Feed additive combinations – the key to fully unlocking healthy poultry potential?

January 16, 2014

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Today's Speakers:

Dr. Kirk Klasing
University of California

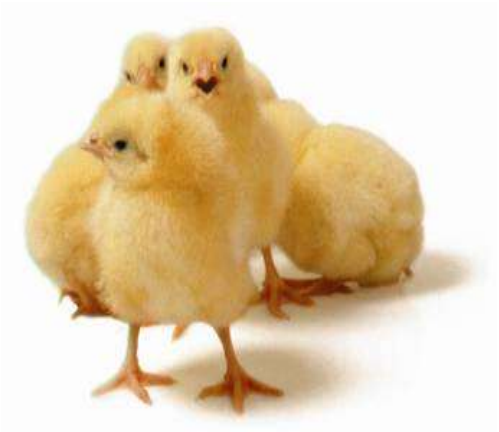
Dr. Ajay Awati
Danisco Animal Nutrition

Dr. Kirk Klasing is an extensively published expert on poultry nutrition and immunology, with more than 175 peer reviewed publications, 10 books and eight awards to his name for his work in poultry nutrition. Specialist topics focus on the interaction between poultry nutrition and immunity, including the effect of diet on immune-competence, the nutritional cost of immunity and the impact of immune responses on growth-related physiology. He is currently Professor of Avian Biology in the Department of Animal Science at the University of California, Davis, where he has worked for over 25 years. Previously, he was Assistant Professor of Animal Science at University of Illinois. He holds a BS in Agricultural Science and an MS in Animal Science from Purdue University and a PhD in Nutritional Biochemistry from Cornell University.

What is the Nutritional Cost of Achieving Immunity

KIRK C. KLASING

UNIVERSITY OF CALIFORNIA, DAVIS



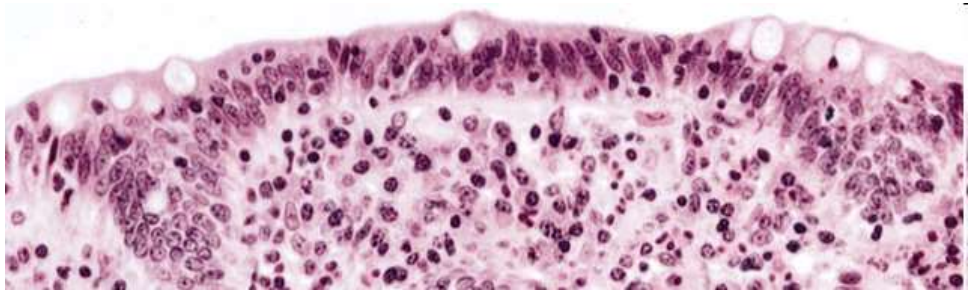
Tackling recognized pathogens is only part of the story in tackling poultry gut health

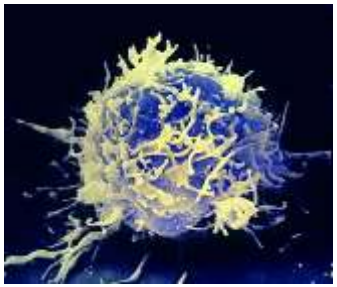
- A 1 kg chicken consumes ≈ 15 g food antigens daily & possesses ≈ 15 g of antigens associated with commensal microflora

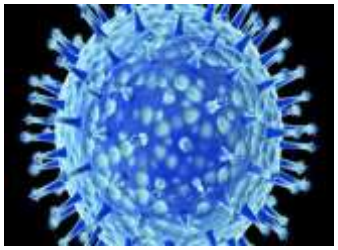
- The immune system has the capacity to detect antigens at the ng/ml level (10^{-9}g/ml)

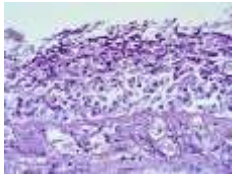
- Pathogen antigens are the proverbial “needle in the haystack”

Bird age, genetics and production conditions impact immunity...

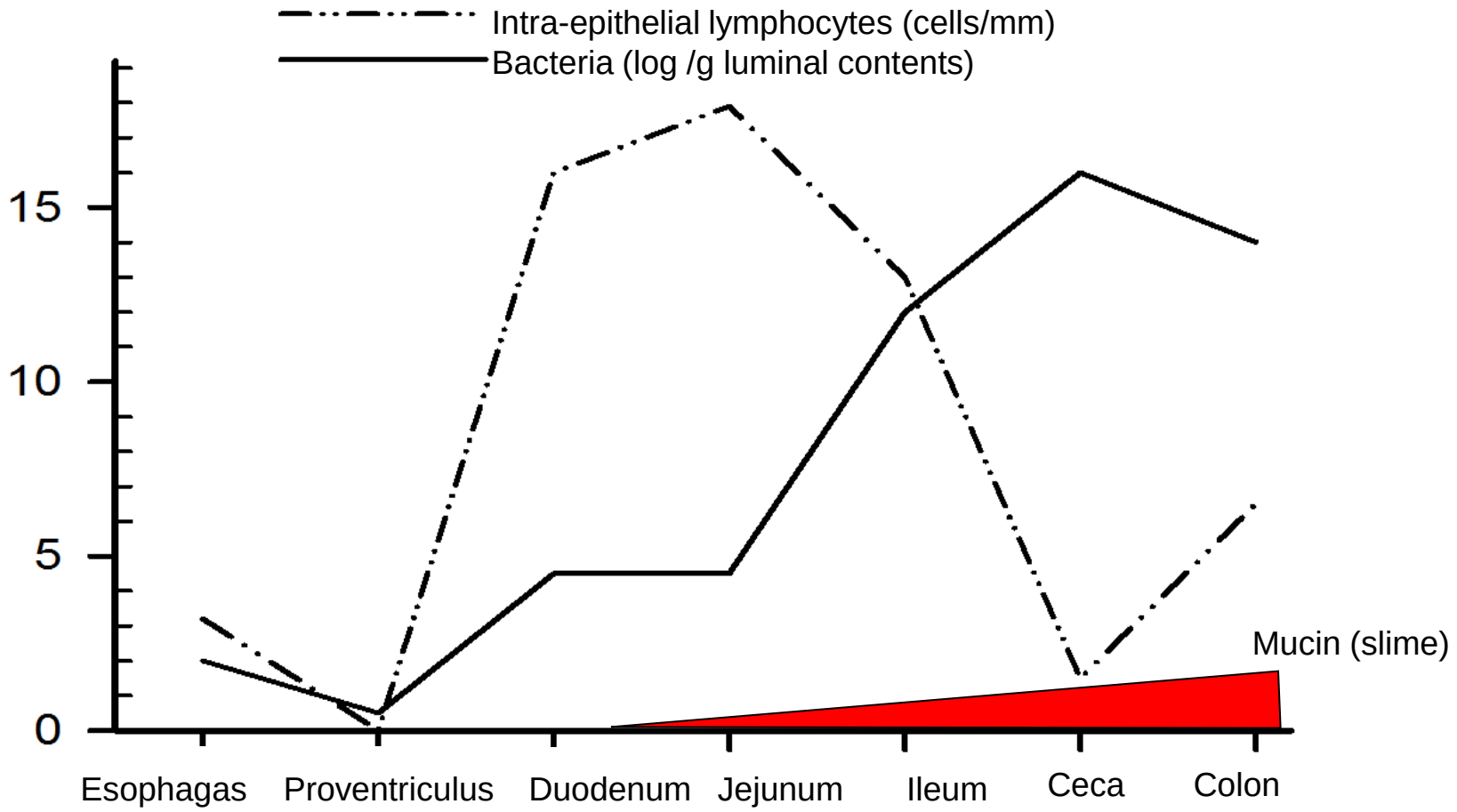








HYGIENE



Intra-epithelial lymphocytes (IEL) in chickens are natural killer (NK), B and T cells (Klasing, 2005).

Microbial ecology

- **Luminal micro-biota** – greatly influenced by chemical and physical composition of digesta and rate of passage.
Typically do not trigger an inflammatory or cellular response (unless toxins released)

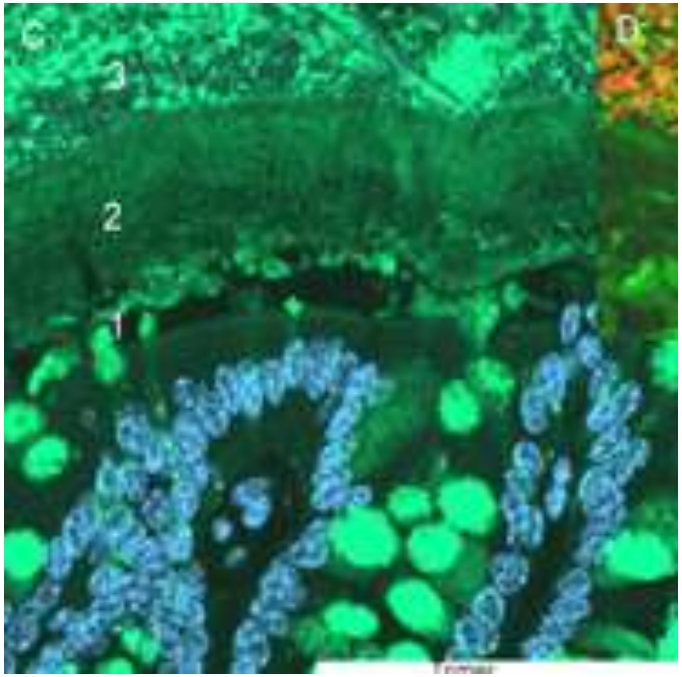
- Secretory IgA response – tethers those that penetrate into mucous layer for removal

- **Mucous micro-biota**

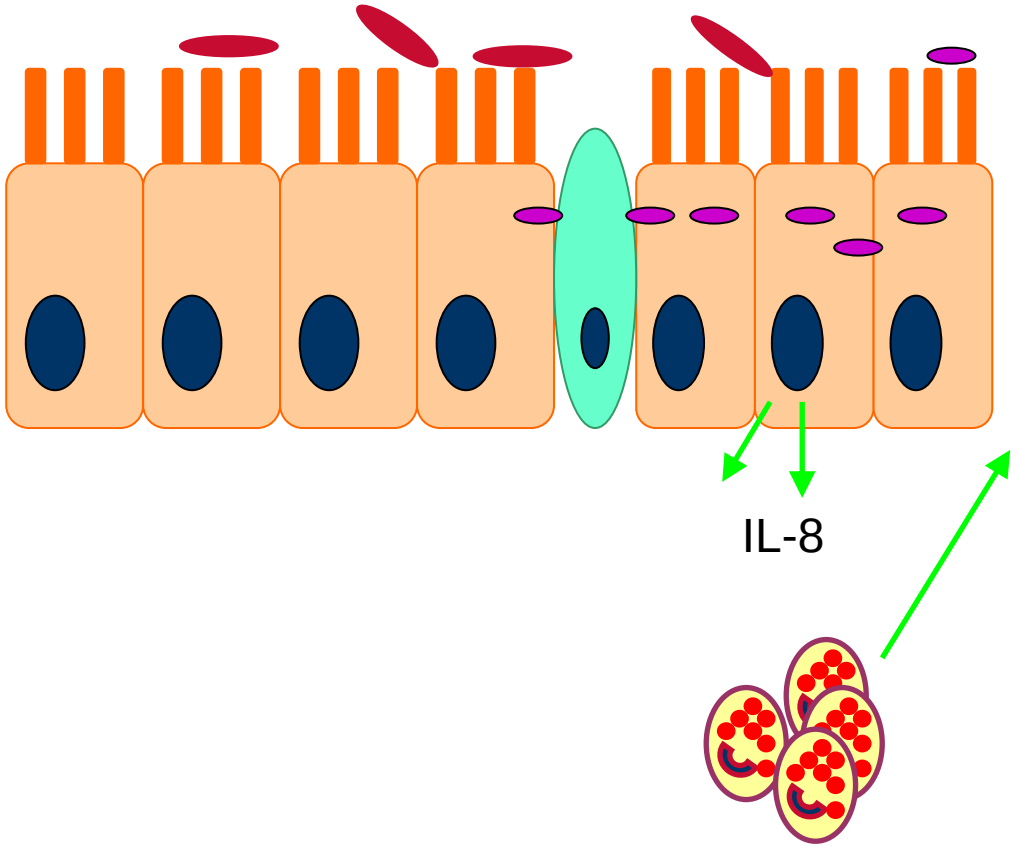
- **Epithelial micro-biota**

- **Mucous micro-biota** – specialized to use mucin as nutrition

- Receptors for mucin
- Trigger benign IgA response
- Nutritional effects?



- **Epithelial microbiota**
- Attach to glycocalyx or apical membrane. May form bio-film



Immune-response relationships

- **Commensal** - relationship between two organisms where one organism benefits but the other is unaffected

- **Mutualism** – both organisms benefit

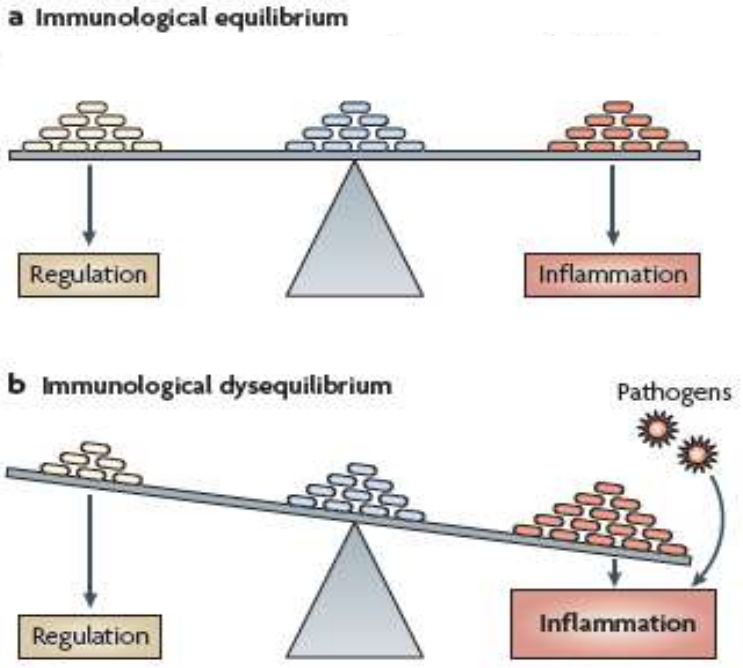
- **Pathogenic** – harmful

Often the same microbial species can have all three of these relationships, depending on circumstance!

Gene expression pattern is often more important than species

Nutrition and Immune response are linked

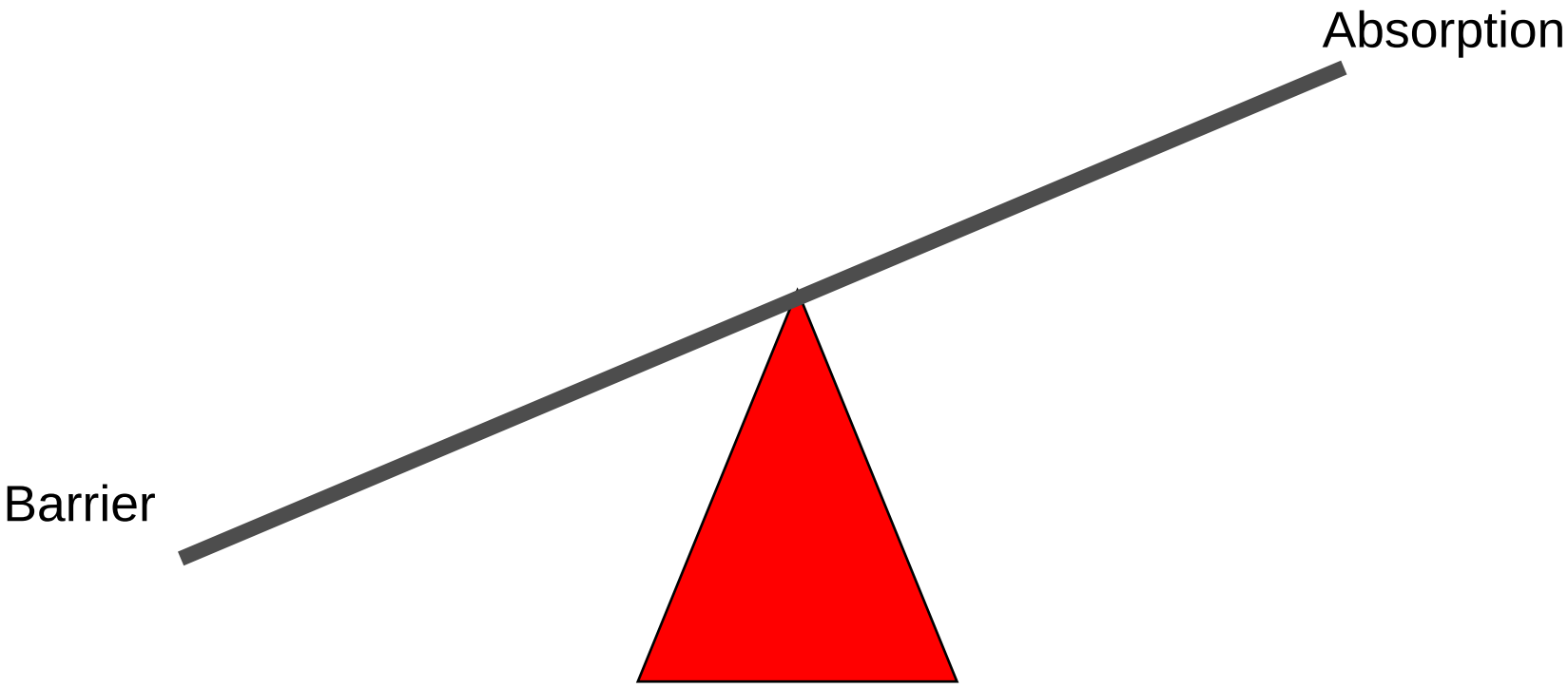
- Almost all nutrients in a diet play a fundamental role in sustaining optimal immune function
- Deficient or excessive nutrient intake can negatively impact immune status or pathogen susceptibility
- Very important to maintain immune activity without inflammation



Kogut & Klasing, 2009

Intestinal Integrity(1)

"The cells and products constituting the barrier against leakage or translocation of feed components, microbial toxins and microorganisms from the lumen to the body"
Jeurissen et al., 2002



Intestinal Integrity(2)

"The cells and products constituting the barrier against leakage or translocation of feed components, microbial toxins and microorganisms from the lumen to the body"
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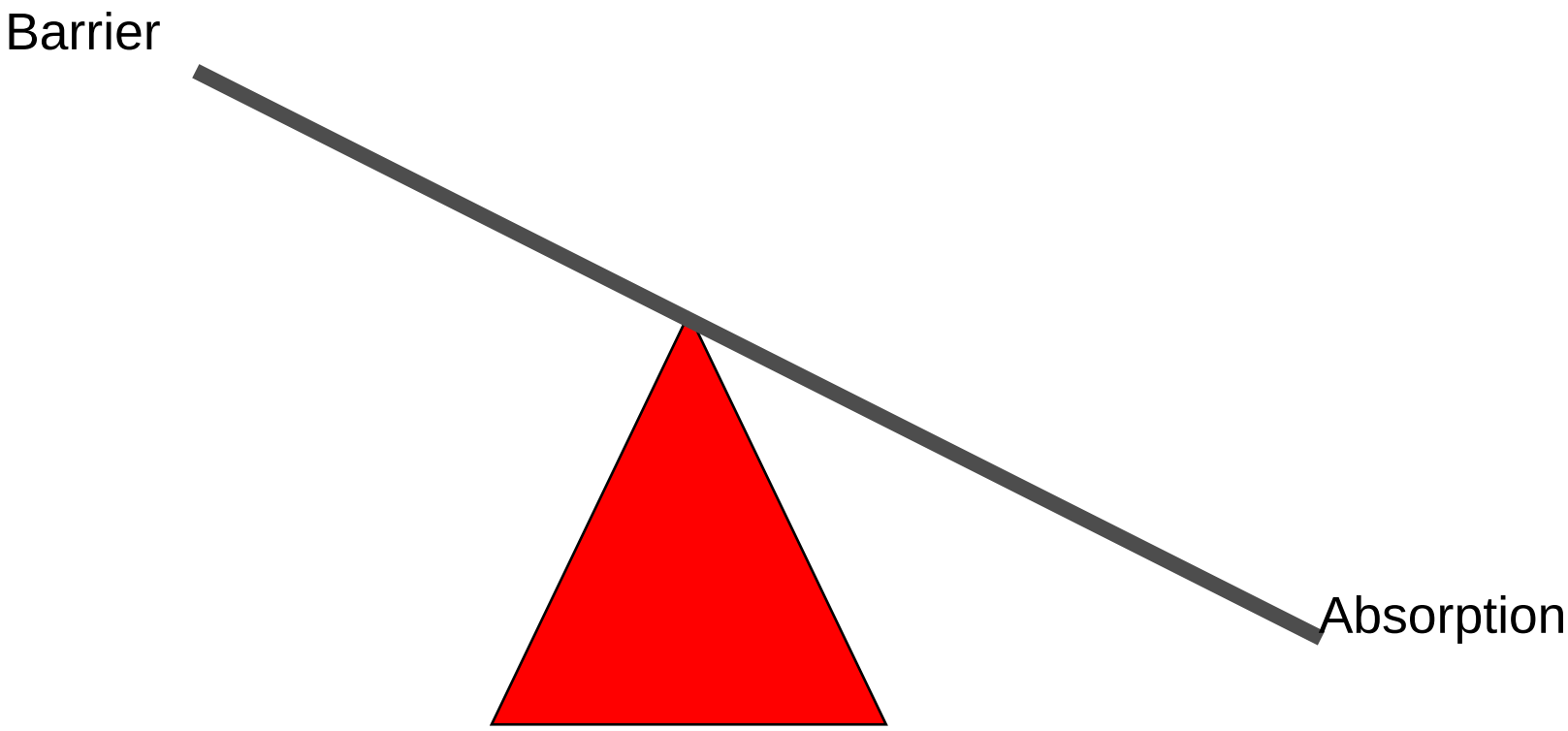


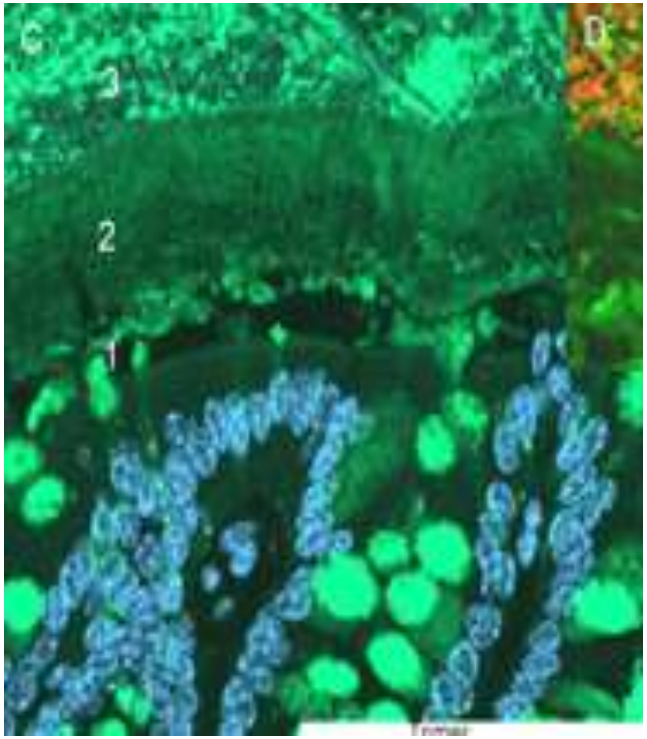
The essential balance

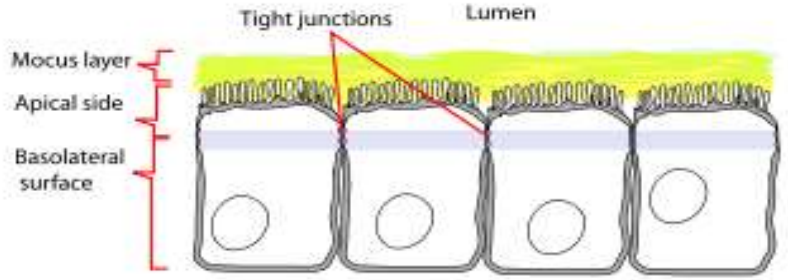
- Positive gut microbes
  - Early colonizers of gut
  - Form protective barrier
  - Provide benign stimulation of innate immune syste
  - Maintain equilibrium by strength of numbers

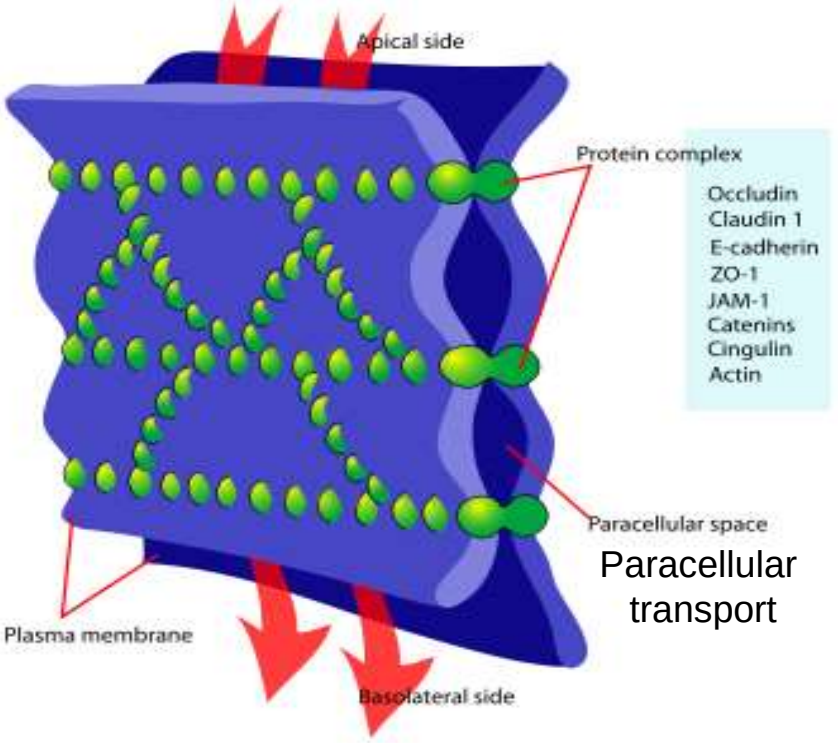
- Solution - make sure the equilibrium is maintained and the positive gut microbes are in control

Barrier integrity: Epithelial tight junctions

Mucin fibril network



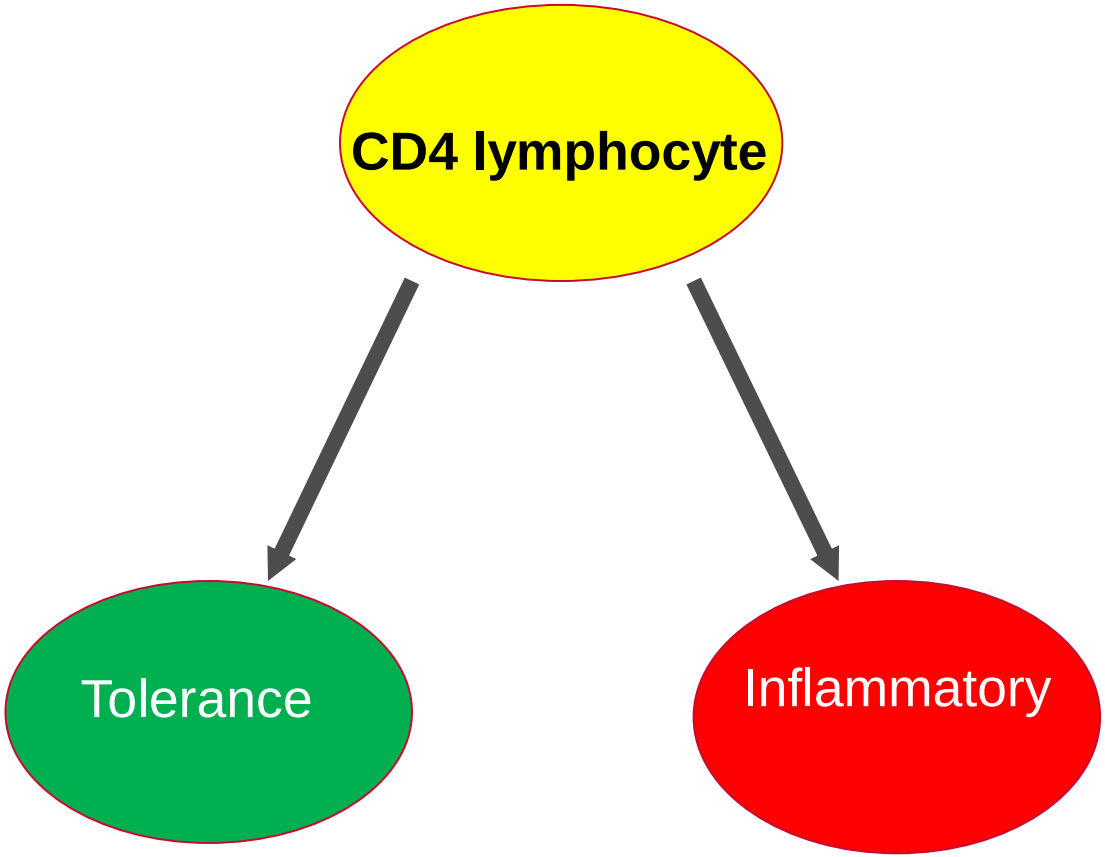




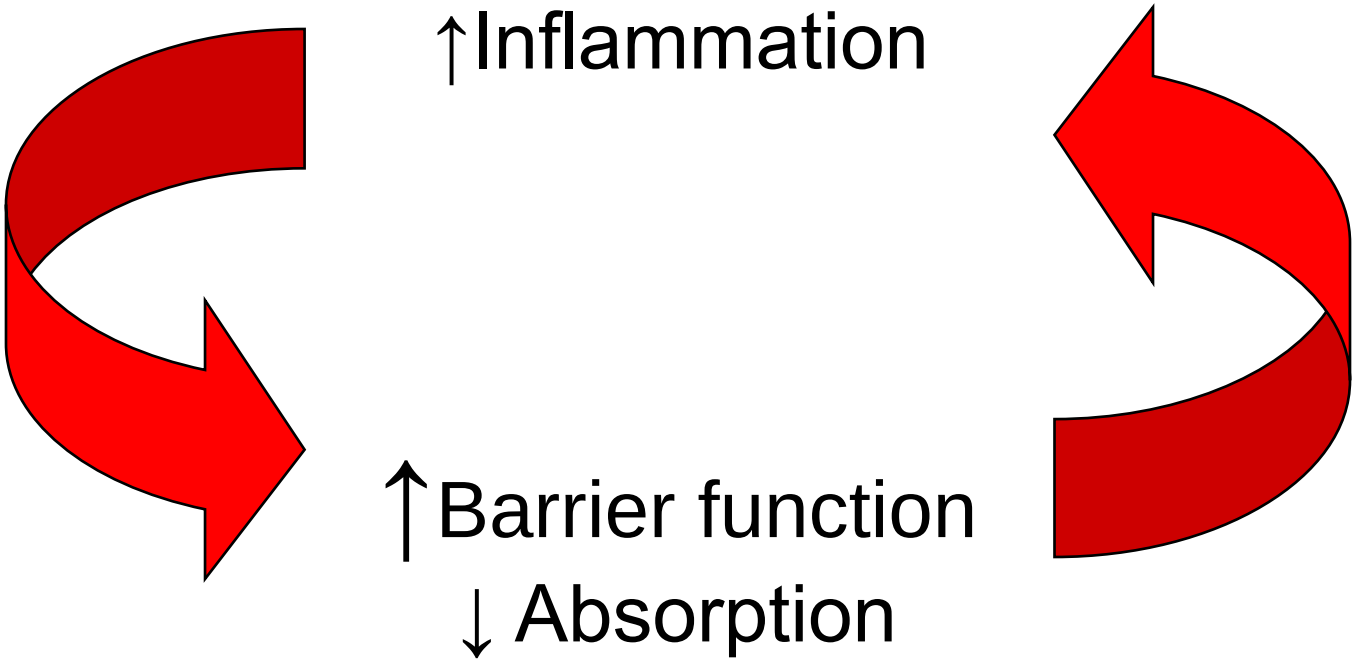
Paracellular transport

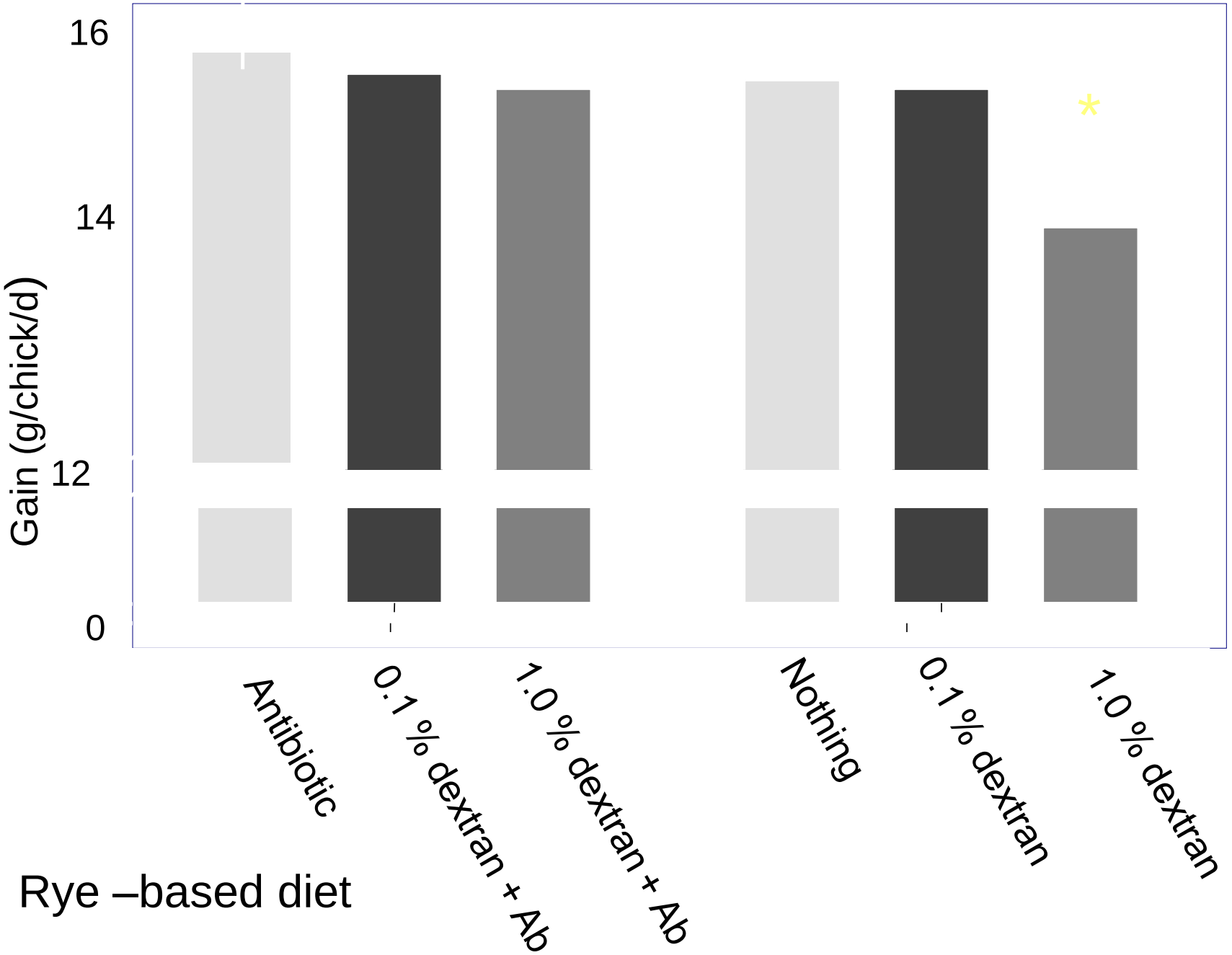
The immune system judges micro-biota by:

- Location
- Looks (MAMPS)
- Damage

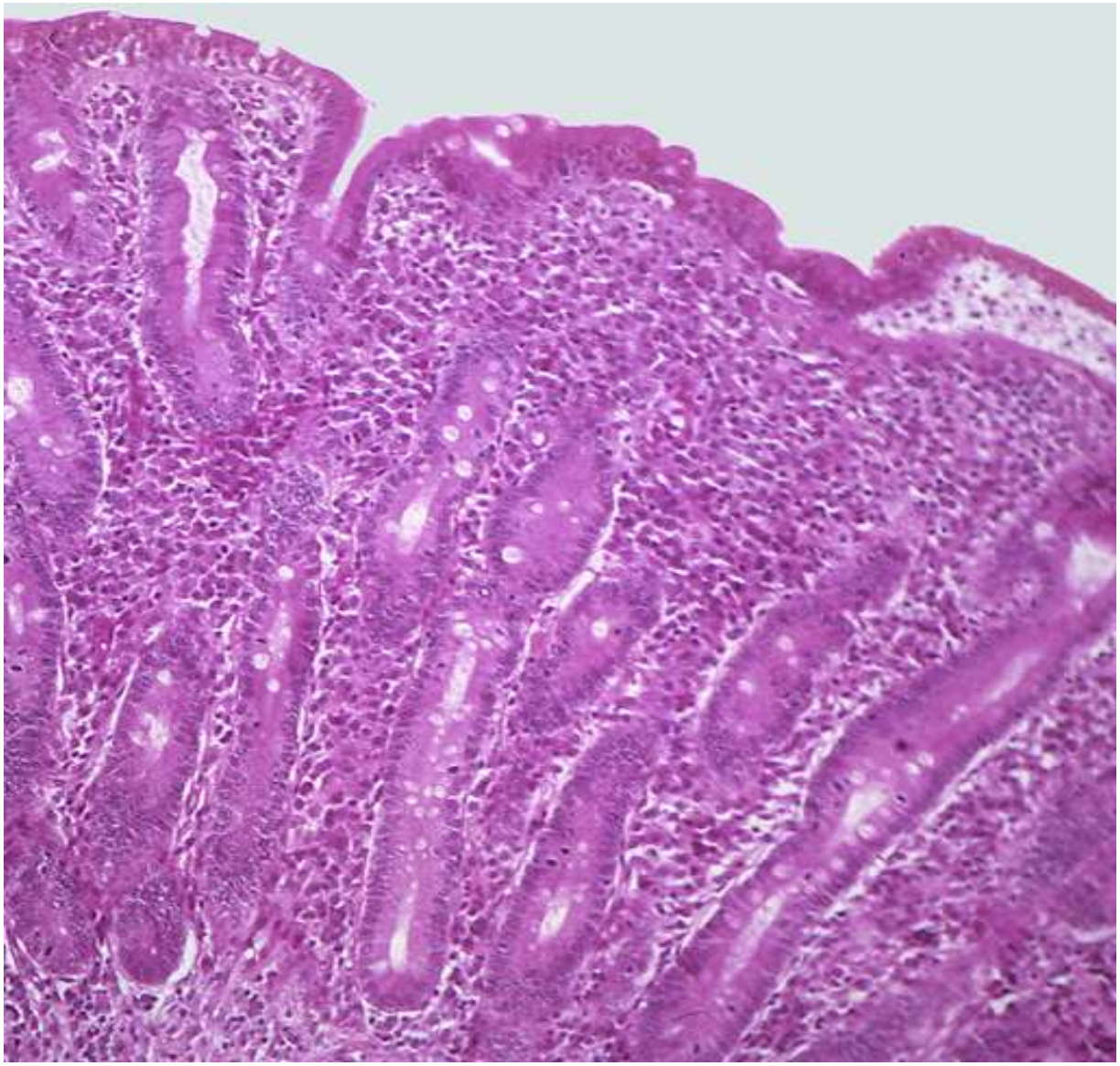


Effects of an inflammatory response on barrier and absorptive functions

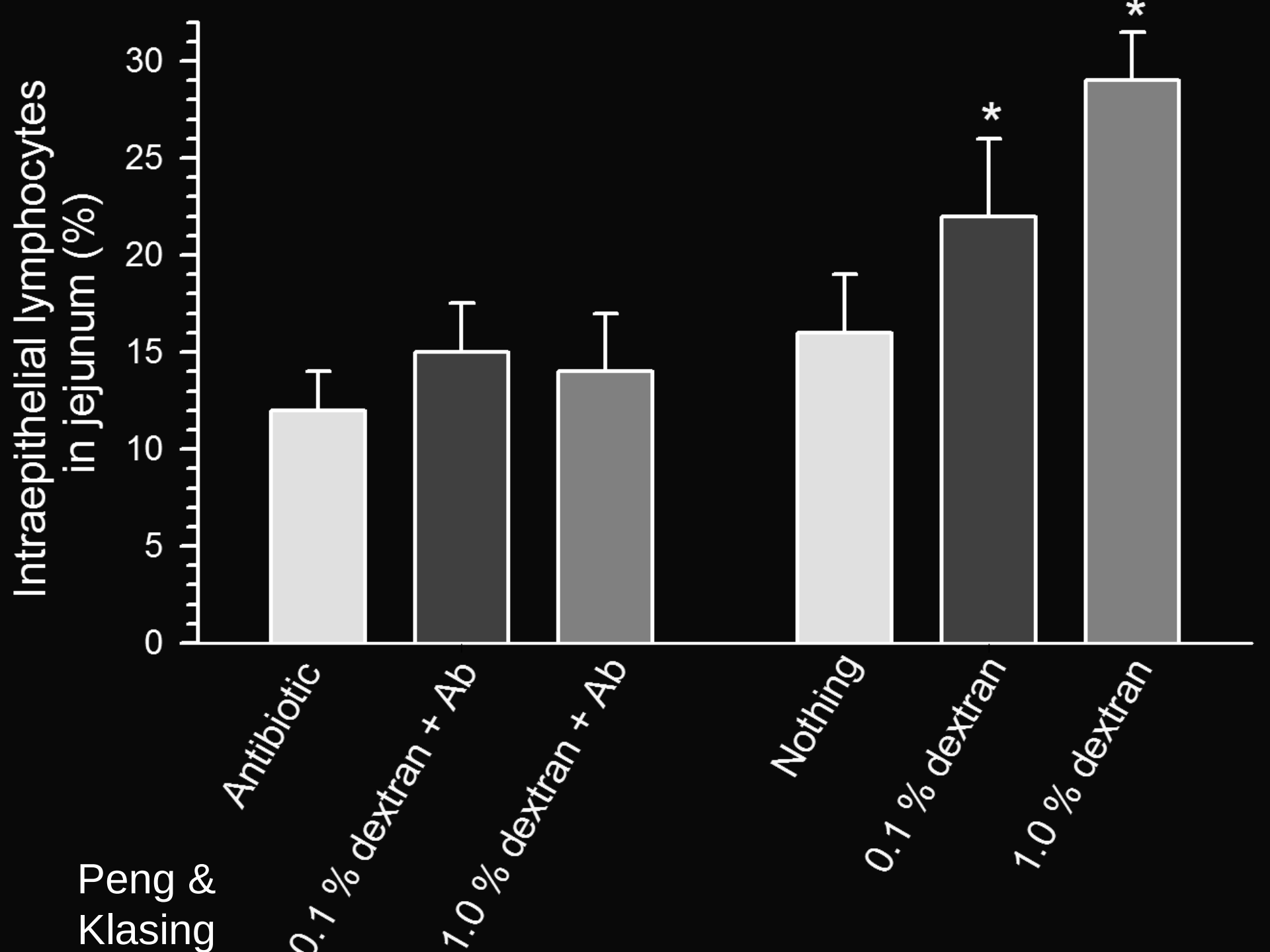


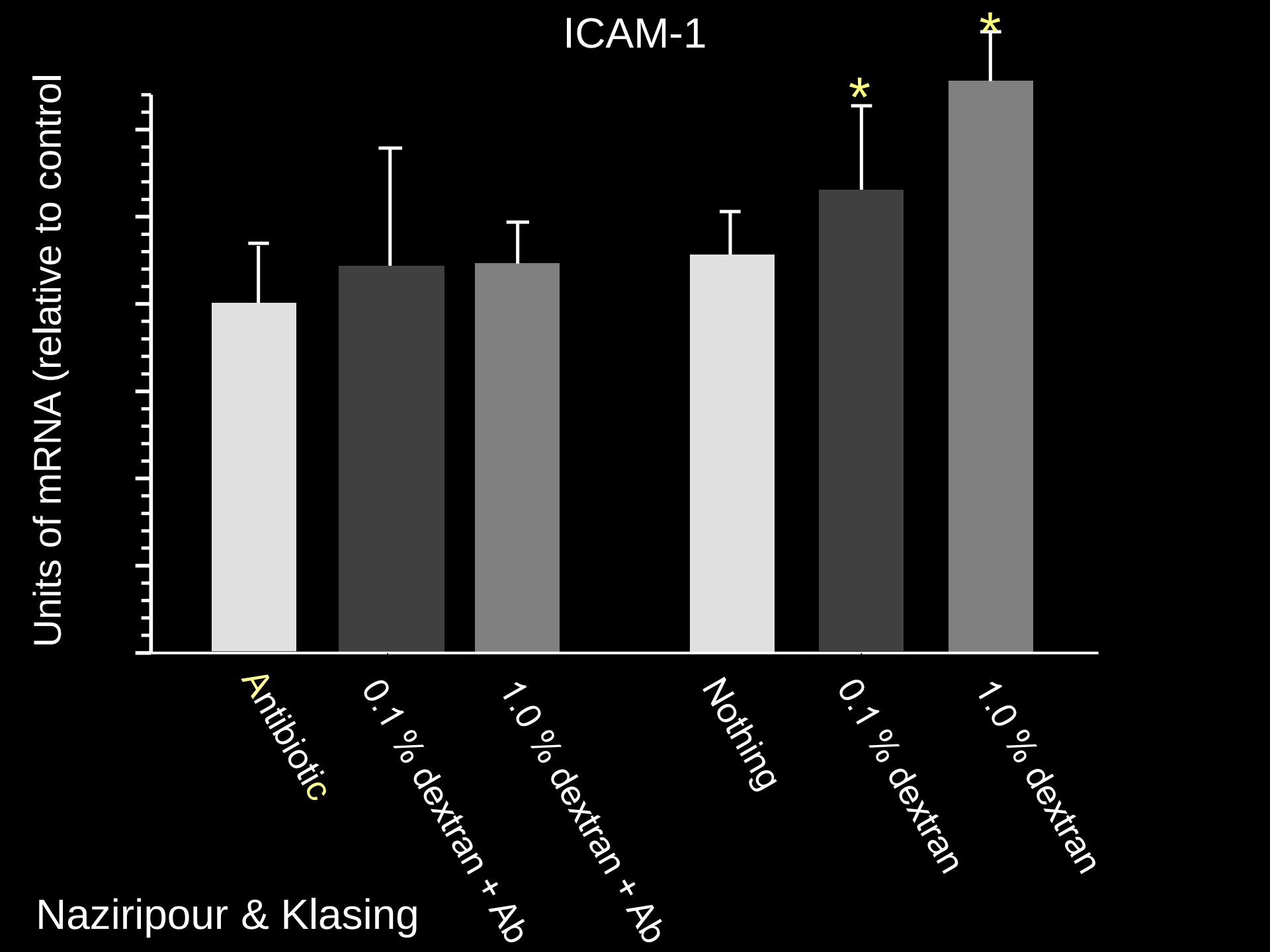


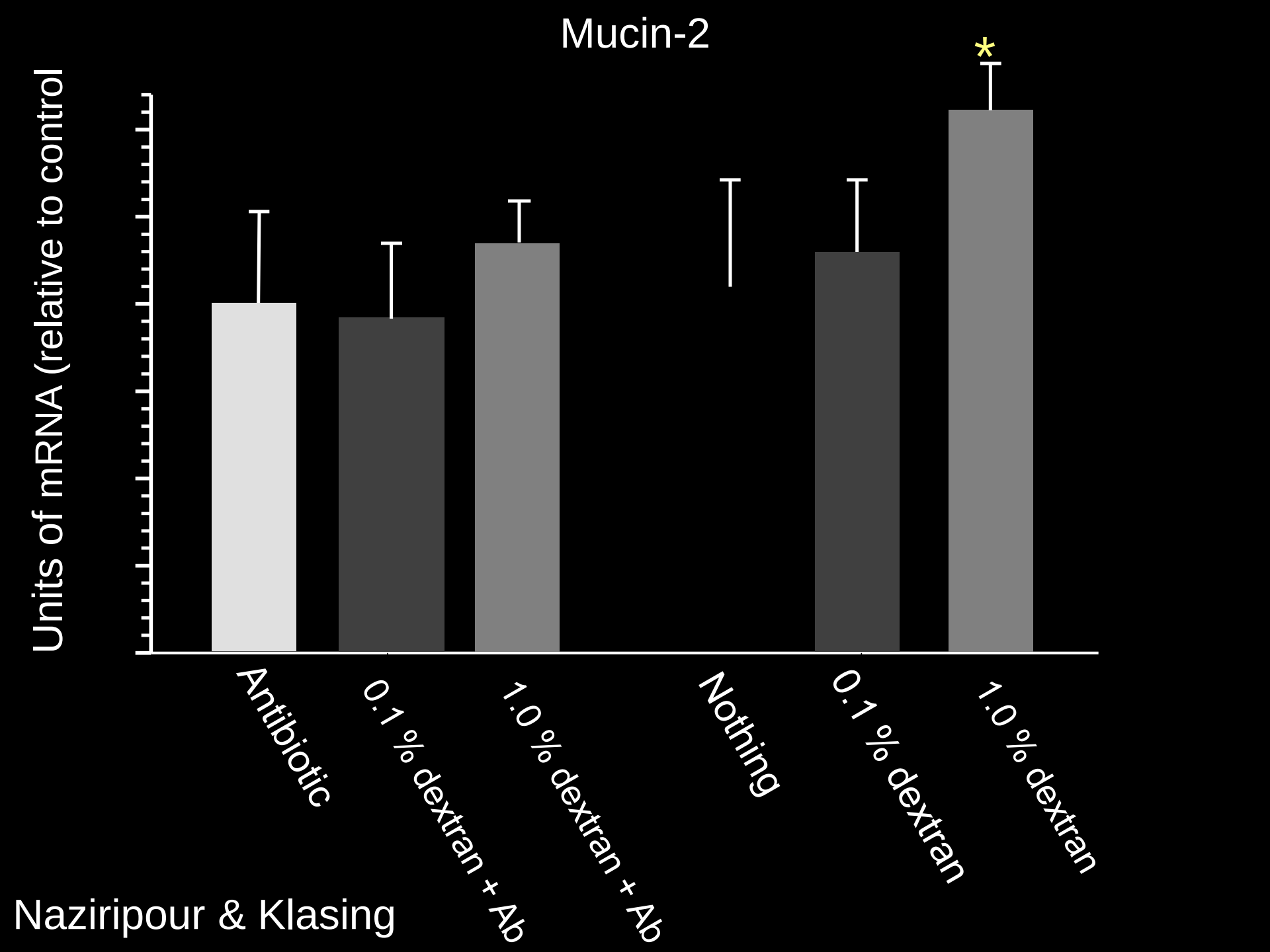
Rye –based diet



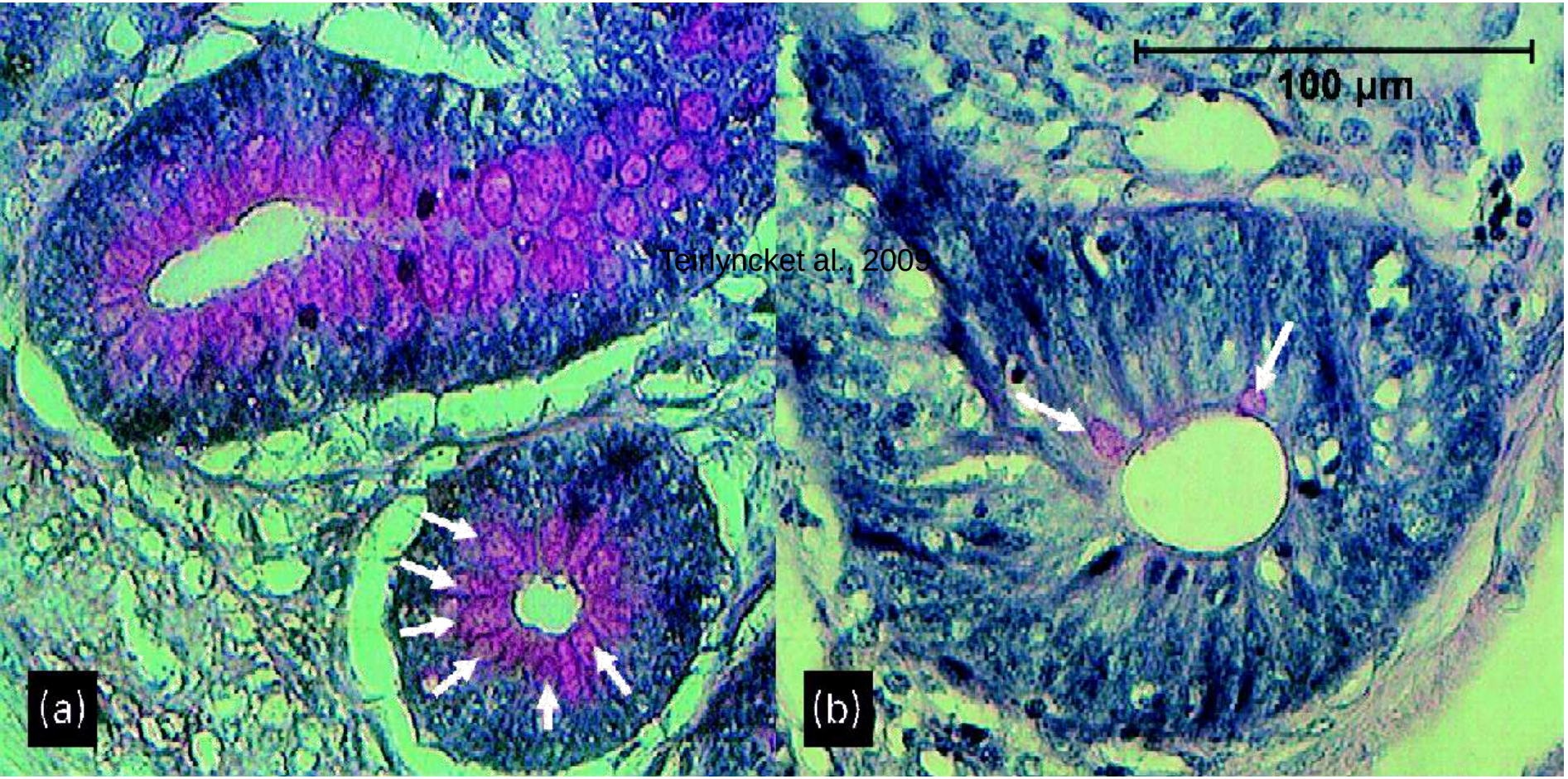
Rye + dextran


Intraepithelial lymphocytes in jejunum (%)
0
5
10
15
20
25
30
*
*
Antibiotic
0.1 % dextran + Ab
1.0 % dextran + Ab
Nothing
0.1 % dextran
1.0 % dextran
Peng & Klasing


ICAM-1
Units of mRNA (relative to control
*
*
Antibiotic
0.1 % dextran + Ab
1.0 % dextran + Ab
Nothing
0.1 % dextran
1.0 % dextran
Naziripour & Klasing


Mucin-2
Units of mRNA (relative to control
*
Antibiotic
0.1 % dextran + Ab
1.0 % dextran + Ab
Nothing
0.1 % dextran
1.0 % dextran
Naziripour & Klasing

Larger goblet cells both on the villi and in the crypts at the age of 29 d in broilers given a wheat/rye diet than in those given a maize diet. Especially in the ileum and caecum.

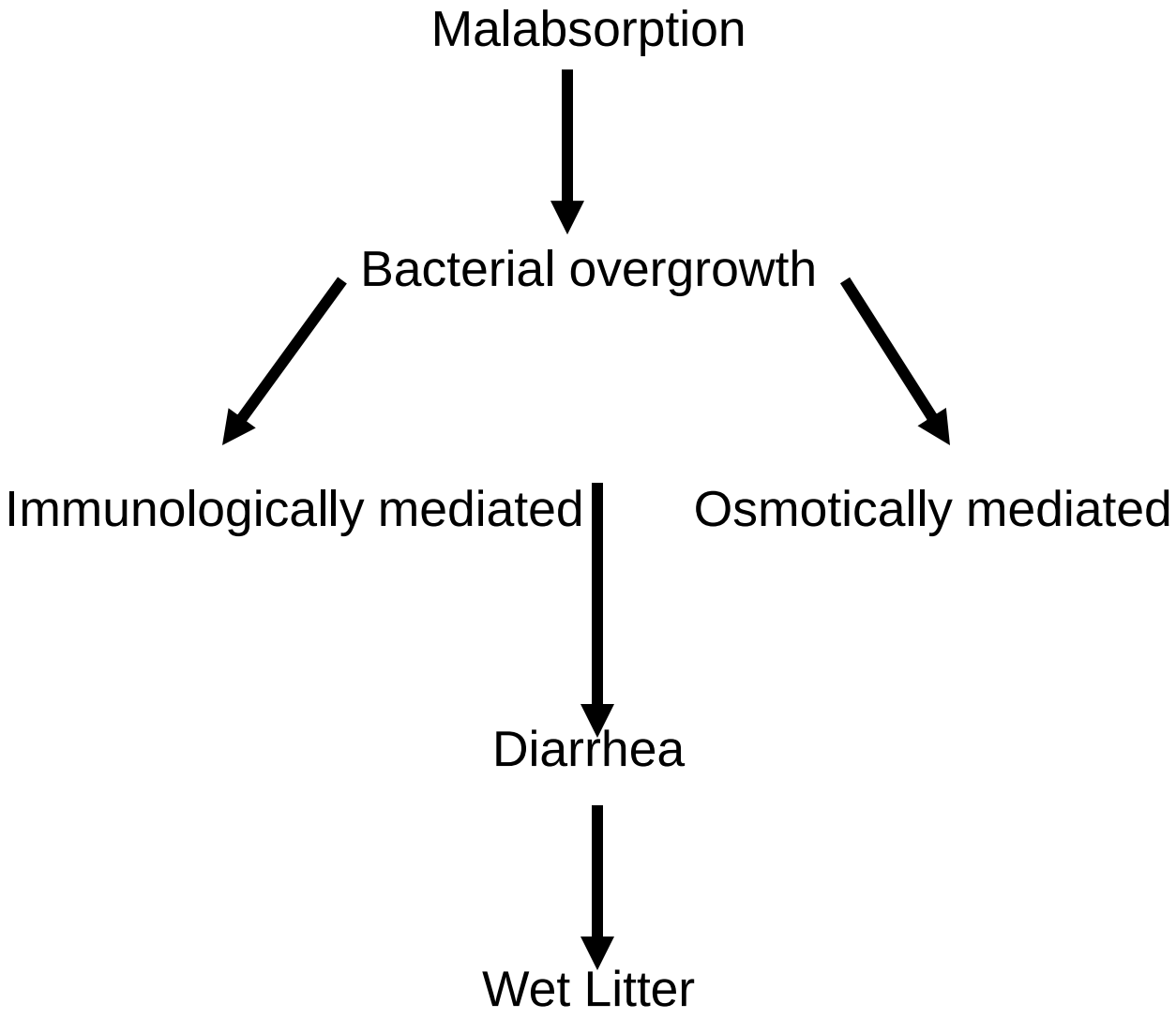


Teirlynck et al., British Journal of Nutrition (2009), 102, 1453–1461

Nutrient absorption during intestinal inflammation

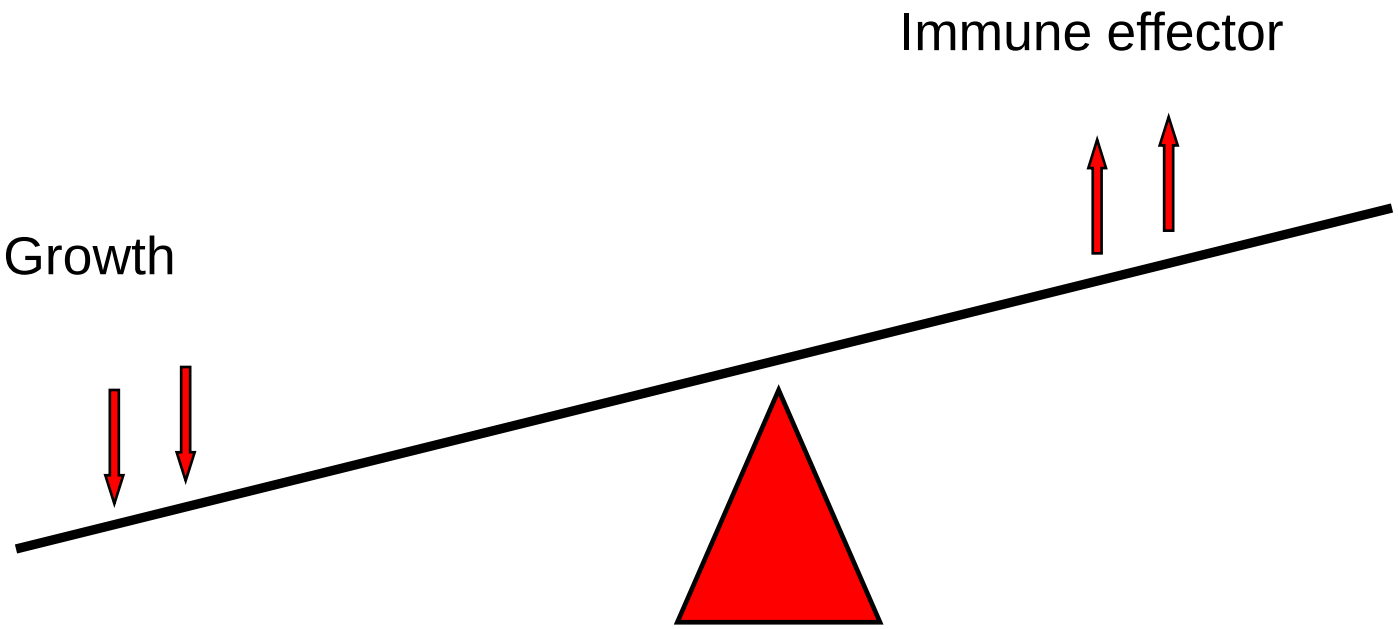
Nutrient	Dietary Treatment					
	Basal	+dextran	+antibiotic	+dextran +antibiotic	SEM	Significant P Value
Nitrogen	100	103	97	102	2.2	D, AxD
Lysine	100	102	97	95	2.9	-
Methionine	100	99	98	103	1.9	-
Glutamine	100	**111**	102	107	2.0	D, AxD
Lipid	100	**88**	105	93	1.8	D, AxD
Retinol	100	**76**	97	76	3.1	D
Lutein	100	**68**	102	79	3.4	D, AxD
Ca	100	100	103	95	2.8	-
Fe	100	94	96	95	2.7	-
Zn	100	**91**	105	96	1.9	D
Cu	100	**90**	97	94	1.8	D

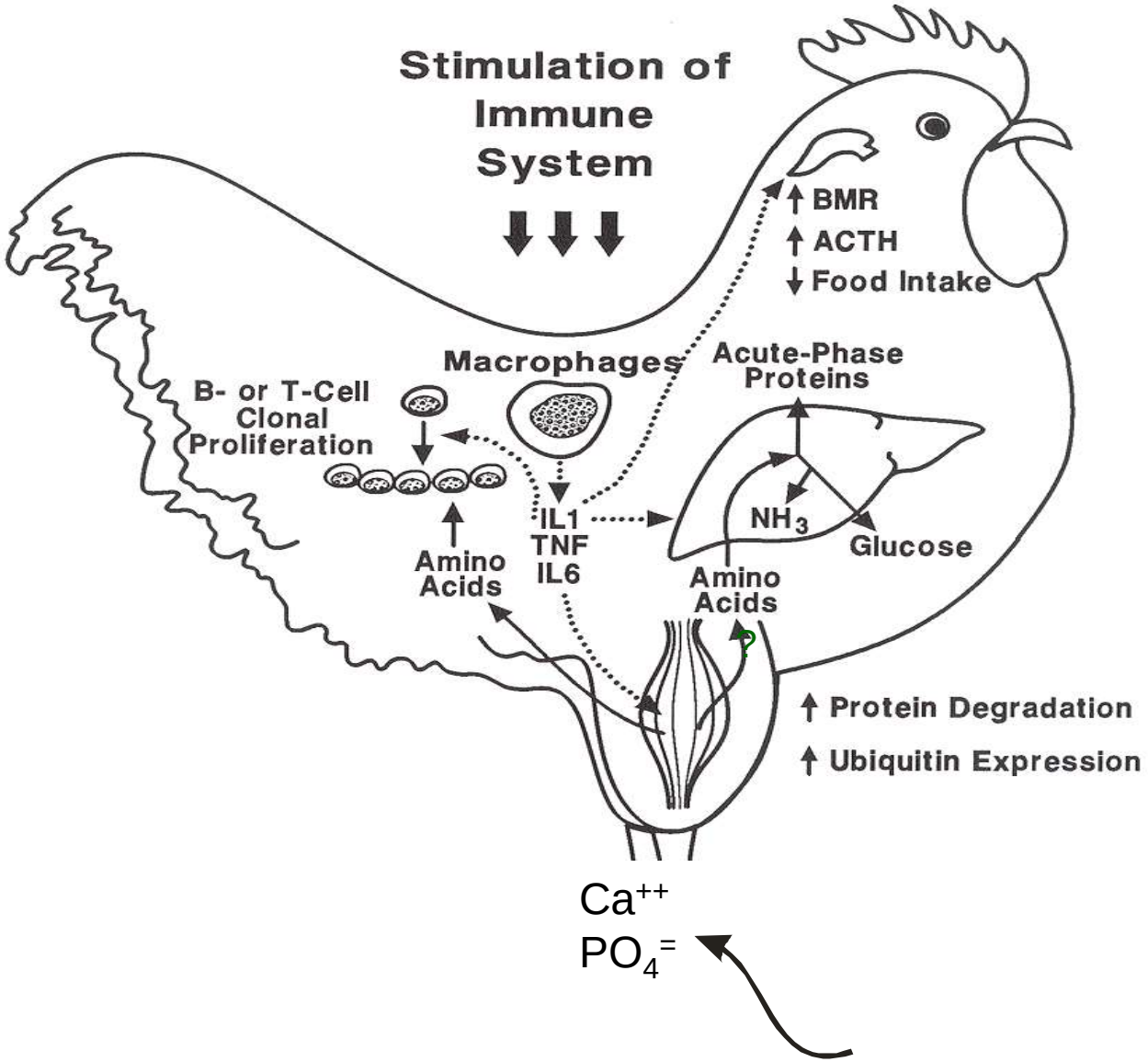
Nutrient remaining in treatment group/control group



Foot pad dermatitis, Yolk sac infections, Cellulitis

The cost of a disease challenge



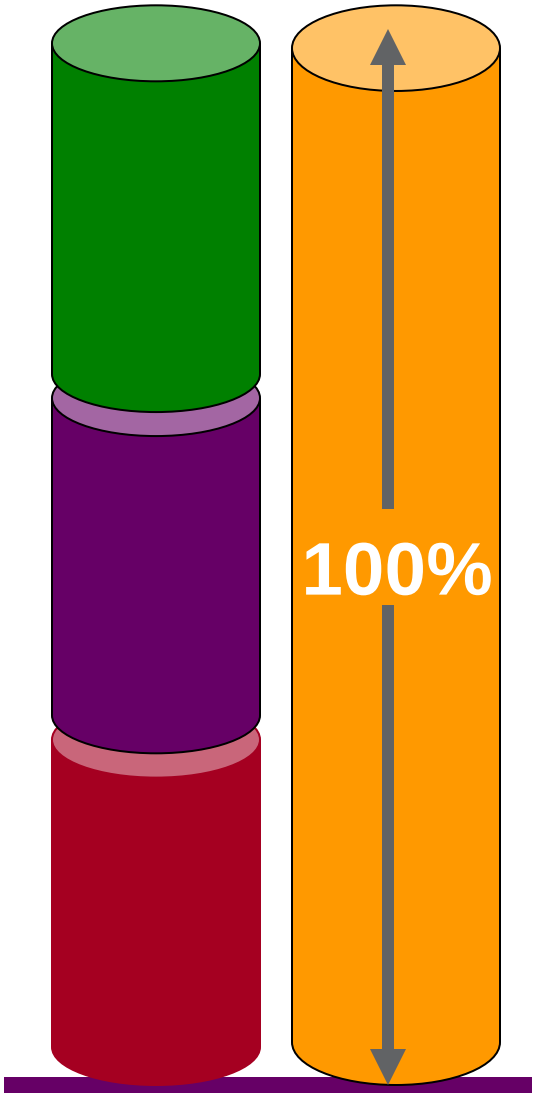

Stimulation of Immune System
BMR
ACTH
Food Intake
Macrophages
Acute-Phase Proteins
B- or T-Cell Clonal Proliferation
IL1
TNF
IL6
NH3
Glucose
Amino Acids
Amino Acids
?
Protein Degradation
Ubiquitin Expression
Ca++
PO4=

Reasons for decreased performance due to an immune response

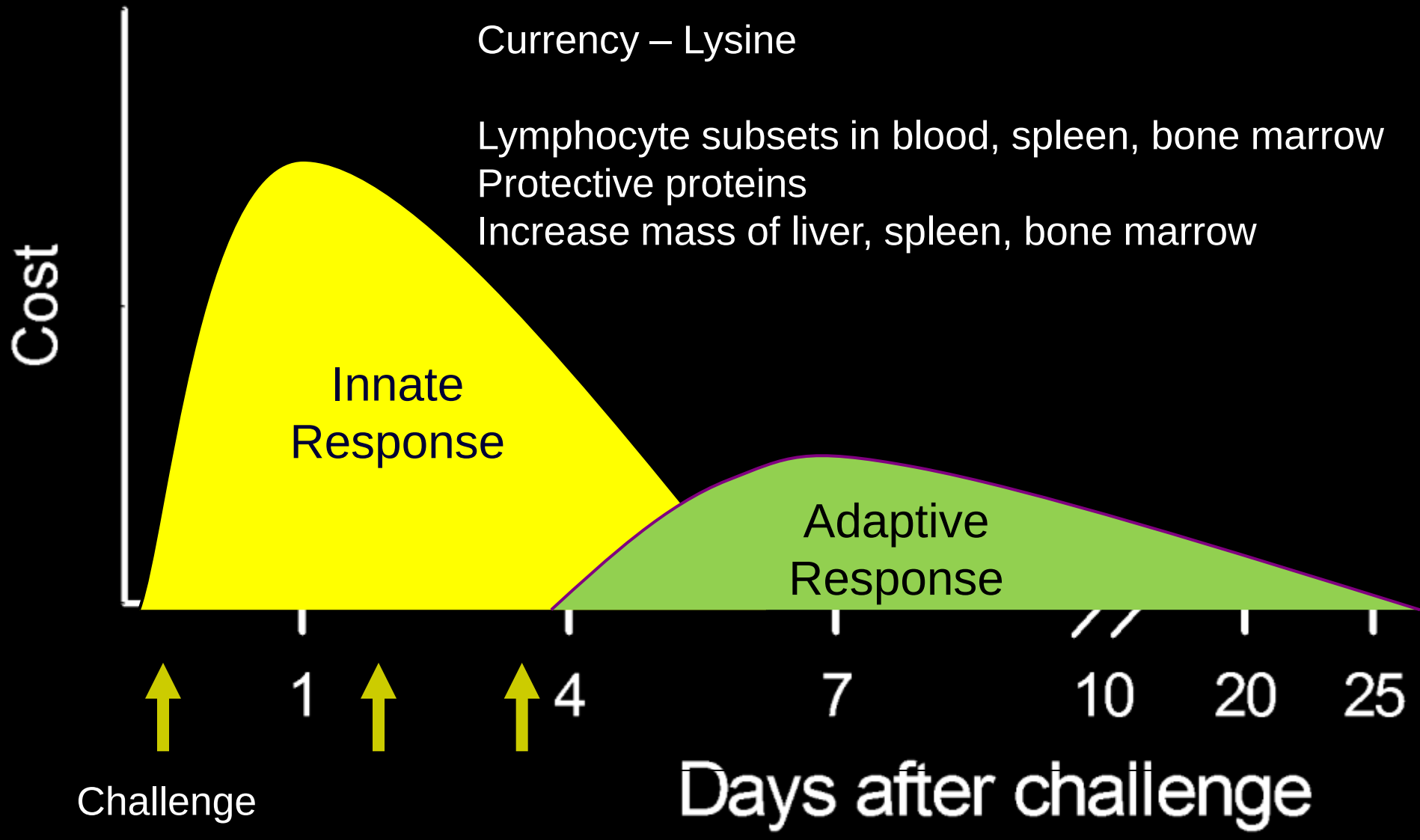
- Diversions?
  - Repartitioning
  - To immune defenses

- Metabolic inefficiencies?
  - Mismatches in supply vs needs

- Digestive inefficiencies?



Evaluation of the size of the cost of an immune response to E. coli

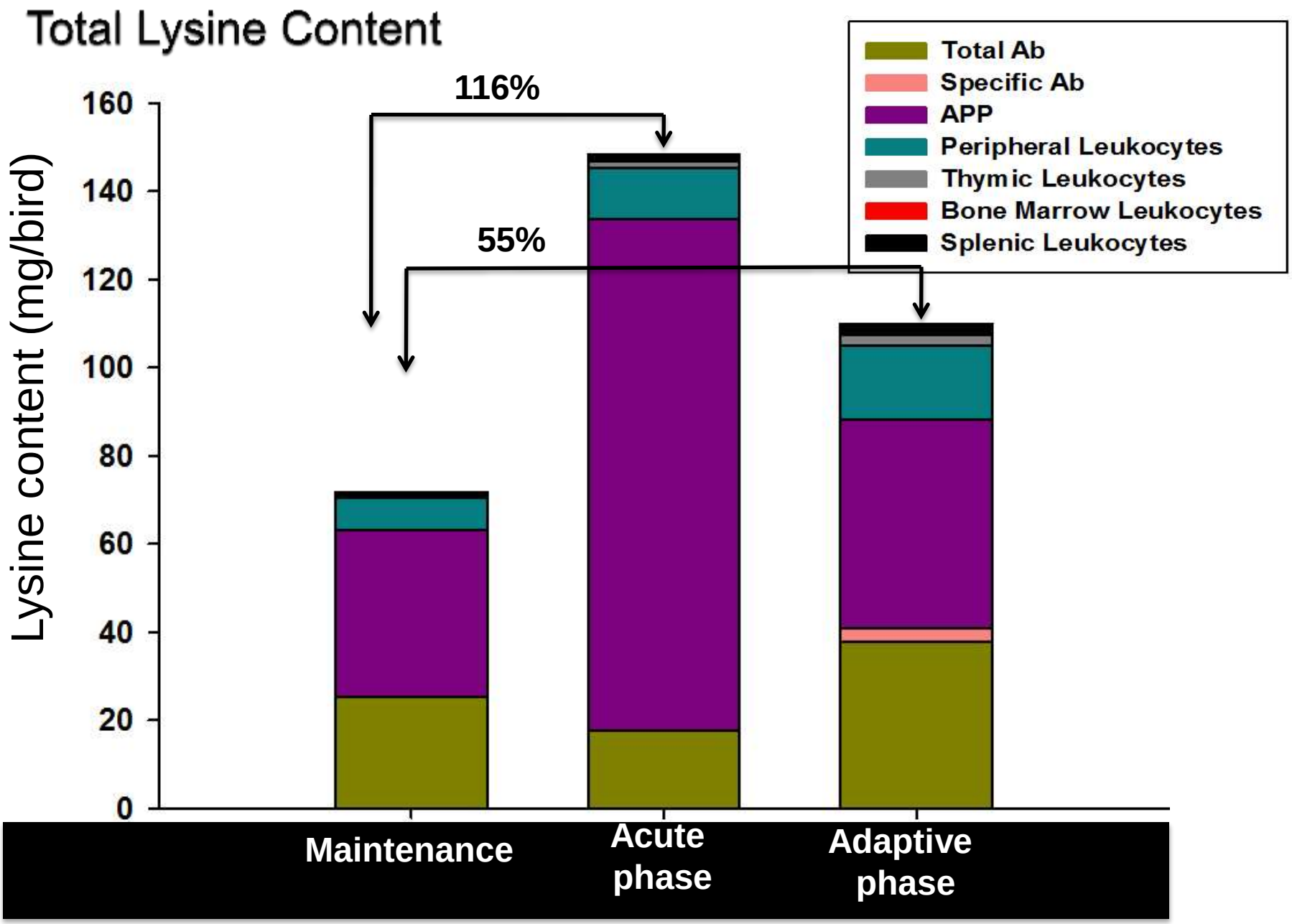


Protective Proteins

Ceruloplasmin
Lysozme
Ferritin
Hemopexin
Transferrin
Haptoglobin
Metallothionein
α-2 Macroglobulin
Avidin
α-1 acid glycoprot
Complement
Mannan binding protein
C-reactive protein
Immunoglobulin – M, Y, A

Leukocytes

B lymphocytes
T lymphocytes
Monocytes/macrophages
Heterophils
Thrombocytes


Total Lysine Content
Lysine content (mg/bird)
160
140
120
100
80
60
40
20
0
116%
55%
Total Ab
Specific Ab
APP
Peripheral Leukocytes
Thymic Leukocytes
Bone Marrow Leukocytes
Splenic Leukocytes
Maintenance
Acute phase
Adaptive phase

Proportion of lysine used for systemic immunity

Component:	% of the Immune System		% of the Whole Chicken	
Innate	Maintenance	Max Response	Maintenance	Max Response
Cells	0.8	0.7	0.04	0.09
Extracellular Proteins	59.5	79.2	0.32	1.01
Adaptive				
Cells	0.7	0.4	0.05	0.02
Immunoglobulins	39.1	19.7	0.22	0.25
Subtotal	100	100	**0.55**	**1.28**
Liver				***1.74***
Total				**3.02**

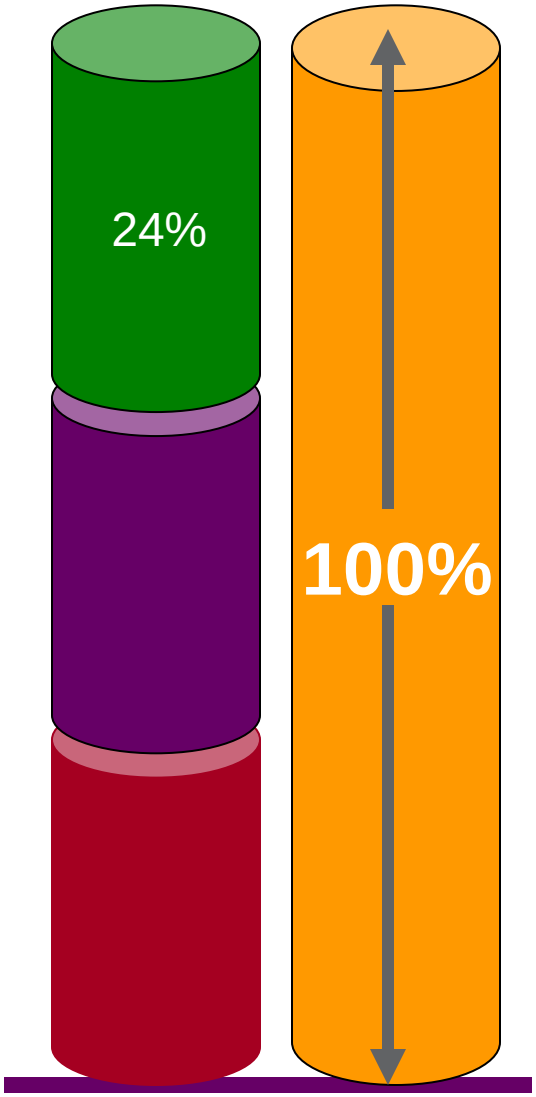
Diversions?

- Repartitioning
- To immune defenses

Metabolic inefficiencies?

- Mismatches in supply vs needs
- Increased energy expenditure

Digestive inefficiencies?



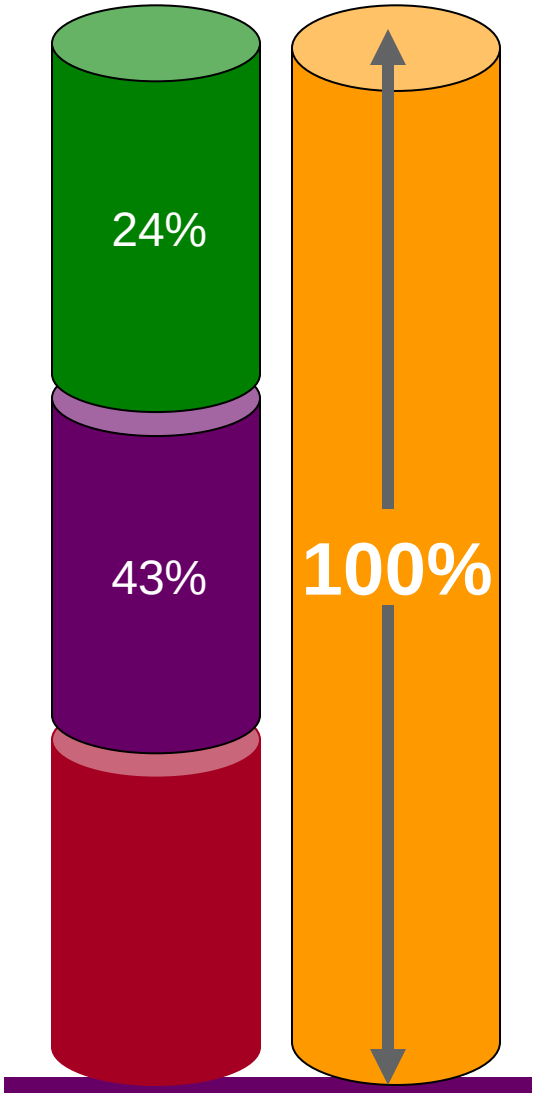
Mismatch between Immune System and Skeletal Muscle Amino Acid Balances

Amino acid	Innate System		Adaptive System
	24hr Ratio[1]	5d Ratio[1]	5d Ratio[1]
Arginine	0.81	0.94	0.82
Cystiene	**1.88**	**1.62**	**1.67**
Glycine	1.53	1.42	1.43
Histidine	0.76	0.79	0.42
Isoleucine	0.83	0.80	0.50
Leucine	0.96	1.00	0.89
Lysine	0.69	0.72	0.42
Methionine	0.72	0.61	0.36
Phenylalanine	0.82	0.90	0.66
Proline	0.99	1.04	2.11
Threonine	1.40	1.29	1.04
Valine	1.09	1.18	**2.26**

- Diversions?
  - Repartitioning
  - To immune defenses

-Metabolic inefficiencies?
  - Mismatches in supply vs needs
  - Increased energy expendature

-Digestive inefficiencies?



Nutrient absorption after an E. coli challenge

Nutrient	Treatment			
	Control (pair fed)	+ E.coli	SEM	Significant P Value
Starch	100	96	2.8	-
Lysine	100	98	2.6	-
Methionine	100	**92**	2.0	0.05
Glutamine	100	101	2.4	-
Lipid	100	**81**	2.2	< 0.01
Retinol	100	**56**	2.1	< 0.01
Lutein	100	**36**	3.0	< 0.01
Ca	100	**89**	2.8	0.03
Fe	100	**63**	2.9	< 0.01
Zn	100	**95**	1.5	0.06
Cu	100	**92**	2.8	0.06

Effect of decrease digestion on growth?

Nutrient	Dietary Treatment	
	Control (pair fed)	Adjusted
Starch	100	96
Lysine	100	98
Methionine	100	**92**
Glutamine	100	101
Lipid	100	**81**
Retinol	100	**56**
Lutein	100	**36**
Ca	100	**89**
Fe	100	**83**
Zn	100	**95**
Cu	100	**92**
Gain	**144±4**	**131±5** = **9%**

-Diversions?

Repartitioning

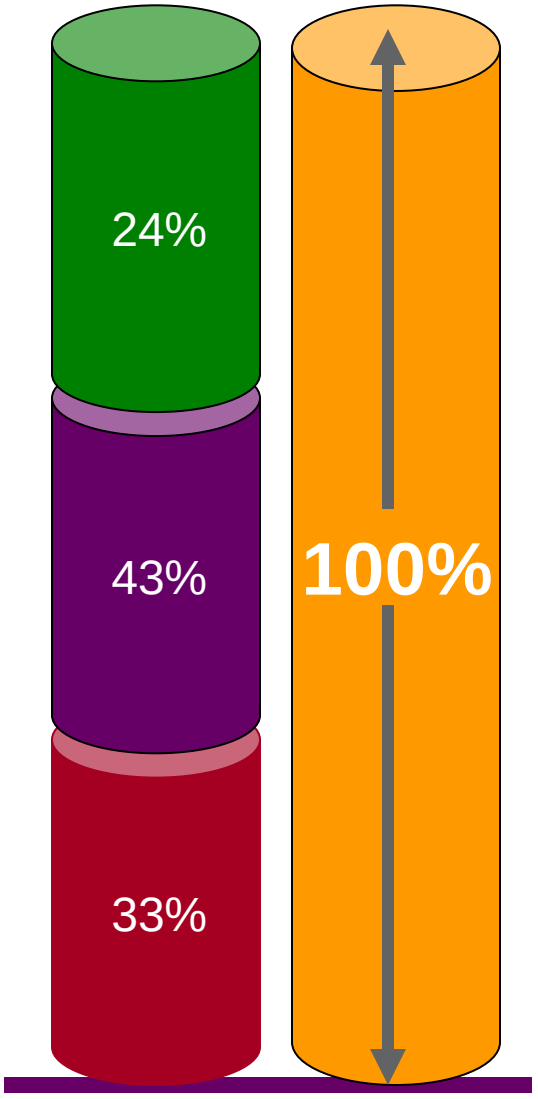
To immune defenses

-Metabolic inefficiencies?

Mismatches in supply vs needs

Increased energy expendature

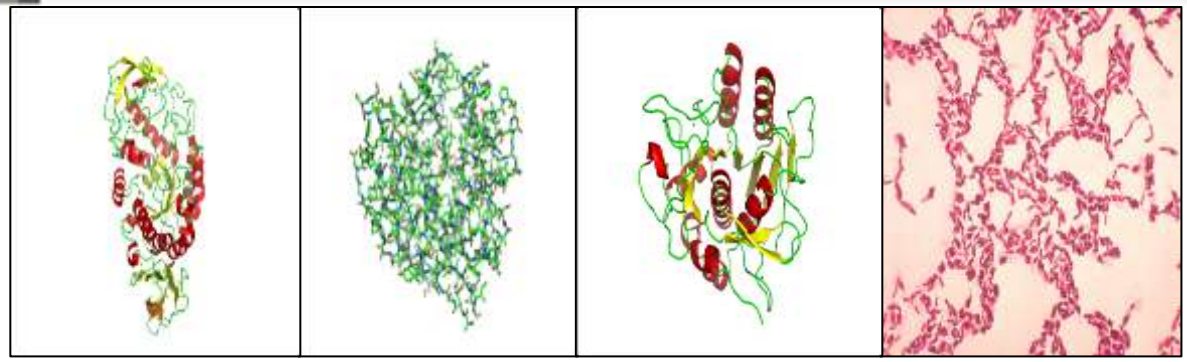
-Digestive inefficiencies?



Dr. Ajay Awati is a qualified vet and a doctor of Animal Sciences with over eight years healthy nutrition experience and an extensive back catalogue of peer reviewed publications, book chapters, trade press articles and conference speaker places. His Masters degree -taken at Wageningen University and Research centre in the Netherlands where he also did his PhD - was focused on nutrition and immunology in poultry. He joined Danisco Animal Nutrition as Senior Scientist and Development team lead in 2012, having previously worked at Nutreco and the Riddet Institute, New Zealand.

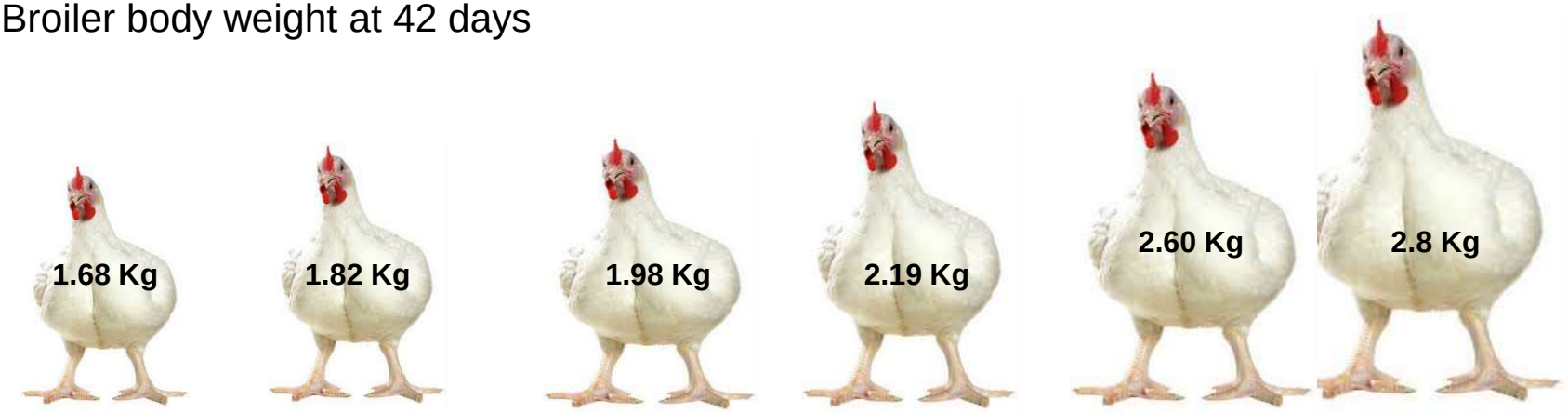
Use of combination containing *Bacillus*, Xylanase, Amylase and Protease to improve broiler performance

AJAY AWATI
DANISCO ANIMAL NUTRITION



The last 50 years….

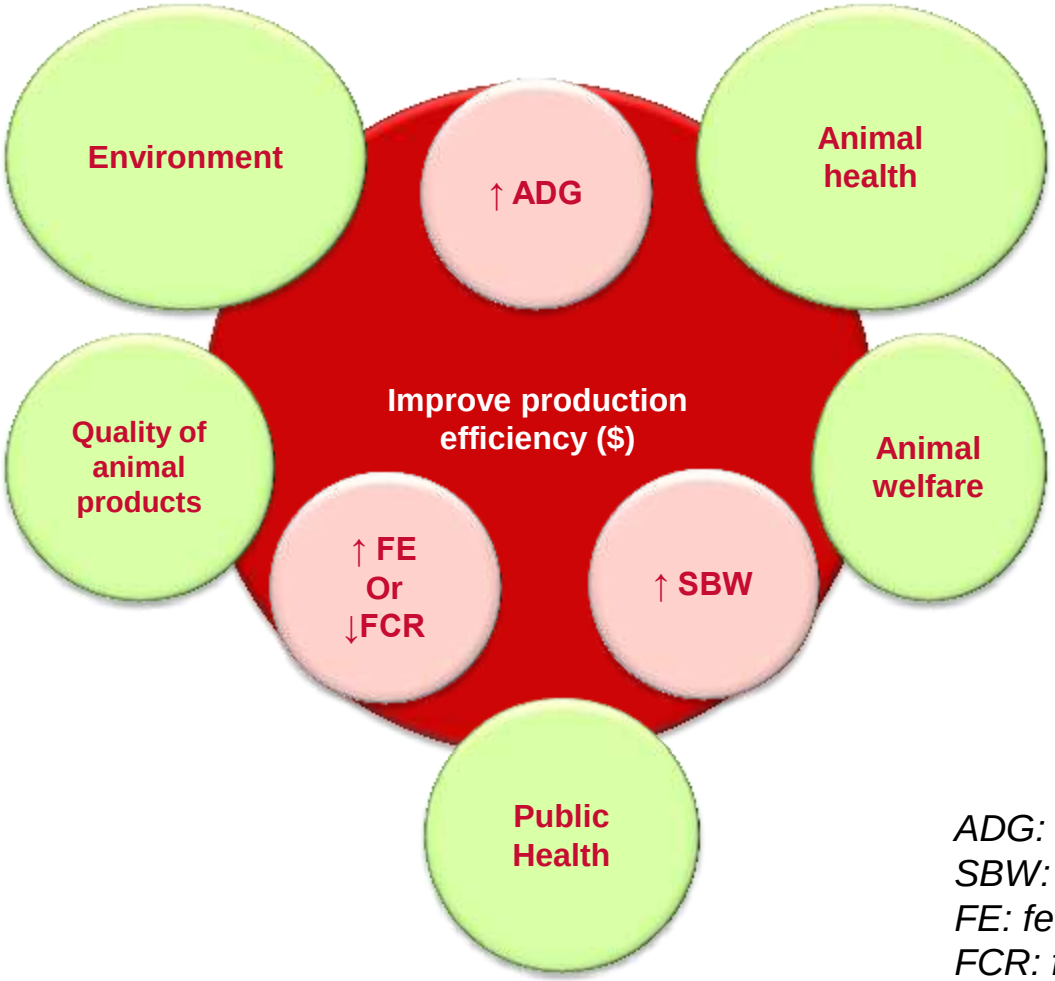
Broiler body weight at 42 days



Feed conversion ratio

Based on Rauw 1998 & Ross recommendations

Today….Things Have Become More Complicated



ADG: average daily gain g/d;
SBW: Slaughter Body weight;
FE: feed efficiency;
FCR: feed conversion ratio

One Possible Solution….Combined Enzymes

Substrate (ANF)	Anti-nutritional effect	Enzyme
Soluble viscous NSPs (e.g arabinoxylans)	↑ viscosity and digesta retention time ↓ nutrient absorption ↑ activity of intestinal microflora	Xylanase
Insoluble, non-viscous NSPs	↓ accessibility of nutrients by physical entrapment	Xylanase
Resistant Starch	↓ ME value of ingredients ↑ substrate for gut microflora	Amylase
Indigestible Proteins (Trypsin Inhibitors, antigens)	↓ ME and AA value of ingredients ↑ substrate for pathogens	Protease
Phytate	↓ Dig AA and minerals, ME ↑ P and Ca excretion	Phytase

Each class of enzyme targets specific substrate
Substrates more accessible by other enzymes ….Interactions

Another Possible Solution…Probiotics

Benefits of using probiotics:

- Production of nutrients and vitamins
- Reduction in meat contamination
- Improved animal performance
- Prevention of inflammatory reactions
- Alternative to AGPs

Ref: Ohimain and Ofongo, 2012

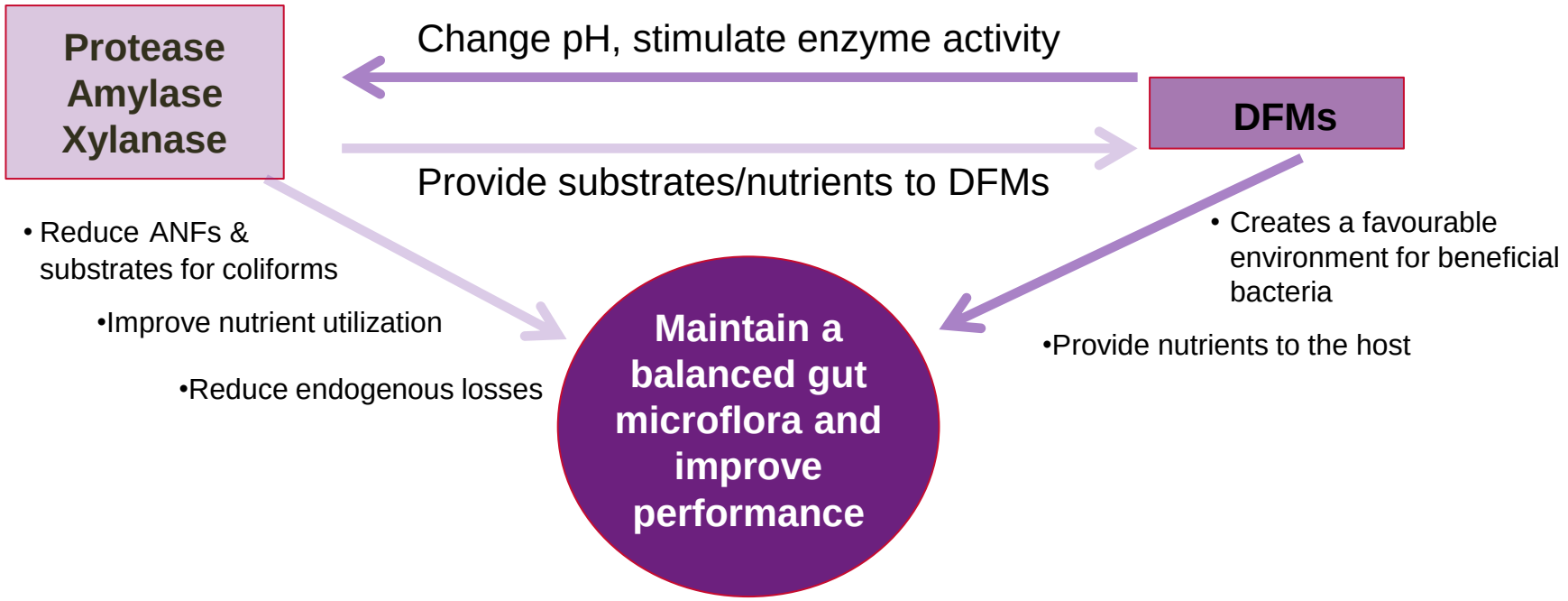
Benefits of using *Bacillus* spores:

- Stable during distribution, feed processing and storage
- Long shelf life
- In the chicken GI tract, germinate rapidly
- Active in the GI tract

Ref: Kiarie *et al*., 2013

There may be a better way…

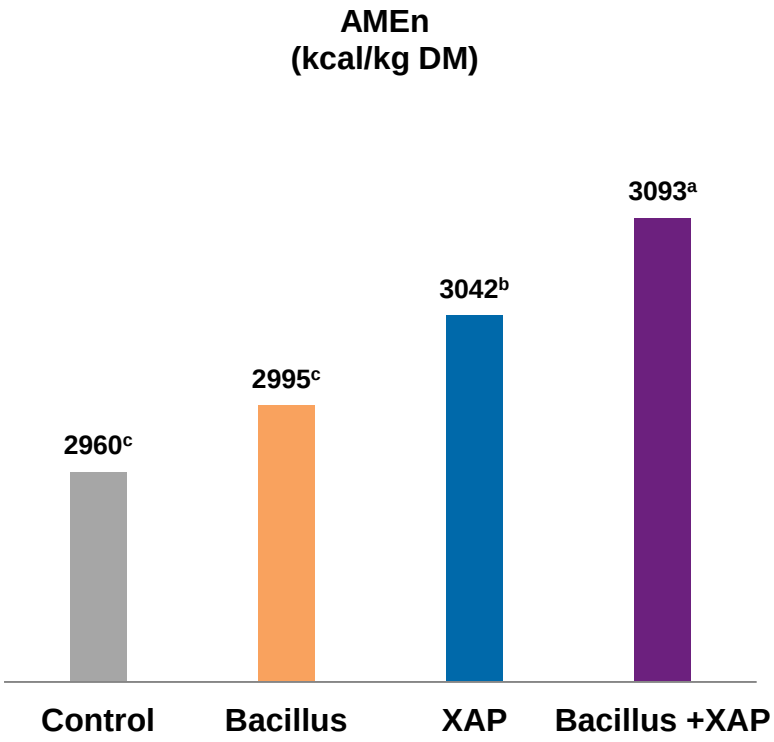
Probiotics + enzymes = better maintained 'gut health'



Combination of 3 strains of *Bacillus* + xylanase, amylase and protease enzymes

Scientific studies showed…(1)

- Corn/SBM/corn DDGs
- Ross 308 male broiler
- 6 replicate pens
- 22 birds/pen
- DFM: 3 *Bacillus* strains
- 21d digestibility study

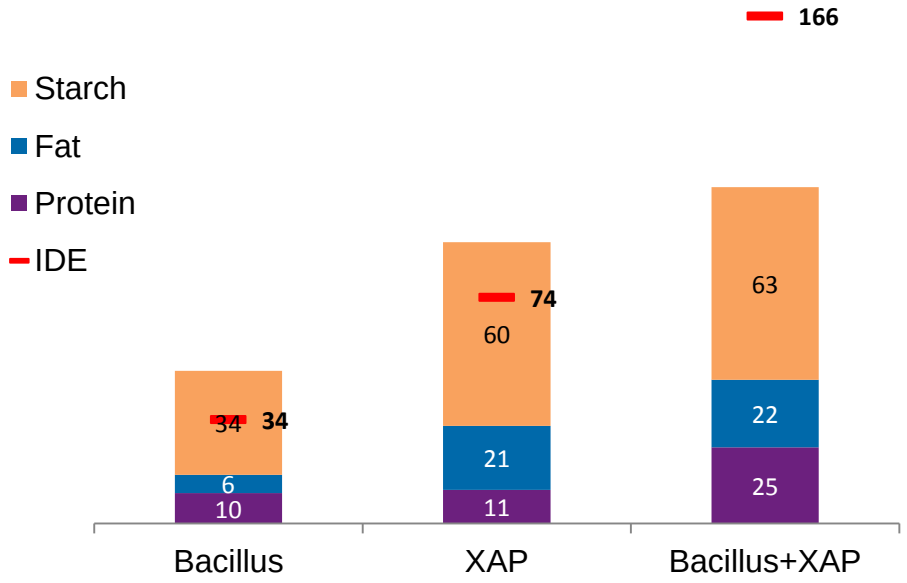


Romero *et al*., 2013

Scientific studies showed…(2)

- Corn/SBM/corn DDGs
- Ross 308 male broiler
- 6 replicate pens
- 22 birds/pen
- DFM: 3 *Bacillus* strains
- 21d digestibility study

Ileal energy digestibility improvement provided by starch, fat and protein
(kcal/kg dry matter)

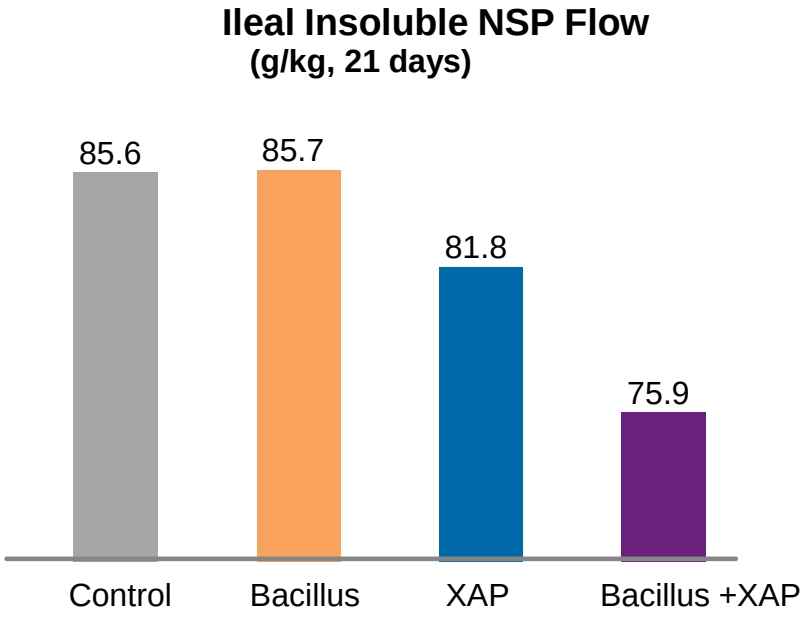


Unchallenged conditions

Romero *et al*., 2013

Scientific studies showed…(3)

- Corn/SBM/corn DDGs
- Ross 308 male broiler
- 6 replicate pens
- 22 birds/pen
- DFM: 3 *Bacillus* strains
- 21d digestibility study



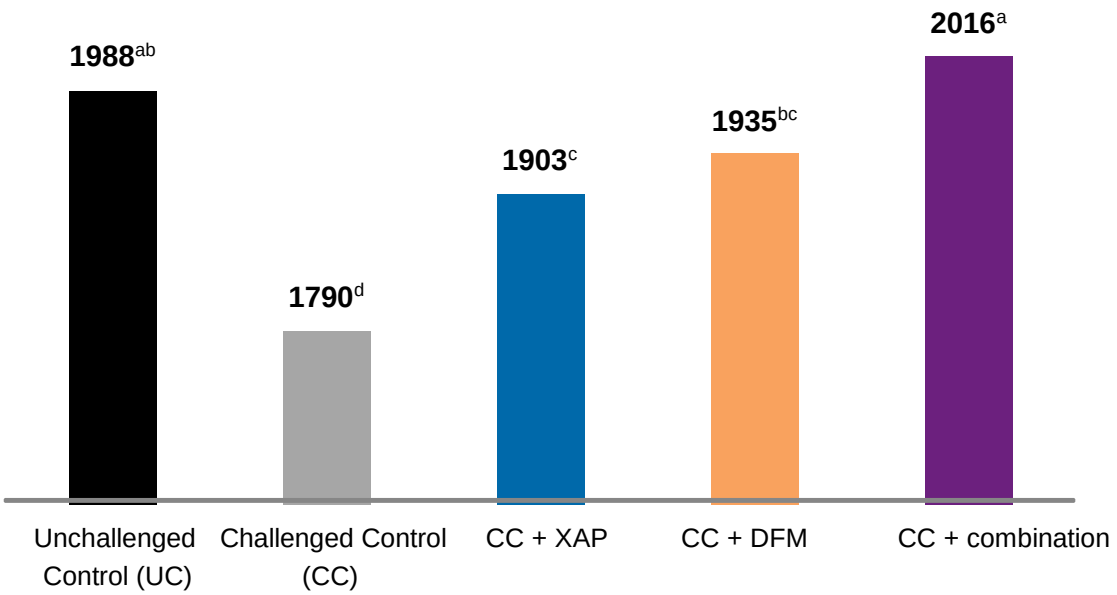
Unchallenged conditions

Romero *et al*., 2013

Scientific studies showed…(4)

- Cobb x Cobb 500 male broilers
  - Corn/SBM, 10% corn DDGs
  - 8 replicates (pen)/treat
  - 50 birds/pen
  - NE (necrotic enteritis) induced by a broth culture of *Clostridium perfringens* during day 20-22

Body weight gain (g, 0-42 days)



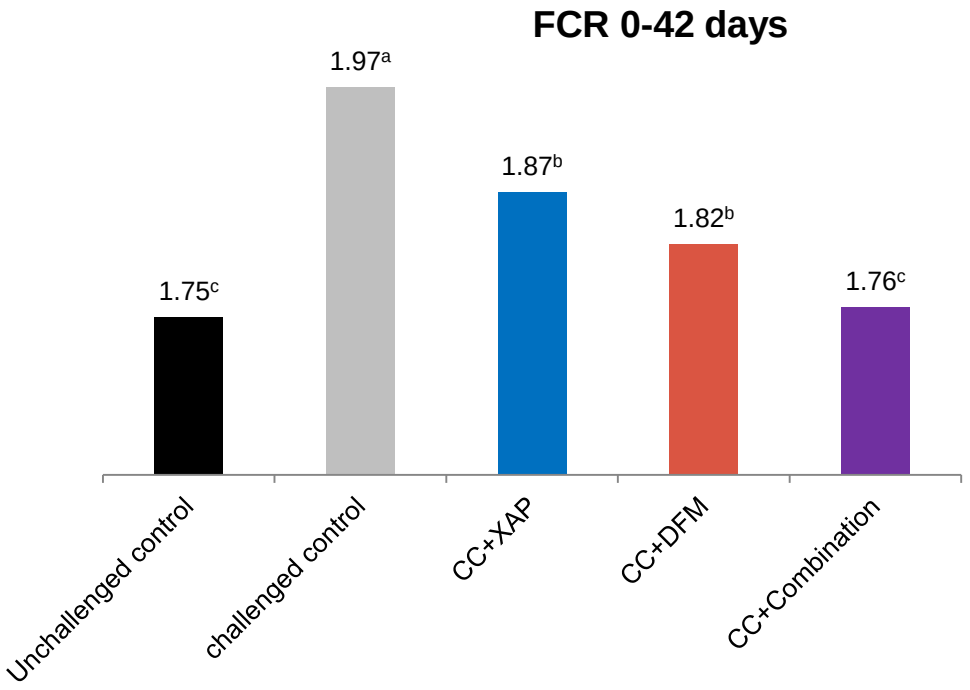
NE-Challenged conditions

abc Values without a common superscript are significantly different (P<0.05)
XAP= xylanase, amylase and protease
DFM = A mixture of 3 *Bacillus* strains

Mathis et al., 2013

Scientific studies showed…(5)

- Cobb x Cobb 500 male broilers
- Corn/SBM, 10% corn DDGs
- 8 replicates (pen)/treat
- 50 birds/pen
- NE (necrotic enteritis) induced by a broth culture of *Clostridium perfringens* during day 20-22



NE-Challenged conditions

abc Values without a common superscript are significantly different (P<0.05)
XAP= xylanase, amylase and protease
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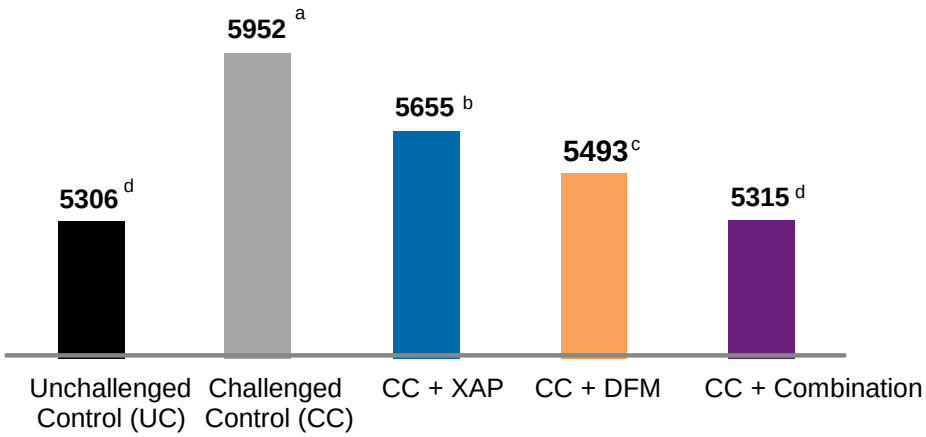
Mathis et al., 2013

Scientific studies showed…(6)

- Cobb x Cobb 500 male broilers
- Corn/SBM, 10% corn DDGs
- 8 replicates (pen)/treat
- 50 birds/pen
- NE (necrotic enteritis) induced by a broth culture of *Clostridium perfringens* during day 20-22

Calorie conversion (kcal/body weight gain)

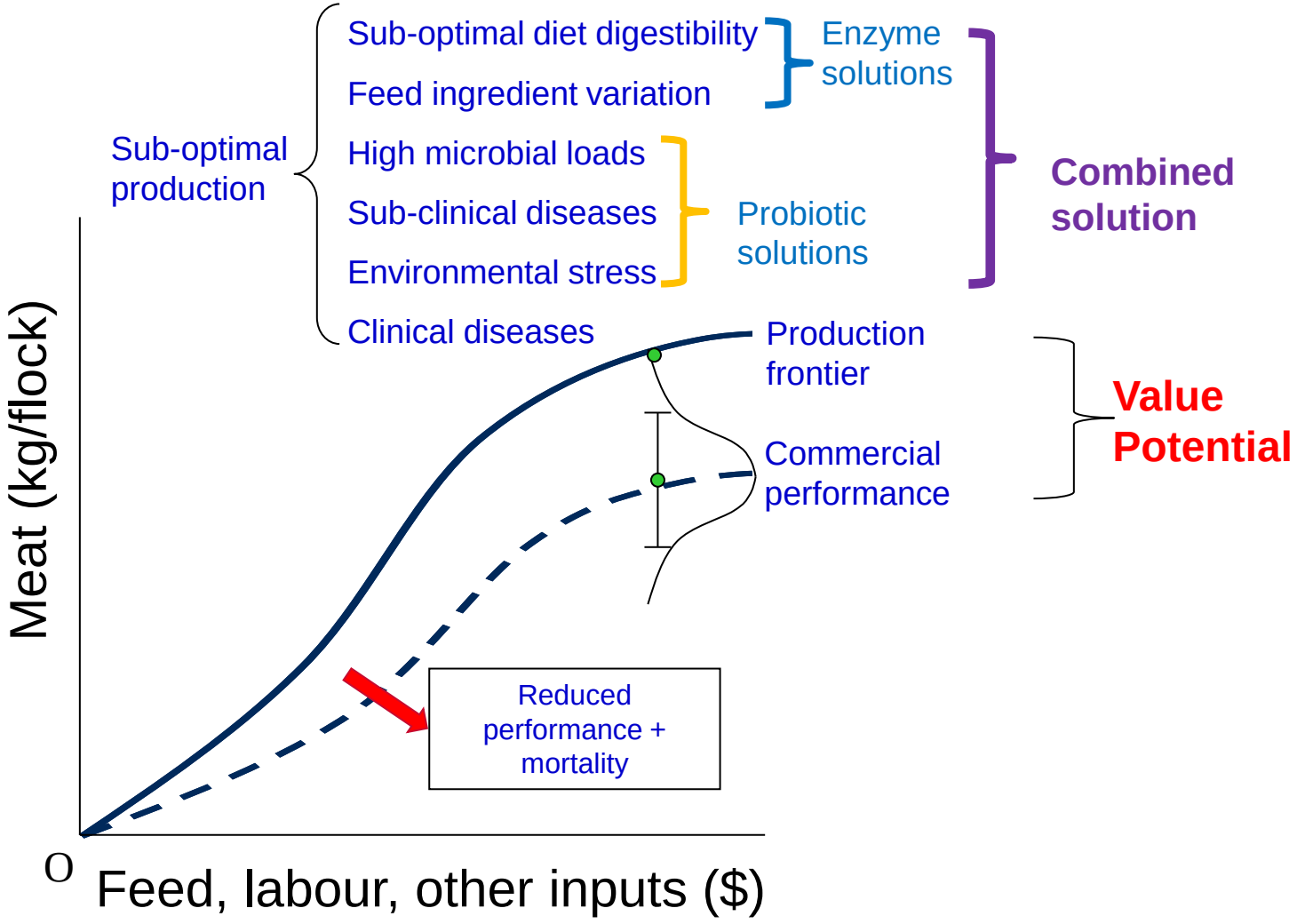


NE-Challenged conditions

[abc] Values without a common superscript are significantly different (P<0.05)
XAP= xylanase, amylase and protease
DFM = A mixture of 3 *Bacillus* strains

Mathis et al., 2013

The gap between genetic potential and actual performance is variable and under-estimated



Conclusions:

- Enzymes are substrate specific and with this specificity they target certain ANFs to improve nutrient digestion in the gut
- DFMs by providing a healthy environment for beneficial bacteria create a better gut condition to improve nutrient absorption
- Combination of enzymes and DFMs can have positive additive effect on feed utilization either in normal unchallenged conditions or under challenge conditions like Necrotic Enteritis.
- http://www.noveltouch.co.uk/dupont-poultry/app.html

Thank You!!

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