Abstract

Our previous studies have shown that supplementation of low-dose antibiotic growth promoter (AGP) exacerbated growth performance and systemic inflammation of weaned pigs infected with pathogenic *Escherichia coli* (*E. coli*). The objective of this experiment, which is extension of our previous report, was to investigate the effect of low-dose AGP on the serum metabolomic profiles of weaned pigs experimentally infected with F18 *E. coli*. Thirty-four pigs (6.88 ± 1.03 kg BW) were individually housed in disease containment rooms and randomly allotted to one of three treatments with 11-12 replicate pigs per treatment. The three dietary treatments were control diet (control) and 2 additional diets supplemented with 0.5 or 50 mg/kg of AGP (carbadox), respectively. The experiment lasted 18 d [7 d before and 11 d after first inoculation (d 0)]. The F18 *E. coli* inoculum was orally provided to all pigs with the dose of 10^{10} cfu/3 mL for 3 consecutive days. Blood samples were collected on d 0 before *E. coli* inoculation and on d 5 and 11 post-inoculation (PI). Serum metabolomics were analyzed by gas chromatography time of flight-mass spectrometer (GCTOF-MS). All processed data were statistically analyzed and evaluated by online MetaboAnalyst tool. No significant differences were observed in the serum metabolites between control and low-dose AGP throughout the experiment. However, further metabolic pathway enrichment analysis showed that the low-dose AGP modified pentose phosphate pathway, DNA synthesis in T and B lymphocytes, pyruvate metabolism and amino acid metabolism compared to high-dose AGP. In conclusion, modification of serum metabolites levels and metabolic pathways by supplementing low-dose AGP may have relevance for pathogenesis, disease activity and clinical manifestations of weaned pigs infected with F18 *E. coli*. These findings may provide a comprehensive understanding of our previous results.
Antibiotic resistance is one of the biggest health concerns that lead to tremendous economical losses and increased mortality of both humans and livestock (WHO, 2018).

Low-dose antibiotics application enhanced bacterial selection for antibiotic resistance genes (Davies et al., 2006).

Supplementation of low-dose antibiotic growth promoter (AGP) exacerbated growth performance and systemic inflammation of weaned pigs infected with pathogenic *E. coli* (Kim et al., 2019a,b).
Effects of antibiotics on serum metabolomic profiles in weanling pigs experimentally infected with a pathogenic *E. coli*

Kwangwook Kim¹, Yijie He¹, Cynthia Jinno¹, Seijoo Yang¹, Minho Song², Peng Ji¹, Yanhong Liu¹
¹University of California, Davis, CA, ²Chungnam National University, Daejeon, Republic of Korea

**Abstract**

**Objective & Methods**

Investigate the effect of low-dose AGP on the serum metabolomic profiles of weaned pigs experimentally infected with F18 *E. coli*.

- **Experimental design**: RCBD (Blocks: BW x Sex)
- **34 weanling pigs (6.88 ± 1.03 kg, 21 d old)**
- **Treatments**: 3 dietary treatments (11-12 pigs/treatment)

**E. coli** challenge

- Nursery basal diet as control (CON)
- CON + 0.5 mg/kg of AGP (Low dose)
- CON + 50 mg/kg of AGP (High dose)

**E. coli** challenge

- Weaning
- E. coli challenge
- Peak infection period
- Recovery period

**Results**

- Serum samples were collected on d 0, 5, and 11 PI.
- Serum metabolomics were analyzed by gas chromatography time of flight-mass spectrometer (GCTOF-MS).
- All processed data were analyzed by MetaboAnalyst (http://www.metaboanalyst.ca) (Chong et al., 2018).
  - Statistical analysis
    - VIP (variable importance projection) > 1
    - Fold change > 2
    - FDR (adjusted P-value) < 0.1
  - Enrichment & pathway analysis
    - *P* < 0.05

*PI= post-inoculation
Effects of antibiotics on serum metabolomic profiles in weanling pigs experimentally infected with a pathogenic *E. coli*

Kwangwook Kim¹, Yijie He¹, Cynthia Jinno¹, Seijoo Yang¹, Minho Song², Peng Ji¹, Yanhong Liu¹
¹University of California, Davis, CA, ²Chungnam National University, Daejeon, Republic of Korea

**Results**

**Synchronized 3D plots:**
Partial least squares – discriminant analysis (PLSDA), d 5 PI

**Heatmap and hierarchical clustering, d 5 PI**

**Metabolite sets enrichment, d 5 PI**

**CON**

**LOW**

**HIGH**

- Serine
- Cystine
- Creatine
- Asparagine
- Glycolic acid
- Pinitol
- Mannonic acid
- Xylitol
- Guanine
- 2,5-dihydroxypyrazine
- Pantothenic acid
- Arabinol
- Glycerol-alpha-phosphate
- Galactonic acid
- Glucose-1-phosphate
- Myo-inositol
- Hypoxanthine
- Propylene glycol
- Arachidic acid
- 6-deoxyglucose

**PLS-DA VIP**

**Fold enrichment**

- METABOLITES AFFECTED BY OXIDATIVE STRESS
- CRITICAL ILLNESS (MAJOR TRAUMA, SEVERE CARDIOGENIC SHOCK)
- EARLY MARKERS OF MYOCARDIAL INJURY
- POST TRANSURETHRAL PROSTATIC RESECTION
- LEIGH’S SYNDROME
- JUVENILE MYOCLONIC EPILEPSY
- PYRUVATE DEHYDROGENASE E3-BINDING PROTEIN DEFICIENCY
- ACUTE SEIZURES
- RIBOSE-5-PHOSPHATE ISOMERASE DEFICIENCY
- SCHIZOPHRENIA
- PYRUVATE DEHYDROGENASE DEFICIENCY (E3)
- HYPERBARIC OXYGEN EXPOSURE
- PYRUVATE CARBOXYLASE DEFICIENCY
- L-ARGININE/GLYCINE AMINOTRANSFERASE DEFICIENCY
- CREATINE DEFICIENCY, GUANIDINOACETATE
Effects of antibiotics on serum metabolomic profiles in weanling pigs experimentally infected with a pathogenic *E. coli*

Kwangwook Kim¹, Yijie He¹, Cynthia Jinno¹, Seijoo Yang¹, Minho Song², Peng Ji¹, Yanhong Liu¹
¹University of California, Davis, CA, ²Chungnam National University, Daejeon, Republic of Korea

**Abstract**

Results

- Supplementation of low-dose AGP modified serum metabolites and associated metabolic pathways of weaned pigs infected with F18 *E. coli* during the peak infection period.

Conclusions

- The most affected metabolic pathways include nucleic acid synthesis and major nutrient metabolism, which may account for the lower growth rate and worse diarrhea of pigs fed low-dose AGP.

**Results**

- *Most affected metabolic pathways*
  - Supplementation of low-dose AGP modified
    - Pentose phosphate pathway (RNA synthesis)
    - DNA synthesis in lymphocytes
    - Pyruvate metabolism
    - Amino acid metabolism
    - Galactose metabolism
    - Glycolysis/gluconeogenesis
  - compared with high-dose AGP on d 5 PI.

- No differences were observed in serum metabolites among dietary treatments on d 0 and 11 PI.

**References**

- Chong, J., O. Sougan, C. Li, L. Caraus, S. Li, G. Bourque, D.S. Wishart, and J. Xia. Metabo Analyst 4.0: towards more transparent and integrative metabolomics analysis, Nucleic Acids Research, 2018;46(W1):W486-494