Phytogenics support gut health

FIAAP ANIMAL NUTRITION CONFERENCE 2017

Phytogenics, modes of action, and application

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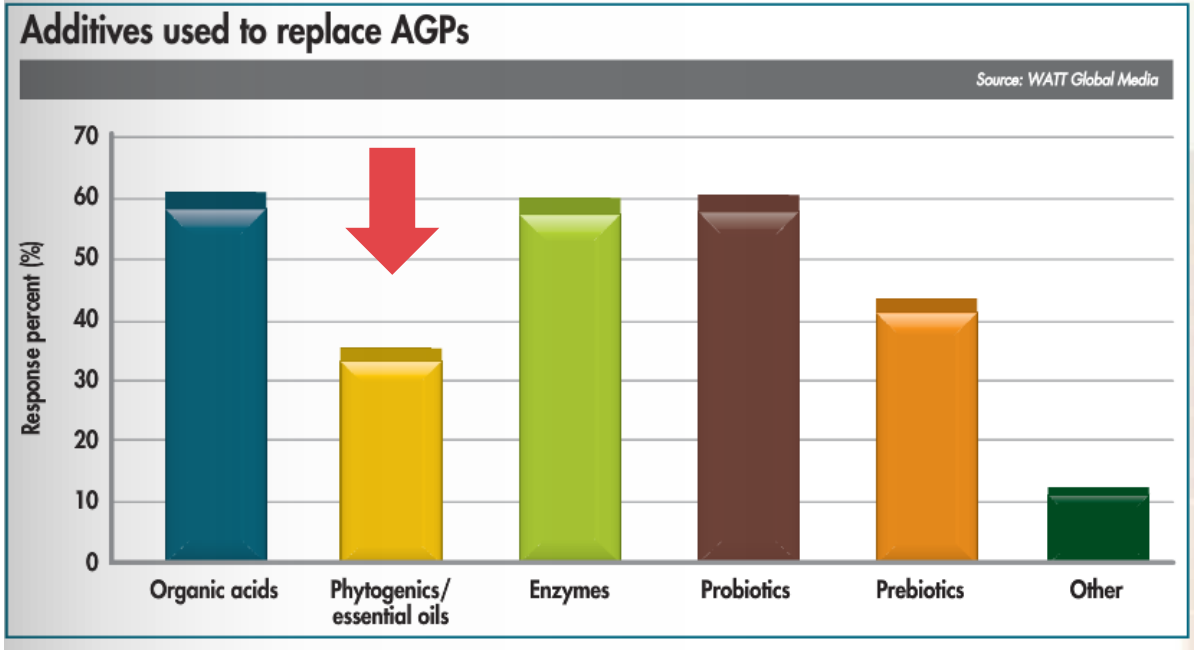
- Phytogenics: A ‘new’ class of feed additives
  - Definition and classification
  - Bio-active compounds and extraction methods
  - Bio-efficacy
  - Natural variation and standardisation
- Gut health challenges in poultry
- Modes of action of phytogenics
- In-feed applications

Phytogenics: A ‘new’ class of feed additives

Phytogenics use in AGP replacement programs



Nutrition & Feed Survey 2016:
'Formulating poultry feed for antibiotic-free production'

Results of 2006 world-wide survey among 286 poultry feed producers and users

Roembke, June 2016

Comments

Reasons for relatively low use of phytogenics:

- ‘Unfamiliar with this type of products’
- ‘Modes of action are still largely unclear’
- ‘Lack of consistency of results’

What are phytogenics?

Definition:
'*Plant-derived, natural bio-active compounds*'

(e.g. Puvaca et al. (2013)

like essential oils, herb extracts, oleoresins, tannins and saponins

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Use of phytogenics as feed additives

Use of plant materials as ingredients for feed additives:

- Europe: Ingredients should be mentioned in Register of Feed Additives *pursuant to Regulation (EC) No 1831/2003*
- US: being 'Generally Recognised As Safe (GRAS)'

In EU botanical feed additives are considered as sensory additives (flavouring substances). Some are registered as zootechnical additives with proven safety and efficacy.

Phytogenics: Sourcing & extraction from plants

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Plant parts: leaves, barks, roots, seeds, fruits, flowers

Sequential steps:

1. Size reduction
2. Extraction
3. Filtration
4. Concentration
5. Drying

Extraction (examples):

I. Cold aqueous percolation
II. Hot aqueous extraction (decoction)
III. Solvent extraction (cold or hot)
IV. Supercritical fluid extraction

Choices for extraction are based on characteristics (eg. solubility in various solvents and heat stability of extract) and price of active component

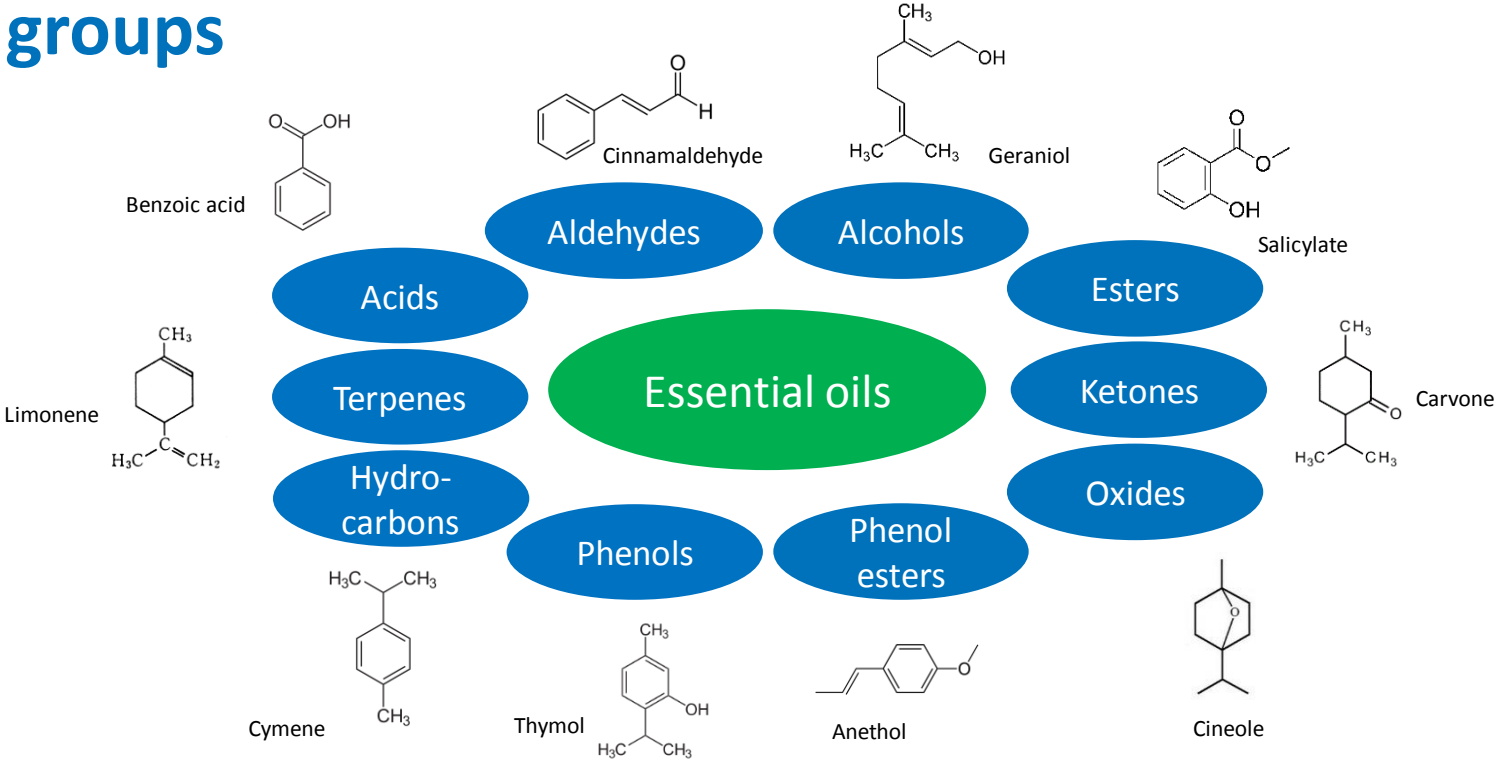
'Extraction Technologies for Medicinal and Aromatic Plants' (2008)

Bioactive plant components

Major class: terpenes and terpenoids

Type	Size of molecule
Monoterpenes: e.g. limonene, linalool, geraniol	C10
Monoterpenoids: e.g. thymol, carvacrol, p-cymene, menthol	C10
Sesquiterpenoids: e.g. artemisinin	C15
Triterpenoids: e.g. saponins	C30
Tetraterpenoids: e.g. carotenes, xanthophyls	C40
Polyterpenoids: e.g. sap, resins, latex	>C40

Phytogenics comprise heterogenous chemical groups



Adapted from 'Extraction Technologies for Medicinal and Aromatic Plants' (2008)

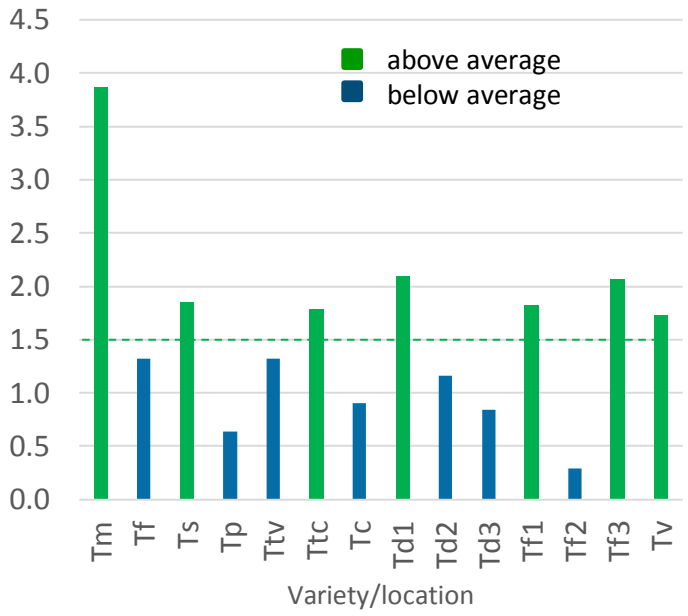
Bio-efficacy of phytogenic feed additives

Bio-efficacy depends on:

- Plants species and plant parts
- Growing conditions (climate, soil, region) and development stage at harvest
- Extraction methods of actives and further processing
- Synergies between bio-active components
- Gastro-intestinal conditions

Phytogenics: Natural variation in *Thymus* spp

Essential oil yield, %



- 14 *Thymus* samples (10 species) from different regions in Iran
- *Thymus* EO content affected by environment (eg. soil type) and *Thymus* species
- Max. EO level at flowering and min. EO level at seed set stage
- Main EO: thymol (12-79%), carvacrol (4.4-42%), geraniol (0.3-22%) and p-cymen (0.9-13%)
- Anti-oxidant capacity variation between samples: 273-694 µg/mL

Tohidi *et al.* (2017), Vaičiulyte *et al.* (2017)

Phytogenics: Natural variation in leaves of *Mentha piperita L.*

Origin	Limonene, %	Cineole, %	Methone, %	Menthol, %
A	**6.7** 5.4-7.7	**5.0** 4.1-6.1	**27.4** 19.3-35.1	**34.9** 31.1-42.1
B	**0.7** 0.2-1.0	**3.6** 3.0-4.2	**43.7** 36.4-49.8	**26.1** 21.5-33.3
C	**1.0** 0.5-1.5	**3.6** 3.0-4.4	**31.2** 27.0-36.8	**34.5** 39.6-39.7

(means values, min – max during 4 years)

Bomme *et al.* (2005)

Phytogenics: Standardisation of bio-efficacy

- Standardisation of contents of active ingredients via analyses of raw materials and blending
- Effective micro-encapsulation procedure
- Understanding of
  - Modes of Action
  - Synergistic and antagonistic effects between actives and between feed additives
- Optimisation of dose levels

Gut health challenges in poultry

Gut health challenges in poultry

Major gut health challenges

(bacteria/ parasites/ viruses:

- Coccidiosis
- Necrotic enteritis
- Dysbacteriosis

NE challenge:
-250 g BW at d35

Consequences

- Inflammation:
  reduced feed intake
  body tissue degradation
- Reduced weight gain
- Poor uniformity
- Impaired intestinal integrity
- Bacterial translocation
- Reduced nutrient digestibility
- Reduced food safety
- Wet litter

Modes of Action

Phytogenics: What can they do?

Phytogenic feed additives

Biological activities

- Anti-bacterial
- Anti-oxidant
- Anti-coccidial
- Anti-inflammatory
- Immunomodulating
- Anti-stress
- Performance enhancer
- Enzyme secretion

Zootechnical additives

Proven efficacy and safety

Sensory additives

- Appetizing effects
- Sensory properties
- Flavor enhancing

Adapted from Hashemi and Davoodi (2011)

Modes of action: antibacterial

Direct

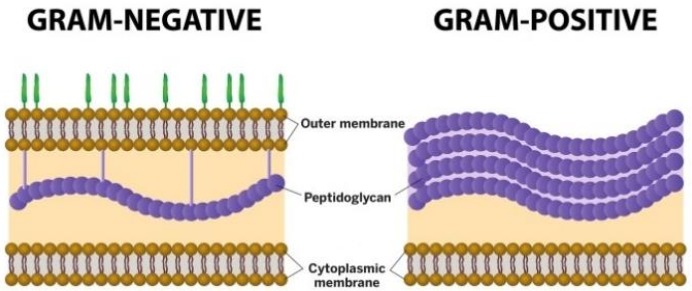
- Antibacterial effects via bacteriostatic/bacteriocidal effects
- Effects seen at high dose levels (> MIC)

Indirect

- Reducing bacterial virulence by Quorum Sensing Inhibition
- Interference via bacterial gene expression, reducing toxin, fimbriae and biofilm production
- Effects seen at levels below MIC

Efficacy depends on bacterium species and phytogenic active

Antibacterial effects



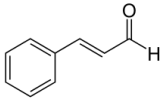
More resistant than Gram+

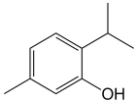
1. Thymol and carvacrol outer membrane disintegrating properties
2. Synergy proven between EO and organic acids
3. Organic acids can pass via porin proteins

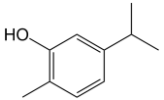
Peptidoglycan cell wall is easily penetrated by hydrophobic compounds

1. Phenolic compounds interfere with bacterial energy metabolism
2. Denature proteins

Combination effects against *S. typhimurium*







	Cinnamaldehyde (mg/L)	Thymol (mg/L)	Carvacrol (mg/L)	Log (delta) @ 37C, 24h
Single compounds	**0**	**0**	**0**	2.1
	100	**0**	**0**	1.1
	0	**200**	**0**	0.6
	0	**0**	**200**	0.5
Combinations	**100**	**200**	**0**	-3.3
	100	**0**	**200**	-3.3
	0	**200**	**200**	-3.2
	0	**200**	**100**	-3.0

max. effects (>-3.0) for single substances were found at 200, 400 and 400 mg/L
thymol and carvacrol had similar antibacterial efficacy

Zhou *et al.* (2007)

Bioactive compounds in garlic extracts

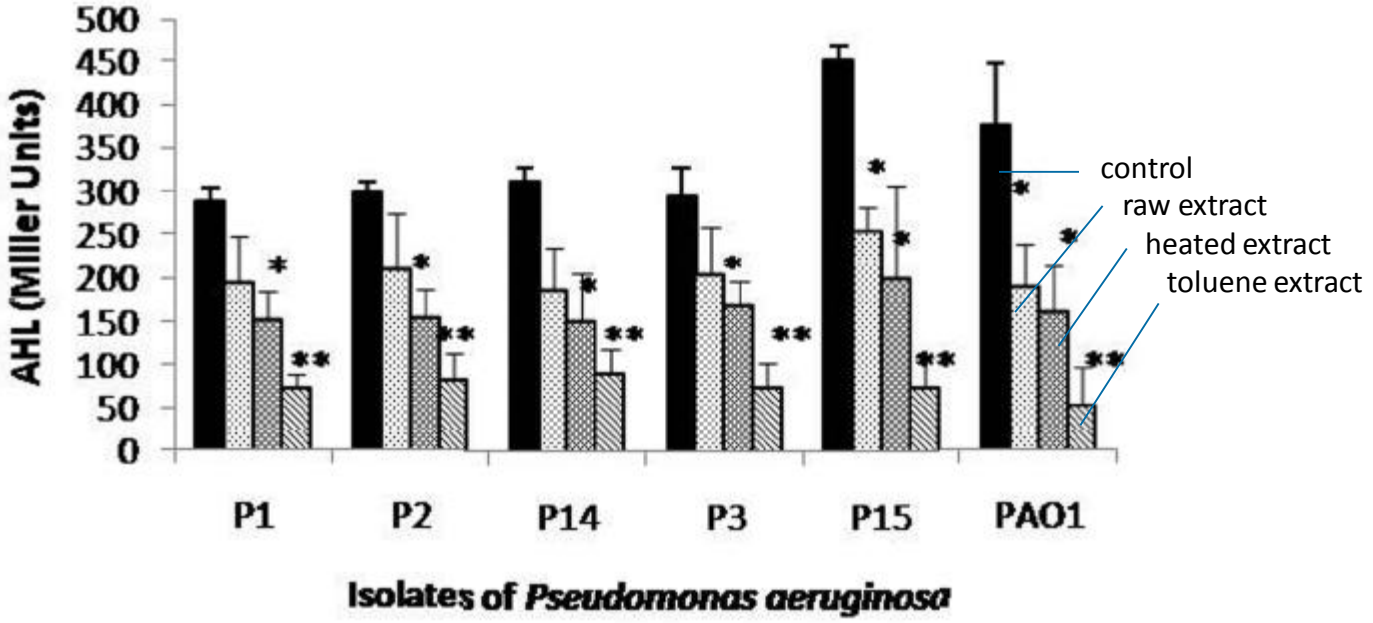
Extraction method	Saponins	Flavonoid	Terpenoid	Alkaloid	Thiosulfinates (mM)
Raw	**+**	**+**	**+**	**+**	**3.5**
Heated	**-**	**+**	**+**	**+**	**3.7**
Toluene	**+**	**-**	**+**	**-**	**4.0**
Ethanol	+	-	+	+	3.1
Methanol	-	+	+	-	3.7
Acetone	-	+	+	+	2.6
Chloroform	-	-	-	-	3.7

Vadekeetil *et al.* (2014)

Effect of garlic extracts* against QS signal molecules (AHL) in *P. aeruginosa*

*tested at sub MIC levels

Acyl-Homoserine-Lactone (AHL): autoinducer mol. of Gram- bacteria

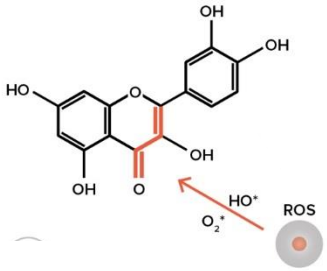


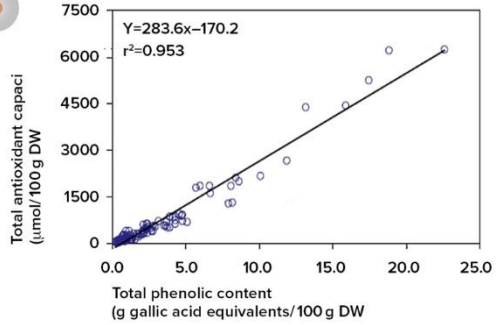
Vadekeetil *et al.* (2014)

Modes of action: anti-oxidant

Direct

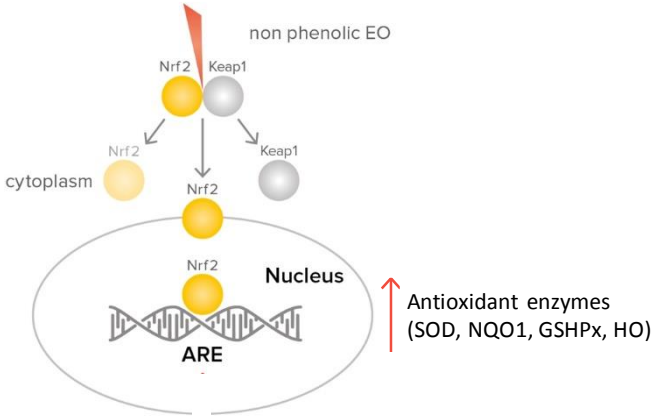
- Radical scavenging



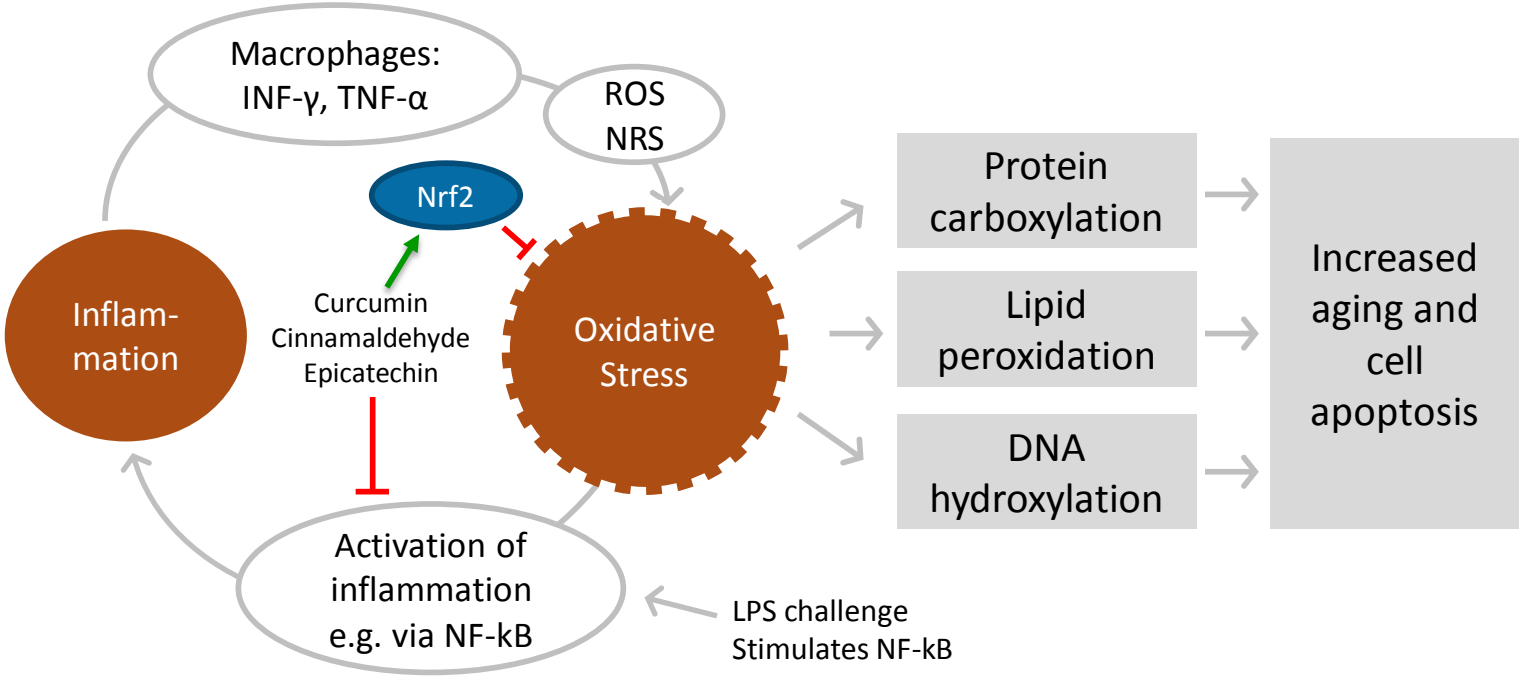


Indirect

- Stimulation of synthesis of anti-oxidant enzymes



Inflammation and oxidative stress cascade



After Okusaga (2014) and Gessner *et al.* (2016)

Improved intestinal barrier function and intestinal defense mechanisms

Ross 308 broilers 0-5 wk old

Treatments:

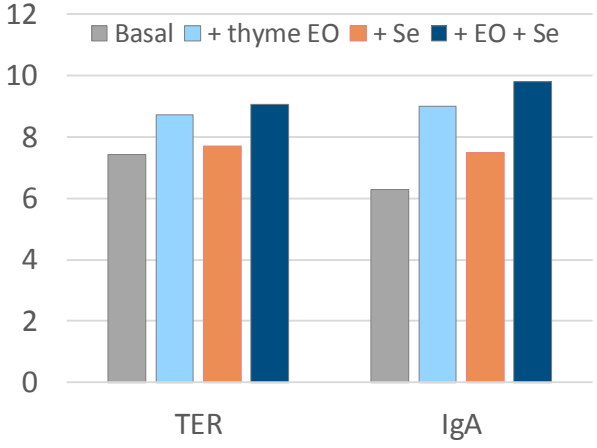
- Basal: 0.2 mg/kg Se
- Added: 0.4 mg/kg Se and/or 0.5 mg/kg Thymus vulgaris EO

Measurements:

- Duodenal Transepithelial Electrical Resistance (MΩ)
- Duodenal IgA (mg/g *0.10)

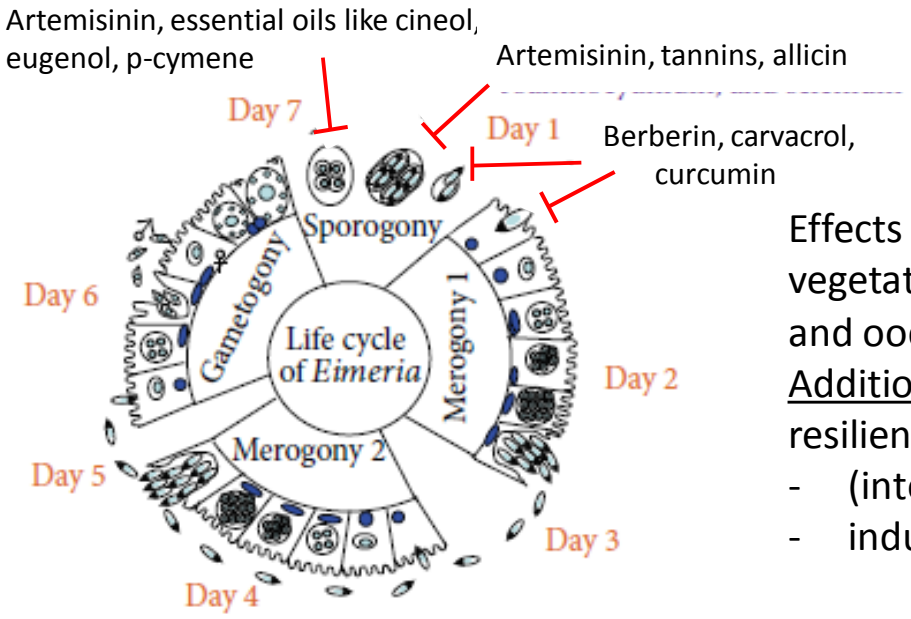
In addition higher phagocytic acitity was found in duodenum by thyme EO

	sign. of main effects	
EO	0.013	0.002
Se	0.558	0.163



Source: Placha et al. (2014)

Modes of action: Anticoccidial effects



Effects on reproductive cycle in vegetative state, reducing lesions and oocysts excretion

<u>Additional effects</u> via improved resilience of the bird:

- (intestinal) immunity
- induction of oxidative stress

Muthamilselvan *et al.* (2016)

Modes of action: Nutrient digestion

Break-down of feed

Increased synthesis of endogenous enzymes (like chymotrypsin), bile acids and brush border enzymes

Absorption of nutrients

Increased expression of membrane transporters of enterocytes

- Improved nutrient digestion
- Increased efficiency of utilisation
- Reduced substrate for intestinal bacteria

In-feed applications

Alternatives to AGPs

Choices for combinations of feed additives as AGP alternatives should be made based on

Intestinal challenges: eg. viscosity

Production restraints: Eg. No Antibiotics Ever?

Efficacy: anti-oxidant, anti-bacterial, immunity, digestibility effects, …

Complementary, additive and/or synergistic effects

Proven Efficacy!

Conclusions

Conclusions

- Phytogenics
  - Comprise large group of active ingredients
  - Diverse modes of actions (moA) with huge potential
  - Standardisation is crucial for successful application
- Systematic research and scientific support
  - Supports understanding of MoA
- Cornerstone of AGP alternatives
  - Phytogenics show synergies with other feed additives

Thank you for your attention!