Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

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Introduction: antibiotic alternatives

- Avian coccidiosis with *Emeria* spp
- Necrotic enteritis
- With AGP withdrawal, there is an urgent need to develop antibiotic alternatives.

Gallucci and Matzinger, 2001; Shirley and Lillehoj, 2012
**Introduction: antibiotic alternatives**

Bacillus subtilis as probiotics in chicken feed
- Shows growth-promoting effect (Gadde et al. 2017)
- Shows a protective role against chicken pathogen (Park et al. 2019)
What is the mechanisms of dietary *Bacillus subtilis* supplementation?

**Introduction: gut microbiota and metabolites**

- **Nutrients**
- **Pathogens**

**Metabolites**

**PAMPs**

**Growth**

**Immunity**

**Anti-stress**

**Anti-oxidant**

“Postbiotics” novel materials promoting gut health as functional additive of diet
Therefore, the current study was undertaken to characterize the metabolic alterations in the chicken gut following dietary supplementation with *B. subtilis* DFMs with the goal of identifying potential chemical compounds that might be directly used to improve poultry growth performance without the use of AGPs.
Materials and Methods

84 male day-old Ross 708 broilers

- Initial body weight
- Allocation to 3 treatments

ileal content from euthanized chicken (2 chickens/pen = total 8 chickens/treatment)

Measurements:
- Metabolomic profiling of the ileal contents by mass spectrometry (Metabolon, Durham, NC)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Supplements</th>
<th>Dose</th>
<th>Chickens/cage</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>Basal diet</td>
<td></td>
<td>7</td>
<td>4</td>
</tr>
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<td>B. subtilis 1781</td>
<td>CON+B. subtilis 1781</td>
<td>1.5 × 10^5 CFU/g feed</td>
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</tbody>
</table>

- B. subtilis strains were obtained from Church & Dwight Co. Inc. (Waukesha, WI).
Materials and Methods

• Statistical Analysis
  • Growth performance
    - PROC MIXED in SAS (SAS Inst. Inc., Cary NC)
    - \( P \) values < 0.05
    - PDIFF option
  • Ileal biochemicals
    - Array Studio software (OmicSoft, Cary, NC)
    - the programs R (R Foundation for Statistical Computing, Vienna, Austria)
    - JMP (SAS Institute)
    - \( P \) values < 0.05
    - Random Forest Analysis (RFA) by computing the Mean Decrease Accuracy
Result: growth effect and biochemical distribution

Body weight gain of chickens

Body weight gain = final body weight – initial body weight

Principal component analysis of ileal biochemicals
Result: hierarchical clustering heatmap

- Total 674 biochemicals

- Con vs *B. subtilis* 1781
  - Increased 209 (25; \( P < 0.05 \))
  - Decreased 461 (58; \( P < 0.05 \))

- Con vs *B. subtilis* 747
  - Increased 265 (12; \( P < 0.05 \))
  - Decreased 402 (38; \( P < 0.05 \))
Result: pathways in this study

- 193 amino acid pathways
- 32 carbohydrate pathways
- 263 lipid pathways
- 42 cofactors and vitamins pathways
- 51 nucleotide pathways
- 12 energy pathways
- 81 unknown pathways
- 498 Human Metabolome Database (HMDB)
- 336 Kyoto Encyclopedia of Genes and Genomes (KEGG) codes
CON vs B. subtilis 1781

**Result: random forest analysis and plot**

- **8 amino acids (26.7%)**
  - 2-hydroxy-4-(methylthio)butanoic acid
  - 3-(4-hydroxyphenyl)lactate
  - beta-hydroxycholate
  - creatine
  - cysteine S-sulfate
  - N-methylproline
  - glutamate gamma-methyl ester
  - dimethylglycine

- **8 lipids (26.7%)**
  - 2,4-dihydroxybutyrate
  - glycerophosphoethanolamine
  - 2R 3R-dihydroxybutyrate
  - chenodeoxycholate
  - 16-hydroxypalmitate
  - beta-sitosterol
  - 1-palmitoyl-digalactosylglycerol (16:0)
  - octadecenedioate

- **5 vitamins and cofactors (16.7%)**
  - carotene diol
  - alpha-tocopherol acetate
  - beta-cryptoxanthin
  - nicotinamide adenine dinucleotide (NAD+)
  - alpha-tocopherol

- **3 nucleotides (10.0%)**
  - 2'-deoxyguanosine
  - N6-methyladenosine
  - N1-methyladenosine
Result: random forest analysis and plot

**CON vs B. subtilis 747**

- **6 amino acids (20.0%)**
  - glutamate gamma-methyl ester
  - betaine
  - taurine
  - dimethylglycine
  - 5-hydroxyindoleacetate
  - methylsuccinate

- **6 peptides (20.0%)**
  - glutaminyleucine
  - alanylleucine
  - valylleucine
  - leucylglycine
  - leucylalanine
  - valylglycine

- **10 lipids (33.0%)**
  - 6-oxolithocholate
  - oleoylcholine
  - glycerophosphoglycerol
  - sebacate (C10-DC)
  - 2-hydroxyglutarate
  - palmitoylcholine
  - linoleoylcholine
  - 3-dehydrodeoxycholate
  - chenodeoxycholate
  - stearoylcarnitine (C18)

- **2 nucleotides (0.1%)**
  - uridine 5'-monophosphate (UMP)
  - guanine
Result: amino acids (dipeptides)

Alanylleucine

Glutaminyleucine

Glycylisoleucine

Valylleucine

Phenylalanylalanine

Valylglutamine
Result: lipids

Miyamoto et al., 2015
Result: nucleotides

- N1-methyladenosine
- N6-methyladenosine
- Guanine
- 2-deoxyguanosine
- Uridine 5-monophosphate
- Cytidine
### Result: others

- **Fructose feeding negatively affects antioxidant capacity in the blood of hypertensive rats but improves this capacity in the liver (Girard et al., 2006, Nutrition).**

- **Lactate ion may prevent lipid peroxidation by scavenging free radicals such as O$_2^-$ and -OH but not lipid radicals (Groussard et al., 2000, J Appl Physiol).**
Conclusions

• Dietary supplementation with *B. subtilis* has profound effects on the levels of a wide variety of chemical metabolites in the chicken gut.

• These altered metabolite levels provide a biochemical signature unique to each *B. subtilis* supplementation group.

• Future studies are warranted to assess the growth promoting properties, if any, of the identified chemical compounds in lieu of antibiotics.
Further study: extra data

40 metabolites of
Up-regulated common metabolites between *B. subtilis* 1781 and 747

Chicken epithelial cells (8E11)

Chicken macrophage cells (HD11)

**Proinflammatory responses**

**N-acetylglucosamine**

**Syringic acid**

**2'-deoxyadenosine**

**β-alanine**
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