

# Monoglycerides-

## Their mode of action on Gut Health

Dr. Adriana Barri

Virtual presentation, September 28, 2021

# The importance of knowing the Mechanism of Action of our products



# Performance results are important, but, are they enough?

## Results Commercial Trial A

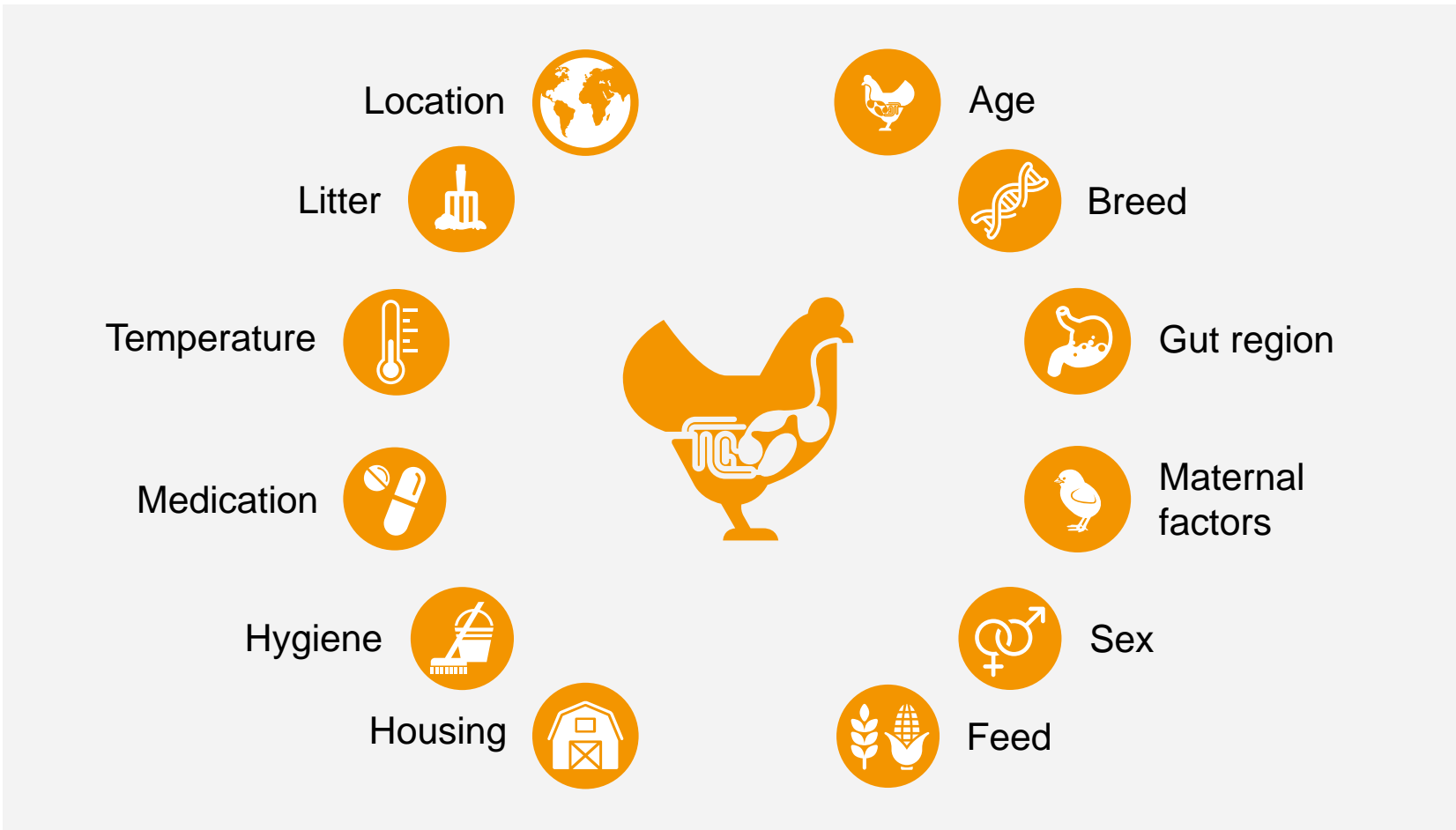
Parameter	Control	BalanGut®	Change
# of chicks (1d old)	22,950	24,200	n/a
# of broilers slaughtered	21,100	22,650	n/a
% mortality	8.20%	6.61%	<b>- 1.59%</b> (- 19 %)
% condemn	1.02%	0.78%	<b>- 0.24%</b> (- 23 %)
Av. final weight per bird (kg)	2.99	3.05	<b>+ 60g</b> (+ 2.2 %)
FCR	1.890	1.845	<b>- 0.045</b> (- 2.4 %)
Adj. FCR**	1.844	1.787	<b>- 0.057</b> (- 3.1 %)

## Results Commercial Trial B

Parameter	Control	BalanGut®	Change
# of chicks (1d old)	212,074	338,800	n/a
# of broilers slaughtered	200,813	324,105	n/a
Day at slaughter	49.21	47.12	<b>- 2.09 d</b> (- 4.2 %)
% mortality	5.31%	4.34%	<b>- 0.98%</b> (- 18 %)
Weight gain per broiler (kg)	2.788	2.815	<b>+ 27g</b> (+ 1.0 %)
ADG (g/d)	56.66	59.75	<b>+ 3.11 g/d</b> (+ 5.5 %)
FCR	1.923	1.873	<b>- 0.05</b> (- 2.6 %)

\*\* Adj. FCR- to mortality and std. BW (2.724kg)

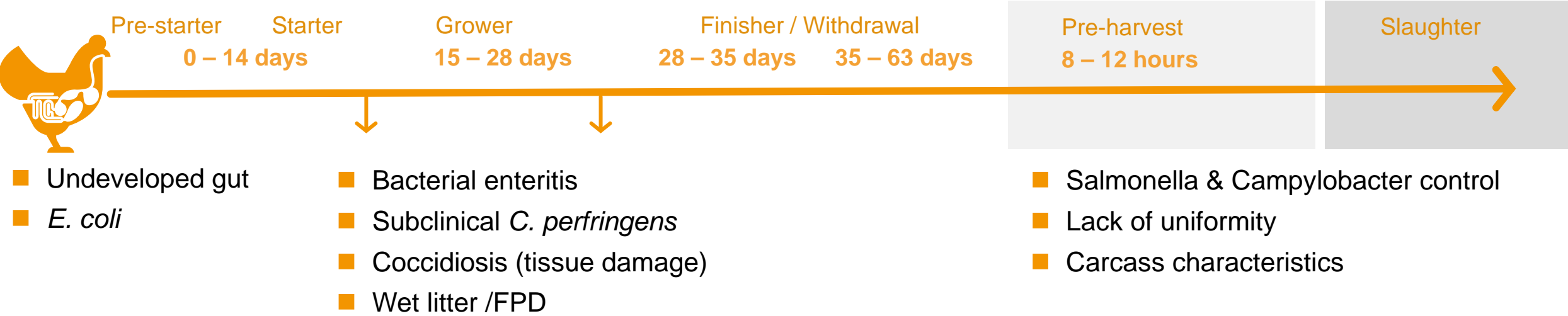
# Animal production is not that simple



- There are **multiple factors** that can affect the health and by consequence the performance of the animals
- Thus, producers **need** recommendations and **tools** to ensure optimal zootechnical performance with economical benefits

# Different challenges throughout the production cycle

## From breeders to broilers

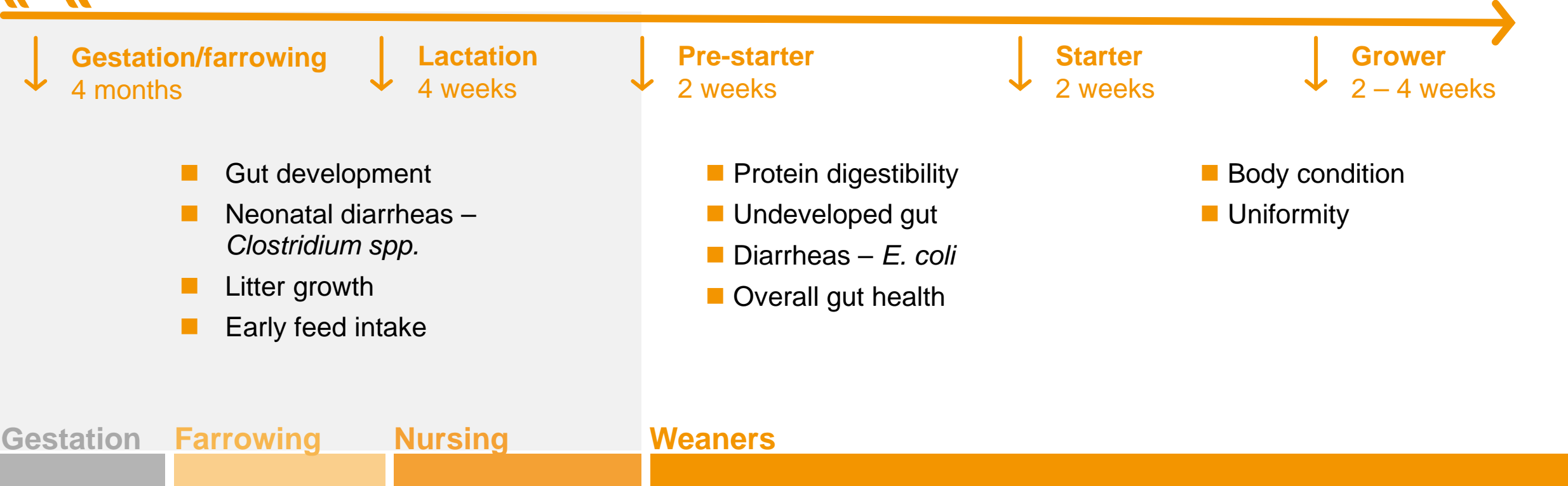
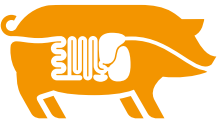


From tissue development & intestinal integrity to performance and control of pathogenic bacteria



# Different challenges throughout the production cycle

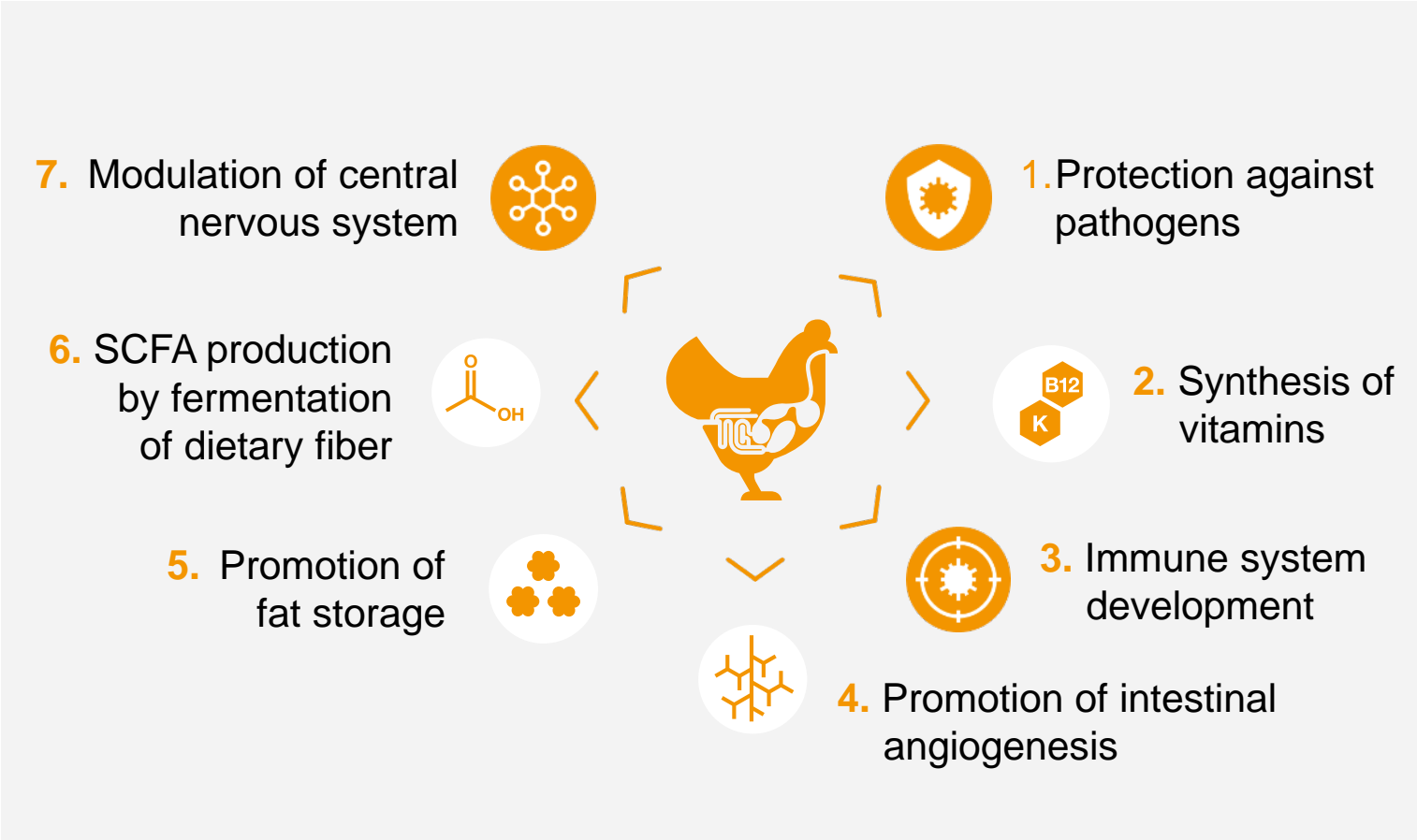
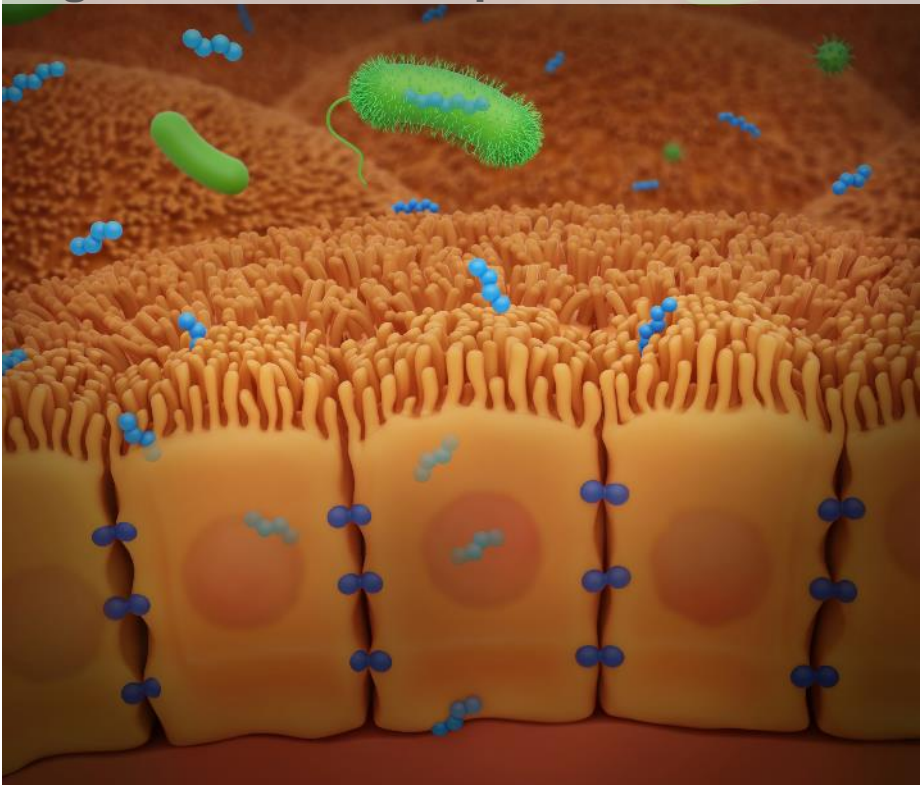
## From sows to fattening pigs



# The gut is more than a digestive organ

## Key for animal health, wellbeing and performance

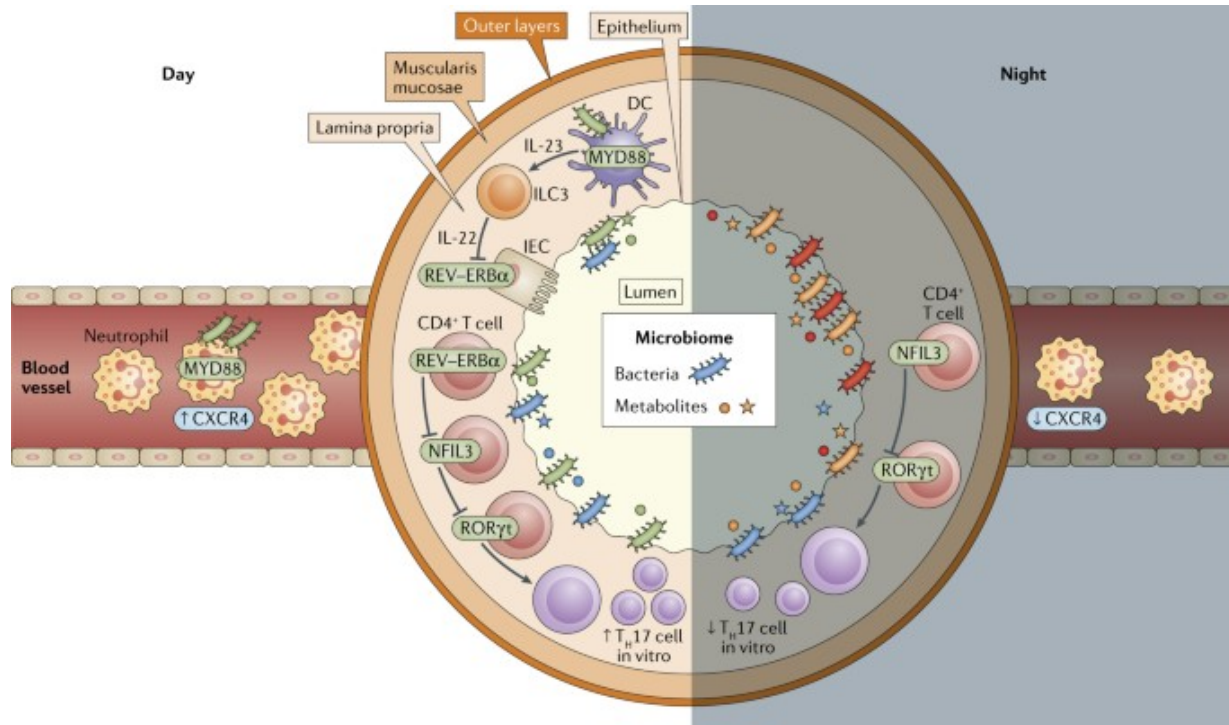
The gut has different functions beyond digestion and absorption of nutrients



Source: [https://commons.wikimedia.org/wiki/File:Microbiota\\_roles.png](https://commons.wikimedia.org/wiki/File:Microbiota_roles.png)

# Chrono-Immunology

Immune cells & molecules not expressed always, nor simultaneously



- Nearly all organisms have adapted their **physiology and behavior** to a daily rhythm.
- **Circadian rhythms** influence virtually all aspects of physiology (from gene expression to organismal behavior).
- Several features of the **immune response** are regulated in a time-of-day dependent manner.

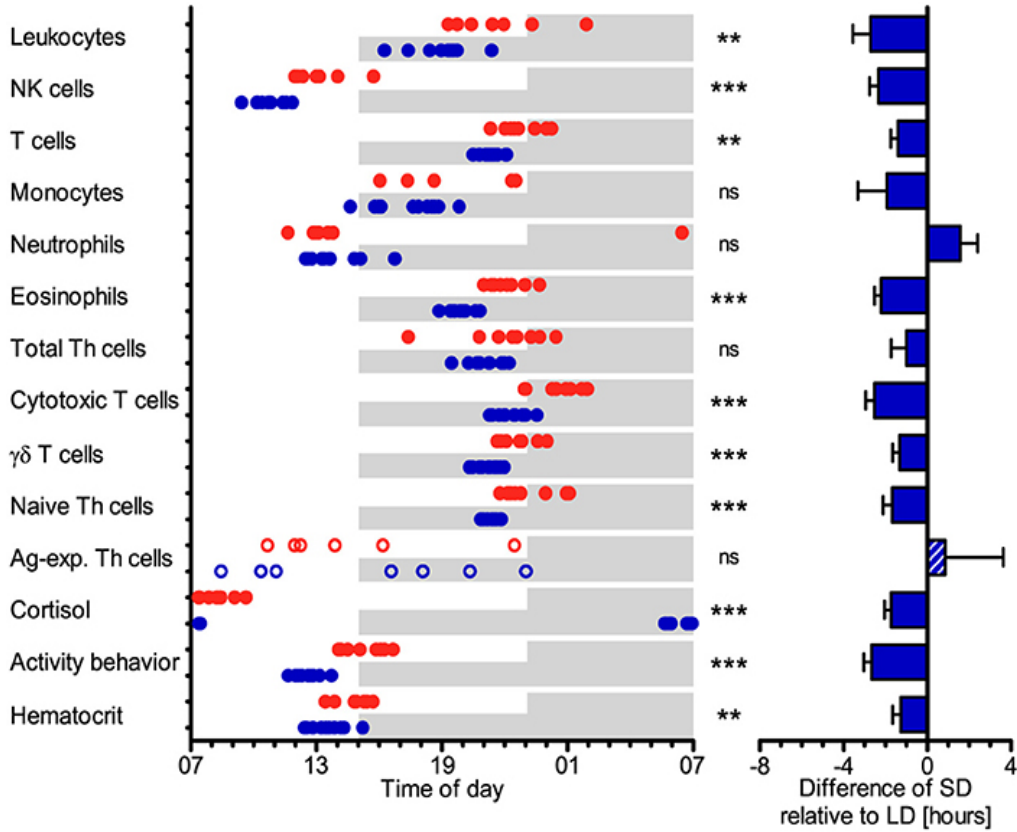
*Nature Reviews Immunology* volume 18, 423–437 (2018)



# Chrono-Immunology

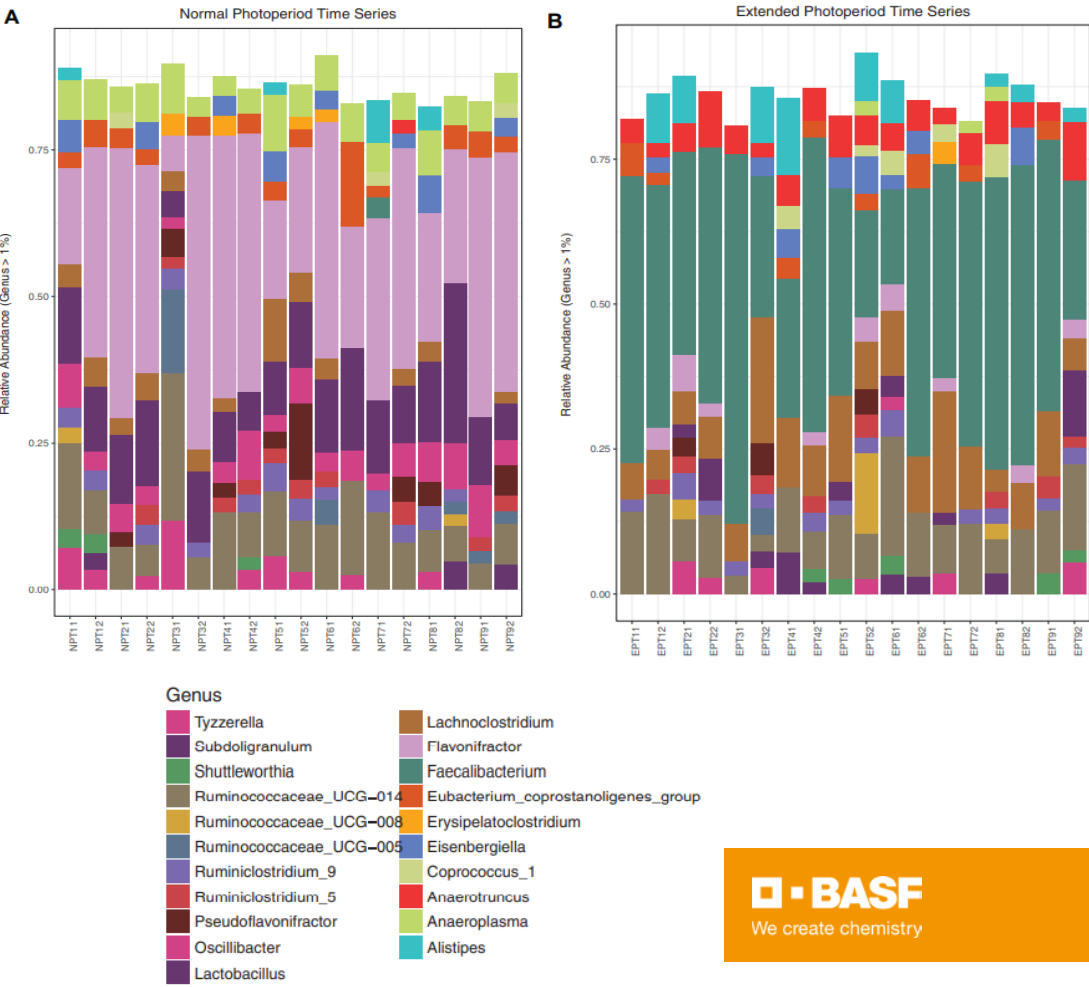
## Critical for immune cell expression & microbiota populations

### 1. Peak times of cell numbers of diff. immune populations in pigs



Engert C. L. *Frontiers in Immunology* 10, 393 (2019)

### 2. Microbiota expression under normal & extended photoperiods in chickens



Hieke A-S.C. et al., *PeerJ Microbiology* (2019)



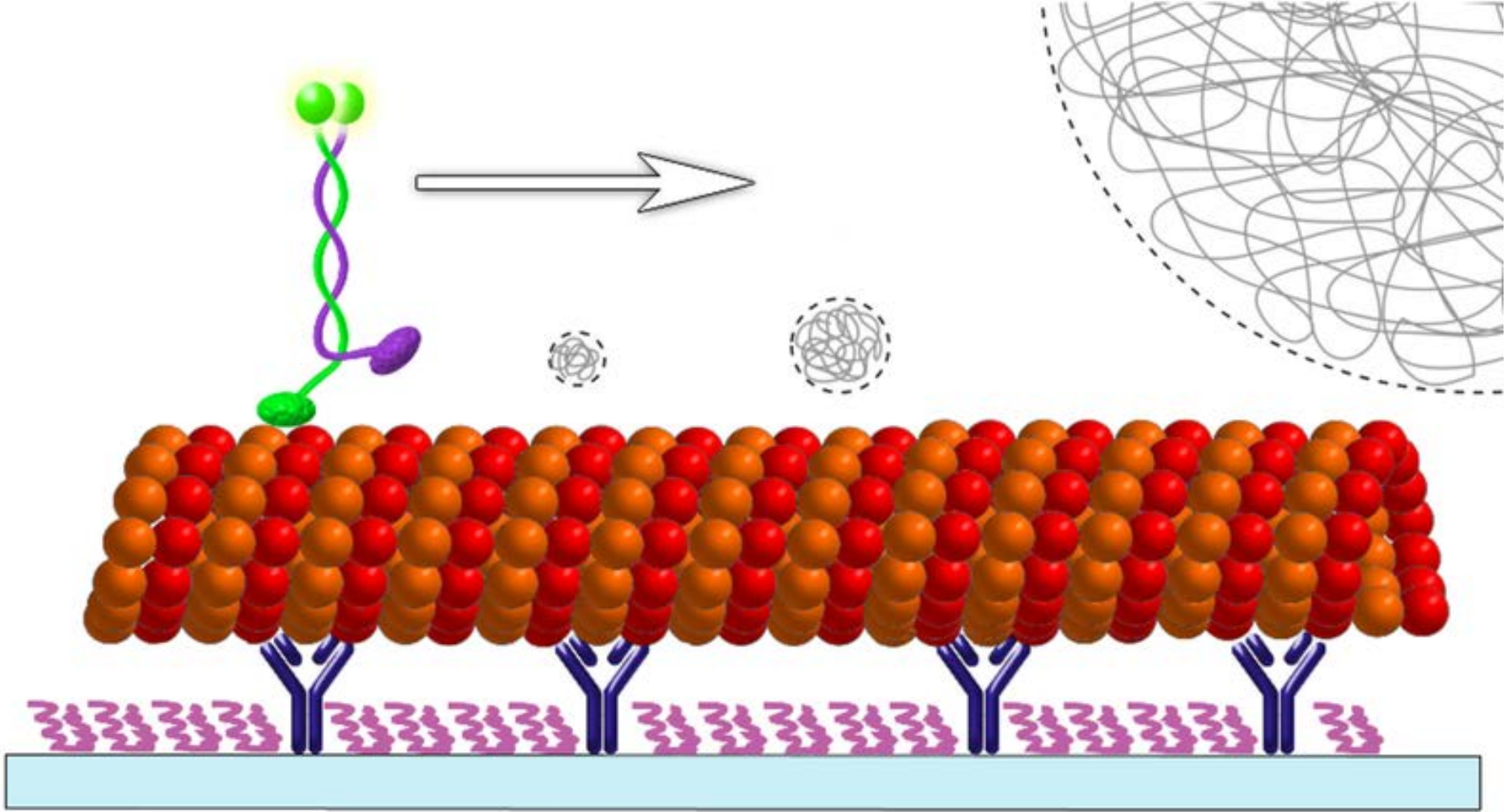
# Urgent need to identify economical solutions through the MoA

- A fully functional immune system is mandatory for
  - Health
  - Welfare
  - Thus, high productivity and safe animal products

Identifying the role of the molecular clock in various gut immune cells as well as the time of peak responsiveness to our products, could have great implications in disease prevention and husbandry practices



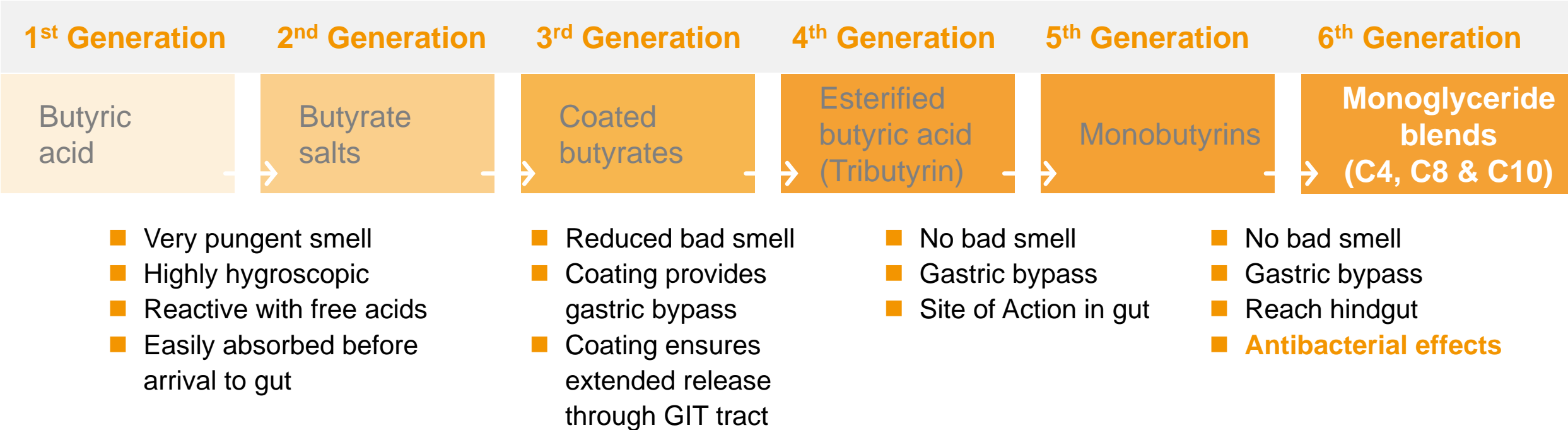
# Moving along



*Physical Review Letters* 115(21) (2015)

# Butyric acid-based products' evolution

## From butyric acid salts to monoglyceride blends



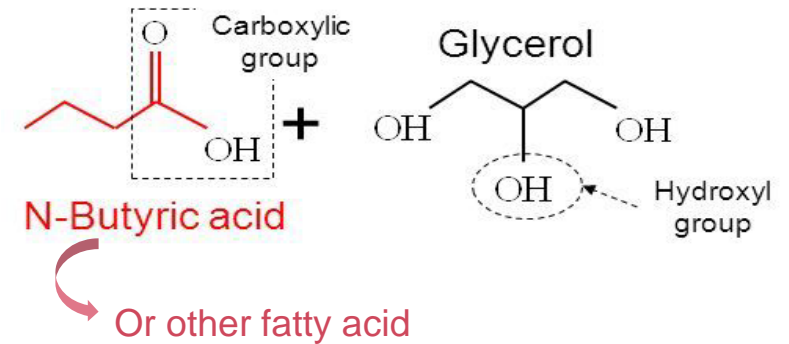
Optimized protection & performance

# Monoglycerides

## What are they and where do they come from?

### ■ What they are

- Class of glycerides composed of 1 glycerol & 1 fatty acid
- Also known as monoacylglycerols
- Different functions depending on the length of the fatty acids
  - Monoglycerides- S/MCFA: antibacterial & emulsifiers
  - Monoglycerides- LCFA: only emulsifiers



### ■ Where do they come from

- Found in maternal milk at low concentrations
- Rarely found in natural fats
- Normally found as by-products of partial hydrolysis from triglycerides

# Esterified butyric acids

## Monobutytrin & tributyrin

4<sup>th</sup> Generation

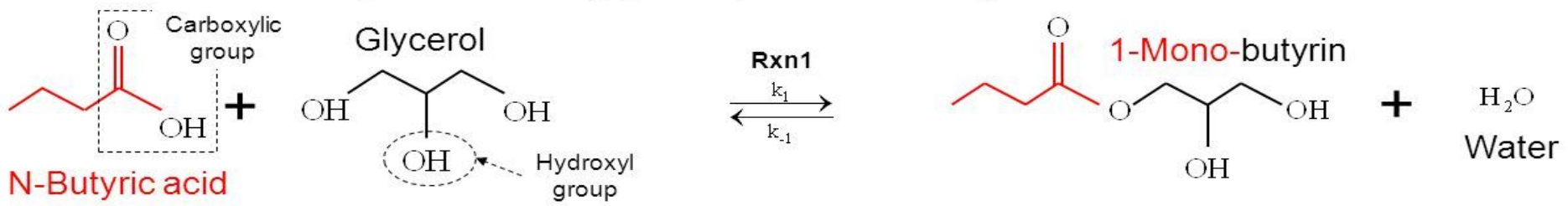
5<sup>th</sup> Generation

Esterified butyric acid (Tributytrins)

Monobutytrins



### Esterification of butyric acid with glycerol (Main Reaction)



# Esterified butyric acids

## Monobutytrin & tributyrin

4<sup>th</sup> Generation

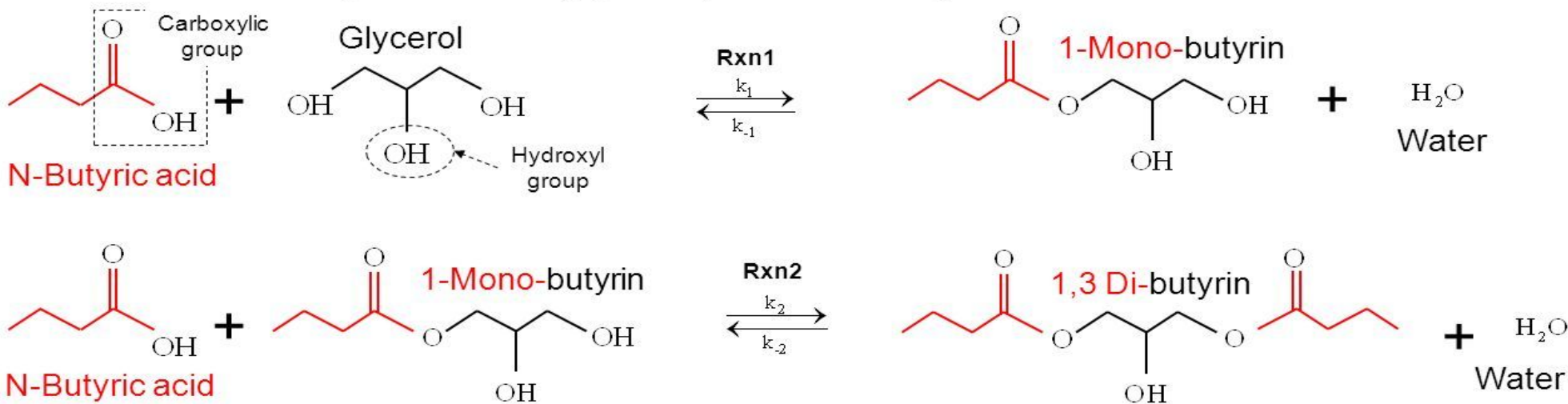
5<sup>th</sup> Generation

Esterified butyric acid (Tributytrins)

Monobutytrins



### Esterification of butyric acid with glycerol (Main Reaction)



# Esterified butyric acids

## Monobutyryn & tributyrin

4<sup>th</sup> Generation

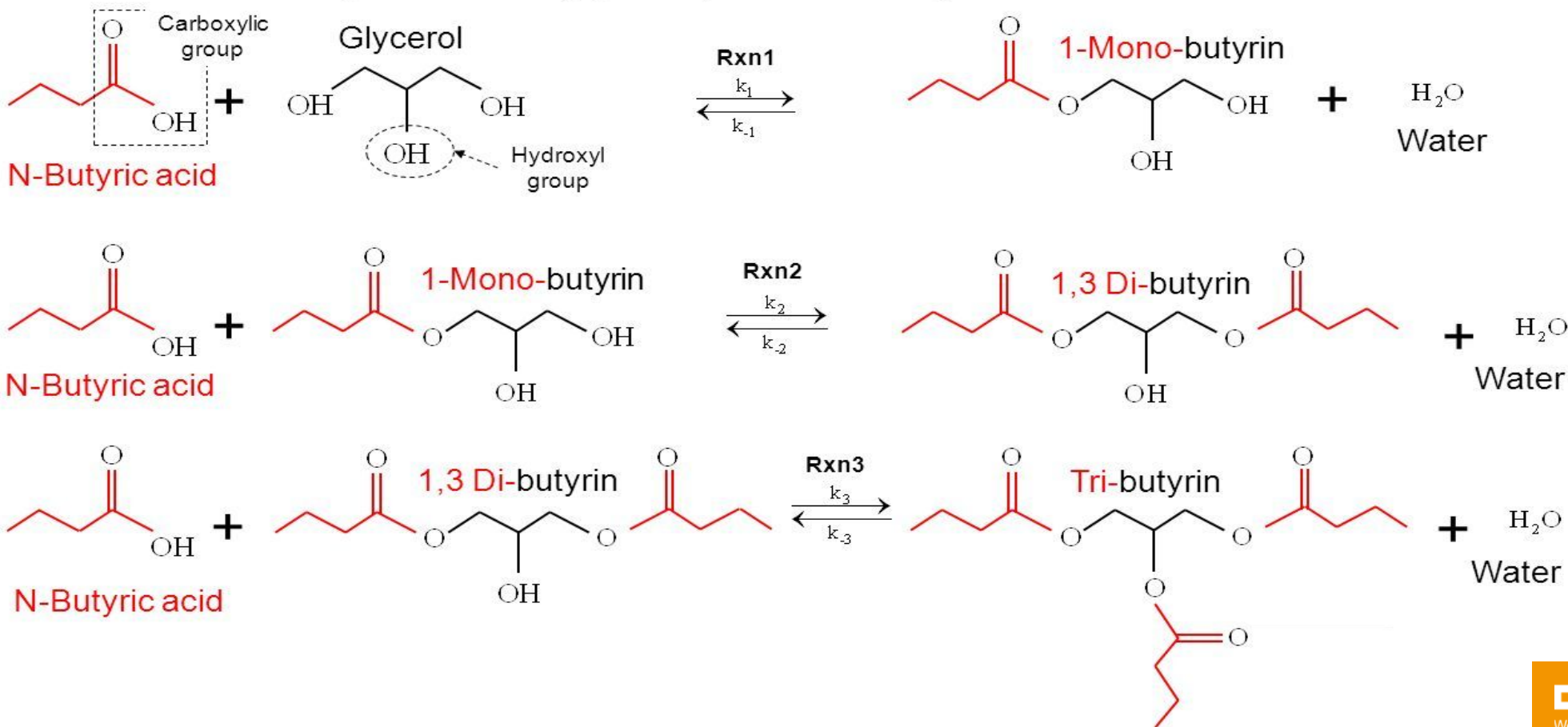
5<sup>th</sup> Generation

Esterified butyric acid (Tributyryns)

Monobutyryns

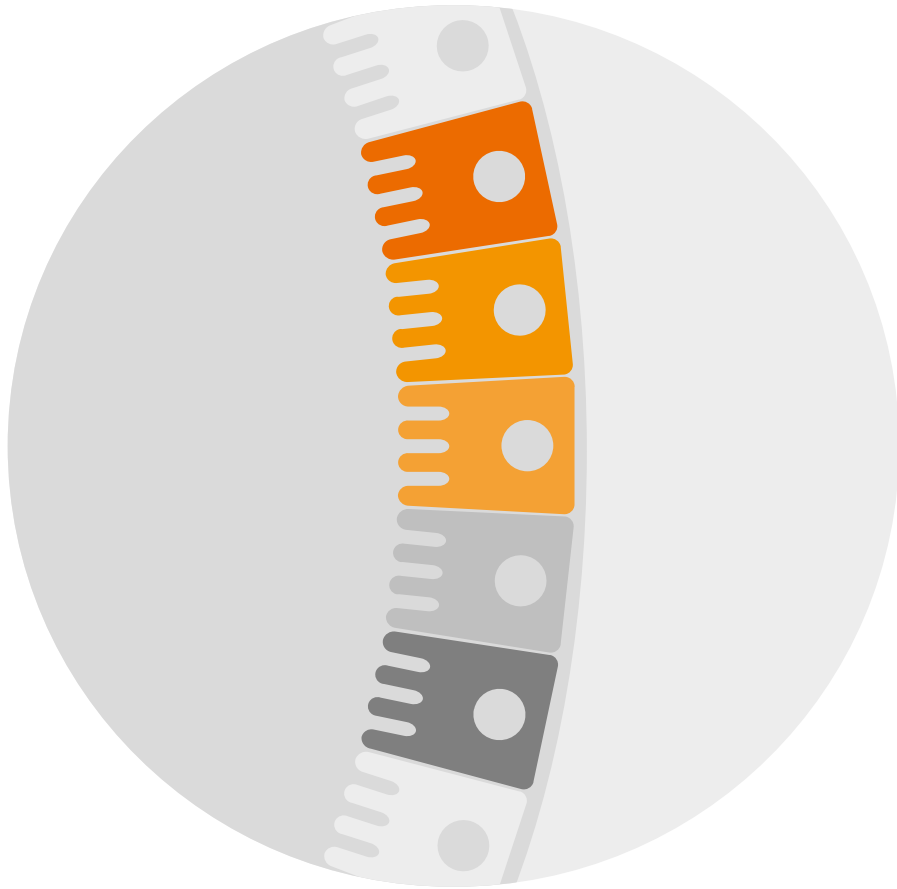


### Esterification of butyric acid with glycerol (Main Reaction)





# BalanGut<sup>®</sup> LS - Properties



- 1 Well protected and easy to handle source of butyric acid in the form of monobutyrim
- 2 Site of action – the gut
- 3 Supports gut integrity and host defense
- 4 Supports beneficial bacteria via monobutyrim & medium chain fatty acid (C8-C10) glycerides
- 5 To be used by poultry and swine producers



# BalanGut<sup>®</sup> LS – Formula

## Source of butyric acid (as monobutyrim) & medium chain fatty acids



1. Well protected & easy to handle

### Blend of mono- di- and triglycerides of short and medium chain fatty acids

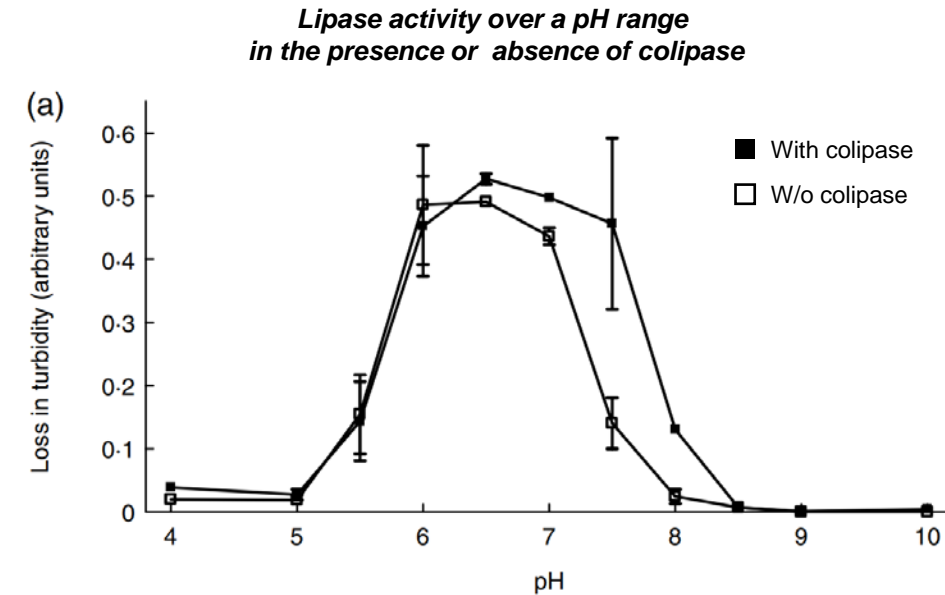
Parameters	Description Characteristics
Composition Liquid 	56 – 63% Mono- di and triglycerides of butyric, caprylic and capric acid 37 – 44% Glycerol <1% Free fatty acids
Composition Powder 	43 – 49% Mono- di and triglycerides of butyric, caprylic and capric acid 16 – 22% Glycerol 31 – 34% Silica <1% Free fatty acids
Butyric acid glyceride distribution	> 70% Monobutyrim > 80% 1-monobutyrim < 12% 2-monobutyrim < 25% Dibutyrim < 5% Tributyrin

# Protection given by ester bonds

## Hydrolyzation / Release of the fatty acids



- Ester bonds need to be hydrolyzed by enzymatic activity (lipases)
- Gastric & pancreatic lipases act mainly on the alpha carbon (1 & 3 positions)
  - Pancreatic lipase activity - pH 4 to 7
  - Bile salts prevent lipase from binding to its substrate
  - Colipase- lipase cofactor- enables lipase activity in presence of bile salts



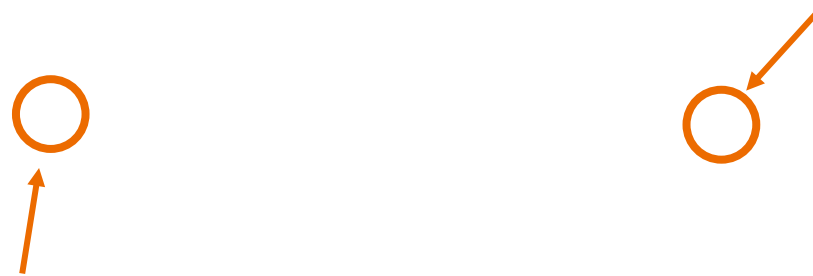
Brownlee I.A. *Nutr. Res. Rev.* 23, 2010

# Protection given by ester bonds

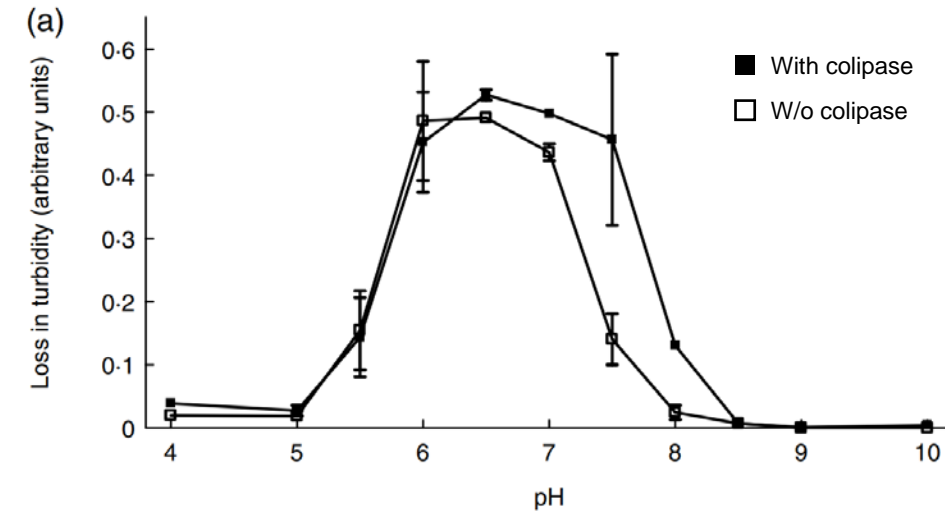
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**Lipase activity over a pH range  
in the presence or absence of colipase**



Brownlee I.A. *Nutr. Res. Rev.* 23, 2010

# Protection given by ester bonds

## *In-Vitro* USDA study

**Objectives:** To determine the hydrolysis of [ $^{14}\text{C}$ ]butyrate from 1- and 2- [ $^{14}\text{C}$ ]-butyryl glycerol monoesters as a function of time when incubated in saline (control), simulated gastric fluid and simulated intestinal fluid.



1. Well protected & easy to handle

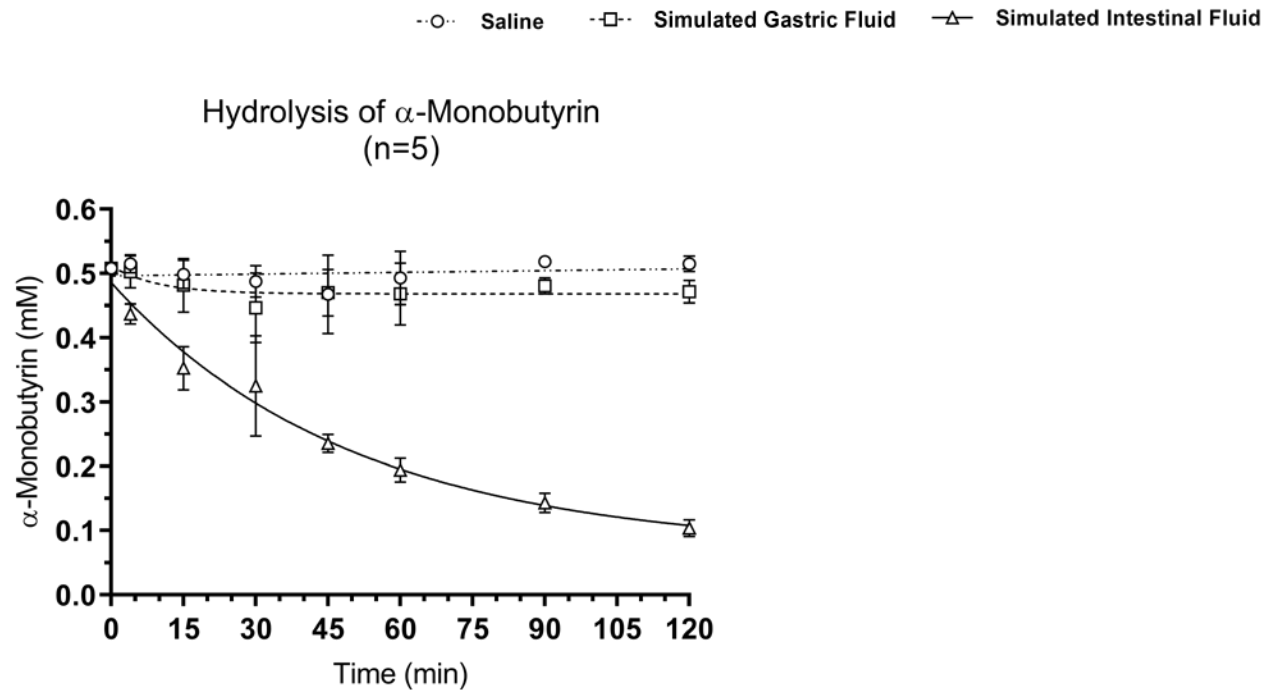
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Source: Animal Metabolism-Agricultural Chemical Research Unit; Dr. David Smith- study ongoing

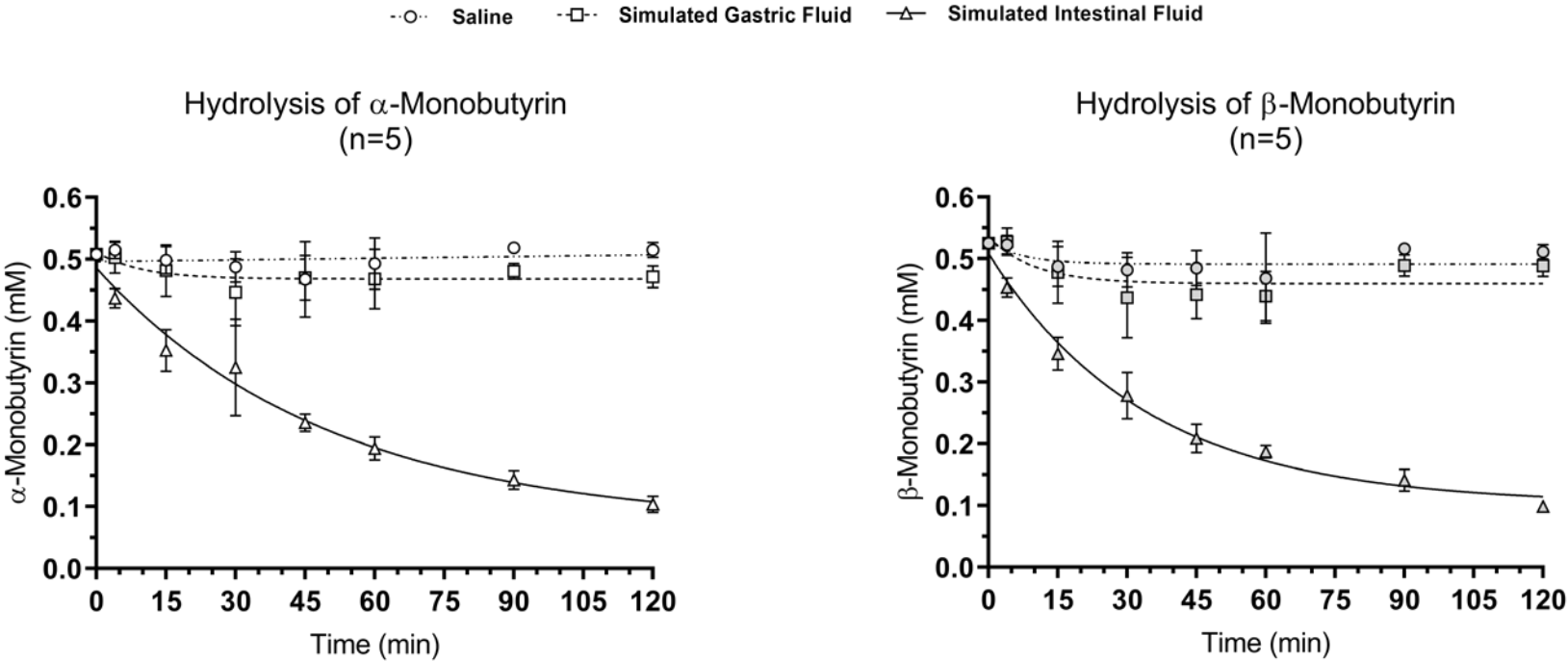
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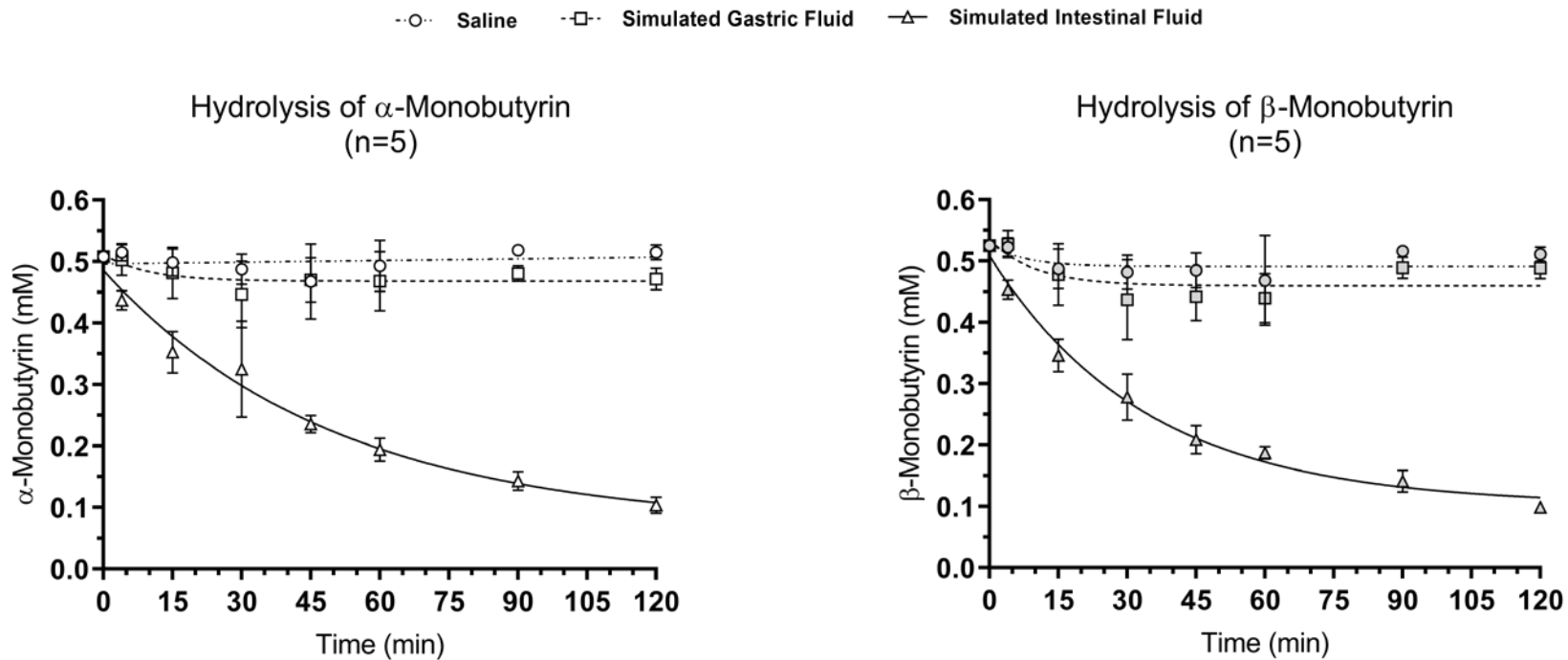
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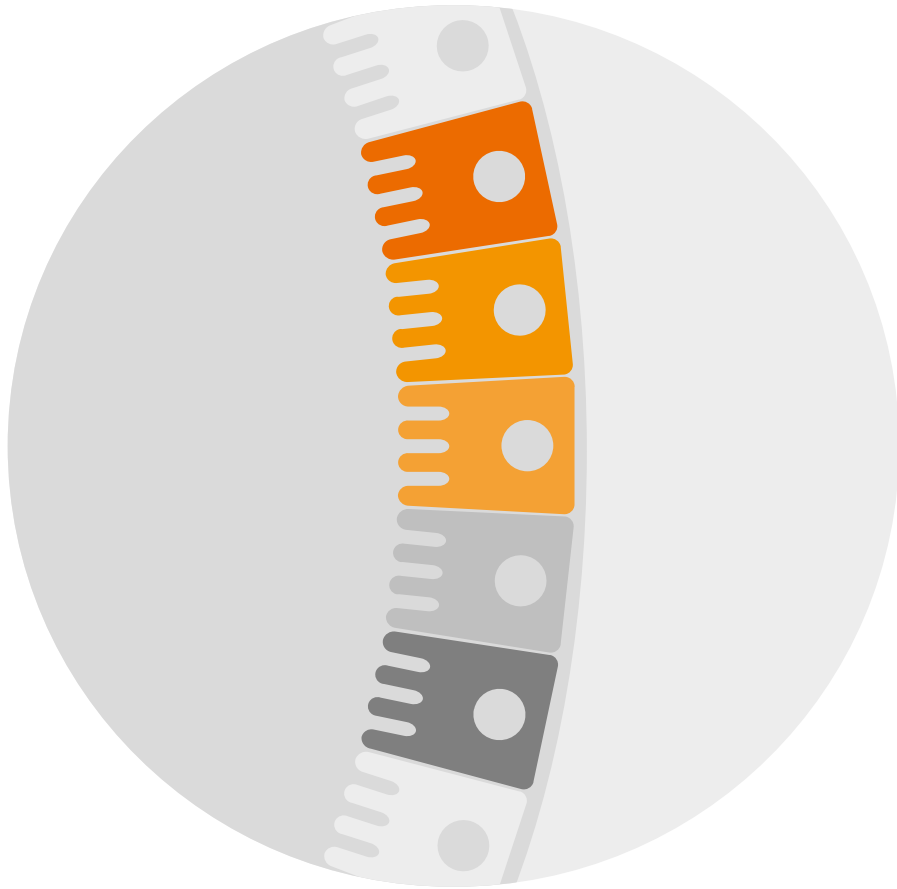


### In-Vitro Conclusions

- 2-monobutyryn rapidly isomerizes to 1-monobutyryn when in the presence of an aqueous matrix.
- 1-monobutyryn is more stable and tends not to isomerize to the 2-monobutyryn.
- Hydrolysis of the 1-monobutyryn by lipases in the duodenum will create free butyric acid for absorption and metabolism.



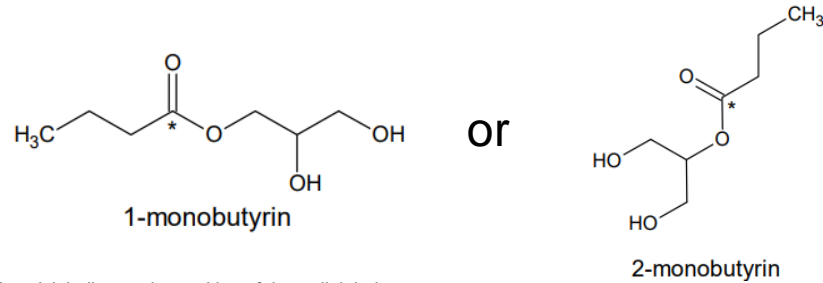
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# BalanGut<sup>®</sup> LS – Site of Absorption / Action

## *In-vivo* USDA study



### Objective

To determine whether two bioactive glycerol fats, 1- monobutylin and 2- monobutylin are absorbed intact, or if they are metabolized in the gastrointestinal tract prior to absorption

### Materials & Methods

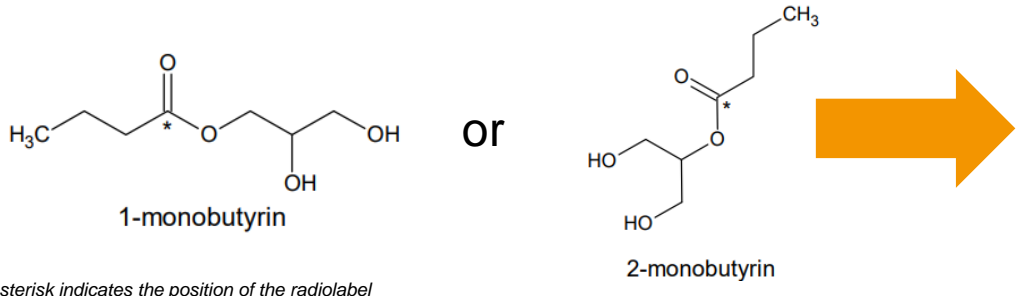
Day of hatch broiler chicks raised in floor cages fed a basic diet containing BalanGut<sup>®</sup> LS @ 2g/kg feed, from arrival until sample collection.



4-5wk

# BalanGut<sup>®</sup> LS – Site of Absorption / Action

## In-vivo USDA study



### 2. Site of action – the gut

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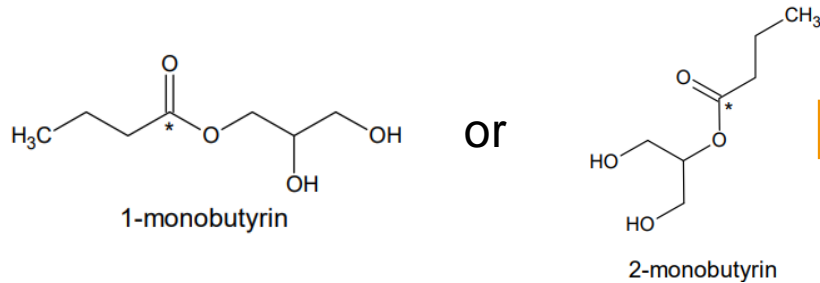
4-5wk

Source: Animal Metabolism-Agricultural Chemical Research Unit; Dr. David Smith- study ongoing

# BalanGut<sup>®</sup> LS – Site of Absorption / Action

## In-vivo USDA study

### 2. Site of action – the gut



Treatments, Replication, and Collection Times

Collection Time h	Treatment	Replicate (week day)					Total
		Mon	Tue	Wed	Thu	Fri	
2	[ <sup>14</sup> C]-1-monobutyryn	1	1	1	1	1	5
	[ <sup>14</sup> C]-2-monobutyryn	1	1	1	1	1	5
4	[ <sup>14</sup> C]-1-monobutyryn	1	1	1	1	1	5
	[ <sup>14</sup> C]-2-monobutyryn	1	1	1	1	1	5
8	[ <sup>14</sup> C]-1-monobutyryn	1	1	1	1	1	5
	[ <sup>14</sup> C]-2-monobutyryn	1	1	1	1	1	5
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Per day totals:		8	8	8	8	8	40

Samples collected: tissue and contents separated from upper, middle & lower GIT, excreta, liver, blood & rest of the carcass.

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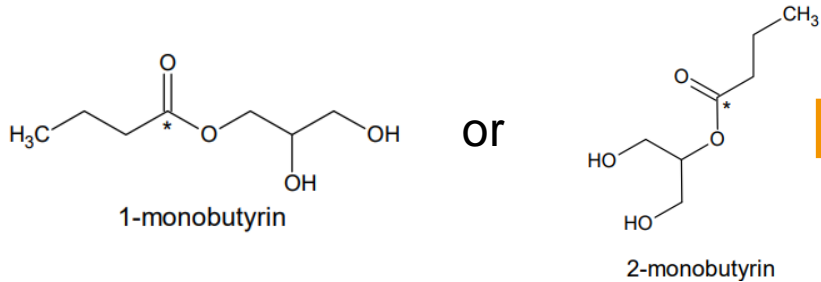
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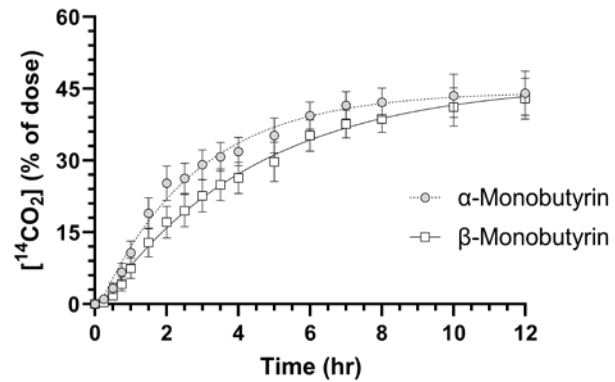
### 2. Site of action – the gut



Cumulative [<sup>14</sup>CO<sub>2</sub>] Elimination

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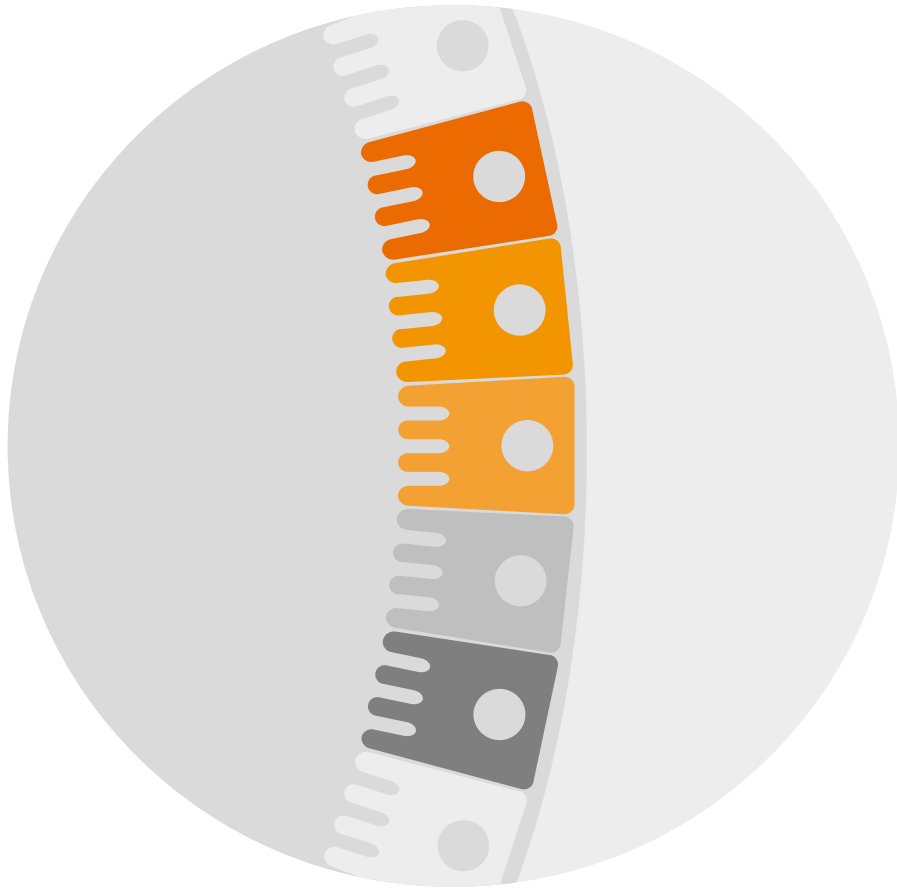
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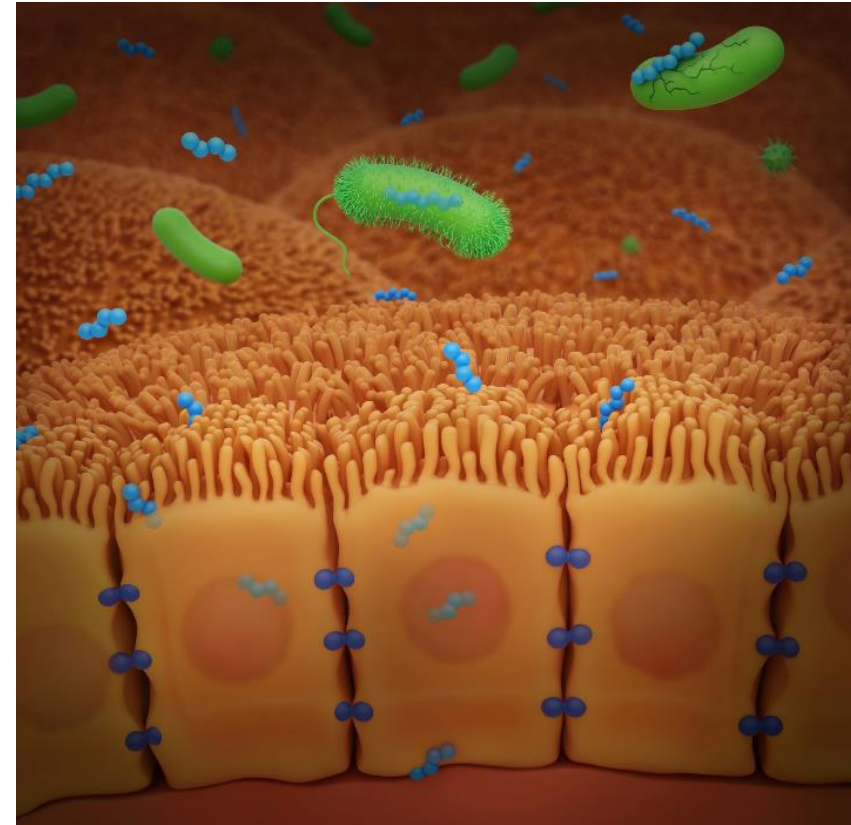
# Benefits from monobutyryn & butyric acid

## Support gut integrity & host defense



### When absorbed into the gut tissue

- Gut development & recovery  
Cell proliferation & renewal of epithelium
- Gut integrity  
Effects on tight junctions
- Host defense  
Antimicrobial peptide production in Paneth cells



# Benefits from butyric acid

## Butyric acid mechanism of action- several studies available



RESEARCH ARTICLE

### Butyrate modifies intestinal barrier function of IPEC-J2 cells through a selective upregulation of tight junction proteins and Akt signaling pathway

Hui Yan, Kolapo M. Ajuwon\*

Department of Animal Sciences, Purdue University, West Lafayette

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;49:190-205

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Original Paper

### Short-Chain Fatty Acids Manifest Stimulative and Protective Effects on Intestinal Barrier Function Through the Inhibition of NLRP3 Inflammasome and Autophagy

Yanhai Feng<sup>a</sup>, Yu Wang<sup>b</sup>, Pei Wang<sup>a</sup>, Yalan Huang<sup>c</sup>, Fengjun Wang<sup>a</sup>

<sup>a</sup>State Key Laboratory of Trauma, Burns, and Combined Injury, Institute of Burn Research, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, <sup>b</sup>Department of Gastroenterology, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China <sup>c</sup>Department of Military Nursing, School of Nursing, Third Military Medical University (Army Medical University), Chongqing, China

Key Words

Short-chain fatty acids • Intestinal barrier • Tight junction • Lipopolysaccharide • NLRP3 inflammasome • Autophagy

### Butyrate Enhances Disease Resistance of Chickens by Inducing Antimicrobial Host Defense Peptide Gene Expression

Lakshmi T. Sunkara<sup>1</sup>, Mallika Achanta<sup>1</sup>, Nicole B. Schreiber<sup>1</sup>, Yugendar R. Bommineni<sup>1,2a</sup>, Gan D. Weiyu Jiang<sup>1</sup>, Susan Lamont<sup>2</sup>, Hyun S. Lillehoj<sup>3</sup>, Ali Beker<sup>1</sup>, Robert G. Teeter<sup>1</sup>, Guolong Zhang<sup>1\*</sup>

<sup>1</sup> Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, United States of America, <sup>2</sup> Department of Animal Science, Iowa State University, United States of America, <sup>3</sup> Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, United States Department of Agriculture/Research Service, Beltsville, Maryland, United States of America



RESEARCH ARTICLE

### Promotion of Intestinal Epithelial Cell Turnover by Commensal Bacteria: Role of Short-Chain Fatty Acids

Jung-ha Park<sup>1\*</sup>, Takenori Kotani<sup>1\*</sup>, Tasuku Konno<sup>1</sup>, Jajar Setiawan<sup>1</sup>, Yasuaki Kitamura<sup>1</sup>, Shinya Imada<sup>1</sup>, Yutaro Usui<sup>1</sup>, Naoya Hatano<sup>2</sup>, Masakazu Shinohara<sup>2</sup>, Yasuyuki Saito<sup>1</sup>, Yoji Murata<sup>1</sup>, Takashi Matozaki<sup>1\*</sup>

<sup>1</sup> Division of Molecular and Cellular Signaling, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe, Japan, <sup>2</sup> The Integrated Center for Mass Spectrometry, Kobe University Graduate School of Medicine, Kobe, Japan

\* Current address: Department of Life Science and Biotechnology, College of Natural Sciences and Human Ecology, Dong-Eui University, Busan, Republic of Korea  
\* [matozaki@med.kobe-u.ac.jp](mailto:matozaki@med.kobe-u.ac.jp) (TM); [kotani@med.kobe-u.ac.jp](mailto:kotani@med.kobe-u.ac.jp) (TK)



click for updates

### Butyrate Enhances the Intestinal Barrier by Facilitating Tight Junction Assembly via Activation of AMP-Activated Protein Kinase in Caco-2 Cell Monolayers<sup>1,2</sup>

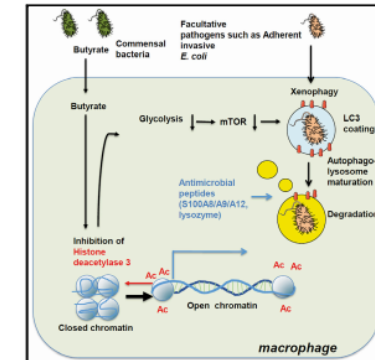
Luying Peng,<sup>3,5</sup> Zhong-Rong Li,<sup>4</sup> Robert S. Green,<sup>3</sup> Ian R. Holzman,<sup>3</sup> and Jing Lin<sup>3\*</sup>

<sup>1</sup>Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029-6574; <sup>2</sup>Department of Pediatric Surgery, Yuying Children's Hospital of Wenzhou Medical College, Wenzhou, China 325027; and <sup>3</sup>Department of Medical Genetics, Tongji University School of Medicine, Shanghai, China 200092

### Immunity

### The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages

Graphical Abstract



Authors

Julie Schulthess, Sumeet Pandey, Melania Capitani, ..., Carolina V. Arancibia-Carcamo, Holm H. Uhlig, Fiona Powrie

Correspondence

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In Brief

Macrophages maintain gut homeostasis by eliminating invasive pathogens and regulating inflammatory responses. Schulthess et al. demonstrate that butyrate, a bacterial fermentation product, imprints potent antimicrobial activity during macrophage differentiation through HDAC3i function.

Highlights

- Butyrate induces differentiation of macrophages with potent antimicrobial function
- Enhanced antimicrobial function is a consequence of glycolysis and mTOR inhibition
- Single-cell RNA-sequencing identifies butyrate-induced antimicrobial peptides
- Butyrate inhibits HDAC3 to drive metabolic changes and microbicidal function

Schulthess et al., 2019, Immunity 50, 432-445  
February 19, 2019 © 2018 The Authors. Published by Elsevier Inc.  
<https://doi.org/10.1016/j.immuni.2018.12.018>





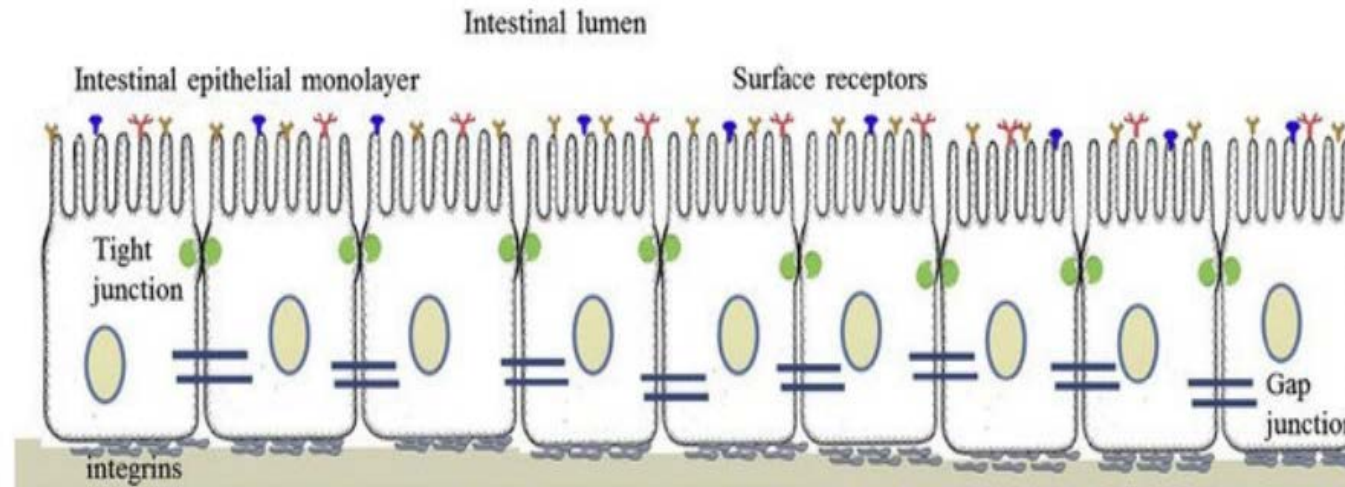
# Benefits from monobutyryn

3. Supports gut integrity and host defense

## Monobutyryn effects on barrier integrity – IPEC-J2 (*In-vitro* assay)

### Barrier function

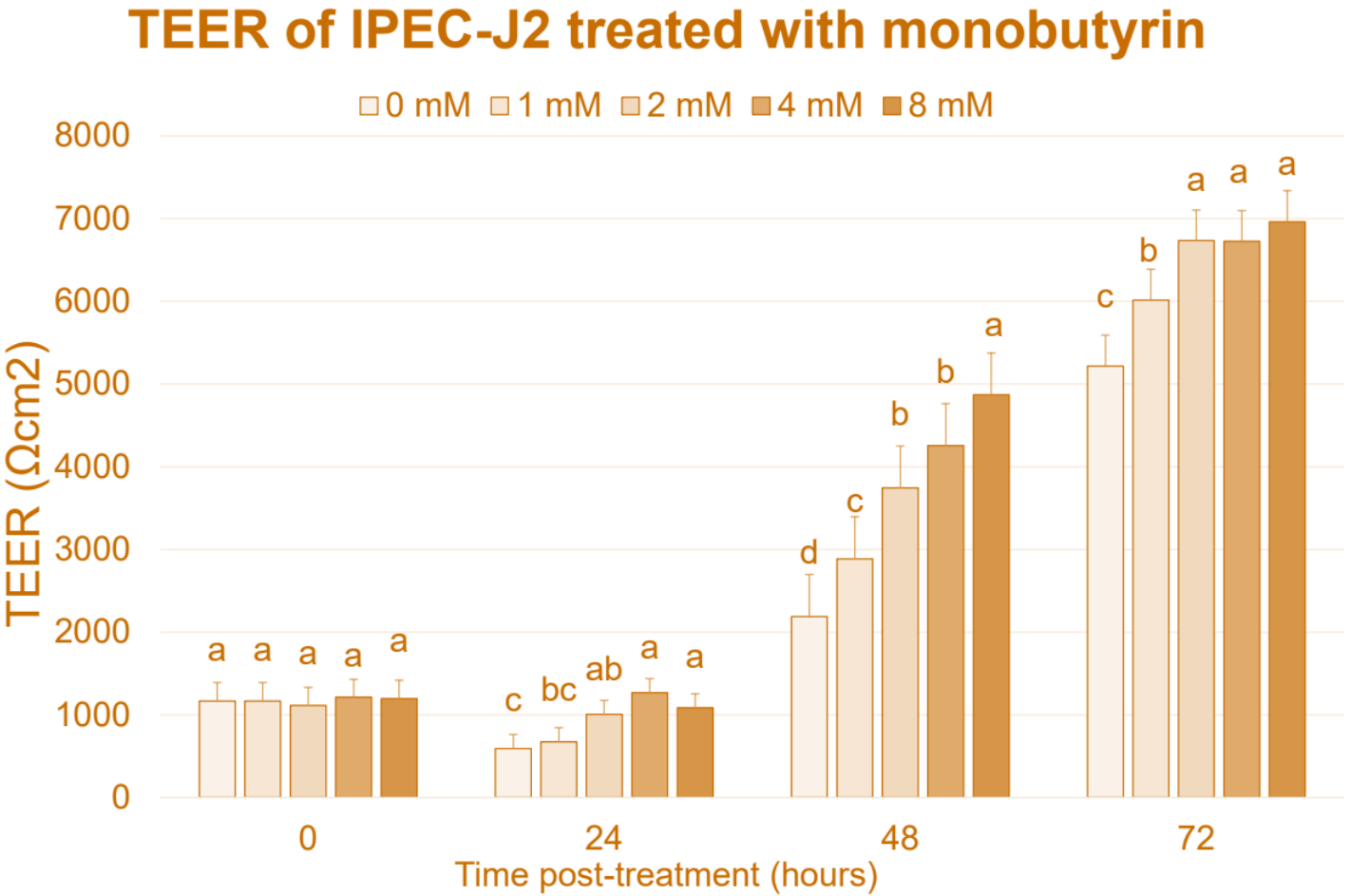
- semi-permeable & vital for nutrient absorption
- H<sub>2</sub>O retention
- prevention of pathogen invasion



Kovanda L., et al., UC Davis; Gokulan et. Al., 2017

# Benefits from monobutyryn

## Increased barrier integrity – IPEC-J2 (*In-vitro* assay)



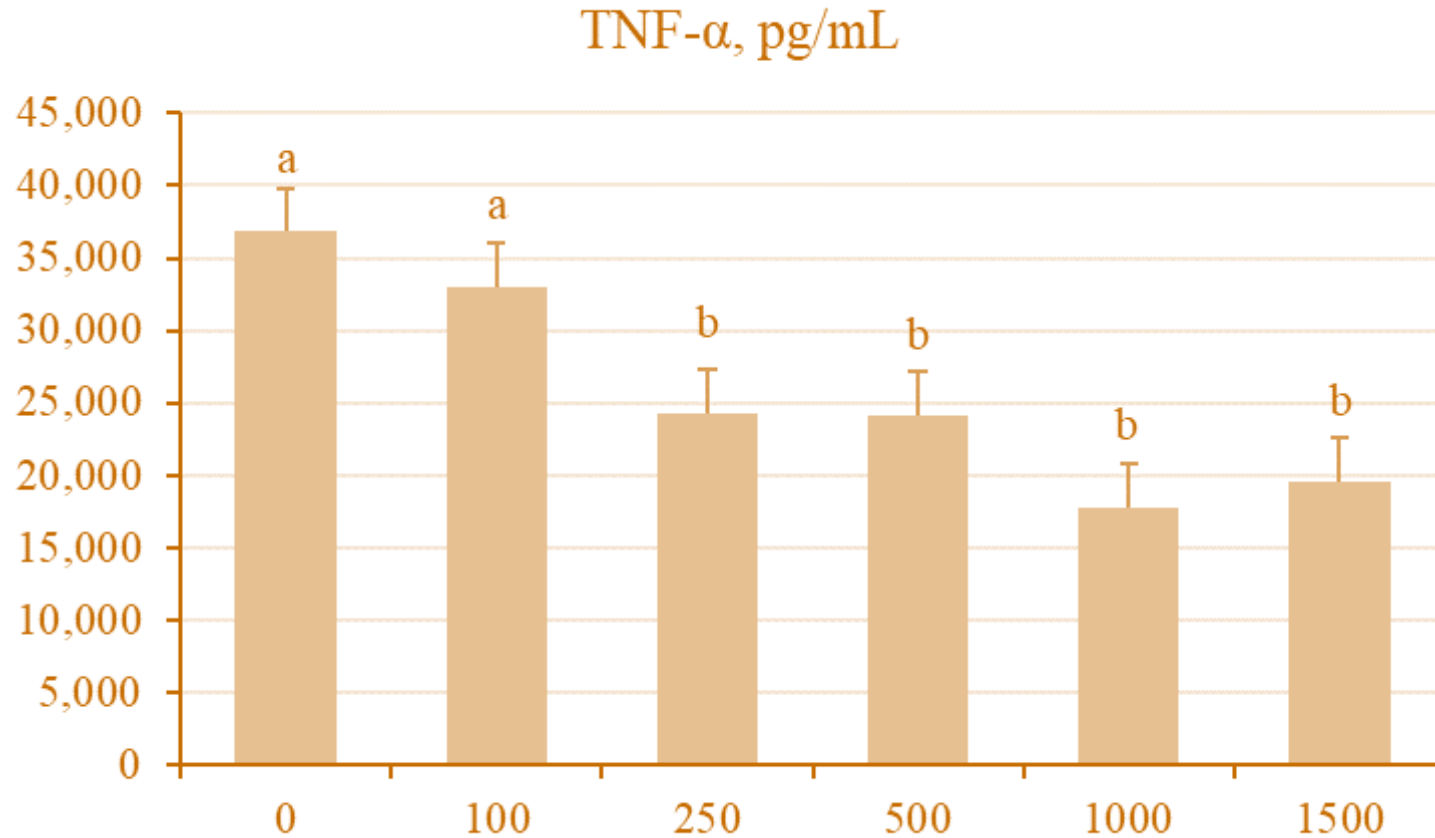
- Transepithelial electrical resistance (TEER) of porcine epithelial cells
- Resistance is inversely proportional to the area of the membrane
- Monobutyryn increased TEER at all timepoints post-treatment in porcine epithelial cell monolayer *in-vitro*

Kovanda L., et al., UC Davis; Gokulan et. Al., 2017; Poenar et al., materials 2020



# Benefits from monobutyryn

## Reduced TNF- $\alpha$ production - PAM\* (*In-vitro* assay)



- Cells were incubated with various concentrations of monobutyryn in the presence of 1  $\mu$ g/mL of LPS\*\* for 24 h.
- Preliminary data show that:
  - *monobutyryn could reduce the overresponses or over-inflammation caused by LPS challenge.*
  - *there was no difference in TNF- $\alpha$  results when the dose was over 250 pg/mL.*

\*PAM= porcine alveolar macrophages  
\*\*LPS= lipopolysaccharide

**Preliminary data: UC Davis**

*a, b means in a column not sharing a common letter are significantly different ( $P < 0.05$ ).*

# Benefits from monobutyryn

## Angiogenesis

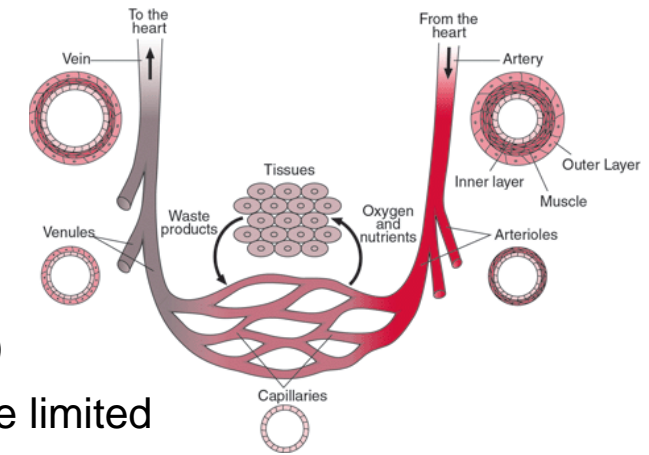


### ■ What is angiogenesis:

- Physiological process through which new blood vessels form.
- Growth of blood vessels from the existing vasculature.
- Occurs throughout life, health and disease (starts in uterus / egg & continues until old age)
- Prominent role in vascular development in embryos where growth is fast and resources are limited

### ■ Why angiogenesis is important:

- Cardiovascular system is the first organ to develop in the embryo
- Capillaries feed every metabolic active tissue in the body
- Capillaries grow and regress in healthy tissues depending on functional demands

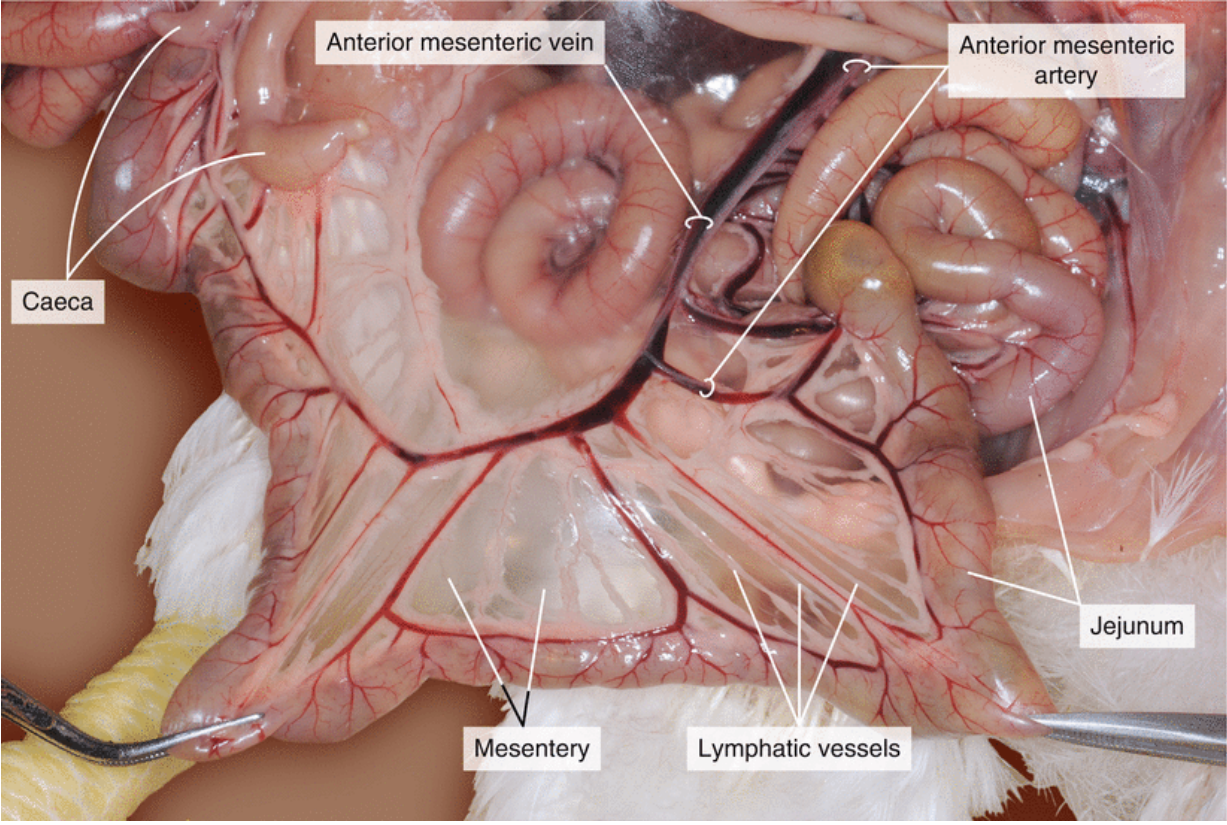
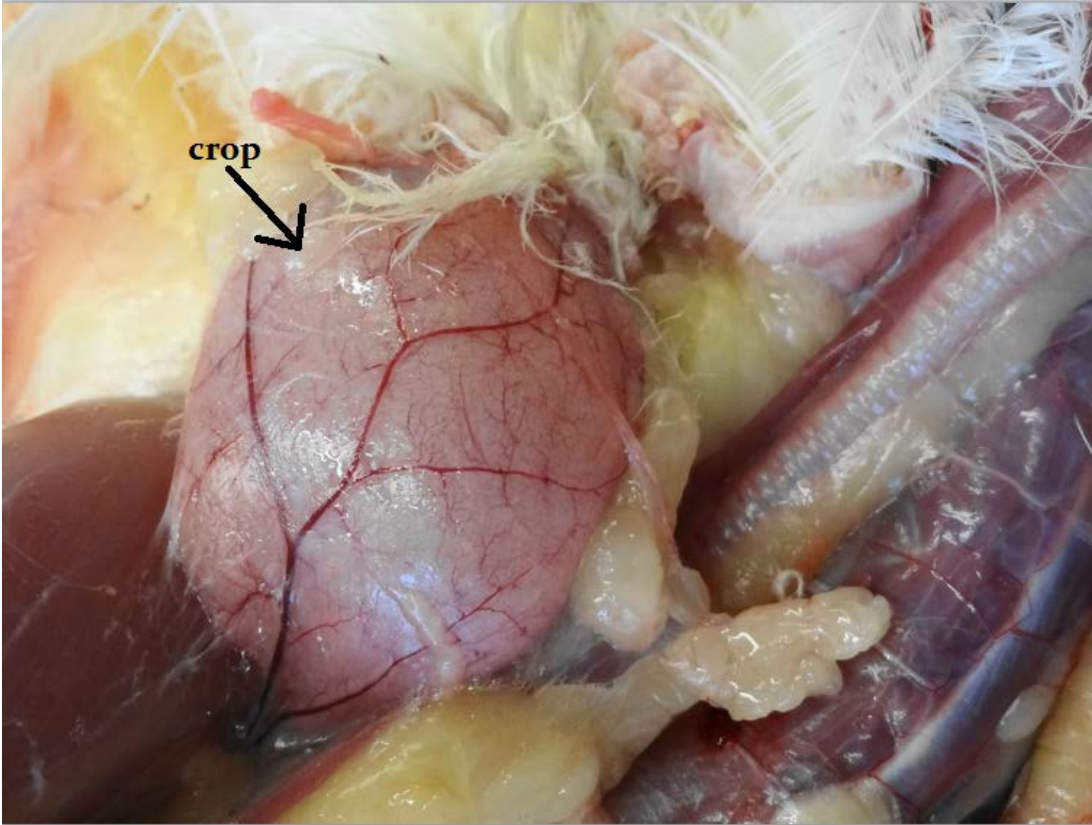


# Benefits from Monobutyryn

## Angiogenesis

Vessels transport nutrients, oxygen, hormones to and from cells

3. Supports gut integrity and host defense



[https://link.springer.com/chapter/10.1007/978-3-319-25172-1\\_11](https://link.springer.com/chapter/10.1007/978-3-319-25172-1_11)

# Benefits from monobutyryn

## Angiogenesis



Vascular endothelial growth factors (VEGF)

- Subfamily of growth factors; signal promotes the growth of new blood vessels.
- Form part of the mechanism that restores blood supply to cells & tissues

Factors that regulate angiogenesis:

- Soluble mediators
  - TGF- $\alpha$  & TGF- $\beta$
  - TNF- $\alpha$
  - Prostaglandin
  - Nicotinamide
  - Monobutyryn
  - Others...
- Membrane bound proteins
  - Integrin

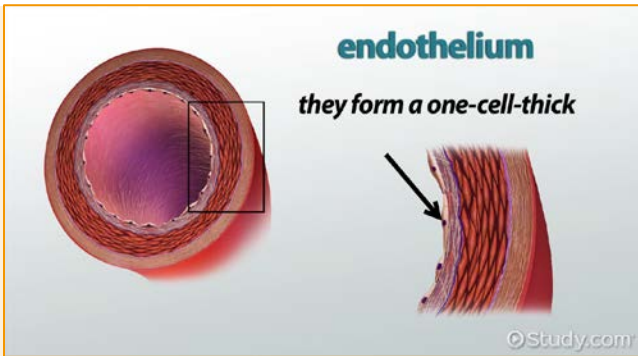
*J. Biol. Chem.* 266 (2) 1991; *Crit. Rev. Oral Biol Med.* 6(3), 1995; *Arterioscler. Thromb. Vasc. Biol.* 24, 2004

# Benefits from monobutyryn

## Angiogenesis



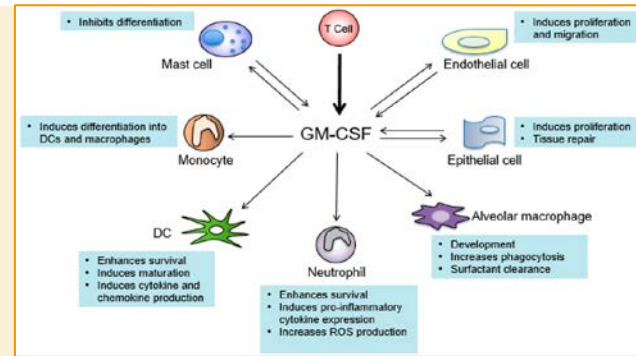
### Endothelial cell proliferation ↑



### Vascular endothelium

- Vascular barrier
- Controls blood coagulation & vascular permeability
- Potent immune regulator
  - Production of inflammatory mediators
  - Upregulation of surface adhesion molecules
  - Recruitment of innate immune cells into damaged / infected tissue

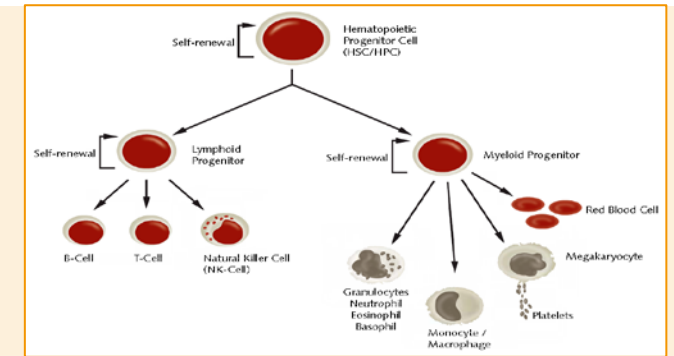
### GM-CSF/G-CSF ↑



### Granulocyte MQ colony stimulating factors

- Hemopoietic growth factor & immunomodulatory cytokine secreted by endothelial cells, T cells, MQs, mast cells
  - Important for monocyte & MQ proliferation, differentiation, maturation & functional activation (phagocytosis & antigen presentation)
  - Promotes leukocyte chemotaxis & adhesion

### Proliferation hematopoietic progenitors ↑



### Proliferation hematopoietic progenitors

- Occurs in bone marrow, liver & spleen
- Continuous replenishment of dead or damaged blood & immune cells
  - Repair after injury
  - Ongoing tissue maintenance
  - Maintaining blood system as a result of their self-renewal & multilineage differentiation capacity

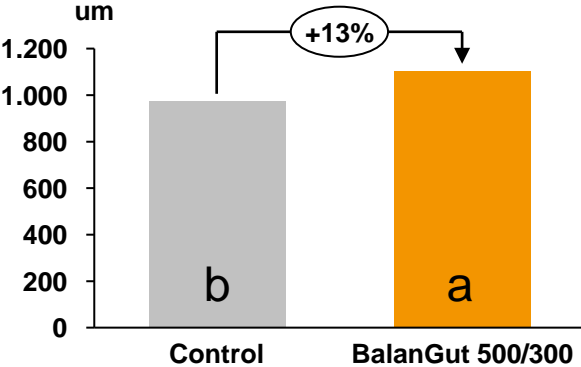
Arterioscler. Thromb. Vasc. Biol. 24, 2004; Mediators of Inflammation, 2015

# Benefits from BalanGut<sup>®</sup> LS

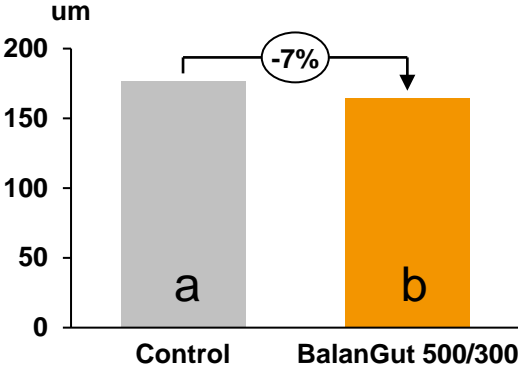
## Improvement of gut health parameters (*In-vivo- broilers*)

3. Supports gut integrity and host defense

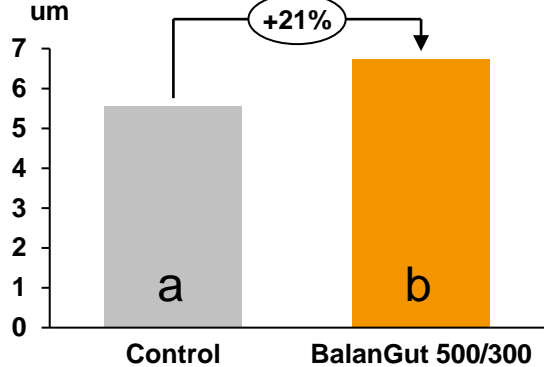
Villus height, ileum [day 21]



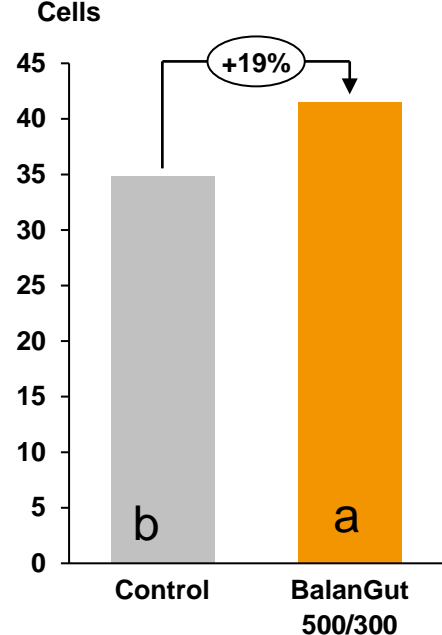
Crypt depth, ileum [day 21]



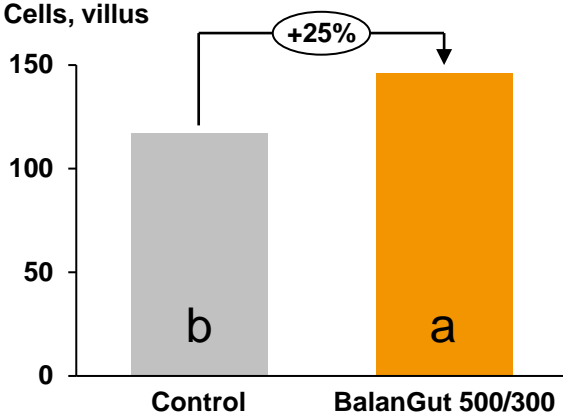
Villus height / crypt depth, ileum [day 21]



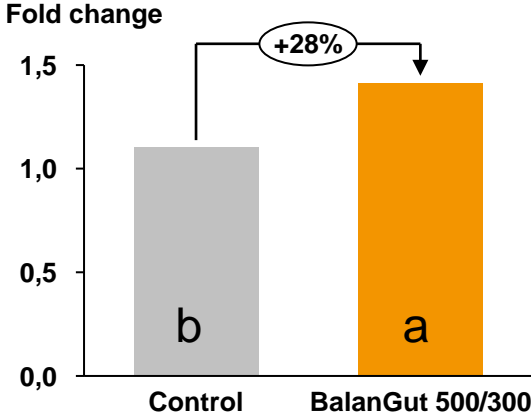
PCNA + cells, ileum [day 21]



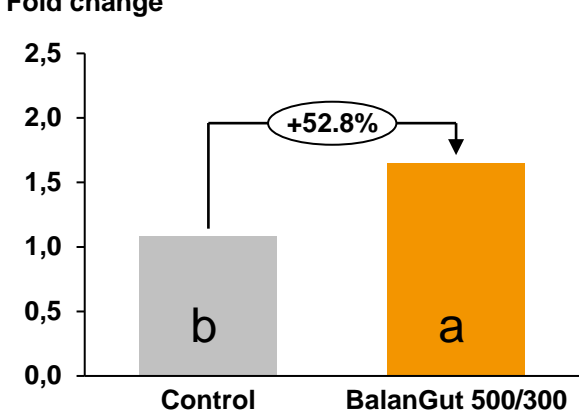
Goblet cells, villus, ileum [day 21]



IL- 1B [day 21]



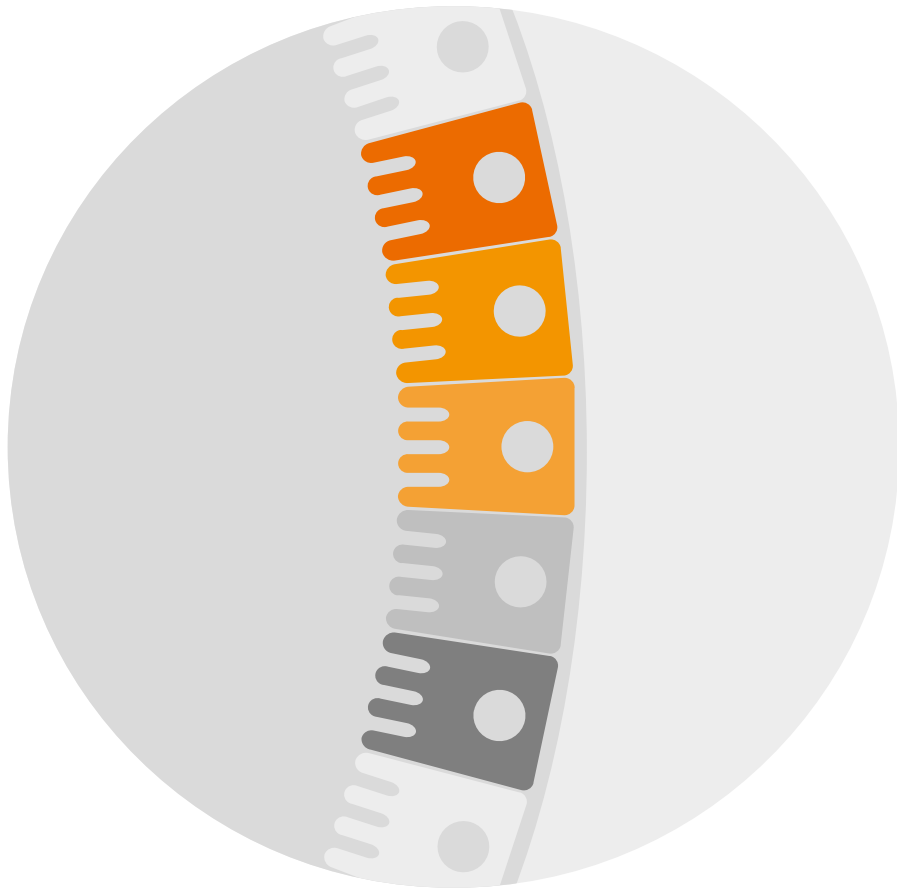
IL- 10 [day 21]



a, b means in a column not sharing a common letter are significantly different (P < 0.05).



# BalanGut<sup>®</sup> LS - Properties



- 1 Well protected and easy to handle source of butyric acid in the form of monobutyryl ✓
- 2 Site of action – the gut ✓
- 3 Supports gut integrity and host defense ✓
- 4 Supports beneficial bacteria via monobutyryl & medium chain fatty acid (C8-C10) glycerides
- 5 To be used by poultry and swine producers

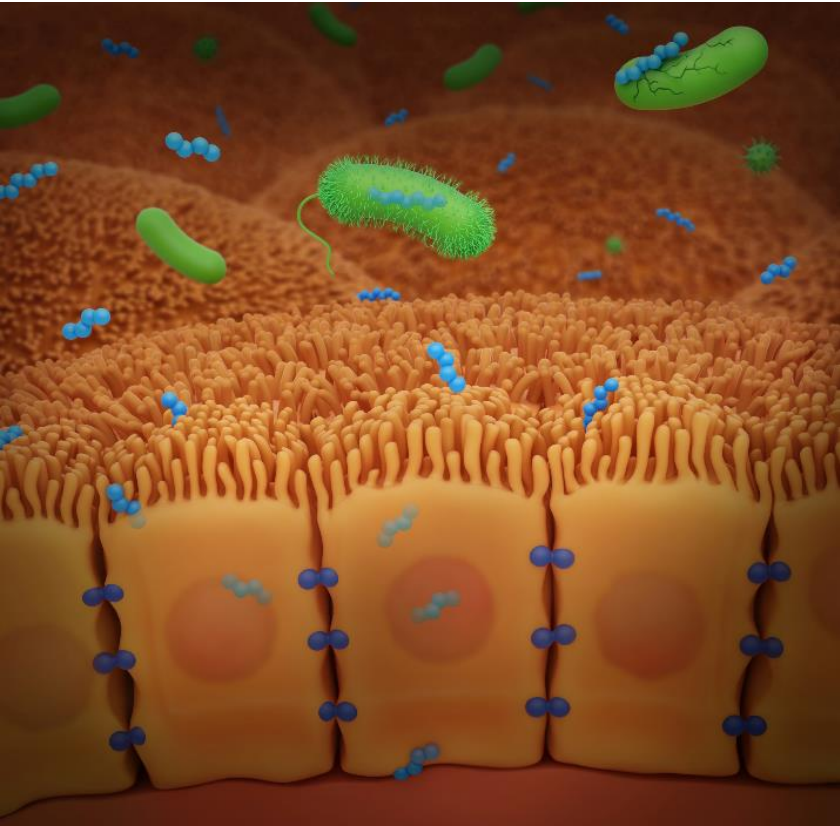
# Benefits from monoglycerides

## Support beneficial bacteria

### When staying in the intestinal lumen

- Enable a balanced enteric microbial population

4. Supports beneficial bacteria via monobutyryl & medium chain fatty acid (C8-C10) glycerides



E. Coli - Control

E. Coli after exposure to monoglycerides



Applied and Environmental Microbiology, 2011

# Benefits from free fatty acids & monoglycerides

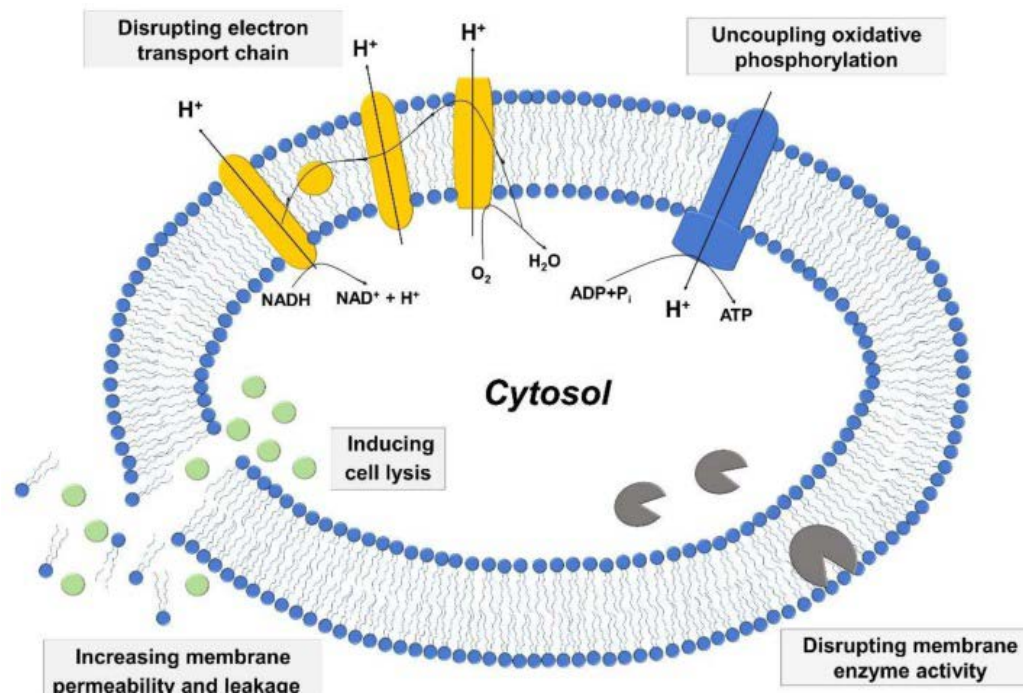
## Effect on bacterial cell membranes



4.

Supports beneficial bacteria via monobutyryn & medium chain fatty acid (C8-C10) glycerides

- Increased membrane permeability & cell lysis
- Disruption of electron transport chain & uncoupling oxidative phosphorylation
- Inhibition of membrane enzymatic activities & nutrient uptake



# Benefits from free fatty acids & monoglycerides

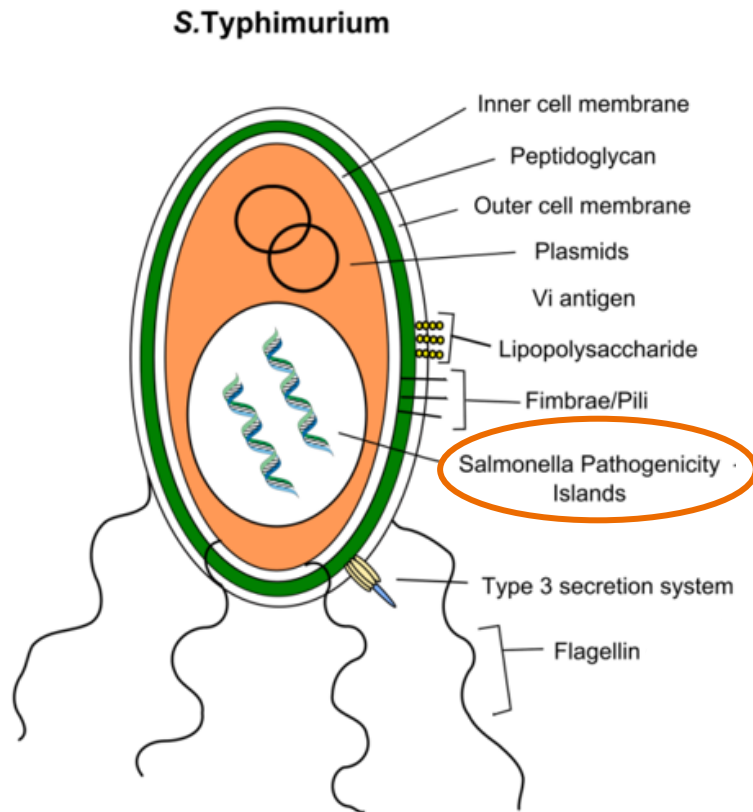
## Downregulation of virulent genes in *S. Typhimurium*



4.

Supports beneficial bacteria via monobutyryl & medium chain fatty acid (C8-C10) glycerides

- Monobutyryl is hydrolyzed by bacterial lipases
- Free butyric acid can enter the nucleus and downregulate gene expression of Pathogenicity island I.



Virulence genes involved in intestinal phase of infection - Invasion

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2006, p. 946-949  
0099-2240/06/\$08.00+0 doi:10.1128/AEM.72.1.946-949.2006  
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

Vol. 72, No. 1

### Butyrate Specifically Down-Regulates *Salmonella* Pathogenicity Island 1 Gene Expression

I. Gantois,<sup>1\*</sup> R. Ducatelle,<sup>1</sup> F. Pasmans,<sup>1</sup> F. Haesebrouck,<sup>1</sup> I. Hautefort,<sup>2</sup> A. Thompson,<sup>2</sup> J. C. Hinton,<sup>2</sup> and F. Van Immerseel<sup>1</sup>

*Department of Pathology, Bacteriology and Avian Diseases, Research Group Veterinary Public Health and Zoonoses, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium,<sup>1</sup> and Molecular Microbiology Group, Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, United Kingdom<sup>2</sup>*

Received 12 August 2005/Accepted 5 October 2005

Invasion of intestinal epithelial cells by *Salmonella enterica* is decreased after exposure to butyric acid. To understand the molecular mechanisms of this phenomenon, a comparative transcriptomic analysis of *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium grown in medium supplemented with butyrate was performed. We found that butyrate down-regulated the expression of 19 genes common to both serovars by a factor of twofold or more, and 17 of these genes localized to the *Salmonella* pathogenicity island 1 (SPI1). These included the SPI1 regulatory genes *hilD* and *invF*. Of the remaining two genes, *ampH* has 91% homology to an *Escherichia coli* penicillin-binding protein and *sopE2* encodes a type III-secreted effector protein associated with invasion but located at a separate site on the chromosome from SPI1.

Downloaded from



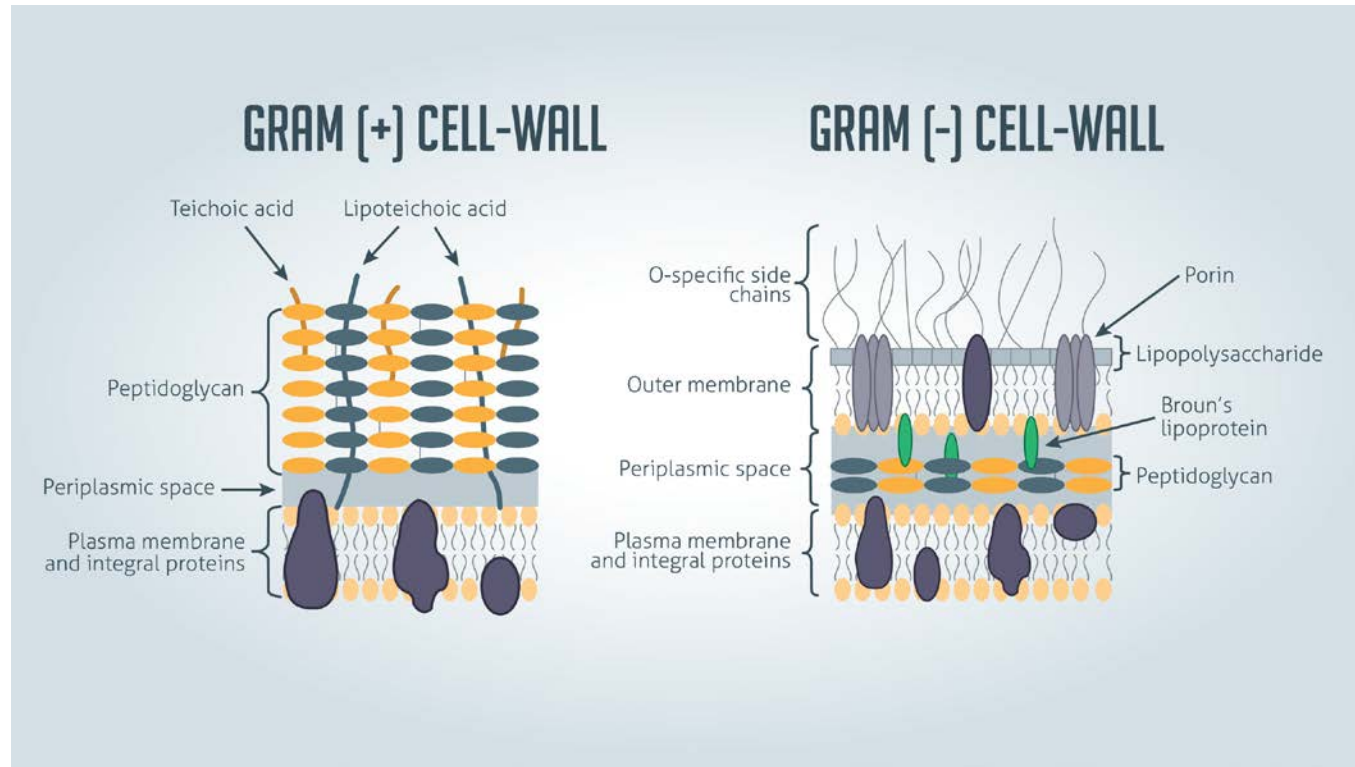
# Benefits from free fatty acids & monoglycerides

## Effect on bacterial cell membranes



Supports beneficial bacteria via monobutyrate & medium chain fatty acid (C8-C10) glycerides

- Fatty acids are known to have good antibacterial effects
- Fatty acids (& their glycerides) can affect both Gram positive (G+) and Gram negative (G-) bacteria
  - Easier to kill G+ bacteria due to their simple single lipid bilayer structure compared to that of G- bacteria?



# Benefits from free fatty acids & monoglycerides

## Effect on bacterial cell membranes



4.

Supports beneficial bacteria via monobutyryl & medium chain fatty acid (C8-C10) glycerides

MIC values of Short & MCFAs tested at pH 6, (mg/mL)

Bacteria	C4	C10	C12
<i>E. coli</i> (K88) (-)	16	4	>16
<i>S. Choleraesuis</i> (DSM4224) (-)	16	4	>16
<i>S. aureus</i> (ATCC43300) (+)	16	8	>16
<i>C. jejuni</i> (CVCC3883) (-)	8	0.5	0.25
<i>C. perfringens</i> type C (CVCC2041) (+)	32	4	2
<i>L. reuteri</i> (ATCC23272) (+)	> 32	4	1
<i>L. johnsonii</i> (AS1.3221) (+)	> 32	2	1

Prof. Guan, 2017

MIC values of Short & MCFA tested at pH 7, (mg/mL)

Bacteria*	C4	C8	C10
<i>E. coli</i> ETEC K88 (F4) (-)	40 ± 0	5 ± 0	40 ± 0
<i>S. Typhimurium</i> (-)	40 ± 0	5 ± 0	40 ± 0
<i>C. jejuni</i> (-)	27 ± 11	10 ± 0	16 ± 5
<i>C. perfringens</i> NetB+ (+)	40 ± 0	5 ± 0	5 ± 0
<i>C. hepaticus</i> (-)	33 ± 11	10 ± 0	10 ± 0
<i>L. salivarius</i> (+)	40 ± 0	5 ± 0	5 ± 0
<i>B. animalis</i> (+)	40 ± 0	5 ± 0	5 ± 0

\* Field strains

± standard deviation (SD based on a mean of 3 replicates)

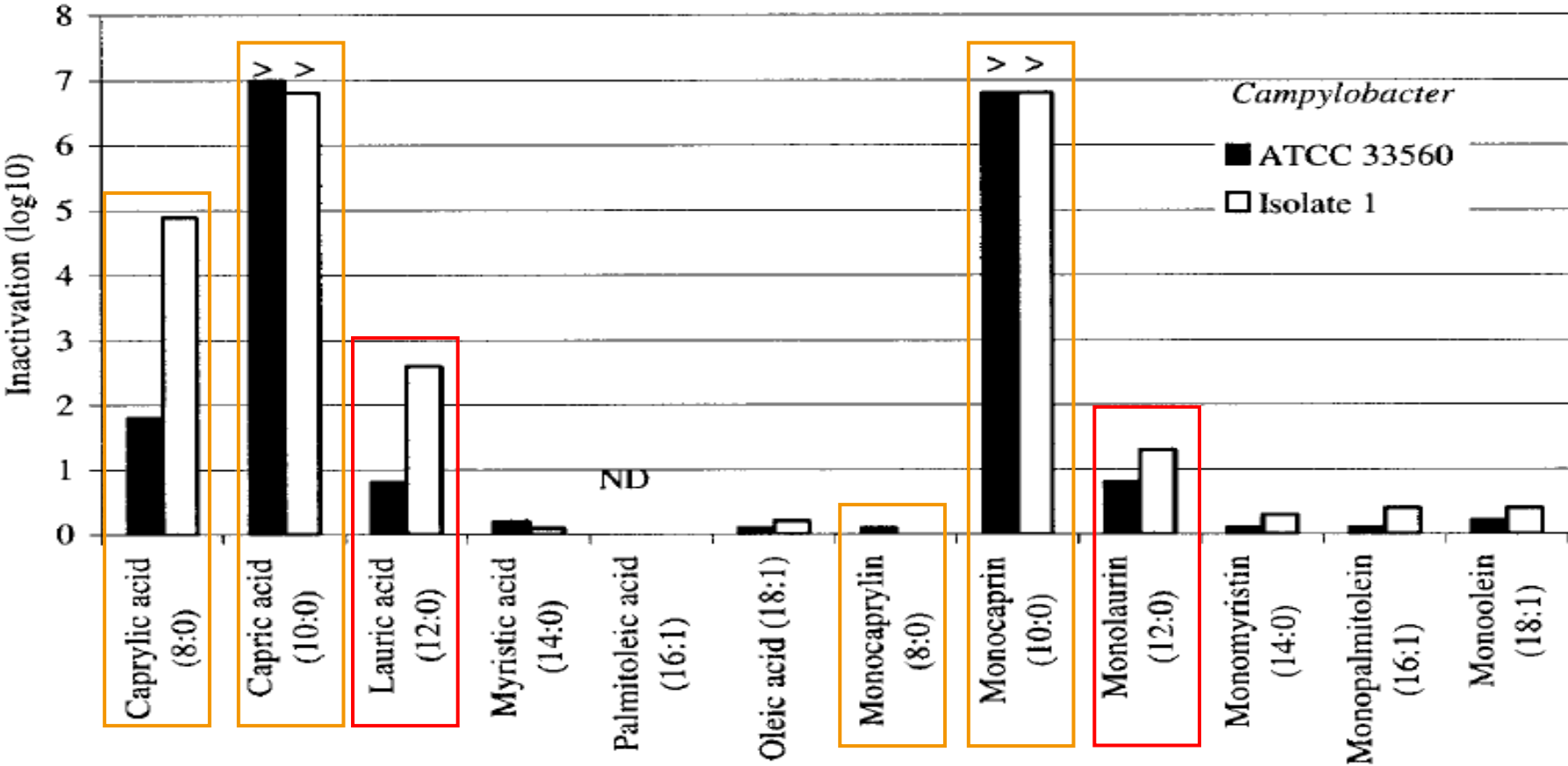
De Smet, 2021

# Benefits from free fatty acids & monoglycerides

## Effect on bacterial cell membranes



4. Supports beneficial bacteria via monobutylin & medium chain fatty acid (C8-C10) glycerides



Applied and Environmental Microbiology, 2006

At the top of the bars, >> indicates that the reduction of the bacterial count was equal to or greater than the value indicated on the left axis. ND, not done.

# Benefits from free fatty acids & monoglycerides

## Effect on bacterial cell membranes



4. Supports beneficial bacteria via monobutyryn & medium chain fatty acid (C8-C10) glycerides

- Glycerol enables bacterial cell membrane penetration
- 1-monoglycerides have stronger antibacterial activities than free fatty acids
- Glycerol increases the antibacterial activity of monobutyryn, monocaprilin and monocaprin

pH 7	<i>E. coli</i>	<i>S. Typhimurium</i>	<i>Campylobacter jejuni</i>
<b>Positive control</b>	1.08E+07	1.20E+07	4.31E+07
<b>Butyric acid</b>	1.06E+06	6.50E+05	1.80E+04
<b>Monobutyryn 43*</b>	7.50E+05	3.50E+04	4.80E+05

\* Monobutyryn 43= monobutyryn + free glycerol

### *Clostridium perfringens* CP27 @ 1.0E5 cfu/mL

ppm	Butyric acid	Monobutyryn 43*	Control +
500	+	+	+
1000	+	++++	+
2000	++	++++	+

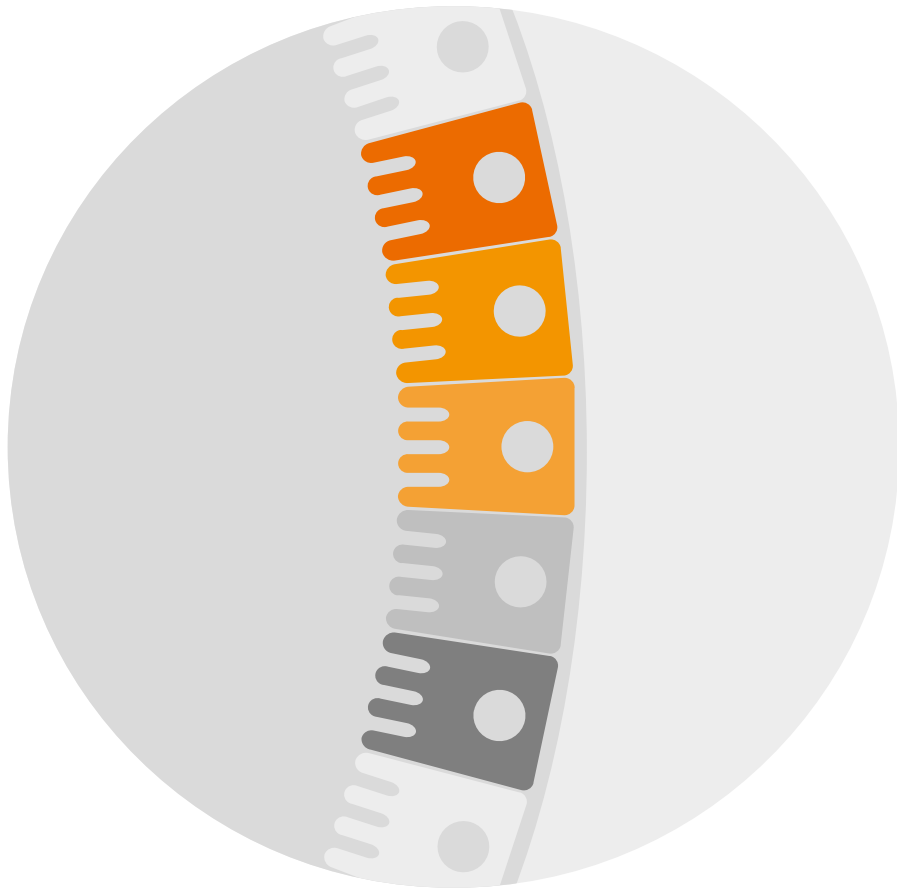
\* Monobutyryn 43= monobutyryn + free glycerol

++++ = No growth

Source: SILO Patent EP 2 410 871 B1



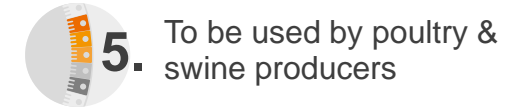
# BalanGut<sup>®</sup> LS - Properties




- 1 Well protected and easy to handle source of butyric acid in the form of monobutyrim ✓
- 2 Site of action – the gut ✓
- 3 Supports gut integrity and host defense ✓
- 4 Supports beneficial bacteria via monobutyrim & medium chain fatty acid (C8-C10) glycerides ✓
- 5 To be used by poultry and swine producers ✓

# Research Trial

## Experimental design




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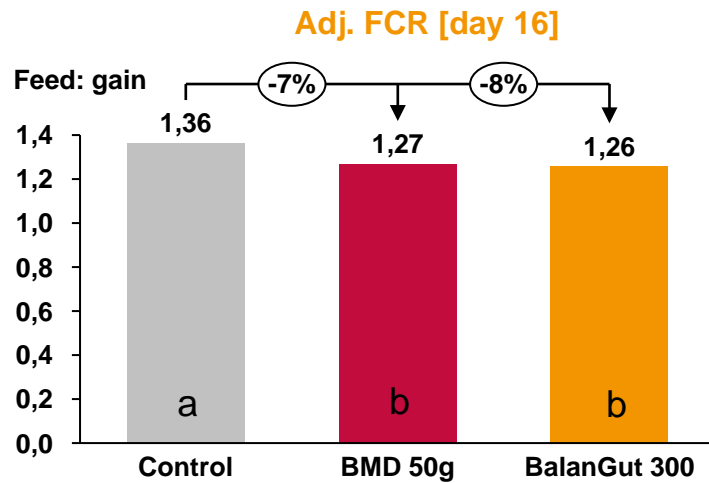
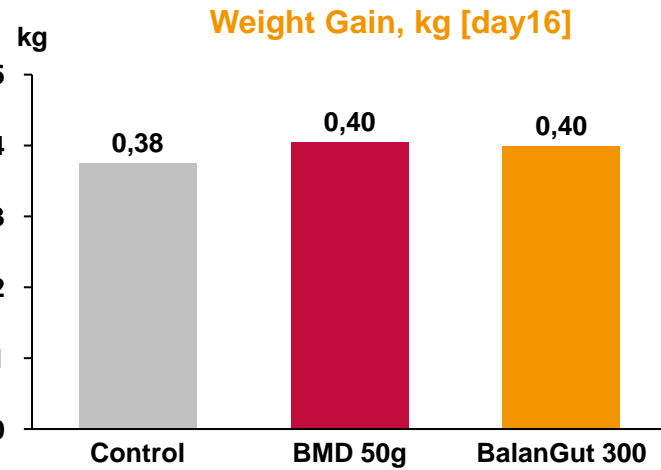
<b>Location</b>	Southern Poultry Research, Inc. 
<b>Animals:</b>	Cobb-Vantress 500 broilers, housed in floor pens with woodshavings
<b>Replication:</b>	3 treatments. 9 pen replicates/ treatment, 7 birds/pen
<b>Trial Length:</b>	49 Days
<b>Basal Diet</b>	3 phases feeding corn/soy diet (pellet). Feed and water permanently <i>ad libitum</i> Starter (0-16) Grower (16-33) Finisher (33-49)
<b>Challenge Model description</b>	No challenge
<b>Treatments:</b>	T1) Negative control, No product no antibiotics T2) BMD: Basal diet, supplemented with BMD at 50 g/MT feed T3) BalanGut® LS L: Basal diet, supplemented with BalanGut® LS L at 300 g /MT feed in starter and grower only
<b>Measurements:</b>	BW, BWG, FI, FCR, and mortality

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# Research Trial

## Performance results

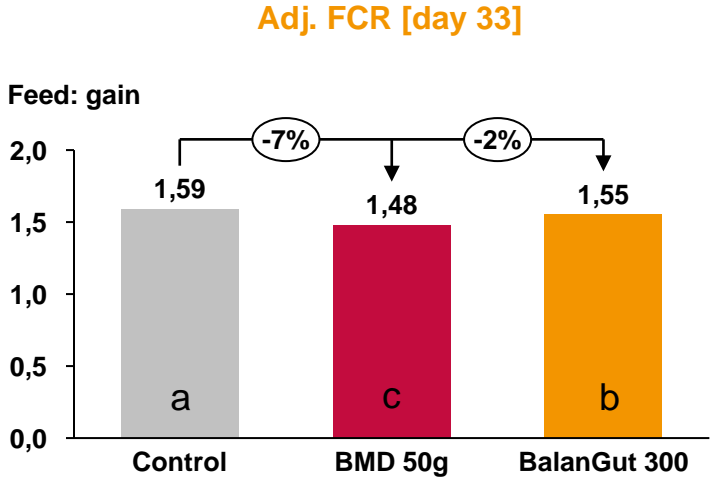
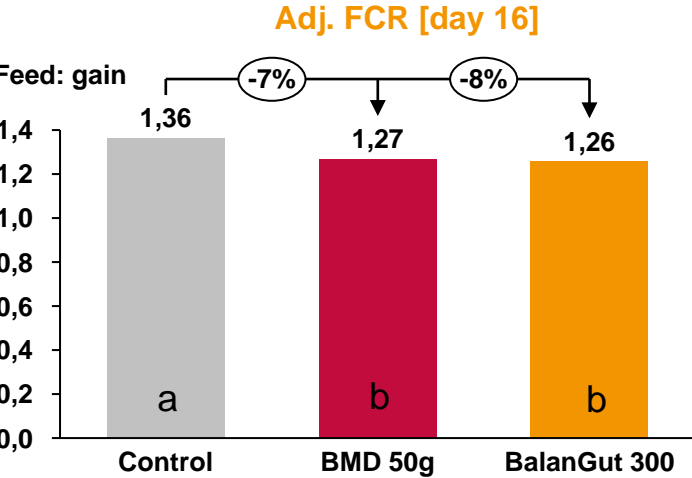
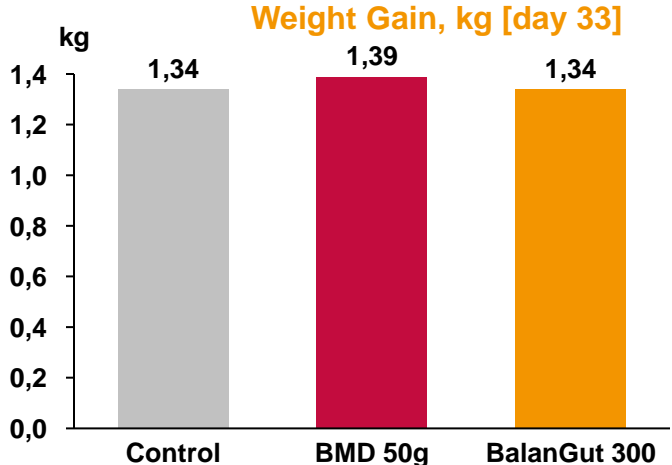
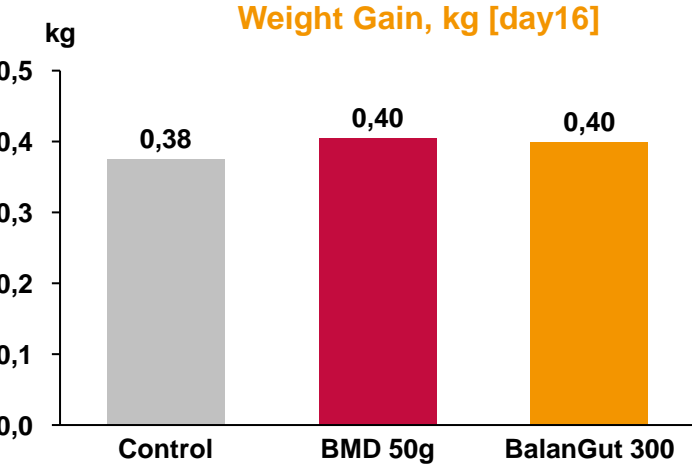
 **5.** To be used by poultry & swine producers



# Research Trial

## Performance results

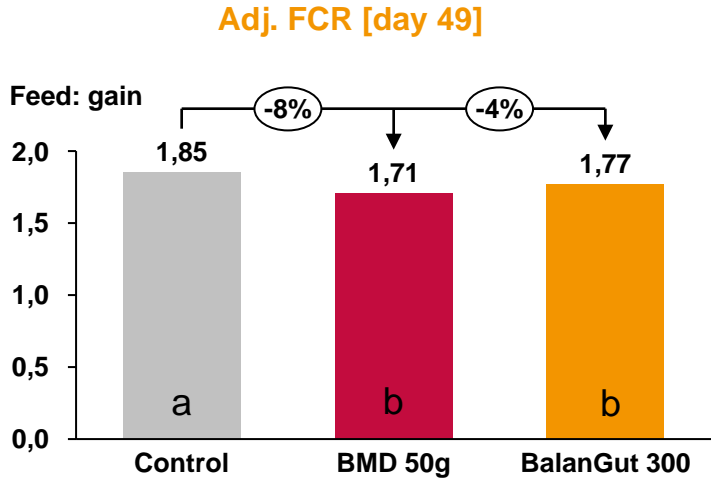
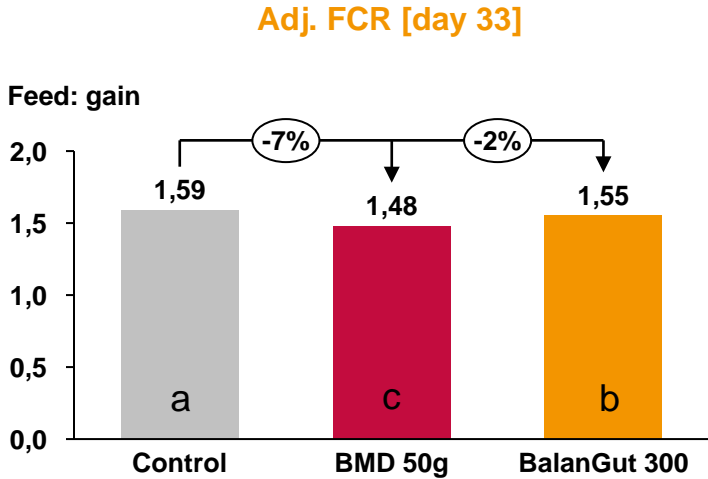
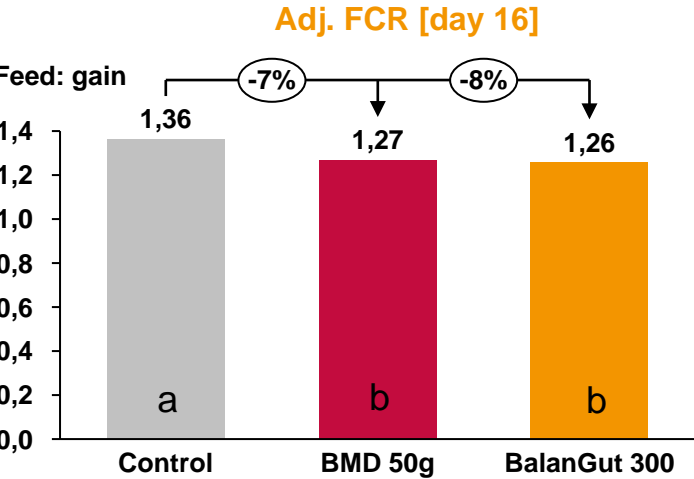
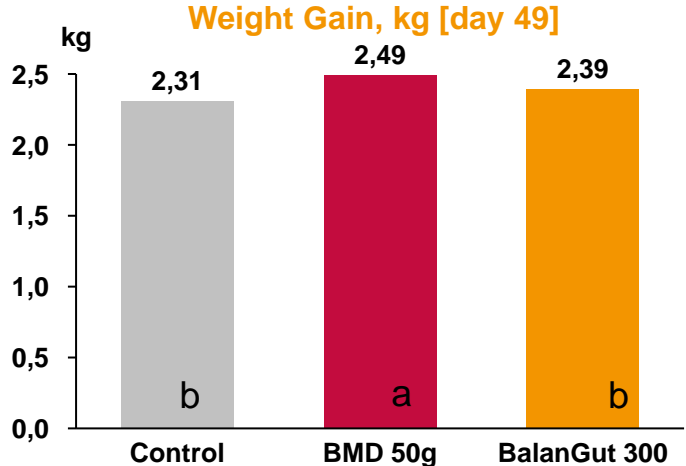
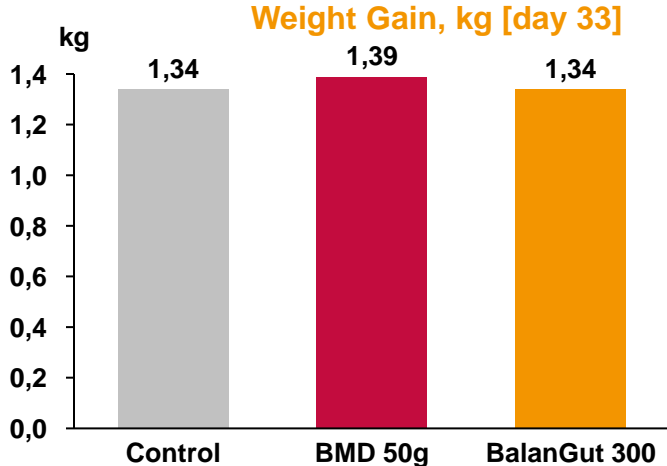
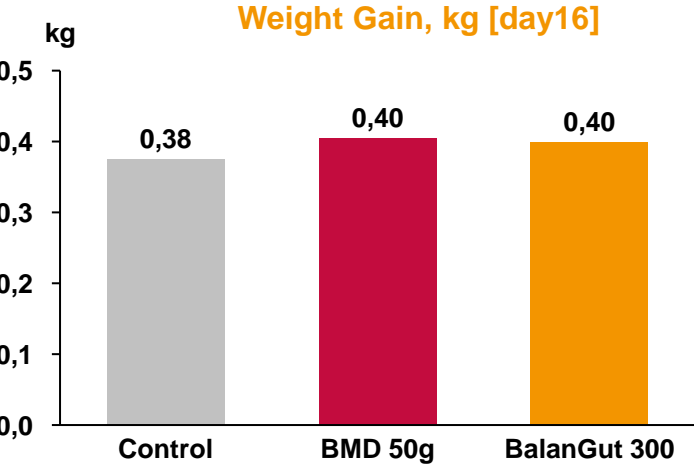
**5.** To be used by poultry & swine producers



# Research Trial

## Performance results

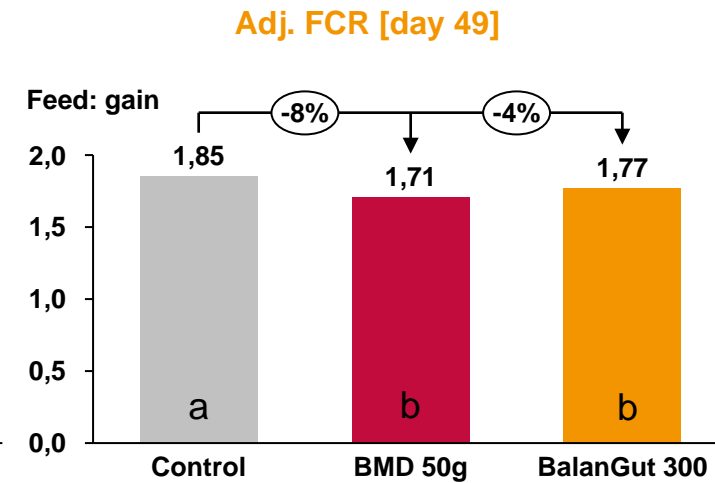
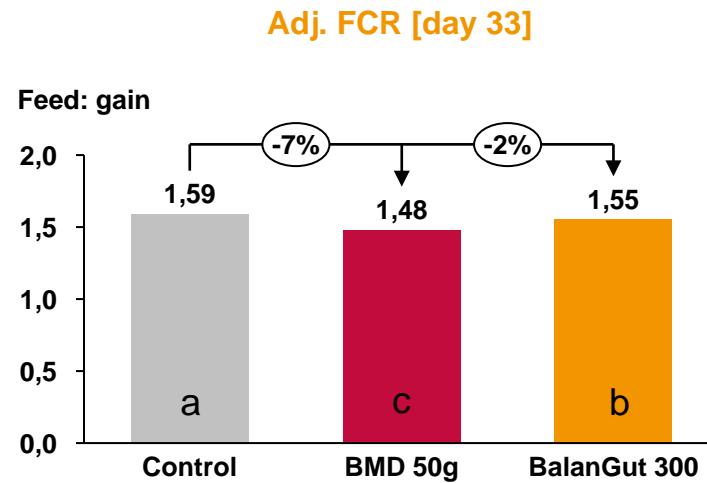
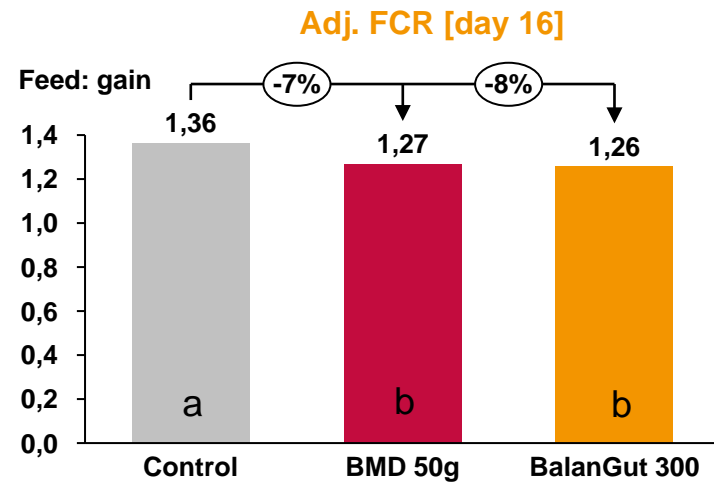
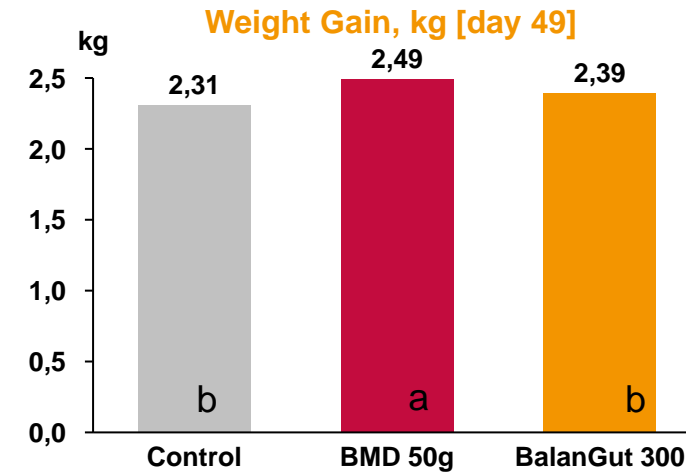
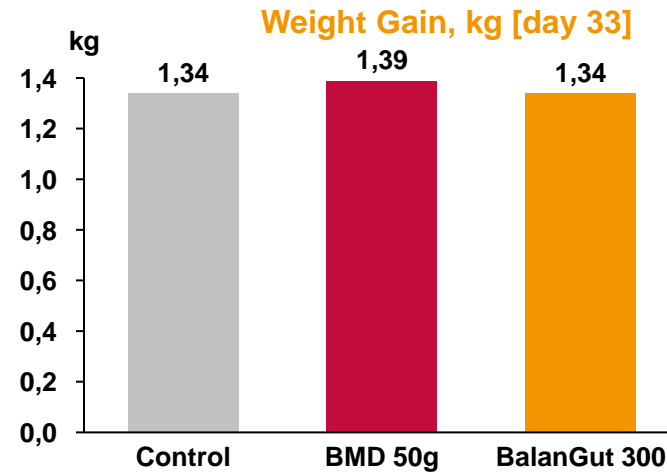
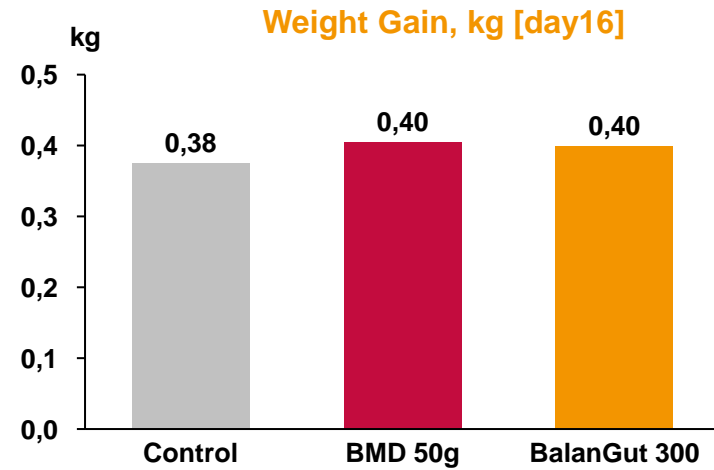
**5.** To be used by poultry & swine producers



# Research Trial

## Performance results

5. To be used by poultry & swine producers



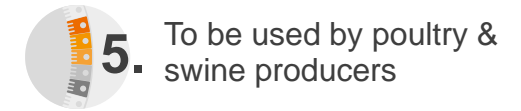
### Conclusions


BalanGut® LS supplemented at 300gMT feed significantly improved FCR of broiler chickens raised under optimal research conditions

BalanGut® LS fed at 300g/MT had comparable results in FCR to birds fed with BMD (AGP)

# Commercial Trial

## Experimental design



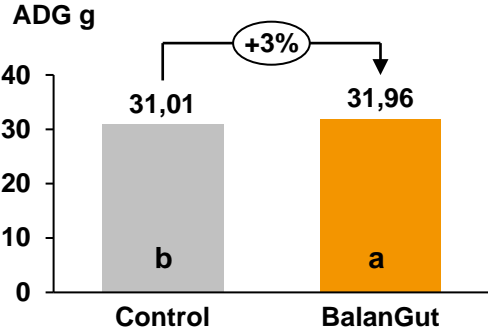
<b>Location</b>	Customer Site (ABF operation) 
<b>Animals:</b>	Cobb 500 broilers, housed in pens with used litter
<b>Replication:</b>	2 treatments. 4 houses: 2 for control & 2 for BalanGut®. One control house contained pens inside; a total of 24 pens (12 pen replicates/ treatment, 18 birds/pen)
<b>Trial Length:</b>	47 Days
<b>Basal Diet</b>	4 phases feeding corn/soy diet (pellet). Feed and water <i>ad libitum</i> Starter (0-17d) Grower (17-28d) Finisher (28-38d) Withdrawal (38-47d)
<b>Challenge description</b>	Commercial conditions; wintertime “Natural” occurrence on infectious bronchitis
<b>Treatments:</b>	T1) Control: Tributyrin (feed supplemented at 500 g/MT in starter, 500 g/MT in grower; 250 g/MT in finisher, 0 g/MT in withdrawal ) T2) BalanGut® LS P (feed supplemented at 500 g /MT in starter, 500 g/MT in grower, 0 g/MT in finisher, 0 g/MT in withdrawal )
<b>Measurements:</b>	BW, BWG, FI, adj FCR ( <i>to mortality and std. BW</i> ), and mortality Data are divided in data collection from the 4 houses; and data collection from the pens (within the control house)

# Commercial Trial

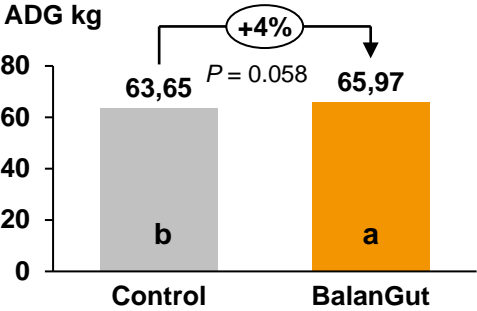
## Performance results & mortality

**5.** To be used by poultry & swine producers

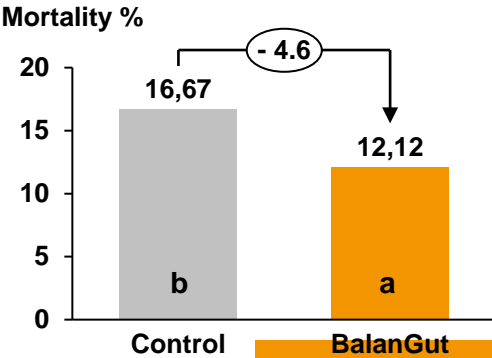
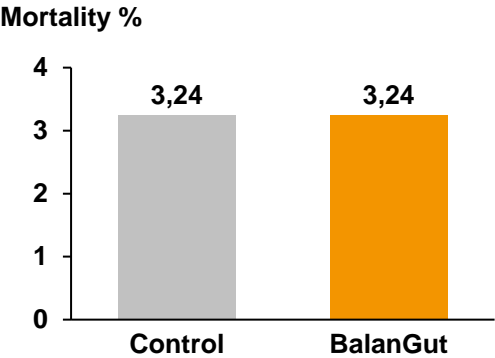
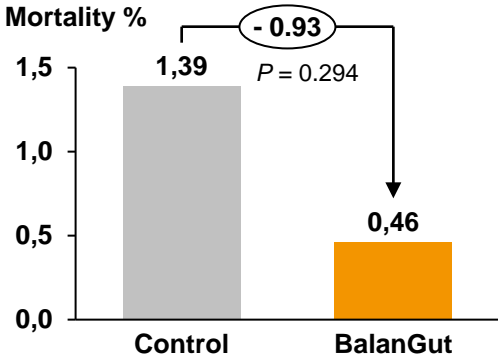
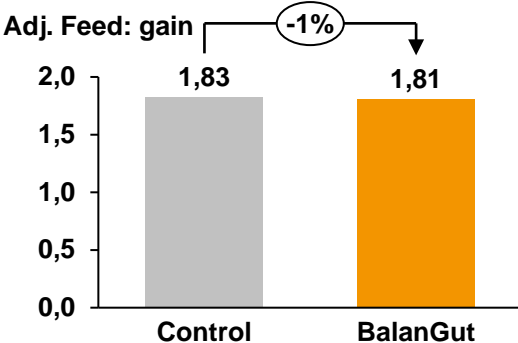
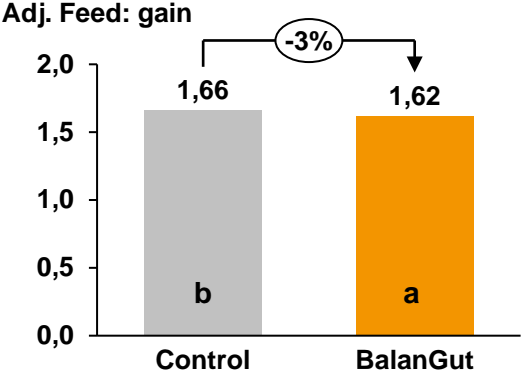
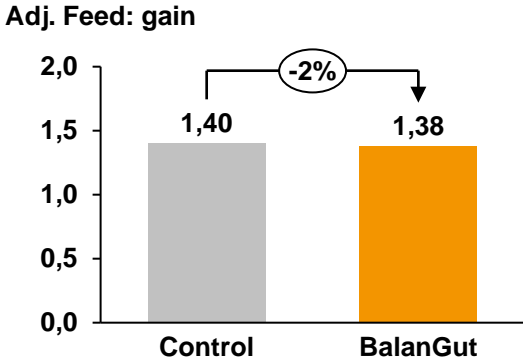
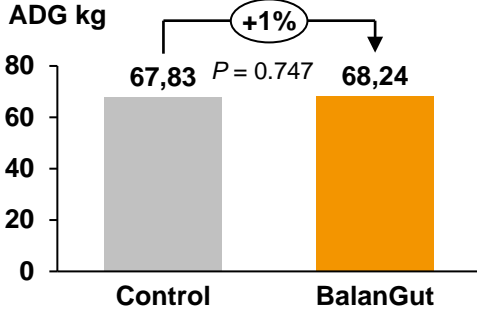
[day 0 - 17]



[day 0 - 38]




[day 0 - 47]



a, b means in a column not sharing a common letter are significantly different ( $P < 0.05$ )



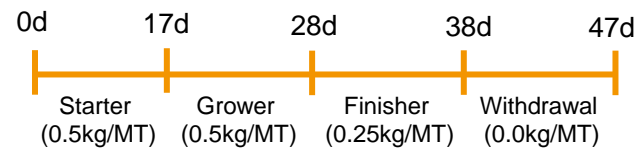
# Commercial Trial Overview

 5. To be used by poultry & swine producers

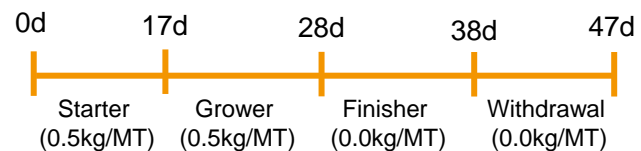
## Set-up

- Comparison of **BalanGut**<sup>®</sup> LS P to a **Control** (Tributylin) group on the same farm
- Trial conducted in winter months with a “natural” occurrence of infectious bronchitis during the study
- Birds were raised in 4 houses (**H**): Control (H1 & 4) and BalanGut<sup>®</sup> (H2 & 3)

### Control (Tributylin)

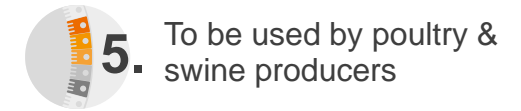


### BalanGut<sup>®</sup>



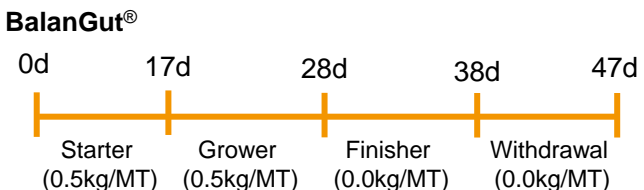
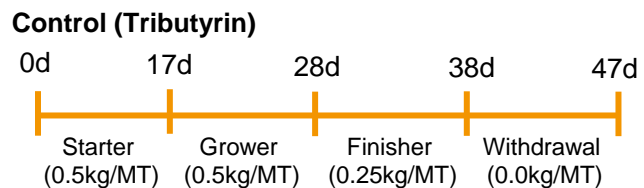
Source: Commercial trial in USA ABF operation

# Commercial Trial Overview & results



## Set-up

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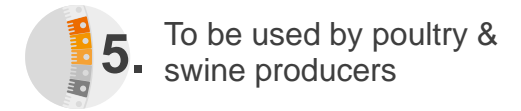
## Results\*

Parameter	Control	BalanGut <sup>®</sup>	Change
# of chicks (1d old)	22,950	24,200	n/a
# of broilers slaughtered	21,100	22,650	n/a
% mortality	8.20%	6.61%	<b>- 1.59%</b> (- 19 %)
% condemn	1.02%	0.78%	<b>- 0.24%</b> (- 23 %)
Av. final weight per bird (kg)	2.99	3.05	<b>+ 60g</b> (+ 2.2 %)
FCR	1.890	1.845	<b>- 0.045</b> (- 2.4 %)
Adj. FCR**	1.844	1.787	<b>- 0.057</b> (- 3.1 %)

\*average of 2 houses/group reported

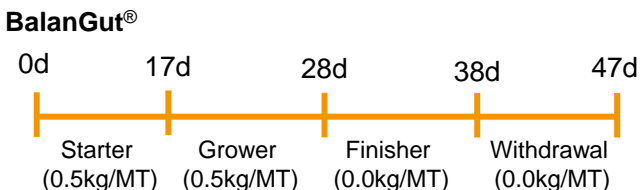
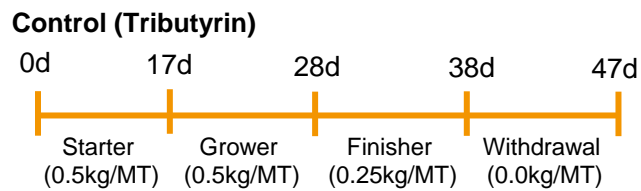
\*\* Adj. FCR- to mortality and std. BW (2.724kg)

# Commercial Trial Overview & results



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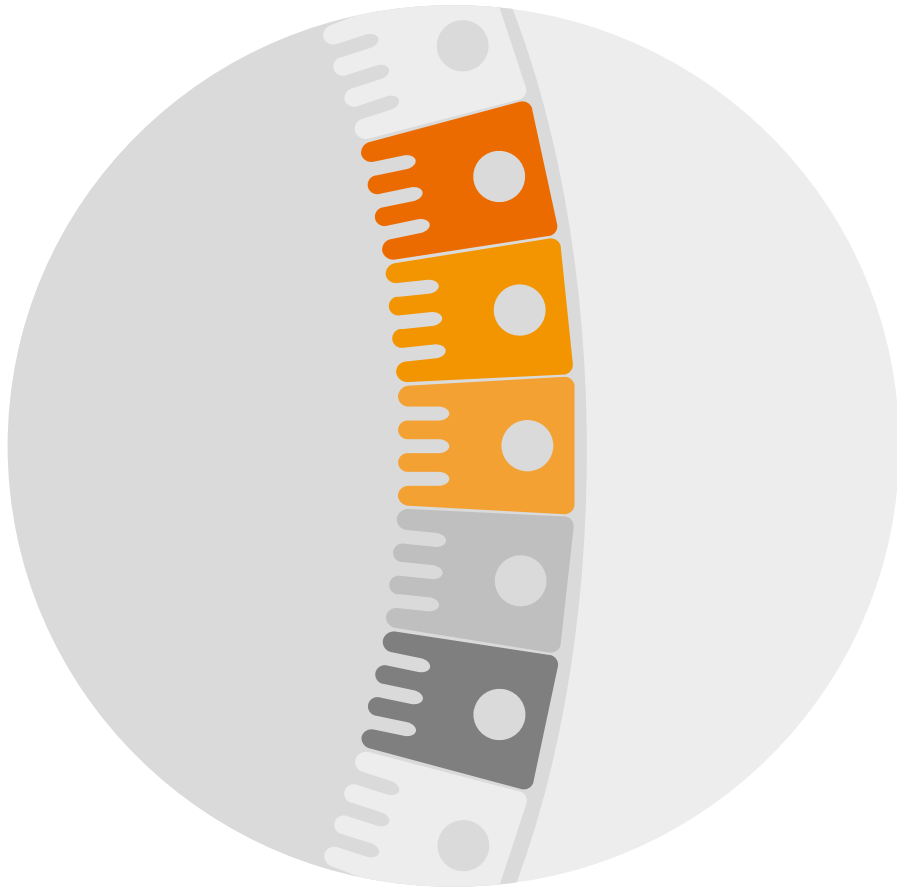
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\*\* Adj. FCR- to mortality and std. BW (2.724kg)

## Conclusions

- BalanGut<sup>®</sup> LS improved all parameters evaluated in comparison to the control group
- BalanGut<sup>®</sup> LS improved the performance of birds during a disease challenge
- BalanGut<sup>®</sup> LS dose at 500g/MT in the starter and grower phase proved effective under current rearing conditions

# BalanGut<sup>®</sup> LS - Properties



- 1 Well protected and easy to handle source of butyric acid in the form of monobutyrim ✓
- 2 Site of action – the gut ✓
- 3 Supports gut integrity and host defense ✓
- 4 Supports beneficial bacteria via monobutyrim & medium chain fatty acid (C8-C10) glycerides ✓
- 5 To be used by poultry and swine producers ✓

# BalanGut® LS

## Conclusions

- As the poultry industry transitions to antibiotic-free production, there is an **urgent need to identify science based economical solutions for promoting gut health.**
- A clear understanding of the **Mechanism of Action** of our products will **allow** us to bring valuable, and economical **recommendations** as an approach to manage gut and metabolic health in livestock.
- While some **monoglycerides** are believed to directly inhibit pathogens, **data show** that they can also enhance the **effectiveness** of the host **immune system.**
- Direct action of monoglycerides on pathogens require gastrointestinal effect, whereas **immune system or other physiologic effects** would **require the absorption of intact fats.**
- Data derived from our studies will allow scientists to understand the relative roles of short chain fatty acid esters on bacterial pathogens before and after absorption from the GI tract. **Increased understanding of post-absorptive processes will allow the design of more effective pre-harvest intervention tools.**

# BalanGut<sup>®</sup> LS

## Conclusions





We create chemistry