WPSA – SILO MEETING

October 8,2010

Ozzano dell'Emilia

Molecular structure and mode of action of Monobutyrin

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Up-dated May. 2010

PRODUCTS LIST

AVAILABLE PRODUCTS, BASED ON:

- MONOPROPIONIN → Butterfly 2010
- MONOBUTYRIN ⟶ Hydro C4 – 30
- MONOCAPRYLIN ⟶ Energy COCO-8
- MONOCAPRIN ⟶ Energy COCO-10
- MONOLAURIN ⟶ Energy COCO-12

PRODUCTS already manufactured on SILO pilot plant

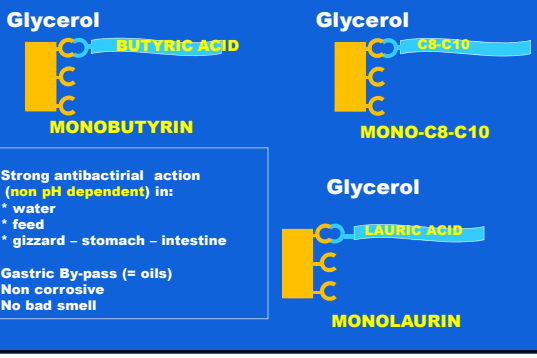
- MONOFORMIN ⟶ Force 10
- MONOACETIN ⟶ Aceto Sweet

PRODUCTS "in fieri..."

- MONOFUMARIN
- MONOLACTIN
- MONOBENZOIN

Patent pending

ESTERIFICATION OF DIFFERENT MONOGLYCERIDES



- Strong antibactirial action (non pH dependent) in:
  - * water
  - * feed
  - * gizzard – stomach – intestine
- Gastric By-pass (= oils)
- Non corrosive
- No bad smell

MONOBUTYRIN HYDRO C4 - 30

HYDRO C4 POWDER C4

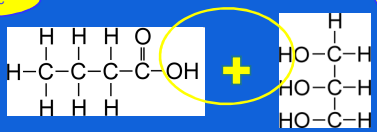
Mix of Monobutyrin + Free Glycerol

LIQUID & POWDER

MONOBUTYRIN

GLYCEROL + BUTYRIC ACID = BUTYRIC ACID MONOGLYCERIDE

lipophilic hydrophilic



Undissociated Butyric acid Molecular weight 88

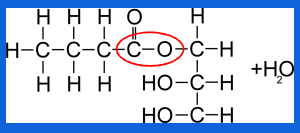
Glycerol Molecular weight 92

The Hydrogen atom (H) of glycerol is bound with the (OH) of the butyric acid

MONOBUTYRIN

A new molecule!!

Molecular weight 162



Covalent bond

Covalent bond = force of attraction between 2 atoms that share a couple of electrons

The chemical bond but. Acid / glycerol is not a ionic bond (pH dependent), but a covalent bond (pH independent)

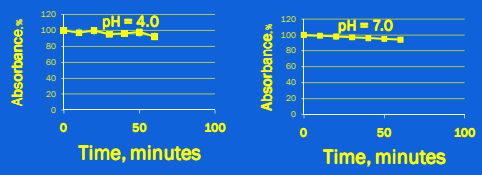
MONOBUTYRIN

Characteristics of the chemical bond butyric acid+glycerol:

- Covalent bond (the attraction force between 2 atoms that share a couple of electrons)
- Very stable (up-to 230° C)
- Stability non-pH dependent (practically no problem of dissociated or undissociated form)
- Odorless

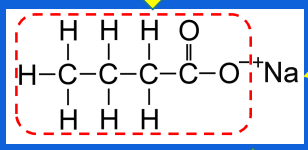
MONOBUTYRIN

STABILITY AT VARIOUS pH VALUES



SODIUM BUTYRATE

pH 7= Dissociated butyric acid



Ionic bond = attraction force between ions charged with opposite signes (+;-)

pH dependent

NON PROTECTED SODIUM BUTYRATE

Non protected sodium butyrate is absorbed very quickly at the level of crop and gizzard

DETERMINATION OF NON PROTECTED SALTS (ACIDS) IN THE GIT

Inclusion in the feed%		Acetic(*)	Propionic(*)	Butyric(*)
		2,4	2,4	2,6
Crop (anathomic part)		99%	40%	60%
Small intestine sections				
Section 1		0	2% (of starting amount= 0,05%)	0,5% (of starting amount= 0,01%)
Section 2		0	1,7% (of starting amount = 0,04%)	0
Section 3		0	0	0
Section 4		0	0	0

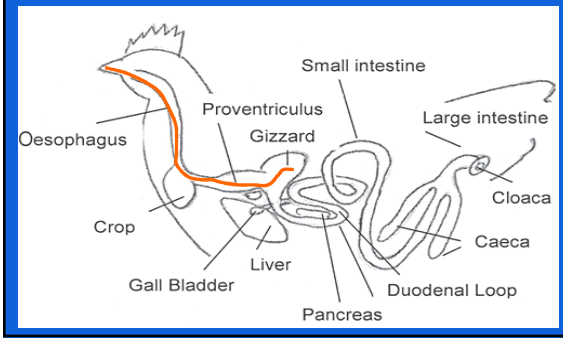
(*)Administered in form of Ca salts

Adapted from "The digestibility of acetic, propionic and butyric acids by the fowl" – Bolton and Dewar 1965

(*) Sodium or calcium butyrate

Na Butyrate – Unprotecte

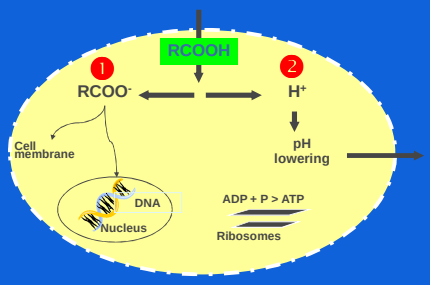
Region where product is a



LIMITS OF PROTECTED SODIUM BUTYRATE

- **If the Sodium Butyrate is protected (micro-encapsulated) it is buffered in the gut by calcium carbonate.**
- **At pH 6 – 7 (pH of the gut) Sodium Butyrate is present in dissociated form losing its antibacterial efficacy**

ONLY UNDISSOCIATED ACIDS KILL BACTERIA



R-COOH = undissociated acid

pH EFFECT ON DISSOCIATION RATE OF ORGANIC ACIDS

		Ratio dissociated: non dissociated		
Acid type	**pK_a**	**pH 4**	**pH 6**	**pH 7**
Formic acid	3.75	1.8:1	178:1	1778:1
Lactic acid	3.83	1.5:1	148:1	1479:1
Benzoic acid	4.19	0.6:1	65:1	646:1
Butyric acid	4.86	0.1:1	15:1	138:1
Propionic acid	4.88	0.1:1	13:1	132:1

Conclusion: organic acids are most active as antimicrobials at low pH

Minimum inhibitory concentrations

Organism	Acid	uM Undissociated	uM Dissociated
E. Coli	Propionic	70	800
Staf. Aureus	Propionic	20	93-200
B. Cereus	Propionic	17	380
E. Coli	Sorbic	1	100-350
Staf.Aureus	Sorbic	0,6	400
Listeria	Lactic	5	1.250

Adapted from Presser *et al.* (1997)

F. Boyen – F.Van Immerseel - Veterinary Microbiology 2007

Overview of the MIC values of formic acid, acetic acid, butyric acid, tested at pH4, 5, 6 using 54 porcine **SALMONELLA TYPHIMURIUM** strains.

	Number of strains with MIC of (mM)										
	0,625	1,25	2,5	5	10	20	40	80	160	320	640
Formic Acid											
pH4		9	45								
pH5							54				
pH6										54	
Acetic Acid											
pH4				54							
pH5						3	51				
pH6									1	48	5
Propionic Acid											
pH4				22	32						
pH5						20	34				
pH6								1	49	4	
Butyric Acid											
pH4	1	3	1	49							
pH5					8	46					
pH6									53	1	

- **Comment to the previous slide:**
- **At pH 4, 5 mM butyric acid, correspondending to 0.04% in water, are sufficient to obtain the MIC on 49 strains of Salmonella Typhymurium**
- **At pH 6, 160 mM butyric acid, correspondending to 1.4% in water, are needed to obtain the MIC on 53 strains of Salmonella Typhymurium**
- **In conclusion, at pH 6 an amount of butyric acid 32 times higher than that at pH 4 is necessary**

MONOGLYCERIDES

NEW IDEAS TO APPROACH THE PROBLEM??

MONOGLYCERIDES proved to have a significantly stronger microbicidial action than relevant acid

MONOBUTYRIN & MONOPROPIONIN

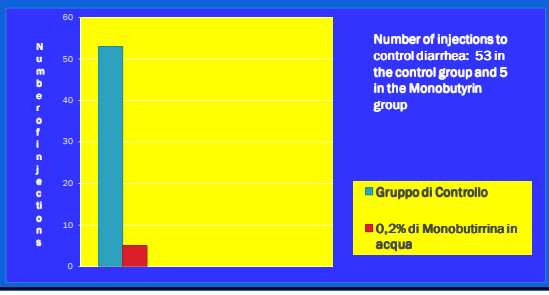
Istituto Zooprofilattico Lombardia - Emilia Romagna "Bruno Ubertini"
Brescia – dr. L. Alborali

MIC DETERMINATION – Dilution expressed as % in water				
	pH	*S. typhimurium*	*S.choleraesuis*	*E. coli O 157*
Monobutyrin Hydro – C4	7	0.06%	0.12%	0.12%
Monopropionin Hydro - C3	7	0.03%	0.12%	0.06%

FARM TRIAL WITH PIGS

Istituto Zooprofilattico Lombardia - Emilia Romagna "Bruno Ubertini"
Brescia – dr. L. Alborali – F. Foresti

Observation period: 6/23 – 8/1/2010; age of pigs: from 41 up to 91 d.
678 pigs in the control group and 653 in the Monobutyrin group



MIC OF ACIDS OR GLYCERIDES

Undissociated form

µmoles/ml

Compounds	Pneumo-cocci	Streptococcus Group A	Streptococcus Beta-Hemolitic non- A	Coryneba cteria	Nocardia asteroides	Micrococci	Candida	S. Aureus	S. epidermis
Lauric acid	0.062	0.124	0.249	0.124	0.124	0.624	2.49	2.49	2.49
1-Monolaurin	0.09	0.045	0.09	0.045	0.09	0.09	0.09	0.09	0.09
1-3 Dilaurin	NI	NI	NI	NI	NI	NI	NI	NI	NI
Trilaurin	NI	NI	NI	NI	NI	NI	NI	NI	NI

+27 fold!

J.J. Kabara - 1972

S.Leeson (Guelph University, personal communication)
CLOSTRIDIUM p.

Inoculum = 10^5

Positive Control	ppm	%	Butyric acid	Monobutyrin Hydro C4 – 30
+	500	0.05%	+	+
+			+	+
++			+	+
	1000	**0.1%**	+	**No growth**
			+	No growth
			+	No growth
	1500	0.15%	+	No growth
			+	No growth
			+	No growth
	2000	0.2%	++	No growth
			++	No growth
			++	No growth

Bacteria Growth
+ = 24 h
++ = 36 h
+++ = 96 h

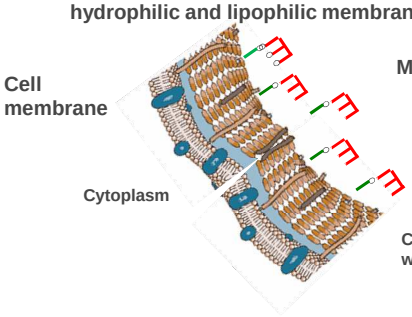
S.Leeson (Guelph University, personal communication)
CLOSTRIDIUM p.

Inoculum = 10^5

Positive Control	ppm	%	Butyric acid	Monobutyrin Hydro C4 – 30
+	2500	0.25%	++	No growth
+			++	No growth
++			++	No growth
	3000	0.3%	No growth	No growth
			No growth	No growth
			No growth	No growth
	4000		No growth	No growth
			No growth	No growth
			No growth	No growth
			No growth	No growth

Bacteria Growth
+ = 24 h
++ = 36 h
+++ = 96 h

One of the modes of action of Monobutyrin is the rupture of cell membranes
Compatibility with hydrophilic and lipophilic membranes



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ANOTHER MODE OF ACTION OF MONOBUTYRIN

Inactivation of pathogenicity island in the bacteria DNA

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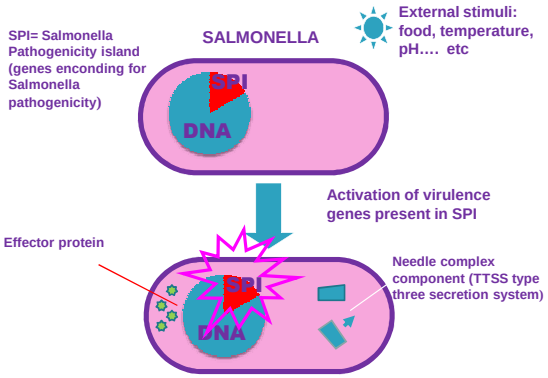
ACTIVATION OF SALMONELLA PATHOGENICITY



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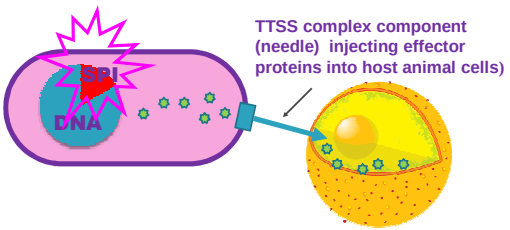

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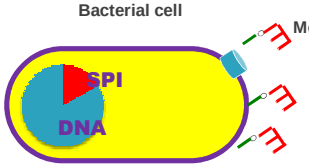
Delivery of Monobutyrin inside the bacterial cell through aquaporins

- Many bacteria can metabolize glycerol as an energy source
- Bacteria possess aquaporins, that is channels designed to absorb glycerol from their surroundings (Glycerol uptake facilitators) (Antonio H. Romano 1990)
- Tactic of theTrojan Horse: employ glycerol as a carrier to transport butyrate into the bacterial cell.



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A non-specific bacterial lipase breacks the gliyerol-butyric acid bond

(R. Gupta et al. 2004)

INACTIVATION OF SALMONELLA PATHOGENICITY

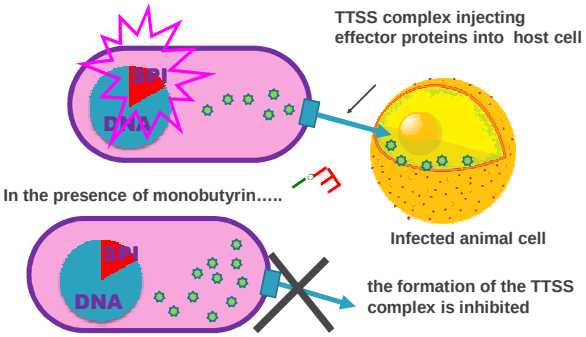
SPI= Salmonella Pathogenicity island

SALMONELLA

Inactivation of SPI genes

Gantois, R. Ducatelle, Butyrate Specifically Down-Regulates Salmonella Pathogenicity Island 1 Gene Expression. Applied and Environmental Microbiology - 2006

The TTSS complex is present in gram negative bacteria: Yersinia, Shigella, Salmonella, Pseudomonas e E. coli



MONOBUTYRIN
ANTIBACTERIAL ACTION

- **Monobutyrin is active at any pH values, from pH 4 to pH 7; it is active in crop, gizzard and intestine conditions**
- **If administered in the drinking water, Monobutyrin activity starts already in the water**

Monobutirrina

Area dove la Monobutirrina è attiva