Alternatives to Antibiotics

International Symposium
Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Production
The World Organization for Animal Health (OIE)
12 Rue de Prony, 75017
Paris, France
25-28 September, 2012

PERSPECTIVES FROM OUTSIDE THE BOX: THE USE OF PHYTONUTRIENTS FOR OPTIMIZING GUT HEALTH AND PRODUCTIVE EFFICIENCY OF POULTRY ANIMALS

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Sept. 27, 2012

INTRODUCTION
PHYTONUTRIENTS

- Government-issued bans and restrictions on the use of antibiotic growth promoters in animal production have presented new challenges.
- This has prompted an interest in the development of drug-independent growth promoting strategies, such as **phytonutrient**-based feed additives.
- Plant extracts, essential oils, phytogenics, phytochemicals... = **plant secondary metabolites**.
- Lee et al. (2004) IJPS; Windisch et al. (2009) JAS; Wallace et al. (2010) BPS; Brenes & Roura (2010) AFST.

INTRODUCTION

PHYTONUTRIENTS

- Although many such additives have been on the market for 10 years or more, we have limited understanding of their efficacy and mode of action.
- Consequently, they are often received with skepticism and there is little acceptance of these products by the animal industry.

PRESENTATION OBJECTIVES

① To present the results of extensive analyses that have been conducted to estimate the efficacy of some phytonutrient-based feed additives in field conditions.

② To describe and discuss the current state of the knowledge regarding the basic mechanisms by which phytonutrients elicit changes in animal health and production. Discuss novel theories.

OUTLINE

- EFFICACY OF PHYTONUTRIENTS
- SUMMARY OF RESEARCH FINDINGS
- MOLECULAR UNDERSTANDING: HOW DO PHYTONUTRIENTS WORK?
- TAKE-HOME MESSAGES

OUTLINE

- **EFFICACY OF PHYTONUTRIENTS**
- SUMMARY OF RESEARCH FINDINGS
- MOLECULAR UNDERSTANDING: HOW DO PHYTONUTRIENTS WORK?
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EFFICACY OF PHYTONUTRIENTS

BACKGROUND

- Conducting a “general” evaluation of the effect of phytonutrients on production performance is difficult.
- Few published studies are available; often papers provide minimal description of additive composition or active ingredients.
- (Very) distinct molecules, different effects, doses, mechanisms of action…
- Encapsulated or not.

EFFICACY OF PHYTONUTRIENTS

BACKGROUND

- Bravo et al. (2011, JAPR): **carvacrol, cinnamaldehyde and capsicum oleoresin** blend.
- Amerah et al. (2011, BPS; 2012, PS) and Tiihonen et al. (2010, BPS): **cinnamaldehyde and thymol** blend.
- Bozkurt M et al., (2012a, PS, 2012b, BPS): **oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil** blend.
- Mathlouthi et al. (2012, JAS): **rosemary EO, oregano EO, blend, commercial product**.

EFFICACY OF PHYTONUTRIENTS

BACKGROUND

- The current analysis was focused on a specific phytonutrient additive with stabilized formula.
- Blend of 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin microencapsulated in 90% hydrogenated fat (**PNB**)
- Dietary inclusion: 100 ppm
- The objective was to evaluate product efficacy across various field conditions.

EFFICACY OF PHYTONUTRIENTS

META ANALYSIS

- **Mixed model**, trial as random variable (St-Pierre, 2001)
- **Effect size** (**ES**) calculation (DeCoster, 2004), taking into account the sample size and the variability of each trial.
- Data from **19 trials** with side-by-side comparisons of PNB to a negative control provided 38 treatments.

EFFICACY OF PHYTONUTRIENTS

META-ANALYSIS

RESULTS OF META-ANALYSIS OF 19 TRIALS
EFFECT OF PNB ON PERFORMANCE OF BROILERS

Outcomes	N	Mixed model			
		Negative control	100 g/t PNB	%	P-value
Daily gain (g/day)	19	48.3	51.3	**+4.9%**	**0.001**
Feed intake (g/day)	19	81.9	84.1	NS	0.352
F:G (g/g)	19	1.73	1.68	**-2.9%**	**0.001**

EFFICACY OF PHYTONUTRIENTS

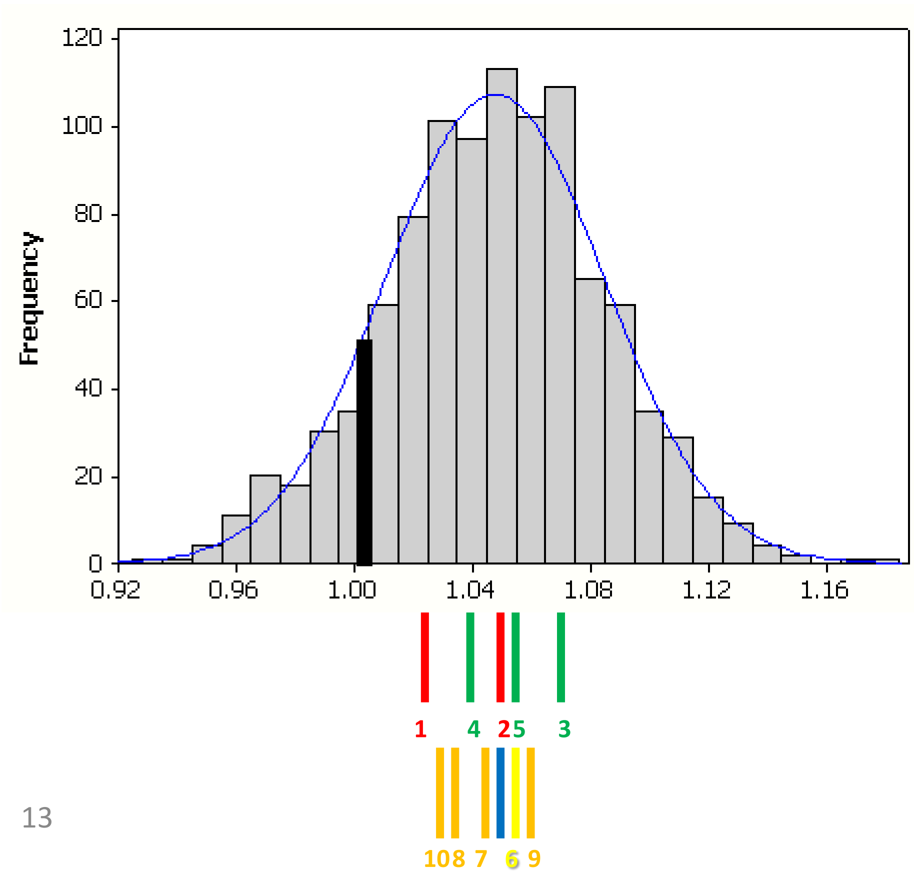
META-ANALYSIS

RESULTS OF META-ANALYSIS OF 19 TRIALS
EFFECT OF PNB ON PERFORMANCE OF BROILERS

Outcomes	N		ES and heterogeneity			Publication bias		
		%	ES (95% CI)	*P*	I^2	*P* Begg test	Missing studies	New ES (95% CI)
Daily gain (g/day)	19	+4.9%	0.150 (0.122, 0.188)	0.001	0	0.001	6 to left	0.140 (0.117, 0.188)
Feed intake (g/day)	19	NS	0.017 (-0.026, 0.061)	0.427	57	0.327		0.017 (-0.026, 0.061)
F:G (g/g)	19	-2.9%	-0.116 (-0.166, -0.066)	0.001	45	0.001	7 to right	-0.076 (-0.126, -0.025)

EFFICACY OF PHYTONUTRIENTS
DISCUSSION

DISTRIBUTION OF STANDARDIZED DIFFERENCE OF MEAN FOR BODY WEIGHT GAIN PNB versus NEGATIVE CONTROL



[1]: +2.4% (Bravo et al., 2011a)

[2]: +4.7% (Bravo et al, 2011b)

[3]: +6.8% (Amerah et al. 2012, PS)

[4]: +3.9% (Amerah et al. 2011, BPS)

[5]: +4.7% (Tiihonen et al., 2010, BPS)

[6]: +4.7% (Bozkurt et al (2012, BPS)

[7]: +4.6% (Mathlouthi et al. 2012, JAS, rosemary EO)

[8]: +3.5% (Mathlouthi et al. 2012, JAS, oregano EO)

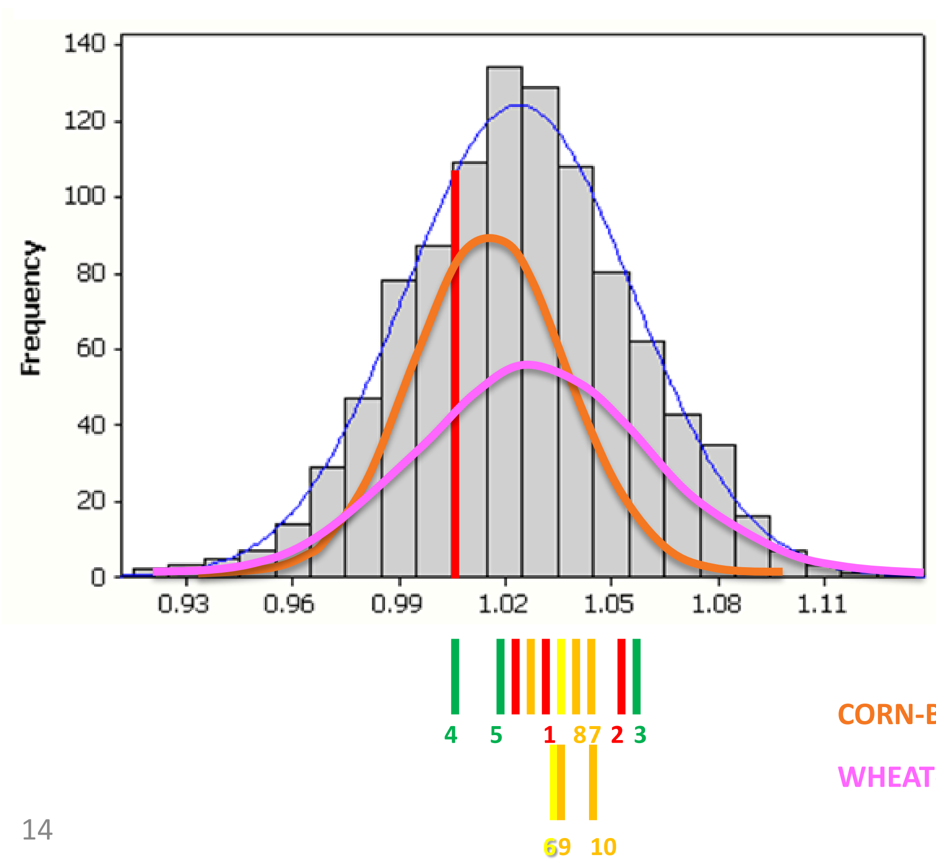
[9]: +5.3% (Mathlouthi et al. 2012, JAS, oreg. + rosem. EO)

[10]: +3.4% (Mathlouthi et al. 2012, JAS, commercial blend)

EFFICACY OF PHYTONUTRIENTS

DISCUSSION

DISTRIBUTION OF STANDARDIZED DIFFERENCE OF MEAN FOR GAIN : FEED
PNB versus NEGATIVE CONTROL



[1]: +2.3% (Bravo et al., 2011a); corn

[2]: +5.3% (Bravo et al, 2011b); corn

[3]: +5.5% (Amerah et al. 2012, PS); wheat

[4]: +1.2% (Amerah et al. 2011, BPS); wheat

[5]: +1.9% (Tiihonen et al., 2010, BPS); wheat

[6]: +2.5% (Bozkurt et al (2012, BPS); corn + wheat

[7]: +4.4% (Mathlouthi et al. 2012, JAS, rosemary EO); corn

[8]: +4.2% (Mathlouthi et al. 2012, JAS, oregano EO)

[9]: +3.6% (Mathlouthi et al. 2012, JAS, oreg. + rosem. EO)

[10]: +4.4% (Mathlouthi et al. 2012, JAS, commercial blend)

CORN-BASED DIETS

WHEAT-BASED DIETS

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EFFICACY OF PHYTONUTRIENTS

DISCUSSION

- Phytonutrients elicit significant improvements in animal health and production performance.
- Observations to date reveal a degree of inconsistency, probably due to environmental effects (eg. corn vs. wheat-based diet; “clean” vs. “dirty”).
- To understand the inconsistencies requires a clear knowledge of the underlying mechanisms by which phytonutrients improve growth.

OUTLINE

- EFFICACY OF PHYTONUTRIENTS
- **SUMMARY OF RESEARCH FINDINGS**
- MOLECULAR UNDERSTANDING: HOW DO PHYTONURTIENTS WORK?
- TAKE-HOME MESSAGES

SUMMARY OF RESEARCH FINDINGS
ASSOCIATED PHYSIOLOGICAL RESPONSES

- Question of interest: what are the physiological changes elicited by phytonutrients when they are included in feed for broilers?

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SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES

GUT LUMEN	GUT MUCOSA	METABOLISM

↑ ENHANCEMENT DUE TO PHYTONUTRIENTS

↓ DECREASE DUE TO PHYTONUTRIENTS

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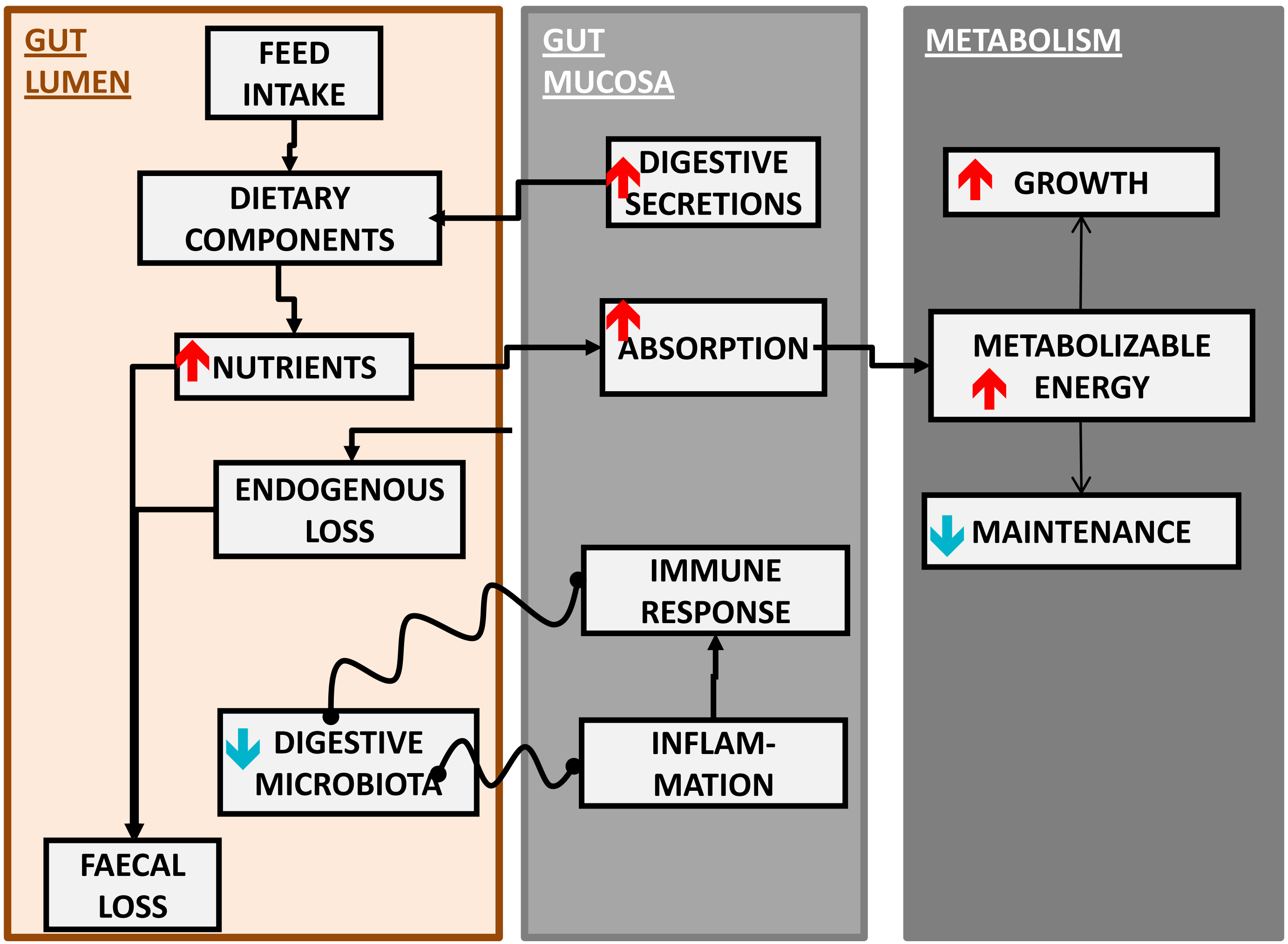
SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES



Rostagno et al. (2001)
Cross et al. (2007)
Wang et al. (2008)
COCCIDIOSIS:
Bravo et al. (2010)
Lee et al. (2011, IJPS)
Bravo et al. (2011)
Lee et al. (2011, BJN)
Dhuley et al. (1999)
Lee et al. (2011, VP)
Gonzalez et al. (1998)
Lee et al. (2012, BJN)
Jamroz et al., (2006)
Manzanilla et al., (2004)
SALMONELLA:
Ganesh et al. (1984)
NECROTIC ENTERITIS:
Basmacioglu Malayoglu et al. (2010, BPS)
Lee et al. (2003)
Bravo et al. (unpublished)

ENHANCEMENT DUE TO PHYTONUTRIENTS

DECREASE DUE TO PHYTONUTRIENTS

SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES

- Protective effects (BWG, lesions score…).
- Often, the protective effects are coincidental with changes to the **innate immune system**.
- Enhanced mucus secretion, upregulation of defensins…
- Anti-inflammatory in high inflammatory context.
- Unclear effect on inflammatory in low inflammatory context.
- Increase of specific antibody, cells changes…

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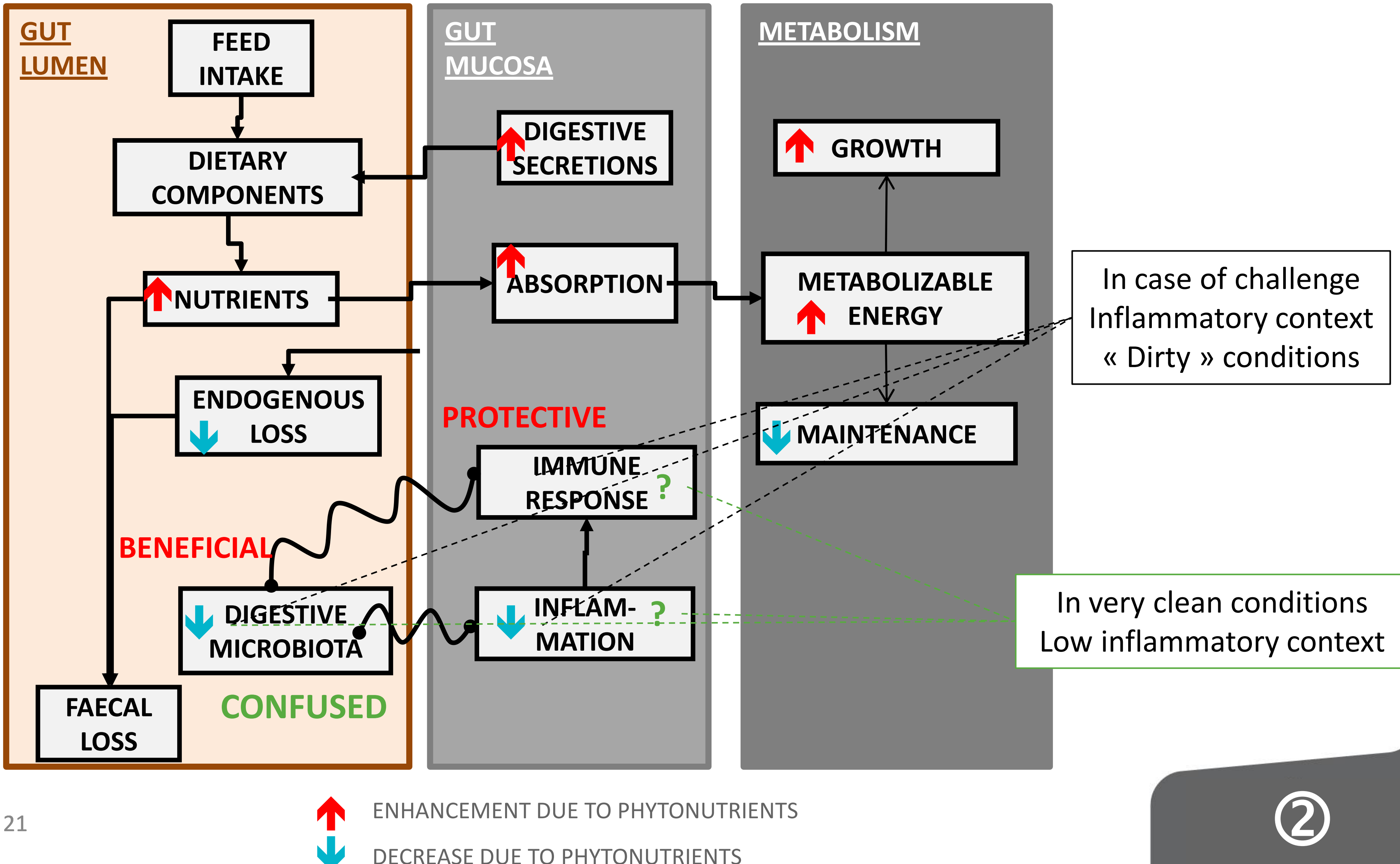
SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES



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SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES

- Taken together, these findings indicate that phytonutrients elicit two types of responses.
- A "**digestive / absorptive process**" response, probably due to increase gut enzymatic secretion.
- An "**innate immune response**" providing protection against complex pathogens such as Eimeria or Clostridium.
- Eventually, this leads to a growth promoting effect which is dependant on environment.

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OUTLINE

- EFFICACY OF PHYTONUTRIENTS
- SUMMARY OF RESESARCH FINDINGS
- **MOLECULAR UNDERSTANDING: HOW DO PHYTONUTRIENTS WORK?**
- TAKE-HOME MESSAGES

MOLECULAR UNDERSTANDING
ANTI-MICROBIAL VS. HOST-MEDIATED

- Questions of interest:
  - What is the molecular understanding of the mode of action of phytonutrients?
  - Do they work the way we think they do?

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MOLECULAR UNDERSTANDING

ANTI-MICROBIAL VS. HOST-MEDIATED

- Because the phytonutrients used in feed applications have been known for a long time to be **anti-microbial** (Cowan, 1999), most of the researchers using phytonutrients for feed applications have investigated **direct killing effects** of phytonutrients on pathogens or bacteria in situ (Lee et al., 2004).

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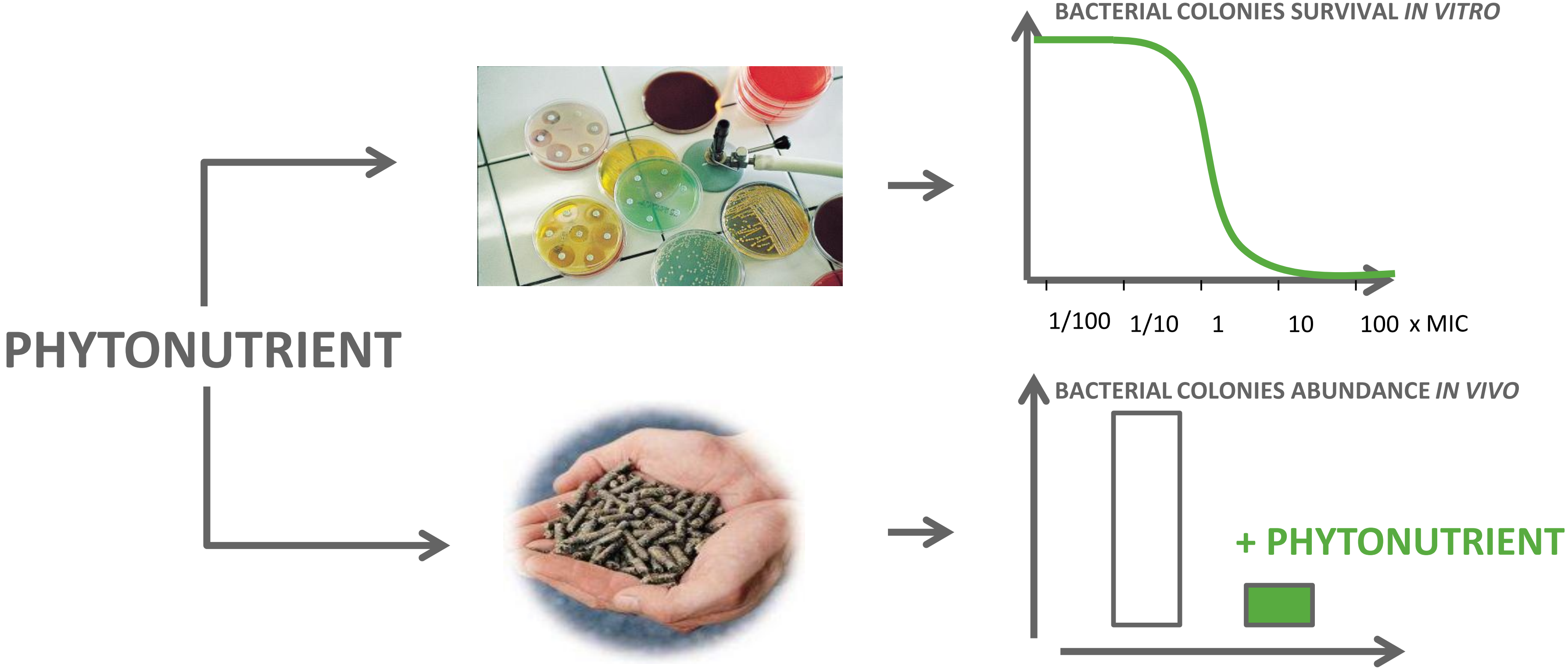
MOLECULAR UNDERSTANDING

ANTI-MICROBIAL VS. HOST-MEDIATED



"**Microflora management theory**" as defined by Niewold (2007, PS) for conventional antibiotic growth promoters

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MOLECULAR UNDERSTANDING

ANTI-MICROBIAL VS. HOST-MEDIATED

Table 3: Minimum inhibitory concentration (MIC, ppm) of carvacrol, cinnamaldehyde and thymol

Microorganisms	Carvacrol	Cinnamaldehyde	Thymol	References
Escherichia coli	450	396	450	Helander *et al.*, 1998
Escherichia coli	225	NT	225	Cosentino *et al.*, 1999
Staphylococcus aureus	450	NT	225	Cosentino *et al.*, 1999
Candida albicans	150	NT	150	Ali-Shtayeh *et al.*, 1997
Candida albicans	113	NT	113	Cosentino *et al.*, 1999
Candida albicans	200	200	NT	Ferhout *et al.*, 1999
Pseudomonas aeruginosa	500	NT	500	Ali-Shtayeh *et al.*, 1997
Pseudomonas aeruginosa	>900	NT	>900	Cosentino *et al.*, 1999
Salmonella typhimurium	150	396	150	Helander *et al.*, 1998
Salmonella typhimurium	225	NT	56	Cosentino *et al.*, 1999
Streptococcus mutans	125	250	250	Didry *et al.*, 1994
Streptococcus mitis	125	125	125	Didry *et al.*, 1994

NT: not tested

SOURCE: LEE, EVERTS & BEYNEN (2004, IJPS)

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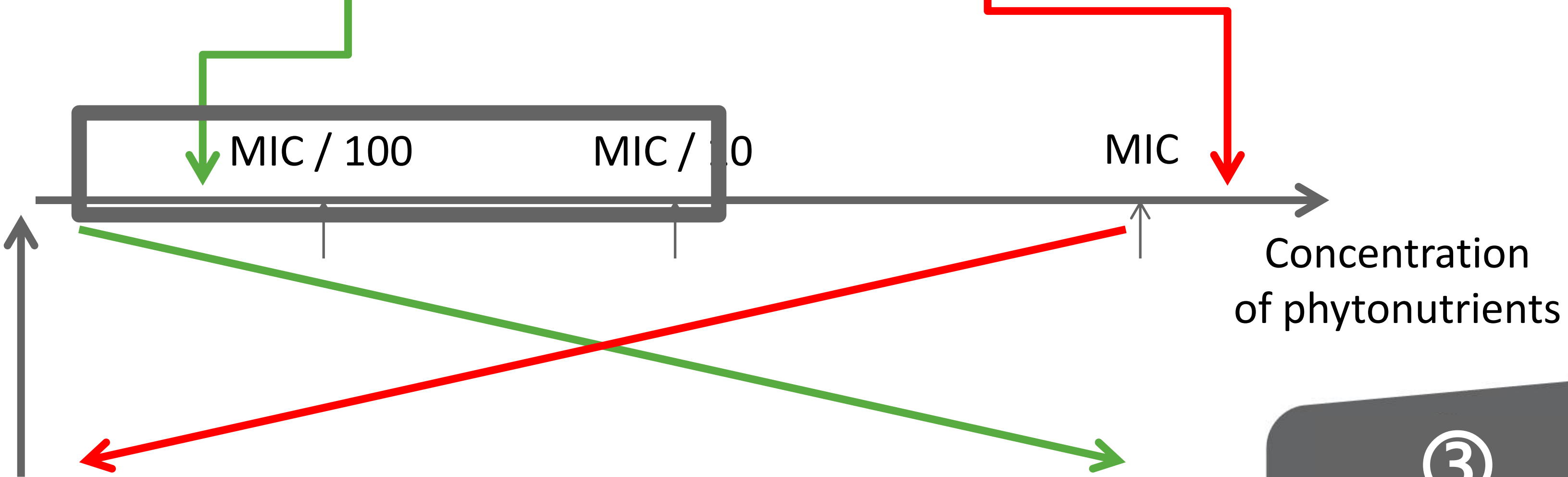
MOLECULAR UNDERSTANDING

ANTI-MICROBIAL VS. HOST-MEDIATED

- Changes in the microflora in the gut of animals fed phytonutrients may be **coincidental** and **not the direct consequence** of the phytonutrient.
- So the question is **Microbiota-mediated effect** or **Host-mediated response**?



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MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

- It is now accepted that the continuous cross-talk between the gut mucosal immune system and the gut microbiota is a major driver of host health and homeostasis.
- The gut microbiota shapes the host's phenotype [1, 2].
- Conversely, the host plays a crucial role in selecting its gut microbiota [3].

SOURCE [1]: LI, WANG, ZHANG, RANTAILAINEN, WANG, ZHOU, ZHANG, SHEN, PANG,ZHANG & AL (2008, PNAS)
SOURCE [2]: ZHAO (2010, NATURE)
SOURCE [3]: RAWLS JF, MAHOWALD MA, LEY RE, GORDON JI (CELL, 2006)

MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

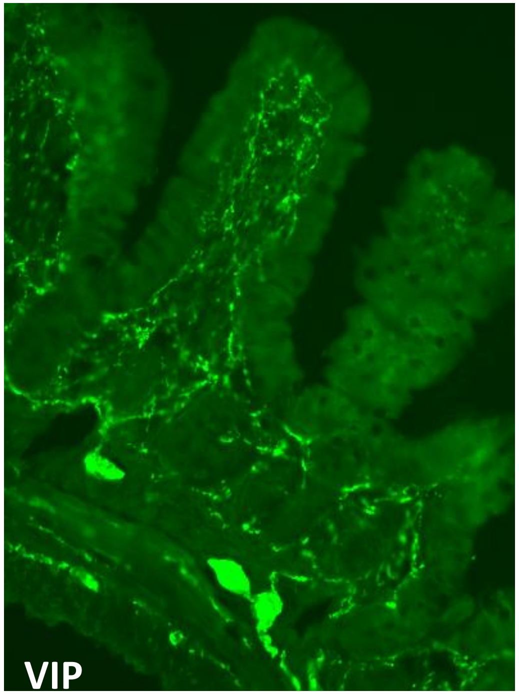
- New technologies have improved our understanding of the pivotal role of the gut in the physiology of the animal.
- The gut hosts an elaborate sensory systems, monitoring processes and feedback mechanisms.
- Let's go back to the **host**!

MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

- The enteric nervous system (ENS) consists of an extensive neural network embedded in the wall of the gut and controls GI functioning to a large extent independently of the central nervous system (CNS).
- Axonal projections do not cross the epithelium. The ENS collaborates with special cells.

DOBLE LABELLING PGP95 AND VIP



SOURCE: BATCHELOR D, MORAN AW, AL-RAMMAHI M, BURRIN D, BRAVO D AND SHIRAZI-BEECHEY SP, (2012, SUBMITTED)

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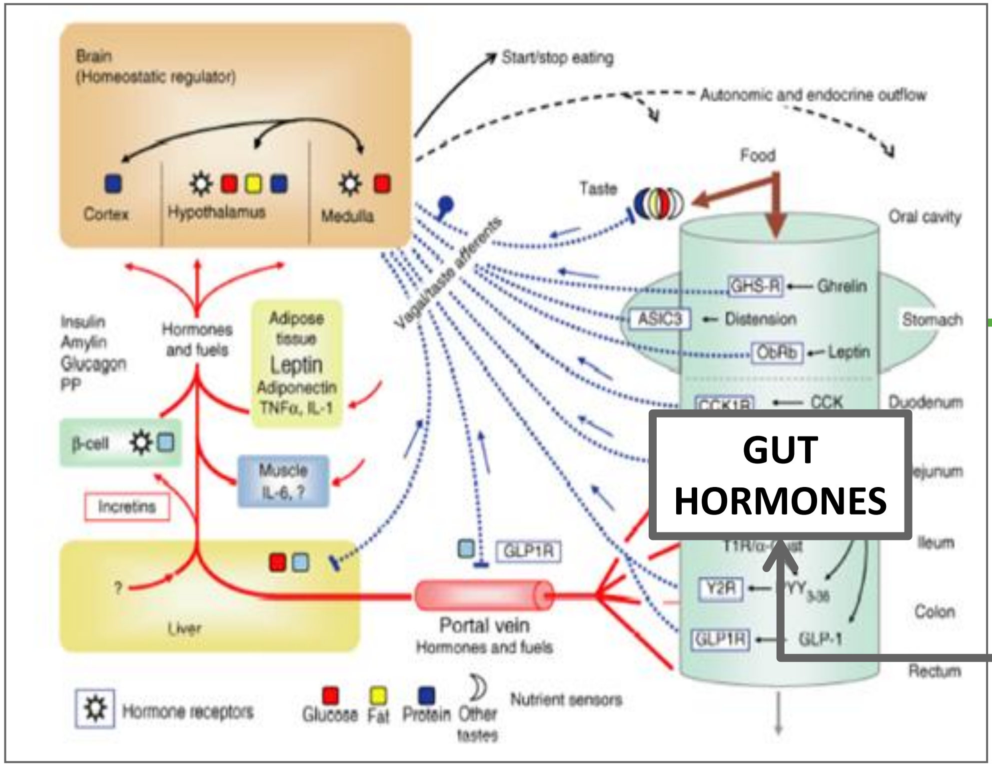
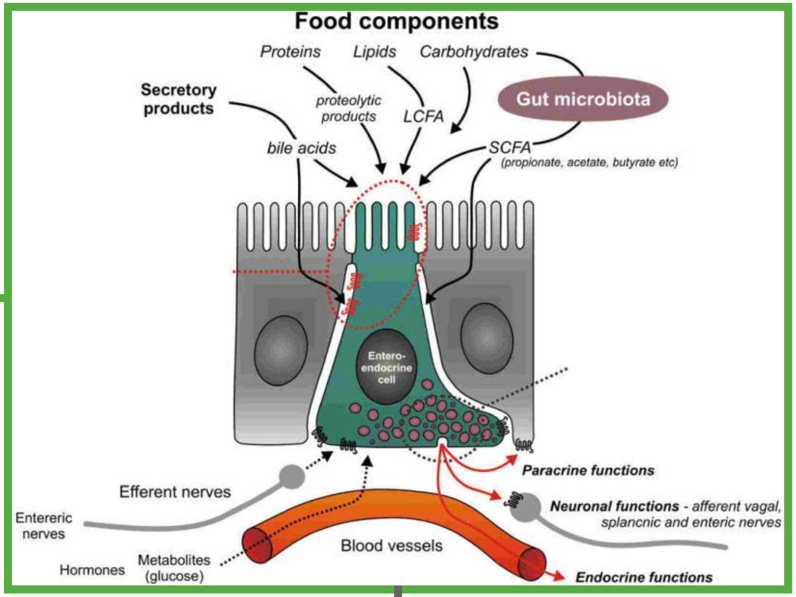
MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING





SOURCE: LENARD & BERTHOUD (2008, OBESITY)
SOURCE: TORTORA AND DERRICKSON (2006)
SOURCE: ENGELSOFT (2008)

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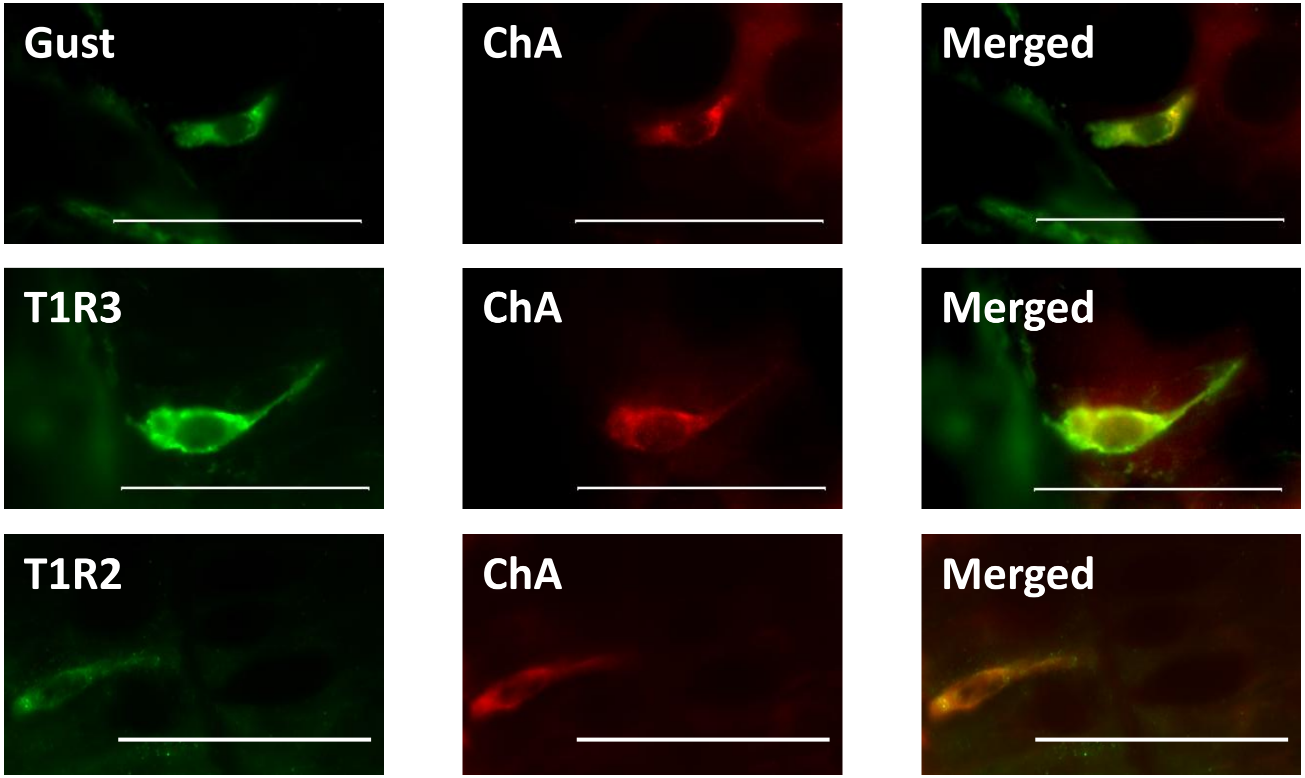
MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

WEANING CALVES



SOURCE: MORAN , AL-RAMMAHI, BRAVO, CALSAMIGLIA & SHIRAZI-BEECHEY (UNPUBLISHED DATA)

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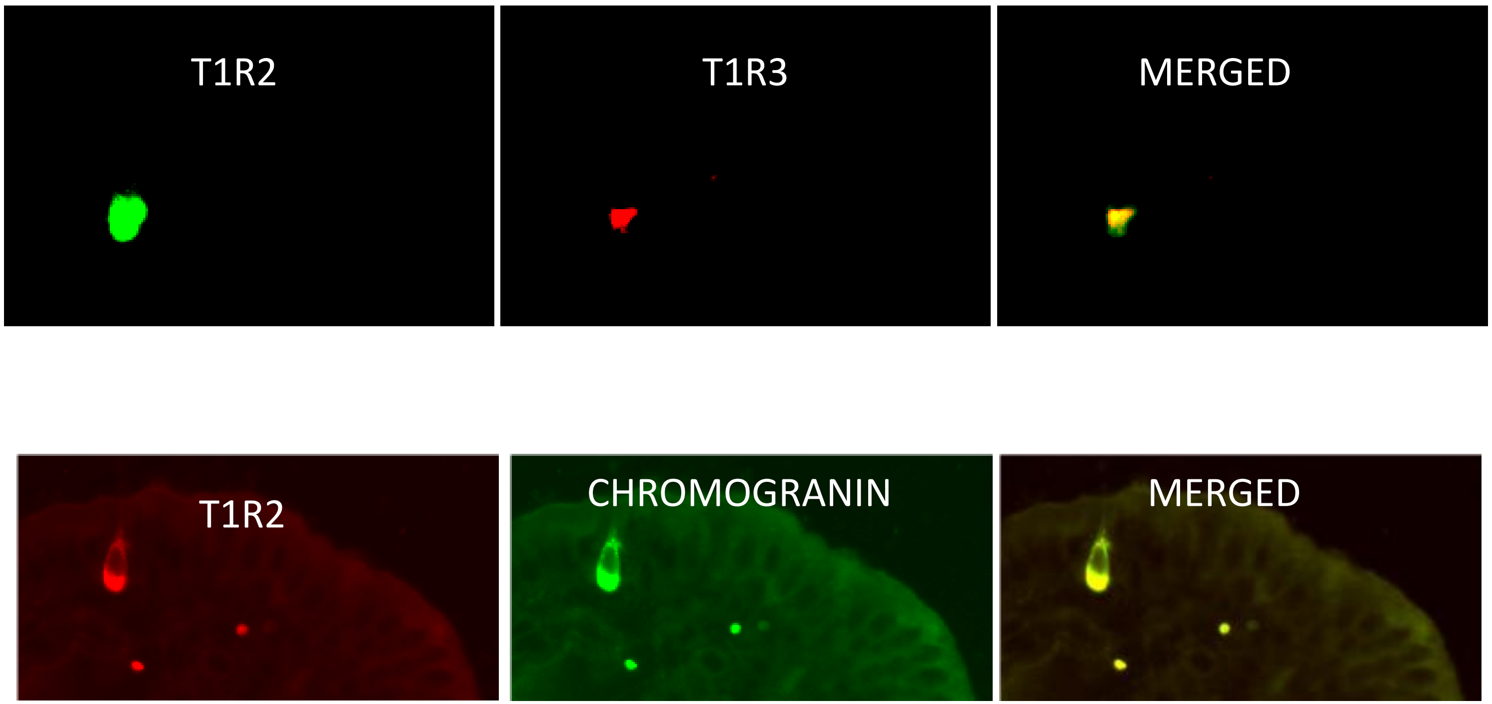
MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

WEANING PIGLETS



SOURCE: MORAN , AL-RAMMAHI, ARORA, BATCHELOR, COULTER, IONESCU, BRAVO & SHIRAZI-BEECHEY (2010, BR. J. NUTR.)

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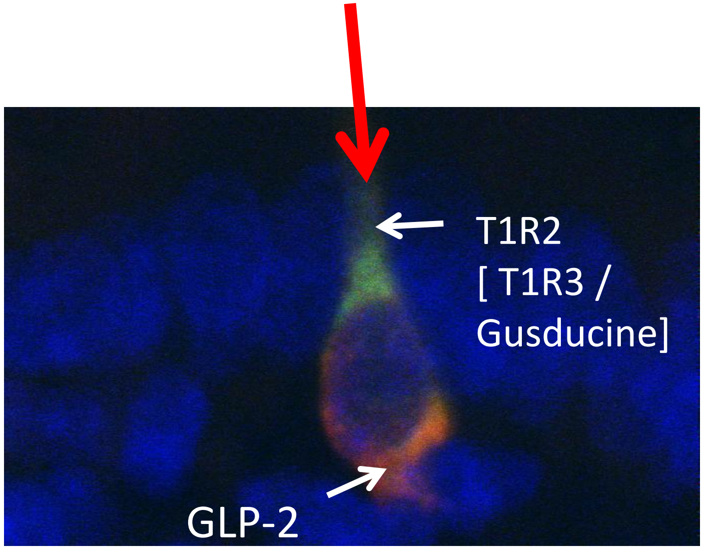
MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

SODIUM SACCHARINE



VIP ENTERIC NEURONS

INTESTINO TROPHIC EFFECT, BETTER GUT ARCHITECTURE, GLUCOSE UPTAKE

SOURCE: MORAN , AL-RAMMAHI, BRAVO & SHIRAZI-BEECHEY (SUBMITTED)

MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

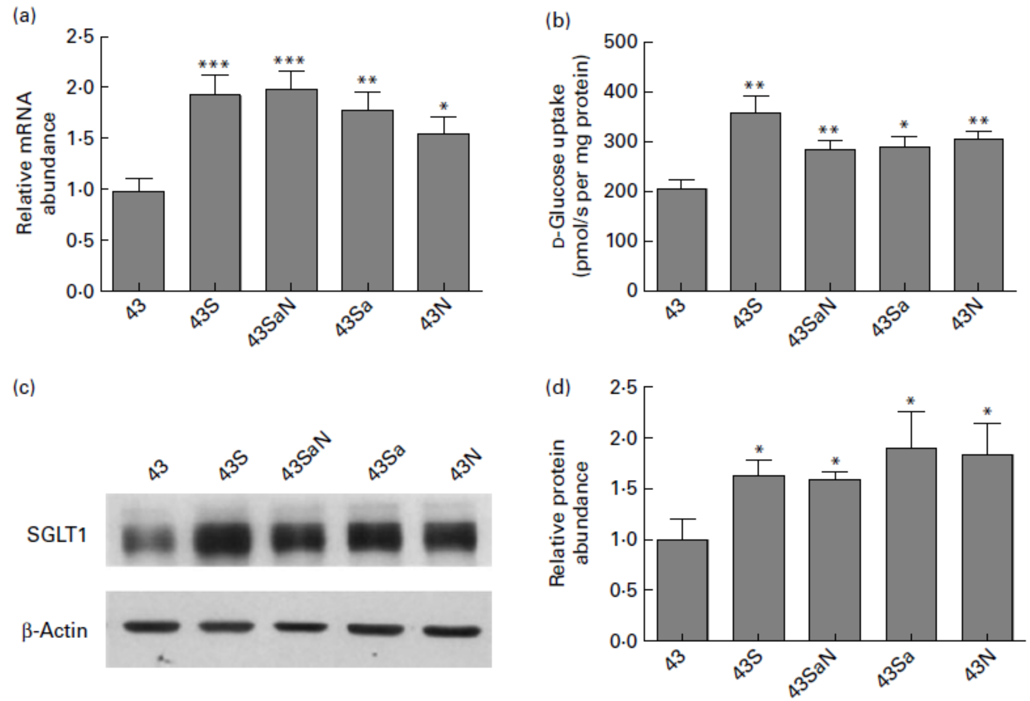


Fig. 4. Expression of Na^{+}/glucose co-transporter 1 (SGLT1) in swine mid-small intestine in response to feed supplementation with the artificial sweeteners, Sucram (43S), saccharin (43Sa), neohesperidin dihydrochalcone (NHDC, 43N) or saccharin and NHDC (43SaN). (a) Steady-state levels of SGLT1 mRNA abundance determined by quantitative PCR were normalised to β-actin mRNA. (b) Initial rates of Na^{+}-dependent D-glucose uptake into brush-border membrane vesicles (BBMV) measured using a rapid filtration technique. (c) Western blot analysis of SGLT1 and β-actin protein abundance in BBMV. (d) Densitometric analysis of Western blots normalised SGLT1 protein expression to that of β-actin. Data were generated in triplicate with *n* 6–12 animals. Results are shown as means with their standard errors. Mean values were significantly different: **P*< 0·05, ***P*< 0·01, ****P*< 0·001 (determined using an unpaired Student's *t* test).

SOURCE: MARGOLSKEE, DYER, KOKRASHVILI, SALMON,K.S.H. ET AL. (2007, PNAS)
SOURCE: MORAN , AL-RAMMAHI, ARORA, BATCHELOR, COULTER, IONESCU, BRAVO AND SHIRAZI-BEECHEY (2010, BR. J. NUTR.)

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MOLECULAR UNDERSTANDING

NUTRIENT AND NON-NUTRIENT SENSING

- Phytonutrients?!
- "**What are these bioactive compounds really doing in their environment**" Prof. J. Davies (ATA, 25.09.2012)
- The chemicals responsible for the gustatory and olfactory pleasures of spices are secondary metabolites of plants… or phytonutrients!
- Recognition of their chemical qualities must have driven the **co-evolution** of a particular categories of sensors in the animal kingdom.

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OUTLINE

- EFFICACY OF PHYTONUTRIENTS
- SUMMARY OF RESEASRCH FINDINGS
- MOLECULAR UNDERSTANDING: HOW DO PHYTONUTRIENTS WORK?
- **TAKE-HOME MESSAGES**

TAKE HOME MESSAGES

- The use of phytonutrients in animal feed is a valuable technology associated with consistent improvements in growth and feed efficiency.
- Phytonutrients are very diverse molecules.
- Increasing number of publications is documenting their efficacy.
- A complete understanding of their mode of action will be key for improved product consistency, consumer acceptance, and global use.

OBRIGADO

ขอบคุณมาก

谢谢

GRAZIE

TEŞEKKÜR EDERIM

DANKE

THANK YOU

MERCI

תודה

GRACIAS

DANK U

ありがとう

감사합니다