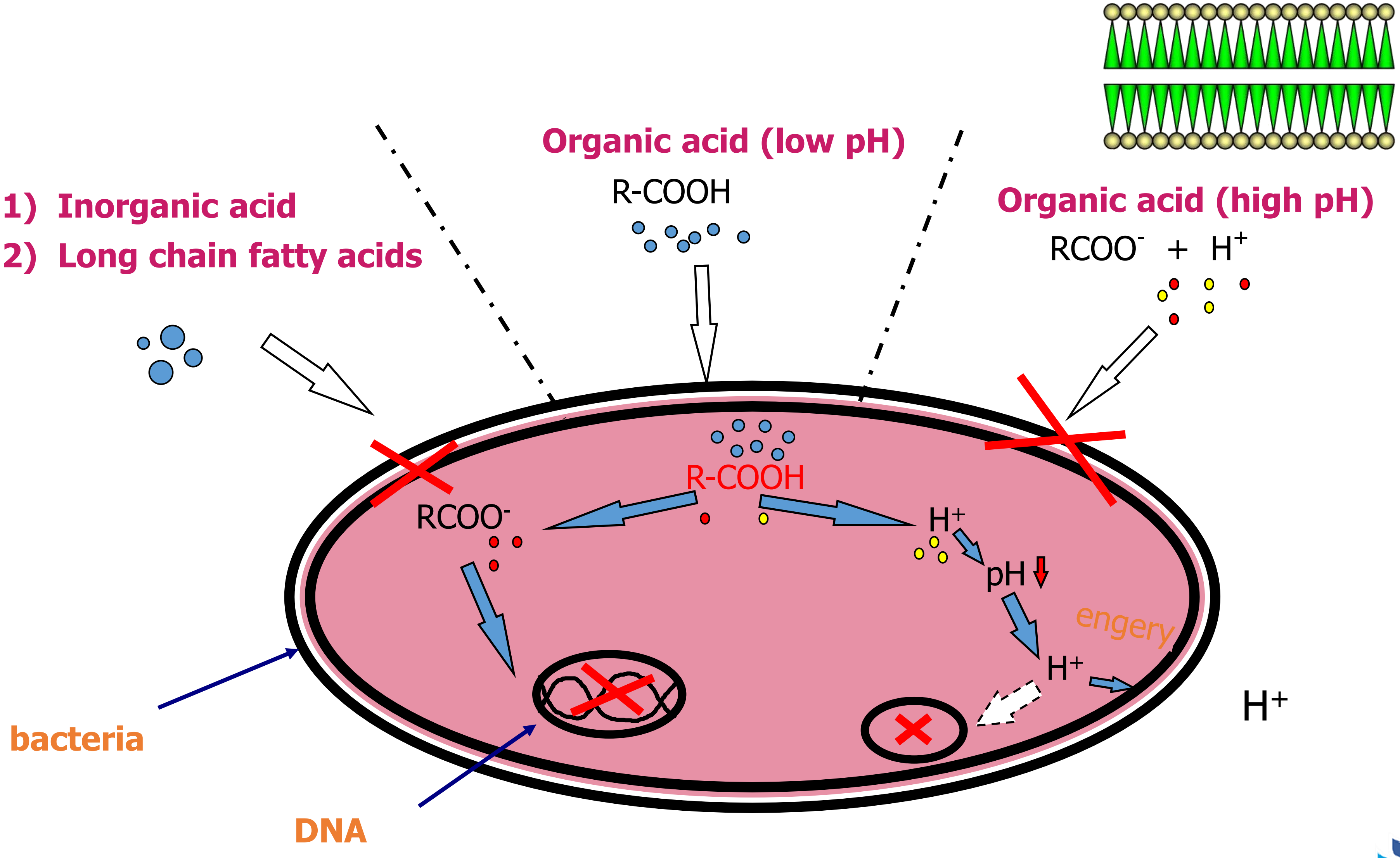
Name	pKa	Name	pKa
Formic acid	3.8	Gallic acid	4.4
Acetic acid	4.8	Malic acid	3.4
Propionic acid	4.9	Fumaric acid	3
Butyric acid	4.8	Lauric acid	5.3
Benzoic acid	4.2	Caproic acid	4.9
Citric acid	3.1	Caprylic acid	4.9
Sorbic acid	4.8	HCl	-7.0

Relationship between % undissociated acid ([HA]%) and pH



University of Manitoba

Mechanisms of pathogen control



(Adapted from R.J. Lambert and M. Stratford, Journal of Applied Microbiology 86, 157-164, 1999)

Experimentally determined values for MICs of undissociated and dissociated organic acids (various authors)

Organism	Acid type	MIC_u[a]	MIC_d[b]
E. coli M23	Lactic	8.32	-
Y. enterocolitica	Lactic	5-10	-
E. coli	Propionic	70	800
Staphylococcus aureus	Propionic	19	830
Bacillus cereus	Propionic	17	380
E. coli	Sorbic	1	100
E. coli	Sorbic	1	350
Staphylococcus aureus	Sorbic	0.6	400
Bacillus cereus	Sorbic	1.2	110
Listeria innocua	Lactic (Na lactate)	4.9	1250

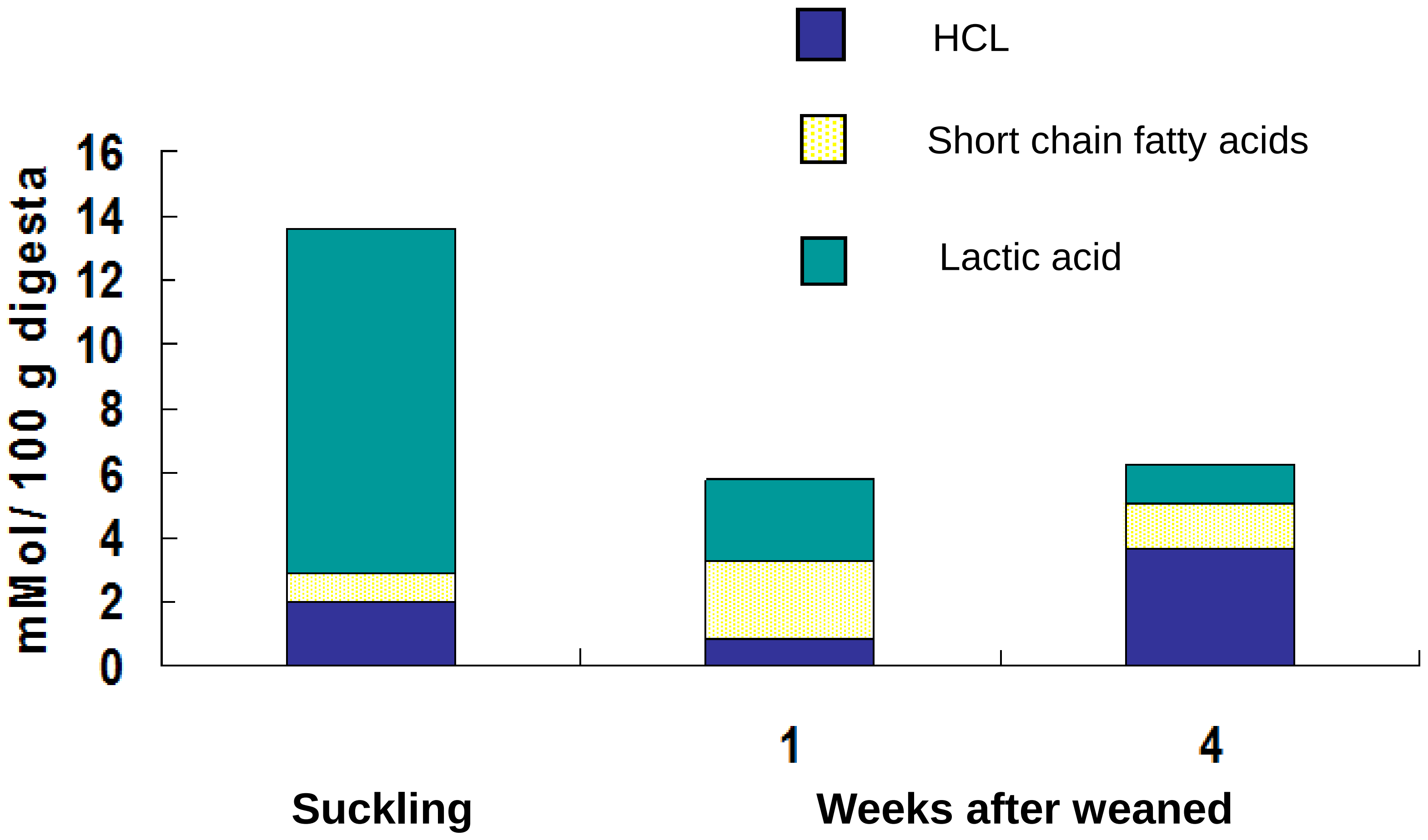
[a] MIC_u, MIC of the undissociated form of acid (micromolar).
[b] MIC_d, MIC of the dissociated for of acid (micromolar).
Adapted from (Presser)

University of Manitoba

Why using organic acids in animal feeding?

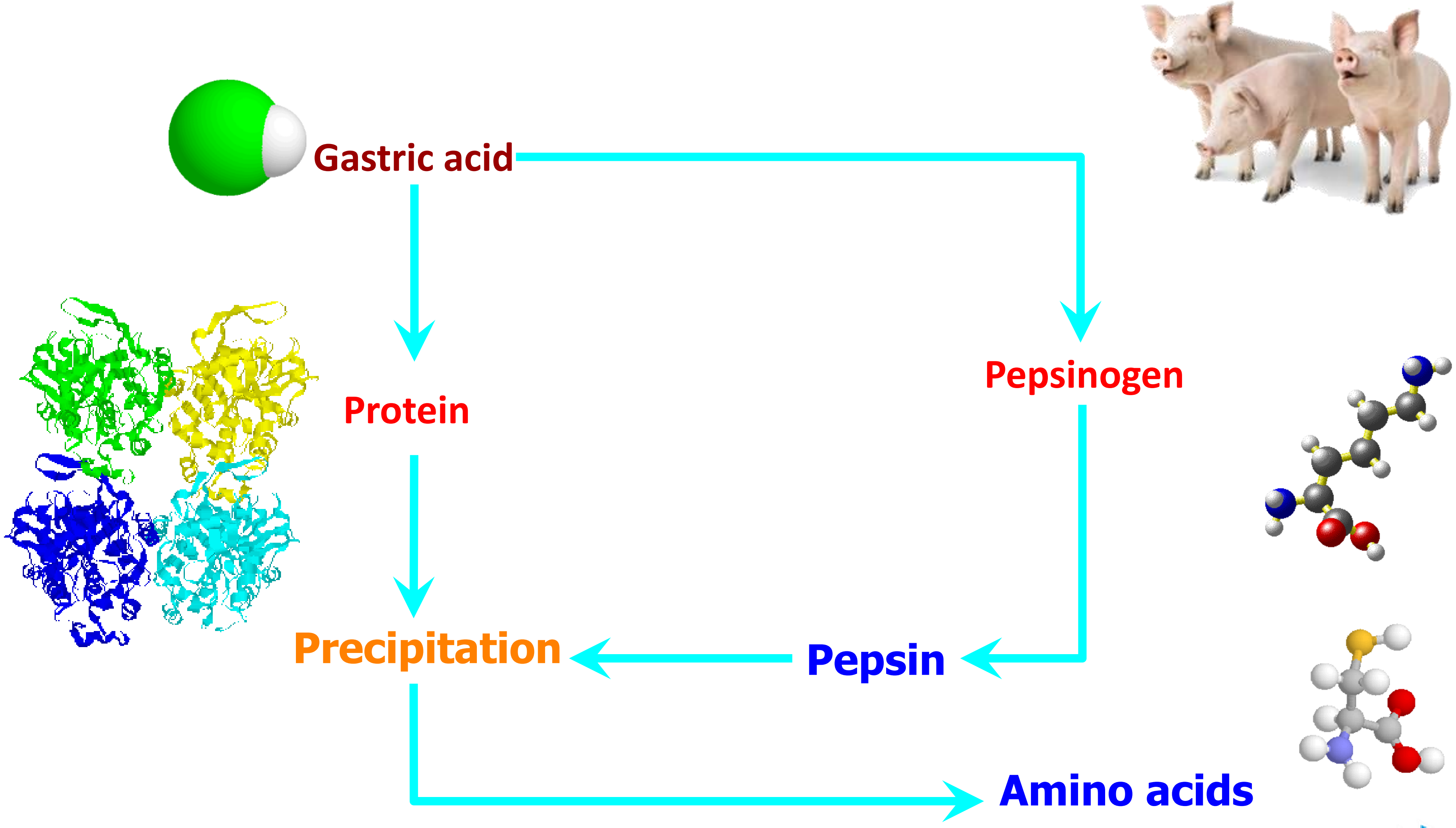
- 1.- Poor HCL secretion
- 2.- Intestinal pathogen control
- 3.- Buffer capacity of feed

Decreased HCl secretion in stomach after weaned



(Weaning at 35 day, Schnabel 1983)

Multiple roles of HCl in the stomach



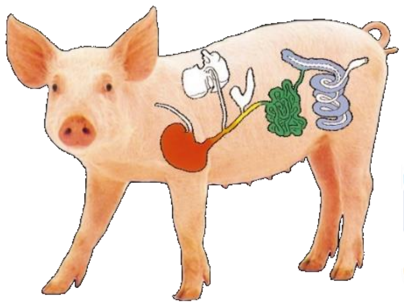
To kill harmful bacteria

To stimulate development of ‘good’ bacteria (Lactobacilli)

Acid binding capacity values for different ingredients in piglets

Ingredient	Buffering Capacity, meq/Kg	Ingredient	Buffering Capacity, meq/Kg
Maize	200	L-Lysine	600
Barley	225	DL-Methionine	1000
Wheat Soft	250	L-Threonine	1100
Wheat bran	500	L-Tryptophan	1025
Soybean 44%	1100	Sweet Whey powder	850-1000
Soybean 48%	1100	Betaine	600
Fishmeal 70/72	1800-2200	Dextrose	140-200
Fishmeal Peru origin	1800-2200	Choline	100-150
Vegetable fat	200	Blood plasma	1150-1350
Limestone	**18500-22000**	**ZnO**	**13000**
Calcium formate	9000	**Citric acid**	**-4000**
MCP	1800-2000	**Fumaric acid**	**-6400**

Definition: Acid Buffering capacity indicates the amount of 1.0 M HCl to lower the pH of feed to a pH =3.



A target of less than 600 meq/Kg

University of Manitoba

(http://www.wattagnet.com/articles/20464-controlling-dietary-buffering-capacity-in-piglet-feeds)

Low calcium levels improve growth in piglets after weaning

	Treatments				
Item	**LCa (0.35%)**	**MCa (0.65%)**	**HCa (0.95%)**	**Pooled SEM**	***P*-Value**
BW d7, Kg	**8.43^{a}**	**8.33^{ab}**	**8.21^{b}**	0.06	**0.041**
BW d14, kg	**10.72^{a}**	**10.6^{a}**	**10.16^{b}**	0.08	**0.0001**
ADG 0–7d, g/d	**106.94^{a}**	**91.25^{ab}**	**74.62^{b}**	8.37	**0.044**
ADFI 0–7d, g/d	162.51	148.45	149.69	8.05	0.411
G:F 0–7d	0.654^{a}	0.616^{a}	0.481^{b}	0.04	0.014
ADG 0–14d, g/d	**219.19^{a}**	**207.25^{a}**	**176.76^{b}**	5.72	**0.0002**
ADFI 0–14d, g/d	342.35	323.01	314.64	9.04	0.113

Different zinc sources may affect the acid buffering capacity of weanling pig diets

	Commercially available protected zinc oxide products			
Acid-binding Capacity	# 1	# 2	# 3	# 4
pH 4	59.43	3785.32	1.91	0.01
pH 3	106.27	3872.62	7.69	0.61
Buffering Capacity				
pH 4	32.92	717.17	0.84	0.01
pH 3	37.82	616.82	2.38	0.15

Acid binding capacity and buffering capacity mean of four zinc products at pH -4 and -3 calculated to 1 mol of the zinc ions.

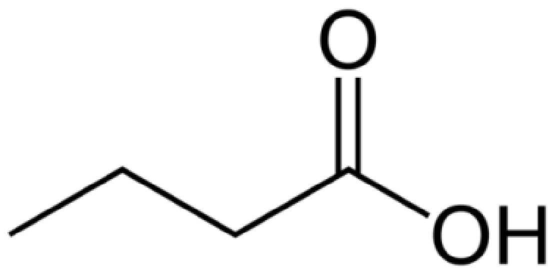
*Brown, N. *, Wang, Y. *, and Yang, C. Different zinc sources may affect the acid buffering capacity of weanling pig diets. Canadian Hog Journal. Special Summer Edition, For the love of science 2019, P 39-41.*

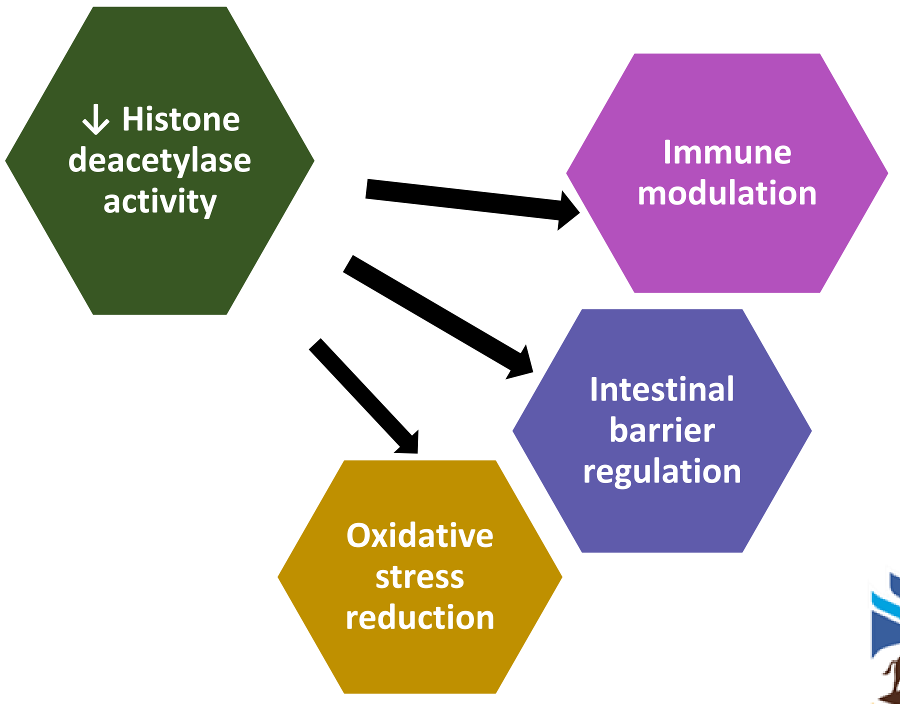
University of Manitoba

Major benefits of butyrate

Potential alternative to antibiotics (gut health):

- Anti-inflammatory activity (more energy for growth)
- Antimicrobials: control of enteric bacterial infection
- ↑Animal growth performance (mainly on young animals)
- Histone deacetylase inhibitor



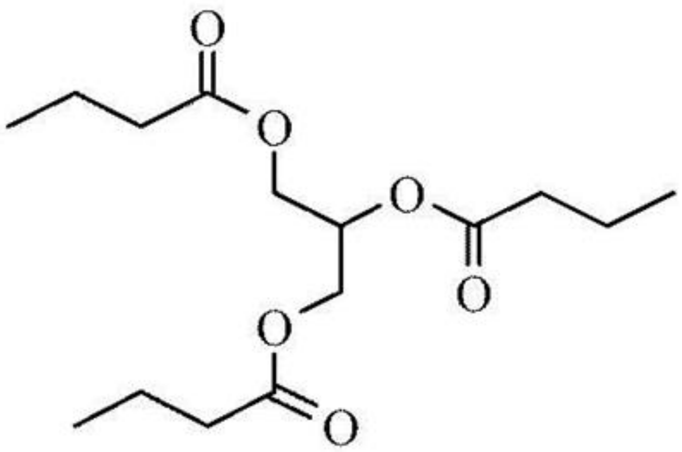


University of Manitoba

Limitations and solutions of butyrate in application

Limitations:

- Offensive odour
- Absorbed to the upper gut



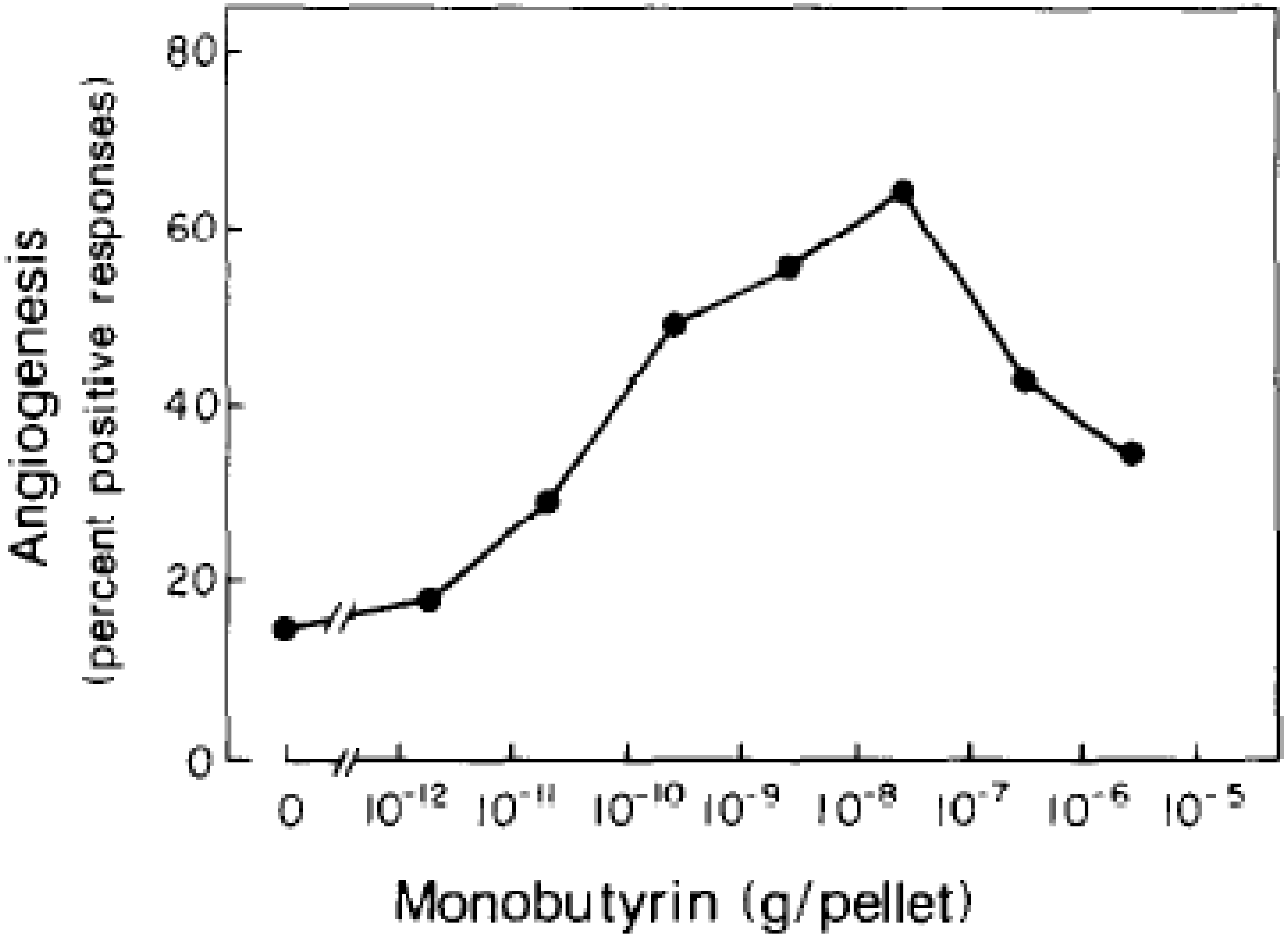
Tri-butyrin

Solutions:

- Sodium butyrate
- Encapsulated butyrate
- Butyrate glycerides (released by lipase)

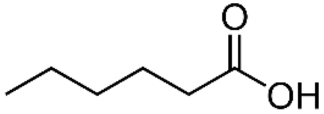
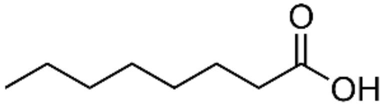
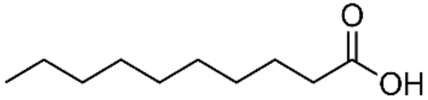
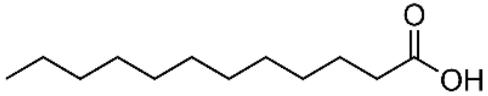
University of Manitoba

Angiogenic activity of synthetic and endogenous monobutyrin



(Dobson et al., 1990; Cell)

Molecular structure and physicochemical properties of medium chain fatty acids used in pig feeds

Name	Systemic name	Formula	Skeletal structure	Melting point (° C)	Boiling point (° C)	Density g/mL
Caproic acid	Hexamoic acid	$C_6H_{12}O_2$		-3.4	205.8	0.929
Caprylic acid	Octanoic acid	$C_8H_{16}O_2$		16.7	239	0.910
Capric acid	Decanoic acid	$C_{10}H_{20}O_2$		27-32	268-270	0.893
Lauric acid	**Dodecanoic acid**	$\mathbf{C_{12}H_{24}O_2}$		**43.8**	**225-297**	**1.007**

(Omonijo et al., 2018)

University of Manitoba

Minimal inhibitory concentrations of 15 fatty acids

Compound	Pneumococci	Streptococcus group A	Streptococcus beta-hemolytic non-A	Corynebacteria	*Nocardia asteroides*	Micrococci	*Candida*	*S. aureus*	*S. epidermidis*	Streptococcus group D
Caproic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Caprilic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Capric	1.45	1.45	2.9	1.45	1.45	2.9	2.9	2.9	2.9	5.8
Lauric	0.062	0.124	0.249	0.124	0.124	0.624	2.49	2.49	2.49	2.49
Myristic	0.218	0.547	2.18	0.437	0.547	0.547	4.37	4.37	2.18	4.37
Myristoleic	0.110	0.110	0.110	0.055	0.110	0.220	0.552	0.441	0.441	0.441
Palmitic	0.48	3.9	3.9	1.9	NI	1.9	NI	NI	3.9	NI
Palmitoleic	0.024	0.098	0.049	0.049	0.049	0.049	0.491	0.983	0.491	0.491
Stearic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Oleic	NI	1.77	NI	NI	NI	NI	NI	NI	NI	NI
Elaidic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Linoleic	0.044	0.089	0.089	0.044	0.089	0.089	0.455	NI	NI	NI
Linolenic	0.179	0.35	0.35	0.179	0.448	0.448	NI	1.79	NI	NI
Linolelaidic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Arachidonic	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

[a] Results are given in micromoles per milliliter. NI = not inhibitory at the concentrations tested (1.0 mg/ml or 3 to 6.0 μmoles/ml).

(Kabara, et al., 1972. Antimicrobial Agents and Chemotherapy)

University of Manitoba

Minimal inhibitory concentrations of free fatty acids when compared with glycerol forms

Compound	Pneumo-cocci	Strep-tococcus group A	Strep-tococcus beta-hemolytic non-A	Coryne-bacteria	*Nocardia asteroides*	Micro-cocci	*Candida*	*S. aureus*	*S. epider-midis*	Streps tococcu- group D
Capric acid...	1.45	1.45	2.9	1.45	1.45	2.9	2.9	2.9	2.9	5.8
1-Mono-caprin......	0.1	0.2	0.2	0.2	0.5	0.1	1.0	1.0	1.0	2.0
1,3-Dicaprin..	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Lauric acid...	0.062	0.124	0.249	0.124	0.124	0.624	2.49	2.49	2.49	2.49
1-Mono-laurin......	0.09	0.045	0.09	0.045	0.09	0.09	0.09	0.09	0.09	NI
1,3-Dilaurin..	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Trilaurin.....	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

[a] Results are given in micromoles per milliliter. NI = no inhibition at the concentrations tested.

(Kabara, et al., 1972. Antimicrobial Agents and Chemotherapy)

University of Manitoba

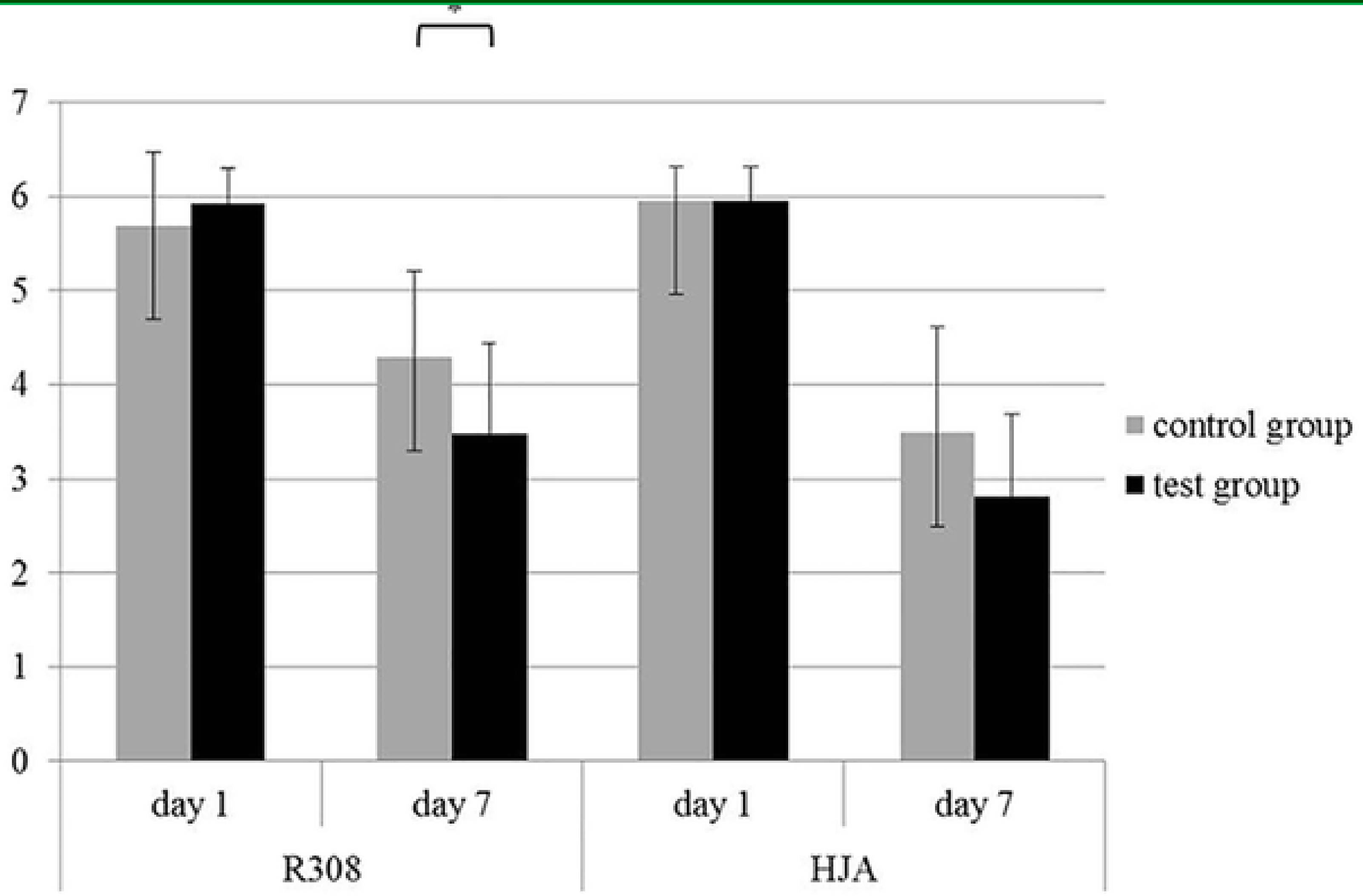
Antibacterial activity of fatty acids against *C. difficile*

	Number of carbon backbone	General name	MIC (mg/ml)
SCFAs	C3	Propionic acid	1.25
	C4	Butyric acid	25
	C4	Isobutyric acid	1.25
	C5	Valeric acid	1.25
	C5	Isovaleric acid	2.5
MCFAs	C6	Hexanoic acid	1.25
	C8	Octanoic acid	2.5
	C10	Capric acid	0.63
	C12	**Lauric acid**	**0.31**
LCFAs	C14	Myristic acid	>10
	C16	Palmitic acid	10

(Yang et al., Front Microbiol. 2017; 8: 2635)

University of Manitoba

Lauric acid reducing *Campylobacter spp.* in broiler meat



Broilers fed with 5% commercial fat addition (control group) and broilers fed with 5% supplement of fat with high lauric acid content (test group).

Zeiger K, Popp J, Becker A, Hankel J, Visscher C, et al. (2017) Lauric acid as feed additive – An approach to reducing Campylobacter spp. in broiler meat. PLOS ONE 12(4): e0175693.

University of Manitoba

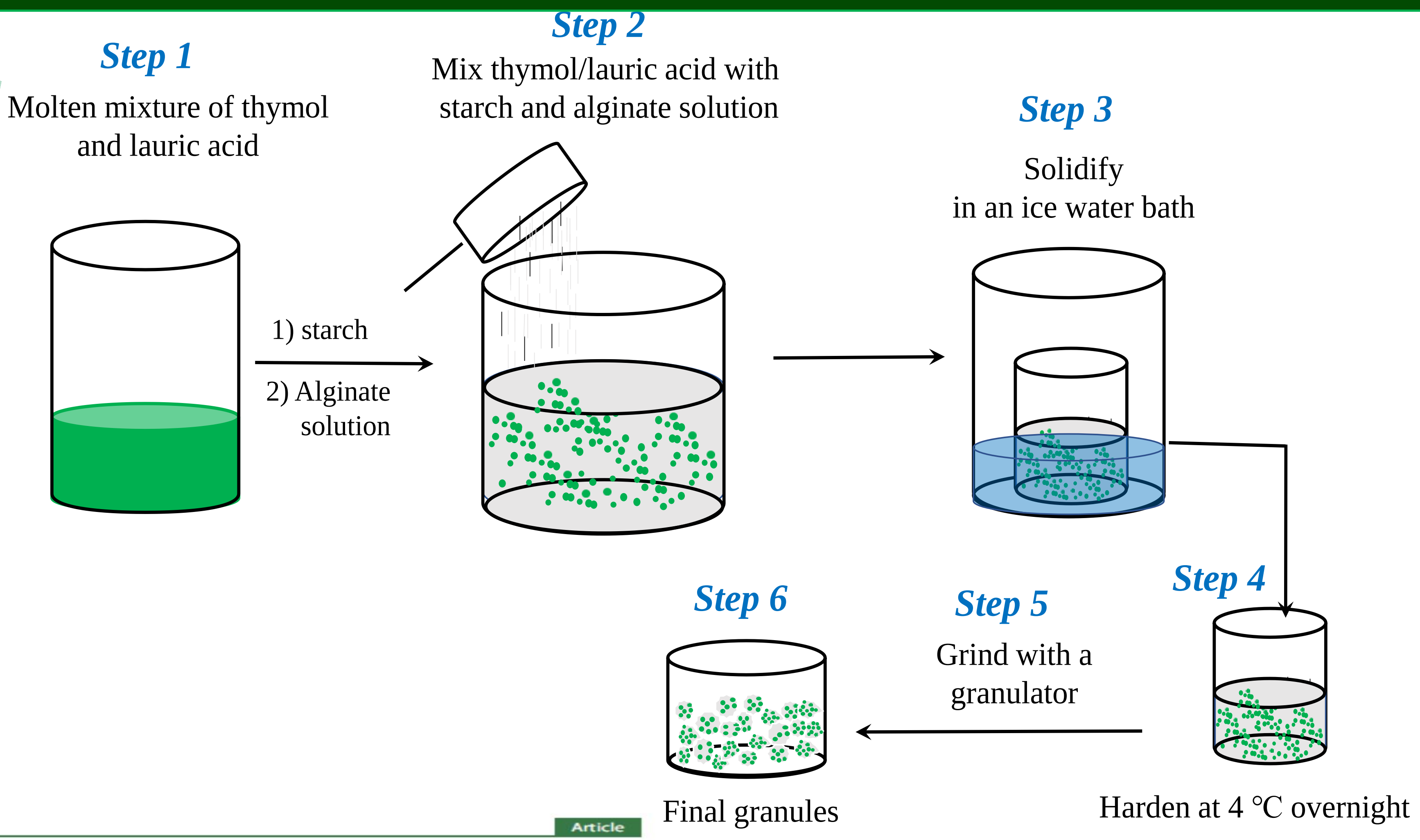
Performance of broiler chickens fed with an experimental diet containing glycerol monolaurate (GML) to replace antibiotic drugs

Variables	Days	T0	T100	T200	T300	*P* value
Weight (g)	1	46.8	48.3	47.7	48.6	0.963
	7	137.9	134.2	135	140.2	0.742
	14	404.4	392	392.2	399.9	0.654
	21	832.9	844.5	800.6	838	0.429
	35	2032.9	2200.6	2153	2204.6	0.175
	42	2598.6b	2756.4ab	2755.6ab	2925.0a	0.001
Feed consumption (g)	42	4196.3	4351.9	4240.5	4422.9	0.098
Weight gain (g)	**42**	**2551.8b**	**2708.1ab**	**2707.9ab**	**2876.5a**	**0.001***
Daily weight gain (g)	**–**	**61.8b**	**65.6ab**	**65.6ab**	**69.6a**	**0.001**
Feed conversion		**1.64a**	**1.60ab**	**1.56ab**	**1.54b**	**0.036**
Mortality (%)	1 to 42	0	0	1.69	5	–

(Fortuos et al., 2019)

University of Manitoba

Production of thymol and lauric acid microparticle



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 9608−9615

pubs.acs.org/JAFC

Development of Novel Microparticles for Effective Delivery of Thymol and Lauric Acid to Pig Intestinal Tract

Faith A. Omonijo,[†] Seungil Kim,[‡] Tracy Guo,[∥] Qi Wang,[∥] Joshua Gong,[∥] Ludovic Lahaye,[⊥] Jean-Christophe Bodin,[⊥] Martin Nyachoti,[†] Song Liu,[‡,§] and Chengbo Yang*,[†]

PCT/CA2019/050599. 2019/05/06

Pictures of the molten mixture of thymol and FAs (lauric acid, stearic acid and palmitic acid respectively)

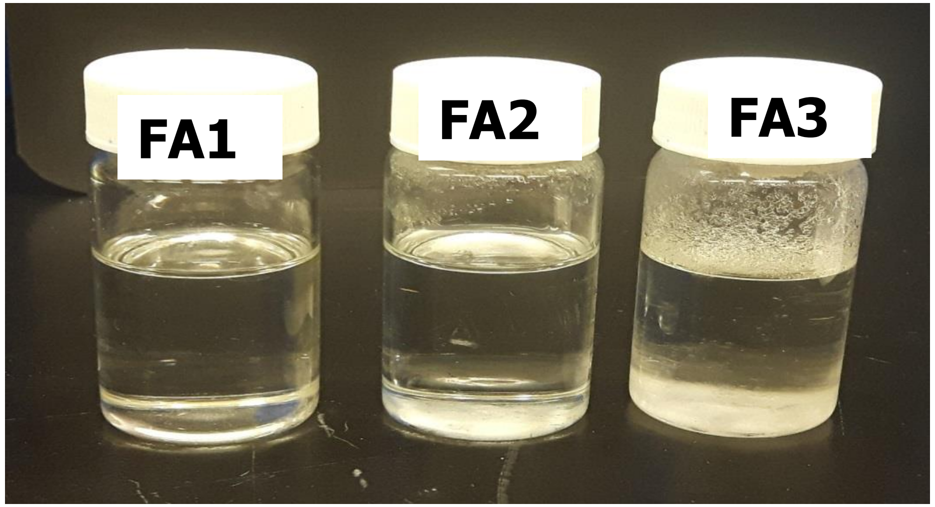


Fig.1. Picture of thymol and FAs mixture set at room temperature at 0 min

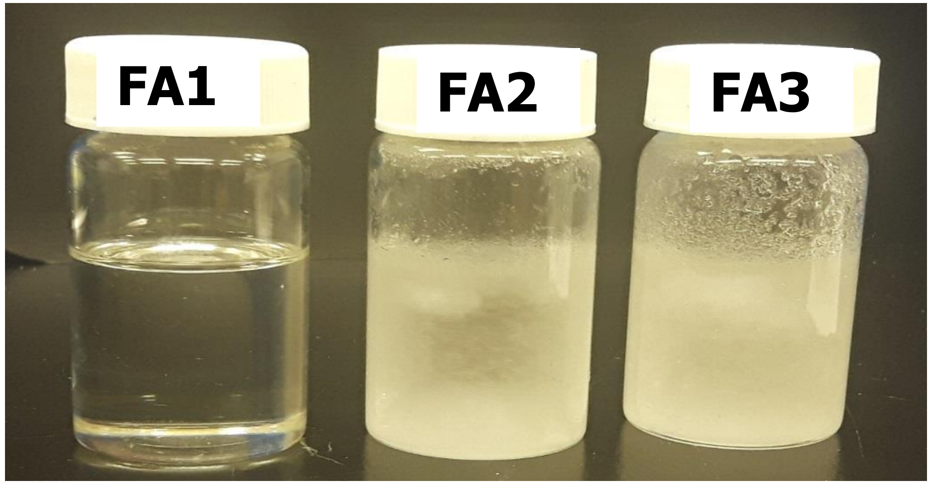


Fig.2. Picture of thymol and FAs mixture set at room temperature at 6 hrs.

At 0 minute:

- no phase separation occurred between the mixture.

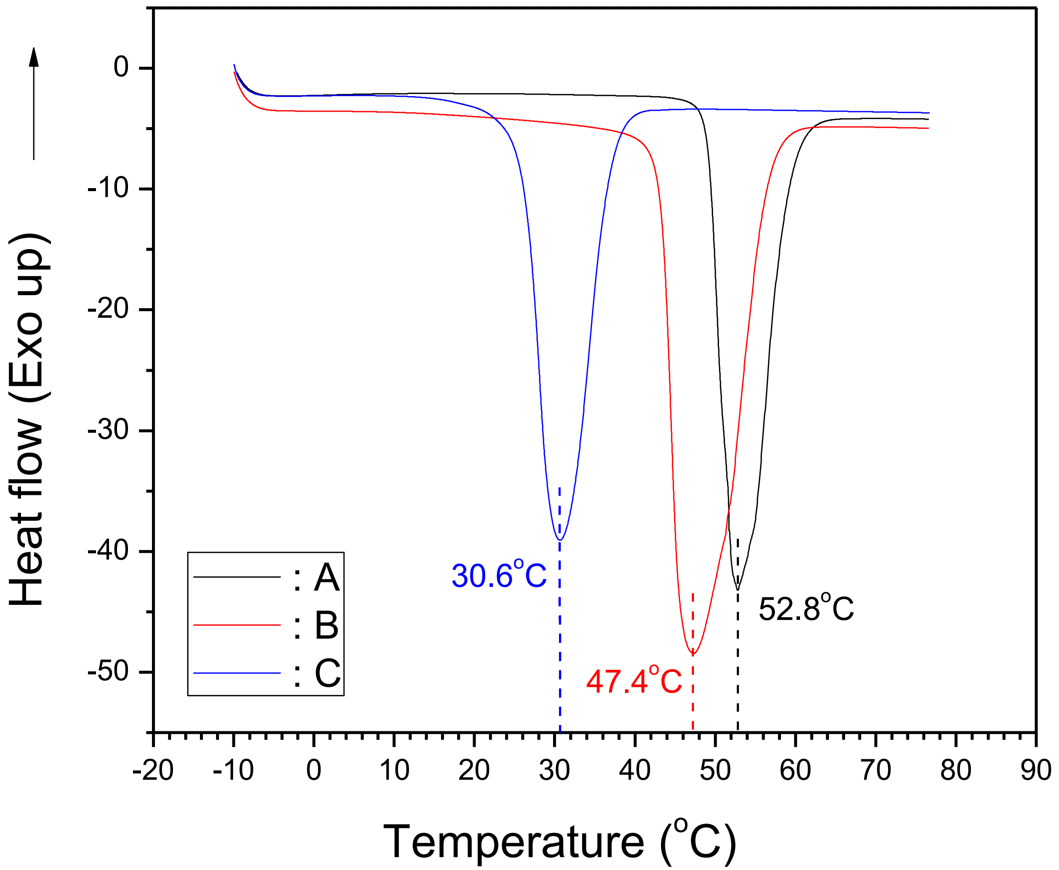
After 6 hours:

- Lauric acid and thymol were in liquid state without phase separation
- FA2 and FA3 solidifies.

(Omonijo et al., 2018)

University of Manitoba

Differential scanning calorimetry (DSC) of (A) thymol, (B) lauric acid, and (C) Mixture of thymol and lauric acid (50: 50wt%). 2nd run with heating rate 10 °C/min from -10 °C to 80 °C.

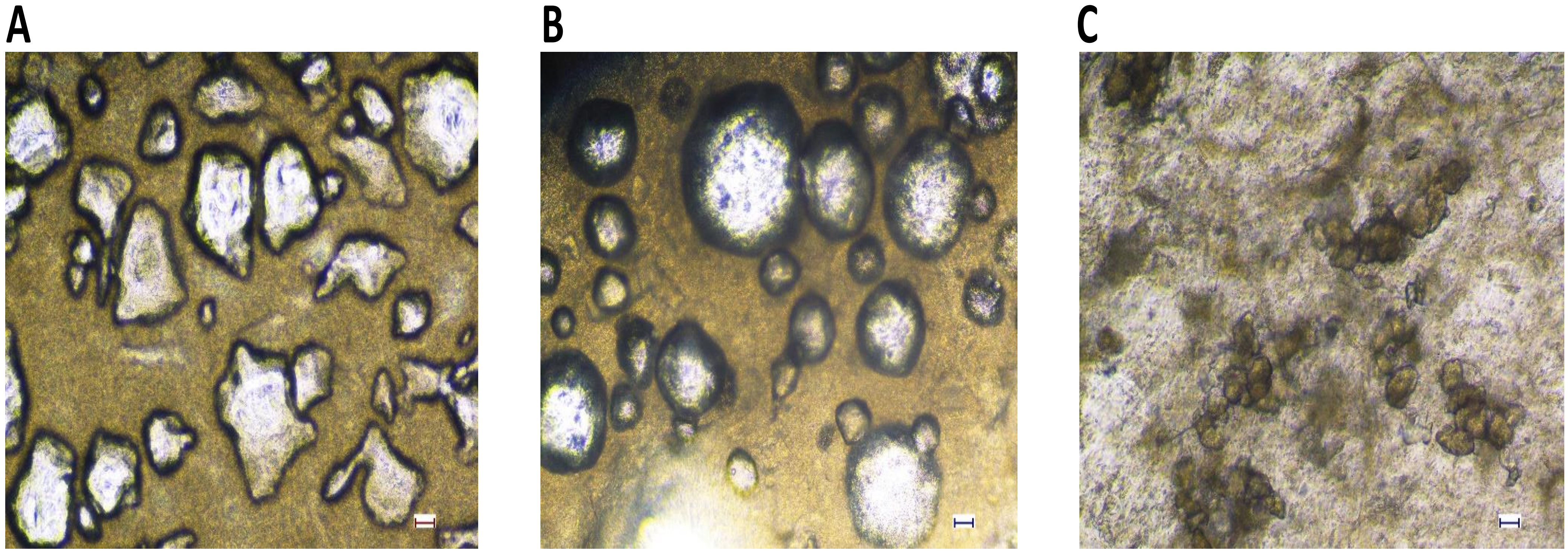


- The melting point of thymol and lauric acid mixture reduced drastically as shown in the graph.
- Explains their eutectic mixture because of their miscibility in liquid state and immiscibility in solid state.

(Omonijo et al., 2018)

University of Manitoba

Morphology of crystals of thymol (A) and lauric acid (B) and a mixture of thymol and lauric acid (C) after crystallization

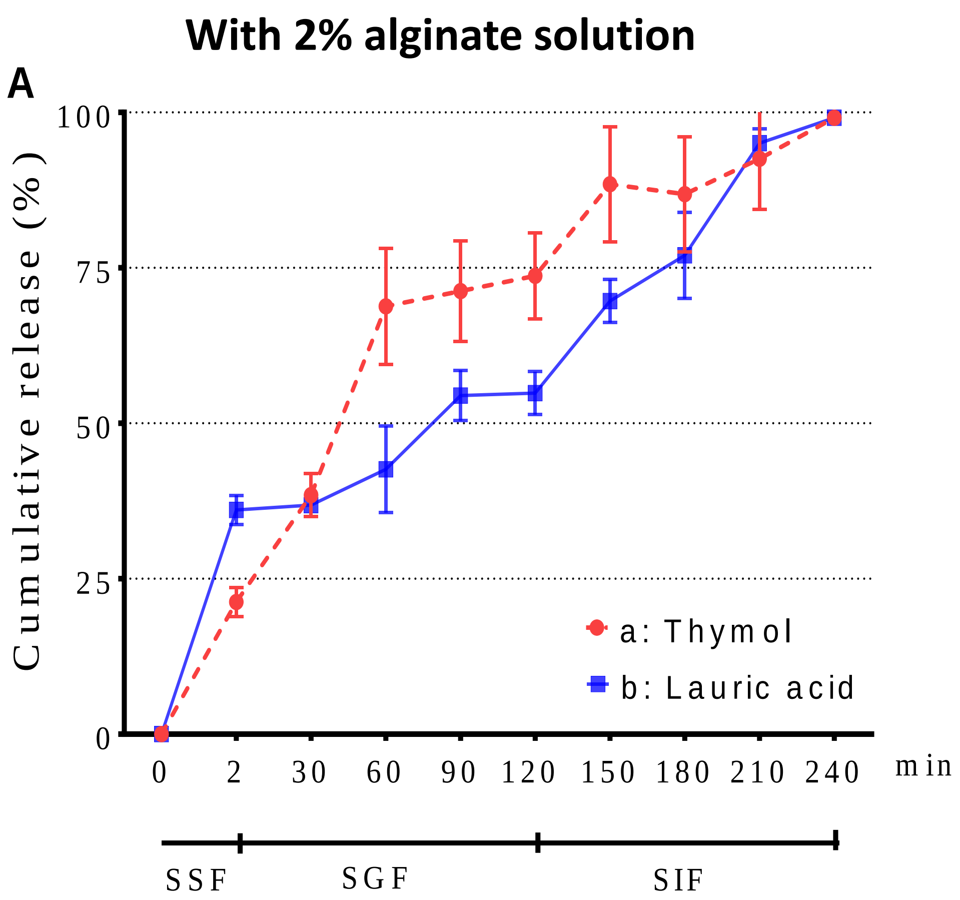


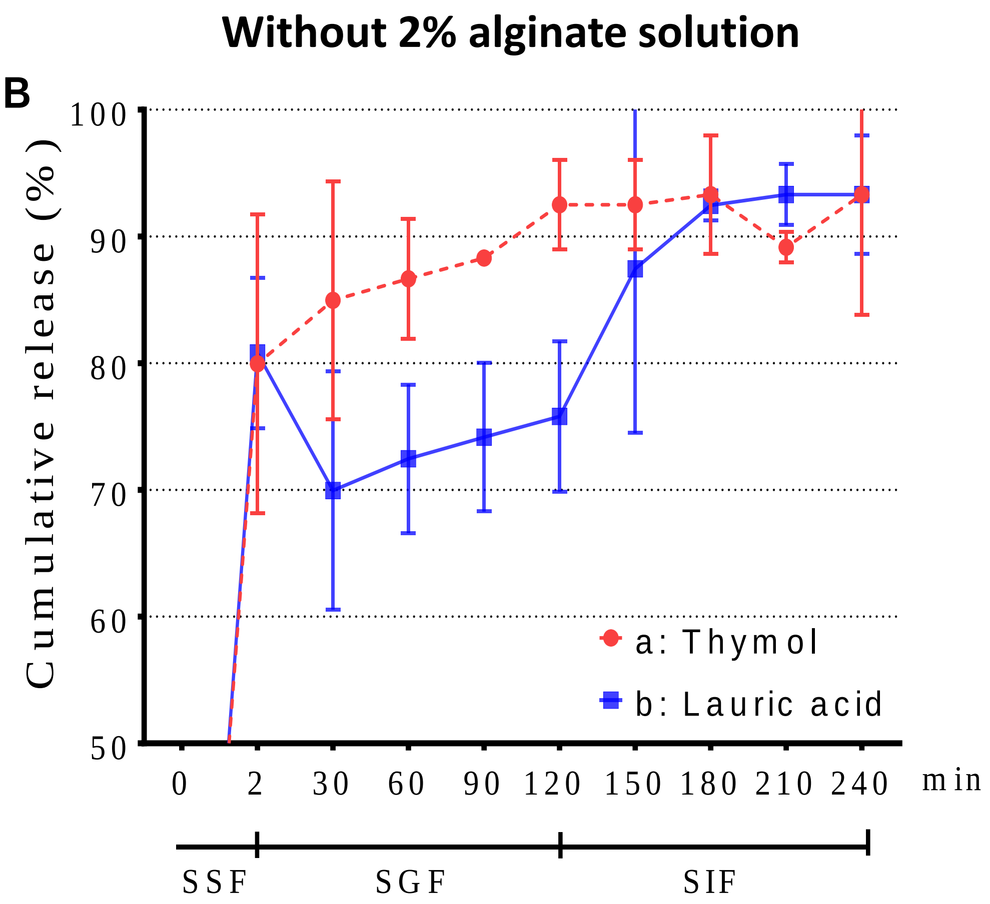
- Thymol crystalized in irregular shapes.
- Lauric acid crystalized in round shapes.
- The mixture crystalized into somewhat ovular shaped particles.
- No visible distinctions between the two individual components.

(Omonijo et al., 2018)

University of Manitoba

In vitro release of thymol and lauric acid from microparticles with/without 2% alginate solution





SSF: Simulated salivary fluid
SGF: Simulated Gastric Fluid
SIF: Simulated Intestinal Fluid

- Microparticles with 2% alginate exhibited slow release compared with microparticles without 2% alginate solution

(Omonijo et al., 2018)

University of Manitoba

Conclusions

- **The antimicrobial activity of organic acids is related to environmental pH;**
- **Manipulating dietary acid-binding capacity using organic acids and selecting proper ingredients is considered a possible approach to reduce the use of antibiotics;**
- **Butyrate can improve gut development, gut integrity and immunity;**
- **Protected organic acids or butyrate glycerides can avoid offensive odour;**
- **Monobutyrate glycerides may represent a therapeutic opportunity for stimulating the growth of intestinal tissue through its angiogenic activity in food-producing animals, especially when there are wounds or damages in the intestinal epithelia;**
- **Lauric acid and its derivatives can be used as antibiotic alternatives in livestock production.**

University of Manitoba

Acknowledgments

Current grant support

Fundamental Research

NSERC CRSNG

- -Discovery Program
- -CRD program
- -Engage program
- -Early Career Researchers (ECR) Supplement

INNOVATION.CA
CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

Seed grants

University of Manitoba

- -Start-up Funds
- -UGRP
- -UCRP

Applied Research

Manitoba Pork

Jefo

Wallenstein Feed & Supply Ltd.

Manitoba Egg Farmers

Topigs Norsvin

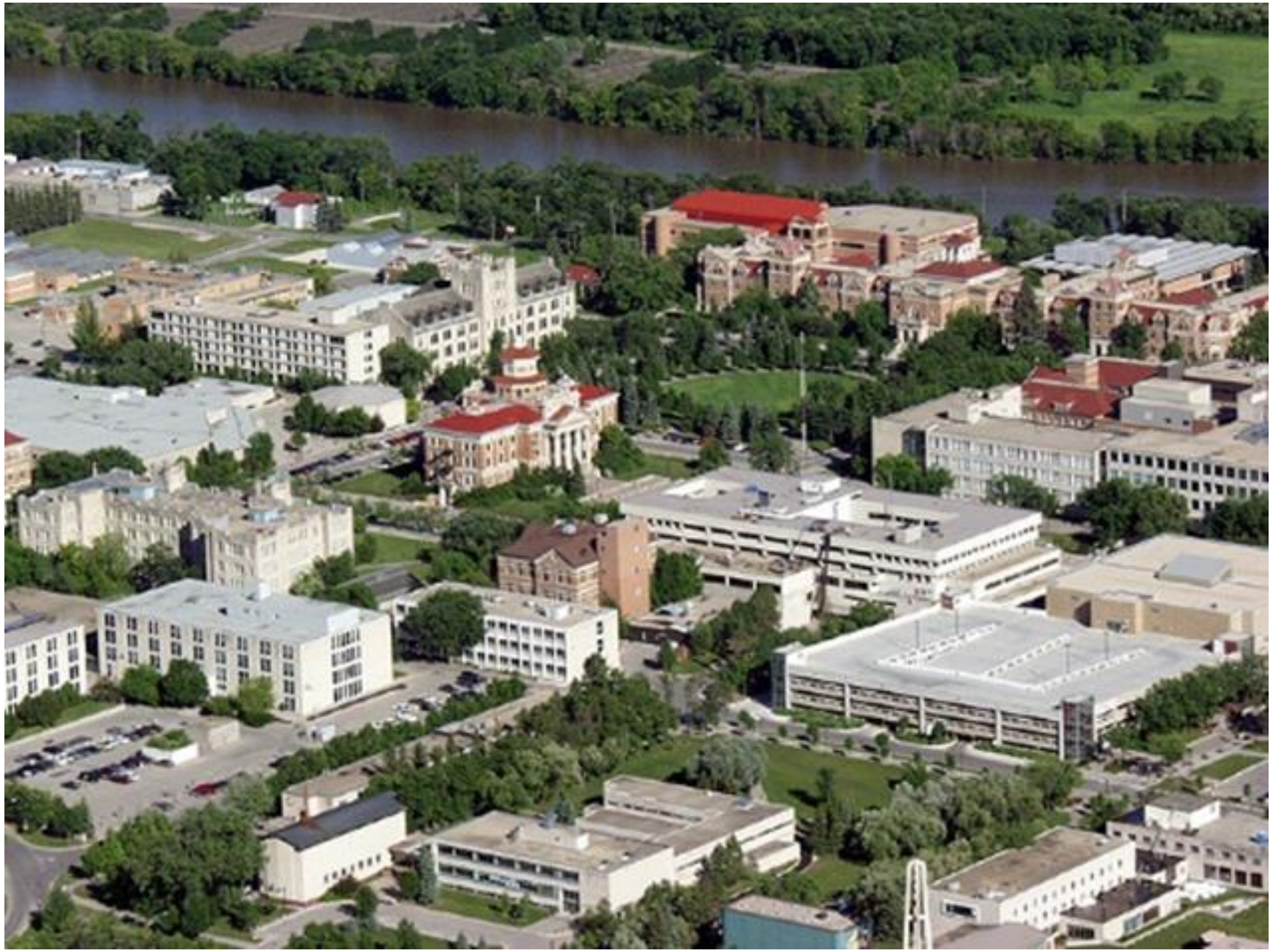
Swine Innovation Porc

Evonik Industries

Acadian Seaplants

Acknowledgments

Thank you



University of Manitoba