Feed additives for enhanced sustainability in animal food chain

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Sustainable agriculture

- Poultry, pig & dairy industry are now in the spotlight to promote sustainable development & an environmental friendly animal harvesting.

- Agriculture (animal sector) is dependent on feed efficiency to sustain growth and profitability.

- Sustainability should be regarded as the crucial factor to match worldwide increased food demands and reserve feed sources; is based on 2 pillars: animal health & productivity.
Competitive environment

- The 4F competition for land
  - Food
  - Feed
  - Fuel
  - Fabric

- Water sustainability is also a crucial factor

- Increase in human population - demand for more food, especially foods of animal origin due to urbanism

- Clothing – water demand for cotton/pesticides/chemicals

- Biofuels – water for oil crops or cereals for ethanol production / chemicals / pesticides / fertilizers
Main Challenge in Agriculture

• A major demand by the international community to the food chain industry is the cancellation of the use of antibiotics during animal growth-breeding,

• Ban of antibiotic non-therapeutic in-feed use in EU, starting from 2006

• This decision took place after the emergence of resistant pathogens in animal and aquatic ecosystems and even participation in human disease outbreaks in last decades
Broiler flocks

- **Acute:**
  - Necrotic enteritis
  - Colibacillosis
  - Mortality

- **Subclinical:**
  - Decreased digestion
  - Reduced weight gain
  - Increased feed conversion ratio

- **Coccidiosis**

Pig industry

- **Weaning problems**
- **Infectious diseases**
- **Production diseases**
- **Raw materials, mycotoxins, feed formulations**
Dairy Animals – health threats

- Infectious
  - Lumpy skin disease
  - Tuberculosis
  - Brucellosis
  - Newborn colibacillosis
  - Contagious agalactia
  - Neonatal diarrhea

- Non-infectious
  - Abomasal displacement
  - Hypocalcemia
  - Ketosis
  - SARAidosis
  - White muscle disease
  - Pregnancy toxemia
Natural feed additives

• Alternative Growth Promoters – Health Support

• Various concepts as non-therapeutic alternatives have been proposed to the animal industry for optimizing performance of their operations:
  • Enzymes, Organic acids, Probiotics, Prebiotics, Minerals, Herbals

• Several feed additives have been used in animal nutrition in order to increase sustainability of the Agrofood sector, in both organic and conventional livestock to alleviate physiological negative effects caused by different challenges
Enzymes to increase sustainability

RESEARCH ARTICLE

Effects of Protease Addition and Replacement of Soybean Meal by Corn Gluten Meal on the Growth of Broilers and on the Environmental Performances of a Broiler Production System in Greece

Ilias Giannenas¹*, Eleftherios Bonos², Vasileios Anestis³,⁴, Georgios Filioussis⁵, Dimitrios K. Papanastasiou⁶, Thomas Bartzanas⁷, Nikolaos Papaioannou⁶, Athina Tzora⁷, Ioannis Skoufos⁷

¹ Laboratory of Nutrition, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, ² Research Institute of Animal Science, ELGO-Dimitra, Paralimni Giannitsa, Pella, Greece, ³ Laboratory of Agricultural Engineering and Environment, Institute for Research and Technology of Thessaly, Centre for Research and Technology Hellas, Volos, Greece, ⁴ Laboratory of Agricultural Constructions and Environmental Control, Department of Agriculture Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, Magnisia, Greece, ⁵ Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, ⁶ Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, ⁷ Department of Agricultural Technology, Division of Animal Production, Technological Institute of Epirus, Arta, Greece
Field trial in broilers, Thessaloniki, Greece

- **Breed**: Ross-308 - 640 males
- **Animals**: 3 groups/6 subgroups of 30 broilers each
- **Duration of trial**: 42 days
- **Diet**: corn/soybean based formulation
- **Bed floor type**: wood shaving
- **Anticoccidials**: No anticoccidial
- **Measurements**: Performance
- **3 treatments**:
  - T1: Control (Standard feed)
  - T2: Soy-Prot
  - T3: Gluten-Prot

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Soy-Prot</th>
<th>Gluten-Prot</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g d1</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>BW, g d42</td>
<td>2424</td>
<td>2389</td>
<td>2259</td>
</tr>
<tr>
<td>FI per bird, kg</td>
<td>3757</td>
<td>3700</td>
<td>3639</td>
</tr>
<tr>
<td>FCR</td>
<td>1.579</td>
<td>1.581</td>
<td>1.653</td>
</tr>
</tbody>
</table>

**Diets**

<table>
<thead>
<tr>
<th>Crude protein, %</th>
<th>22</th>
<th>21</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metab.energy, Kcal/kg</td>
<td>3000</td>
<td>3100</td>
<td>3200</td>
</tr>
</tbody>
</table>
Trial Results

• Reducing the protein concentration in the diets for broilers with the addition of protease enzyme or the substitution of SBM by CGM together with the use of a protease could be potential dietary strategies to lower feeding cost and improve environmental impact.

• The substitution of SBM by CGM together with a protease marginally retained growth performance parameters, affected positively the intestinal microflora of broiler chickens and retained gut integrity.

• The LCA performed indicated that the N related emissions due to litter handling are the most important farm-level flows to be dealt with in order to reduce the acidification, eutrophication and global warming effects caused by the studied partial life-cycles.
A combination of organic acid, essential oil compounds and protease on growth performance of chickens
## Enzymes plus organic acids

<table>
<thead>
<tr>
<th>Ingredients, g/kg</th>
<th>Control 1-12d</th>
<th>Control 13-24d</th>
<th>Control 25-40d</th>
<th>CRI + Protease 1-12d</th>
<th>CRI + Protease 13-24d</th>
<th>CRI + Protease 25-40d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat grain, hard 13.4</td>
<td>631.0</td>
<td>655.0</td>
<td>675.0</td>
<td>651.0</td>
<td>685.0</td>
<td>690.0</td>
</tr>
<tr>
<td>Soybean meal, 46.8</td>
<td>296.0</td>
<td>256.0</td>
<td>232.0</td>
<td>268.0</td>
<td>230.0</td>
<td>210.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>25.0</td>
<td>15.0</td>
<td>10.0</td>
<td>25.0</td>
<td>11.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Fat</td>
<td>18.0</td>
<td>35.0</td>
<td>45.0</td>
<td>15.0</td>
<td>35.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>11.0</td>
<td>11.0</td>
<td>10.0</td>
<td>11.0</td>
<td>11.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>8.0</td>
<td>7.0</td>
<td>7.0</td>
<td>8.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Vit, min, aa premix</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

### Calculated analysis

<table>
<thead>
<tr>
<th></th>
<th>Control 1-12d</th>
<th>Control 13-24d</th>
<th>Control 25-40d</th>
<th>CRI + Protease 1-12d</th>
<th>CRI + Protease 13-24d</th>
<th>CRI + Protease 25-40d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>22.1</td>
<td>21.0</td>
<td>20.1</td>
<td>21.5</td>
<td>20.2</td>
<td>19.5</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.25</td>
<td>1.1</td>
<td>1.05</td>
</tr>
<tr>
<td>Methionine+Cystine, %</td>
<td>1.0</td>
<td>0.96</td>
<td>0.94</td>
<td>0.98</td>
<td>0.95</td>
<td>0.92</td>
</tr>
<tr>
<td>Metabolisable energy, Kcal/kg</td>
<td>3150</td>
<td>3200</td>
<td>3250</td>
<td>3150</td>
<td>3200</td>
<td>3250</td>
</tr>
</tbody>
</table>
Trial Results – Protease

The tested enzyme, a purified mono component serine protease which is expressed in *B. licheniformis*. SDS-PAGE of the heat stable formulated product revealed a single band at approximately 20 kDa verifying the purity of the enzyme used in this study.

Lanes 1: Molecular weight markers.

2&3: Starter diet for Control and CRI+Prot

4&5: Grower diet for Control and CRI+Prot

The three major bands at 70, 35 and 25 kDa are indicated with arrows.

Each lane was loaded with 20 μg of protein
Effect of feed supplementation with protease on protein digestion (II) & solubilisation (III)

Samples of starter diet Control and CRI+Prot (A and A+, respectively) and samples of grower diet Control and CRI+Prot (B and B+)

Feeds were incubated at pH 2.0 for 30 min at 40°C and subsequently at pH 8.0 for another 30 min at 40 °C.

Insoluble materials were removed by centrifugation while the protein content of the resulted supernatants was calculated in order to define the solubilisation degree.

Digestion degree was defined by subjected 20 μg of soluble proteins of each soluble fraction in an SDS-PAGE.
Probiotics

“Live microbial cultures given to animals with the intention of improving health or production parameters.”

Bacteria e.g.

- Lactic acid bacteria (e.g. *Lactobacillus* spp.)
- *Bacillus*
- *Enterococcus*

Yeast - *Saccharomyces*
Cocci -challenge trial with probiotics

- Laboratory of Animal Nutrition and Husbandry, Veterinary Faculty, University of Thessaly, Greece, 2009-2010
- 300 Cobb500 broiler, 1 day old
  - 10 groups
  - 3 replications per group
  - 10 birds per replication
- challenge with *E. tenella* 2E+04 oocysts per bird at day 14 (crop intubation)
Performance parameters

Body weight d 35

Body weight [g]

<table>
<thead>
<tr>
<th>Group</th>
<th>UU</th>
<th>UI</th>
<th>LI</th>
<th>EF std.</th>
<th>EF high</th>
<th>BA</th>
<th>LR</th>
<th>BS</th>
<th>M3 std.</th>
<th>M3 high</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>d</td>
<td>abc</td>
<td>cd</td>
<td>ab</td>
<td>bcd</td>
<td>abc</td>
<td>a</td>
<td>a</td>
<td>abc</td>
</tr>
</tbody>
</table>

Different letters in data series indicate different significances (p > 0.05, Tukey’s test)
## Parasitological parameters

<table>
<thead>
<tr>
<th>Lesion score d</th>
<th>UU</th>
<th>UI</th>
<th>LI</th>
<th>EF std.</th>
<th>EF high</th>
<th>BA</th>
<th>LR</th>
<th>BS</th>
<th>M3 std.</th>
<th>M3 high</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0</td>
<td>d</td>
<td></td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.75&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.08&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.58&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.08&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Faecal score</td>
<td>0</td>
<td>3+</td>
<td>2+</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Oocyst reduction [%]</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>89.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total mortality</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Lesion score according to Johnson and Reid, 1970
Faecal score according to Youn and Noh, 2001
Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with *Eimeria tenella*

I. Giannenas\(^a\), E. Papadopoulos\(^b\), E. Tsali\(^c\), El. Triantafillou\(^d\), S. Henkl\(^e\), K. Teichmann\(^e\), D. Tontis\(^c\)

**Avian Pathology**
Publication details, including instructions for authors and subscription information:
[http://www.tandfonline.com/loi/cavp20](http://www.tandfonline.com/loi/cavp20)

Assessment of probiotics supplementation via feed or water on the growth performance, intestinal morphology and microflora of chickens after experimental infection with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*

I. Giannenas\(^a,b\), E. Tsali\(^c\), E. Triantafillou\(^d\), S. Hessenberger\(^e\), K. Teichmann\(^e\), M. Mohnl\(^e\) & D. Tontis\(^c\)
Combination of probiotics, prebiotics and organic acids in fattening pigs

Effects of *Enterococcus faecium*, mannan oligosaccharide, benzoic acid and their mixture on growth performance, intestinal microbiota, intestinal morphology and blood lymphocyte subpopulations of fattening pigs

I. Giannenas\textsuperscript{a,*}, D. Doukas\textsuperscript{b}, A. Karamoutsios\textsuperscript{c}, A. Tzora\textsuperscript{c}, E. Bonos\textsuperscript{a}, I. Skoufos\textsuperscript{c}, A. Tsinas\textsuperscript{c}, E. Christaki\textsuperscript{a}, D. Tontis\textsuperscript{b}, P. Florou-Paneri\textsuperscript{a}

\textsuperscript{a} Laboratory of Nutrition, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
\textsuperscript{b} Laboratory of Pathology, Faculty of Veterinary Science, University of Thessaly, 43100, Karditsa, Greece
\textsuperscript{c} Department of Agricultural Technology, Division of Animal Production, Technological Institute of Epirus, 47100, Arta, Greece
Effects of combination of feed additives on performance and immunity of pigs

Table 3
Effect of different feed additives as alternatives for antibiotics on body weight, weight gain, feed intake and feed conversion ratio of fattening pigs.

<table>
<thead>
<tr>
<th></th>
<th>Groups 1</th>
<th>SEM 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>EntFaec</td>
<td>MOS</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 113</td>
<td>55.1</td>
<td>54.8</td>
<td>54.8</td>
</tr>
<tr>
<td>Day 169</td>
<td>114.2a</td>
<td>115.8a</td>
<td>116.0a</td>
</tr>
<tr>
<td>Weight gain, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 113–169</td>
<td>59.2a</td>
<td>61.0ab</td>
<td>61.2ab</td>
</tr>
<tr>
<td>Feed intake, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 113–169</td>
<td>177.3</td>
<td>171.3</td>
<td>167.8</td>
</tr>
<tr>
<td>FCR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 113–169</td>
<td>3.00a</td>
<td>2.81ab</td>
<td>2.75ab</td>
</tr>
</tbody>
</table>

Table 4
Effect of different feed additives as alternatives for antibiotics on blood lymphocyte sub populations of fattening pigs measured by flow cytometry.

<table>
<thead>
<tr>
<th></th>
<th>Groups 1</th>
<th>SEM 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>EntFaec</td>
<td>MOS</td>
</tr>
<tr>
<td>CD3 (%)</td>
<td>73.04</td>
<td>72.00</td>
<td>75.10</td>
</tr>
<tr>
<td>CD79a+ SLA-DR+ (%)</td>
<td>17.44</td>
<td>18.00</td>
<td>17.70</td>
</tr>
<tr>
<td>CD4a+ (%)</td>
<td>9.13</td>
<td>7.28</td>
<td>8.97</td>
</tr>
<tr>
<td>CD8a+ (%)</td>
<td>20.02</td>
<td>21.23</td>
<td>19.23</td>
</tr>
<tr>
<td>CD4a/CD8a</td>
<td>0.46ab</td>
<td>0.35ab</td>
<td>0.47a</td>
</tr>
</tbody>
</table>
Pen trial (384 broilers)

- Supplementation of Iron chelate in feed. 4 Treatment groups:
  - control (no additive),
  - iron chelate at 0.02, 0.05 and 0.2 g/kg
  - 6 Replicates per treatment, total number of replicates: 24

- Protocol
  - Continuous administration: days 0 – 42
  - Challenge: litter naturally contaminated with *Campylobacter* infected litter at day 20, no coccidiostats

- Assessment of impact on birds
  - Caecal samples tested for *Campylobacter* at day 42
  - Recorded weight gain and feed conversion ratio
  - FOODSCAN for chemical content
  - Taste Panel

Minerals against campylobacter, a trial supervised by our colleagues in TEI of Epirus, Skoufos et al, 2017
Results on growth performance

Minerals against campylobacter, a trial supervised by our colleagues in TEI of Epirus, Skoufos et al, 2017
... and *Campylobacter* reduction

<table>
<thead>
<tr>
<th></th>
<th>Log$_{10}$ CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control</td>
<td>9.19</td>
</tr>
<tr>
<td>T2 TYPLEX 0.02 g/Kg</td>
<td>8.59</td>
</tr>
<tr>
<td>T3 TYPLEX 0.05 g/Kg</td>
<td>8.85</td>
</tr>
<tr>
<td>T4 TYPLEX 0.20 g/Kg</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Iron chelate

Error bars are for standard error.

Minerals against *campylobacter*, a trial supervised by our colleagues in TEI of Epirus, Skoufos et al, 2017
Iron to support growth of pigs

Effect of Dietary Iron Sulfate and Iron Chelate on Growth Performance, Hematological Traits, Intestinal Microbial Flora of Fattening Pigs and Quality Parameters of Porkmeat

Table 4: Effect of dietary supplementation of iron sulfate and iron chelate in fattening pigs, in the final body weight (age 165 days)

<table>
<thead>
<tr>
<th>Group</th>
<th>S200</th>
<th>S800</th>
<th>C200</th>
<th>C800</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (112th d), kg</td>
<td>54.3</td>
<td>53.8</td>
<td>53.5</td>
<td>54.2</td>
<td>0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Final body weight (165th d), kg</td>
<td>100.3*</td>
<td>104.2*</td>
<td>106.4*</td>
<td>106.2*</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Feed intake, kg</td>
<td>163.8</td>
<td>173.5</td>
<td>183.9</td>
<td>179.0</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>3.560</td>
<td>3.442</td>
<td>3.447</td>
<td>3.443</td>
<td>0.079</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 8: Effects of dietary supplementation of iron sulfate and chelate in fattening pigs on the trace element concentrations (mg or μg/kg) of parts of pork meat

<table>
<thead>
<tr>
<th>Group</th>
<th>S200</th>
<th>S800</th>
<th>C200</th>
<th>C800</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe, mg/kg</td>
<td>2.4*</td>
<td>3.3*</td>
<td>4.3*</td>
<td>4.5*</td>
<td>0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Zn, mg/kg</td>
<td>2.9</td>
<td>2.8</td>
<td>2.7</td>
<td>3.3</td>
<td>0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Se, μg/kg</td>
<td>45.5</td>
<td>44.1</td>
<td>44.3</td>
<td>44.3</td>
<td>0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mn, μg/kg</td>
<td>55.2</td>
<td>53.9</td>
<td>54.8</td>
<td>51.3</td>
<td>4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mo, μg/kg</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
<td>0.9</td>
<td>0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>
1st field trial, with herbals in broilers, Katerini

- **Breed**: Ross 308
- **Animals**: 640 males
  - 5 groups/8 subgroups of 16 broilers each
- **Duration of trial**: 42 days
- **Diet**: corn/soybean based formulation
- **Bed floor type**: wood shaving
- **Anticoccidials**: No anticoccidial
- **Measurements**: zootechnical performance
- **5 treatments**:
  - T1: Neg. Control (Standard feed without eubiotic)
  - T2: control + product A 300 ppm
  - T3: control + product B 500 ppm
  - T4: control + product C 500 ppm
  - T5: control + product D 1000 ppm

### Basal diet

<table>
<thead>
<tr>
<th></th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>22.0</td>
<td>21.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Metabolisable energy, Kcal/kg</td>
<td>3000</td>
<td>3100</td>
<td>3200</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group T1 Control</th>
<th>Group T2</th>
<th>Group T3</th>
<th>Group T4</th>
<th>Group T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g d1</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>BW, g d14</td>
<td>430</td>
<td>429</td>
<td>427</td>
<td>437</td>
<td>435</td>
</tr>
<tr>
<td>BW, g d28</td>
<td>1347</td>
<td>1328</td>
<td>1271</td>
<td>1413</td>
<td>1393</td>
</tr>
<tr>
<td>BW, g d42</td>
<td>2474</td>
<td>2451</td>
<td>2536</td>
<td>2549</td>
<td>2540</td>
</tr>
<tr>
<td>FI per bird, kg</td>
<td>4312</td>
<td>4189</td>
<td>4295</td>
<td>4344</td>
<td>4321</td>
</tr>
<tr>
<td>FCR</td>
<td>1.775</td>
<td>1.741</td>
<td>1.724</td>
<td>1.732</td>
<td>1.734</td>
</tr>
</tbody>
</table>
2nd field trial with herbals in broilers, Arta

- **Breed**: Ross-308
- **Animals**: 400 as hatched chicks
  - 5 groups /4subgroups of 20 broilers each
- **Duration of trial**: 42 days
- **Diet**: corn/soybean based formulation
- **Bed floor type**: wood shaving
- **Anticoccidials**: +anticoccidial
- **Measurements**: zootechnical performance
- **5 treatments**:
  - T1: Neg. Control (Standard feed without herbals)
  - T2: control + herb product
  - T3: control + herb product
  - T4: control + herb product
  - T5: control + herb product

### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group T1 Control</th>
<th>Group T2</th>
<th>Group T3</th>
<th>Group T4</th>
<th>Group T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g d14</td>
<td>393</td>
<td>405</td>
<td>416</td>
<td>418</td>
<td>425</td>
</tr>
<tr>
<td>BW, g d28</td>
<td>1353</td>
<td>1418</td>
<td>1382</td>
<td>1422</td>
<td>1421</td>
</tr>
<tr>
<td>BW, g d42</td>
<td>2425</td>
<td>2557</td>
<td>2444</td>
<td>2437</td>
<td>2510</td>
</tr>
<tr>
<td>FI per bird, kg</td>
<td>4341</td>
<td>4326</td>
<td>4514</td>
<td>4383</td>
<td>4417</td>
</tr>
<tr>
<td>FCR</td>
<td>1.823</td>
<td>1.772</td>
<td>1.881</td>
<td>1.833</td>
<td>1.791</td>
</tr>
<tr>
<td>Mortality,%</td>
<td>3.6</td>
<td>2.5</td>
<td>2.2</td>
<td>2.5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

### Diets

<table>
<thead>
<tr>
<th></th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>22.0</td>
<td>21.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Metabolisable energy, Kcal/kg</td>
<td>3000</td>
<td>3100</td>
<td>3200</td>
</tr>
</tbody>
</table>
3rd field trial with herbal products in broilers, Arta

- **Animals:**
  - 240 day-old broiler chicks

- **Location:**
  - Arta (39°09; 20°59), Epirus, Greece

- **Experiment design:**
  - Randomly distribution of chicks into 5 treatments with 4 replicates (20 chicks per pen)

- **Experiment duration:**
  - 42 days

- **Treatments (groups):** 3
  1. Control
  2. Herb product (0.5 g/kg)
  3. Herb product (1.0 g/kg)

- **Drinking water and feed:**
  - *ad libitum*

- **Measurements:**
  - Performance parameters
  - Intestinal microbiota & morphology
  - Meat chemical composition

Total Phenolics, mg GAE/g dry mass

- Control feed: 0.48
- Feed with herb B: 10.31
- Feed with herb C: 38.22
Antioxidant effect of herbal feed additives

Values in the same row with no superscript in common differ at (P<0.05)
Microbial populations in the broiler jejunum and ceca (log CFU/ g) at day 42 of age

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jejunum</td>
<td>Control</td>
<td>Group B</td>
<td>Group C</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>6.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total Aerobs</td>
<td>6.85</td>
<td>7.25</td>
<td>6.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceca</td>
<td>Control</td>
<td>Group B</td>
<td>Group C</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>7.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total Aerobs</td>
<td>8.77</td>
<td>8.81</td>
<td>8.90</td>
<td></td>
</tr>
</tbody>
</table>
Intestinal morphometry

Group C group
Increased villous height

Group C group
Increased goblet cells
Cocci -Challenge trial with herbals

*Eimeria* mix in vivo

We did a trial with broilers challenged at a moderate level with $3.5 \times 10^4$ *E. acervulina*, $7.0 \times 10^3$ *E. maxima* and $5.0 \times 10^3$ *E. tenella* oocysts per bird at 14d (crop intubation).

Coccidia -challenge

*Challenge rooms in Unit of Avian Medicine, Clinic of Farm Animals, Aristotle University*
Eimeria mix in vivo ::: challenge trial

Diarrhoea scoring according to Youn and Noh (2001)
Lesion scoring according to Johnson and Reid (1970)
Results

**Body weight d 35**

- UU
- UI
- LI
- PS1
- PS3

**FCR d 35**

- UU
- UI
- LI
- PS1
- PS3

*a,b,c* significant differences (*p < 0.05*, ANOVA+Duncan test)
Oocyst shedding

- Oocysts in feces (× 1000/g)
- Age of birds (days)

- Uninfected - Untreated
- Infected - Untreated
- Infected - Lacalosid
- Infected - Coccihar 100
- Infected - Coccihar 200

Herbal products
- 100
- 200
Histology evaluation

Normal villae

Cocci affected villae
Herbals downregulated ileal IL-6 and tonsil LITAF, IFN-γ, TLR4 and IL-10 gene expression & exerted a significant anti-inflammatory effect.
Experimental trial in dairy cows, Komotini, Greece

- A total of 100 dairy cows (Holstein Friesian breed) were randomly selected for recent birth from a flock consisting of 600 dairy cows on Georgakoudi Bros dairy farm located in the area of Thrace, Greece.

- The Latitude and Longitude of Komotini is 41.12 and 25.40.

- Cows were allocated into 5 equal groups.
EFFECTS OF ESSENTIAL OILS ON MILK PRODUCTION, MILK COMPOSITION AND BLOOD TRAITS IN DAIRY COWS FED A TOTAL MIXED RATION UNDER HEAT STRESS

Ilias Giannenas¹, Nektarios Giadinis², Konstantinos Katsoulis³, Costantinos Georgakoudis⁴, Praful Kumar⁵, Maini Shivi⁶, George Kontopidis⁷, Ioannis Skoufos⁸

¹Laboratory of Nutrition, ²Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece ³Laboratory of Biochemistry, Faculty of Veterinary Science, University of Thessaly 43131, Karditsa Greece, ⁴Georgakoudi Bros S.A., Dairy Cow Farm, Komotini, Rodopi,

Keywords: dairy cows; plant extracts; milk production; milk composition

Milk production

Milk quantity, Lt

Weeks

Control
Herbal

Keywords: dairy cows; plant extracts; milk production; milk composition
Herbal EOs trial in dairy sheep
Effects of essential oils on ruminal pH, NH$_3$-N and microbial population of dairy ewes$^{1,2}$

$^{1,2}$TVB = Total viable bacteria, Cellul B = Cellulolytic bacteria, HAPB = Hyper Ammonia producing bacteria
Herbals: Synergistic effect

- Refers to an increased intensity caused by the combination of two or more substances

Antimicrobial / Antioxidant
Algae

- There are about 320,000 species of algae
  - Edible algae can find uses in animal nutrition
- Microalgae
  - *Spirulina platensis*
- Macroalgae
  - *Ascophyllum nodosum*
Field trial with spirulina in broilers

- **Animals**: 120 chicks, day-old, as hatched
- **3 treatments**:
  - Control: commercial control diets
  - Spir-5: commercial diets + spirulina at 5 g/kg
  - Spir-10: commercial diets + spirulina at 10 g/kg
- **Experiment duration**: 42 days
- **Diet**: maize and soybean meal based formulation

**Drinking water and feed**: *ad libitum*

**Evaluated parameters**:
- Body Weight
- Feed intake and FCR
- Mortality
- Breast and thigh meat lipid oxidation (TBARS) after refrigerated storage at 4°C

### Table 2 Effect of dietary spirulina on broiler performance parameters and lipid oxidation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Spirulina: 5 g/kg</th>
<th>Spirulina: 10 g/kg</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight at 21 d (kg)</td>
<td>0.774</td>
<td>0.733</td>
<td>0.740</td>
<td>0.013</td>
<td>N.S.</td>
</tr>
<tr>
<td>Final live weight at 42 d (kg)</td>
<td>2.458</td>
<td>2.328</td>
<td>2.381</td>
<td>0.047</td>
<td>N.S.</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>2.070</td>
<td>2.132</td>
<td>2.103</td>
<td>0.023</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>0.833</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

**Breast muscle**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Spirulina: 5 g/kg</th>
<th>Spirulina: 10 g/kg</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 d refrigeration</td>
<td>0.148</td>
<td>0.140</td>
<td>0.113</td>
<td>0.016</td>
<td>N.S.</td>
</tr>
<tr>
<td>5 d refrigeration</td>
<td>0.285</td>
<td>0.433</td>
<td>0.334</td>
<td>0.048</td>
<td>N.S.</td>
</tr>
<tr>
<td>Increase between 2 d and 5 d</td>
<td>0.137</td>
<td>0.311</td>
<td>0.221</td>
<td>0.042</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Conclusions

• There is experimental evidence that specific feed additives can be effectively used in animal nutrition, aiming to:

  • Increase growth performance
  • Support gut health
  • Improve product quality (meat, milk, egg)
  • Increase sustainability
Future research

• Additional research is needed to examine:

  • Mechanisms of action for promising compounds & bioavailability and pharmacokinetics of feed additives in healthy and challenged animals

  • Possible beneficial or detrimental interactions:
    • Among different feed additives
    • Among feed additives and feed components
    • .......in the absence of chemotherapeutics
Thank you for your attention!