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

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

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>N</th>
<th>Negative control</th>
<th>100 g/t PNB</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily gain (g/day)</td>
<td>19</td>
<td>48.3</td>
<td>51.3</td>
<td>+4.9%</td>
<td>0.001</td>
</tr>
<tr>
<td>Feed intake (g/day)</td>
<td>19</td>
<td>81.9</td>
<td>84.1</td>
<td>NS</td>
<td>0.352</td>
</tr>
<tr>
<td>F:G (g/g)</td>
<td>19</td>
<td>1.73</td>
<td>1.68</td>
<td>-2.9%</td>
<td>0.001</td>
</tr>
</tbody>
</table>
# EFFICACY OF PHYTONUTRIENTS

## META-ANALYSIS

## RESULTS OF META-ANALYSIS OF 19 TRIALS

**EFFECT OF PNB ON PERFORMANCE OF BROILERS**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>N</th>
<th>%</th>
<th>ES and heterogeneity</th>
<th>Publication bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ES (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Daily gain (g/day)</td>
<td>19</td>
<td>+4.9%</td>
<td>0.150 (0.122, 0.188)</td>
<td>0.001</td>
</tr>
<tr>
<td>Feed intake (g/day)</td>
<td>19</td>
<td>NS</td>
<td>0.017 (-0.026, 0.061)</td>
<td>0.427</td>
</tr>
<tr>
<td>F:G (g/g)</td>
<td>19</td>
<td>-2.9%</td>
<td>-0.116 (-0.166, -0.066)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
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)
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 PHYTONUTRIENTS 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?
SUMMARY OF RESEARCH FINDINGS
ASSOCIATED PHYSIOLOGICAL RESPONSES

GUT LUMEN

GUT MUCOSA

METABOLISM

ENHANCEMENT DUE TO PHYTONUTRIENTS
DECREASE DUE TO PHYTONUTRIENTS
SUMMARY OF RESEARCH FINDINGS

ASSOCIATED PHYSIOLOGICAL RESPONSES

Rostagno et al. (2001)
Cross et al. (2007)
Wang et al. (2008)
Bravo et al. (2010)
Bravo et al. (2011)
Cross et al. (2007)
Wang et al. (2008)
Bravo et al. (2010)
Bravo et al. (2011)
Dhulay et al. (2009)
Gonzalez et al. (2008)
Lee et al. (2012, BJN)
Jamroz et al., (2006)
Jamroz et al., (2005)
Hernandez et al. (2004)
Basmacioglu Malayoglu et al. (2010, BPS)
Lee et al. (2003)
Bravo et al. (unpublished)

COCIDIOSIS:
Lee et al. (2011, IJPS)
Lee et al. (2011, BPS)
Lee et al. (2012, BJN)
Jamroz et al., (2006)
... and others...

SALMONELLA:
Amerah et al. (2011, BPS)
Platel et al. (2001, BPS)
Ganesh et al. (1984)
Mitsch et al. (2004, PS)
Oviedo-Rondon et al. (2006, PS)
Tiihonen et al. (2010, BPS)
... and others...

NECROTIC ENTERITIS:
Mitsch et al. (2004, PS)
Lee et al. (2012, JAS)
Jerzsele et al. (2012, PS)

FAECAL LOSS

GUT MUCOSA

ENDOGENOUS LOSS

DIGESTIVE SECRECTIONS

ABSORPTION

IMMUNE RESPONSE

INFLAMMATION

GUT LUMEN

FEED INTAKE

DIETARY COMPONENTS

NUTRIENTS

FAECAL MICROBIOTA

FAECAL LOSS

METABOLISM

GROWTH

METABOLIZABLE ENERGY

MAINTENANCE

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...
SUMMARY OF RESEARCH FINDINGS
ASSOCIATED PHYSIOLOGICAL RESPONSES

GUT LUMEN
- FEED INTAKE
  - DIETARY COMPONENTS
    - NUTRIENTS
      - ENDOGENOUS LOSS
        - BENEFICIAL
          - DIGESTIVE MICROBIOTA
            - FAECAL LOSS
              - CONFUSED

GUT MUCOSA
- DIGESTIVE SECRETIONS
  - ABSORPTION
    - METABOLISABLE ENERGY
      - GROWTH
        - PROTECTIVE
          - IMMUNE RESPONSE
            - INFLAMMATION
              - MAINTENANCE

METABOLISM

ENHANCEMENT DUE TO PHYTONUTRIENTS
DECREASE DUE TO PHYTONUTRIENTS

In case of challenge
Inflammatory context « Dirty » conditions
In very clean conditions
Low inflammatory context
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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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?
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).
MOLECULAR UNDERSTANDING
ANTI-MICROBIAL VS. HOST-MEDIATED

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

ANTI-MICROBIAL VS. HOST-MEDIATED

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Carvacrol</th>
<th>Cinnamaldehyde</th>
<th>Thymol</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>450</td>
<td>396</td>
<td>450</td>
<td>Helander <em>et al.</em>, 1998</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>225</td>
<td>NT</td>
<td>225</td>
<td>Cosentino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>450</td>
<td>NT</td>
<td>225</td>
<td>Cosentino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>150</td>
<td>NT</td>
<td>150</td>
<td>Ali-Shtayeh <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>113</td>
<td>NT</td>
<td>113</td>
<td>Cosentino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>200</td>
<td>200</td>
<td>NT</td>
<td>Ferhout <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>500</td>
<td>NT</td>
<td>500</td>
<td>Ali-Shtayeh <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>&gt;900</td>
<td>NT</td>
<td>&gt;900</td>
<td>Cosentino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>150</td>
<td>396</td>
<td>150</td>
<td>Helander <em>et al.</em>, 1998</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>225</td>
<td>NT</td>
<td>56</td>
<td>Cosentino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em></td>
<td>125</td>
<td>250</td>
<td>250</td>
<td>Didry <em>et al.</em>, 1994</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>Didry <em>et al.</em>, 1994</td>
</tr>
</tbody>
</table>

NT: not tested
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?
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!
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.
MOLECULAR UNDERSTANDING
NUTRIENT AND NON-NUTRIENT SENSING

SOURCE: LENARD & BERTHOUD (2008, OBESITY)
MOLECULAR UNDERSTANDING
NUTRIENT AND NON-NUTRIENT SENSING

WEANING CALVES

SOURCE: MORAN, AL-RAMMAHI, BRAVO, CALSAMIGLIA & SHIRAZI-BEECHEY (UNPUBLISHED DATA)
MOLECULAR UNDERSTANDING
NUTRIENT AND NON-NUTRIENT SENSING

WEANING PIGLETS

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)
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: MORAN, AL-RAMMAHI, ARORA, BATCHelor, COULTER, IONESCU, BRAVO AND SHIRAZI-BEECHEY (2010, BR. J. NUTR.)
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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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.
THANK YOU
OBRIGADO
TEŞEKKÜR EDERIM
THANK YOU
DANKE
TEŞEKKÜR EDERIM
DANK U
GRACIAS
MERCİ
DANK U
GRACIAS
감사합니다
ありがとう
תודה
谢谢