Efficacy of *Bacillus subtilis* based probiotic growth performance, fecal microbiota and intestinal morphology of broiler chickens

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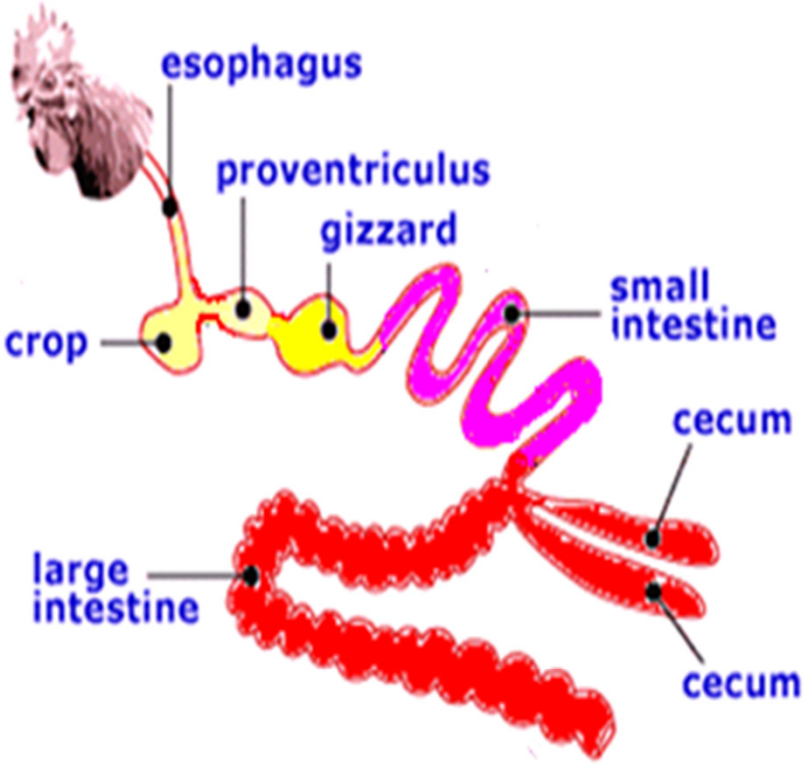
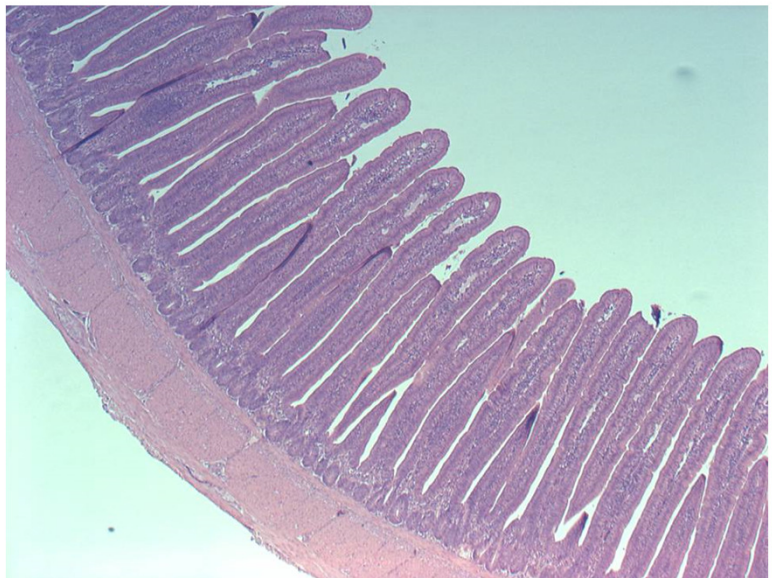
Outline

- Importance of probiotic in digestive trace
- What makes *Bacillus subtilis* different that other microbes
- Materials and methods
- Results and discussion
- Conclusions

Significance of gut

- Barrier to ingested chemicals, feed contaminants and pathogens
- Absorption of nutrients and water
- Components: physical, chemical, immunological and microbiological
- Intestine is the largest immune organ
- Gut microbes perform functions such as:
    - nutrient digestion and absorption
    - gut health and integrity
    - competitive exclusion of pathogens
    - immunomodulation





Development of the microflora

The GIT is sterile

Lactobacilli and *Enterococci* dominate the crop, duodenum and ileum & *Coliforms, Lactobacilli* and *Enterococci* dominate the caeca

Lactobacilli dominate the crop, duodenum and ileum anaerobic bacteria dominate the caeca

The microbial community starts to stabilize indicated by the stable bacterial fermentation

Unstable micro flora

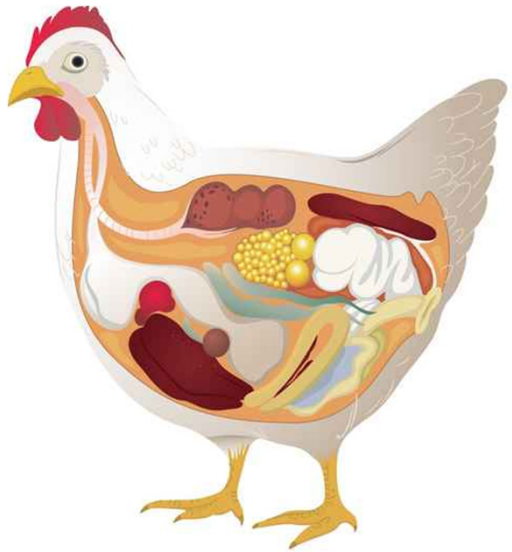
Hatch | 1 week | 2 weeks | 3 weeks

Microbes in GIT

Under commercial rearing conditions birds are exposed to more stressful situations like transportation, vaccination, high flock density, and heat stress.

(Beneficial bacteria) **Imbalance** **(Pathogenic bacteria)**

Lactobacillus sp, *Bifidobacteria*, etc…	*E.coli, Listeria* sp, *Salmonella*, etc…



Definition of probiotics

"Live microorganisms which when administered in adequate amounts confer a health benefit on the host“

FAO/WHO, 2002

The most frequently used organisms for probiotic preparations are:

- *Bacillus*
- Lactic acid bacteria (LAB)
- Live yeast

Probiotics… Mode of Action?

1. Competitive exclusion of pathogens

2. Improved immune status: production of antibacterial defensins and mucin

3. Maintain epithelial integrity and barrier function

4. Nutritional effects:
   - Production of enzymes
   - Production of vitamin B_{12} and K

O'Toole and Cooney (2008)

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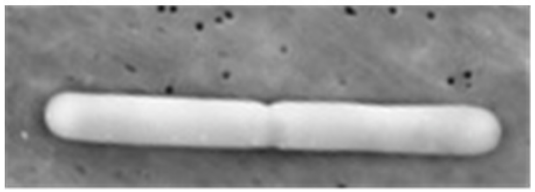
Bacillus

- Definition of *Bacillus*
  - Gram positive organisms
  - Spore formers

- *Bacillus* are found in environmental samples obtained from virtually anywhere on Earth (soil, plants, fresh and salt water, rocks etc.).

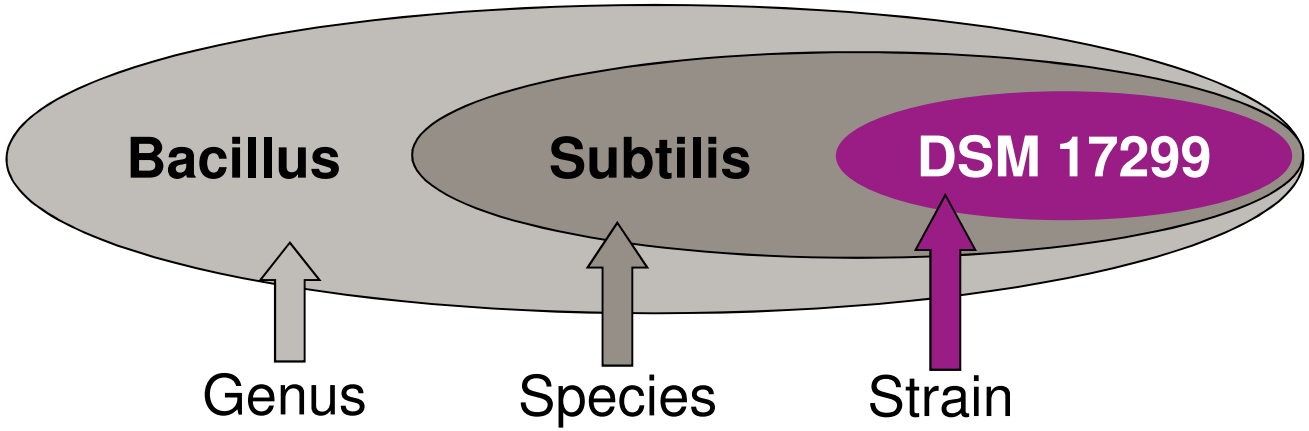
- *Bacillus* are administered in the form of spores, a dormant resistant stage which transform to vegetative cells when entering the intestinal tract of the animal.

- *Bacillus* spores are
  - pH stable
  - Trypsin stable
  - Thermo-tolerant
  - Storage and in-feed stable



Strain selection

Spores of a carefully selected *Bacillus subtilis* with the following major characteristics



- Heat tolerance
- pH stability, even at pH 2-3
- Compatibility with other feed ingredients including organic acids, coccidiostats and antibiotic growth promoters
- Approved by the European Food Safety Authority (EFSA) for application in feed

Rationale for the experiment

- Under commercial feeding conditions birds are exposed to a variety of stress factors which make them more susceptible to gastrointestinal and metabolic disturbances.

- The inclusion of sub-therapeutic antibiotic growth promoters (AGPs) into poultry diets is common to overcome these gastrointestinal challenges. However, as the use of AGPs is being more and more limited and/or banned throughout the world, there is continuous search to identify alternative strategies

- Probiotics, prebiotics, synbiotics, organic acids and phytogenic feed additives could be such alternatives with probiotics being one of the most favorable.

- A *Bacillus subtilis* (DSM 17299) spore-forming probiotic seem to be most suitable candidates for in-feed applications because of their spore forming ability. These spores are tolerant to heat, harsh pH conditions, pressure, coccidiostats and antibiotics..

Objective

- To evaluate the effects of feeding *Bacillus subtilis* (DSM 17299) or antibiotic growth promoters (AGP) alone or in combination on growth performance, fecal microbiota and gut morphology of broiler chickens.

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Experimental Design

- **Birds:** 480 Cobb male broilers
- **Housing:** Open house-Floor pen
- **Growth phases :** 0-21 d (starter), and 22-42 d (grower)
- **Duration of experiment:**0-42 days of age
- **Feed form:** Mash
- **Temperature and humidity:** 26-34^{o}C and 82-93%
- **Diets of each growth phase:**
    - Diet 1: Control
    - Diet 2: Control + AGP
    - Diet 3: Control + Bacillus subtilis (500 g/ton)
    - Diet 4: Control + AGP* + Bacillus subtilis (500 g/ton)

*AGP = a combination of oxytetracyclin and neomycin at 100 ppm (w/w)

- **Replication**: 6 Replicates/treatment (20 birds/replicate)
- **Parameters:** Growth performance, Fecal microbial counts [Lactic acid bacteria (LAB) and *Enterobacteriaceae* (ENT)] and Intestinal morphology

Ingredient and nutrient composition of basal diets

Ingredients	Dietary	
	Starter	Finisher
Corn	506.00	549.00
Soybean	293.82	269.00
Wheat Pollard	60.72	63.75
CPO	36.00	32.00
Fish Meal 55%	76.00	50.00
L-Lysine	2.50	2.50
DL-Methionine	2.00	2.00
Monodicalciumphosphate 21	10.00	14.00
Calcium carbonate	6.80	9.92
Choline chloride	0.60	0.58
Salt	2.50	2.50
Other (Minerals, Vitamin and toxin binder)	3.05	3.05
Calculated analysis:		
Crude protein, %	22.50	20.34
ME, MJ/kg	12.22	12.18

Measurements and analyses

- Body weight and feed intake on weekly basis and FCR was calculated accordingly.

- A total of 18 samples from each treatment in triplicates were determined for the faecal LAB and ENT population using the method as described by Foo et al. (2003b).

- A total of 20 samples from each treatment were used to study the intestinal morphology. The procedure was a modified method as described by Hair-Bejo (1990).
  - Segments of 5 to 6 cm long were removed from the duodenum, jejunum, and ileum collected and flushed with 10% neutral buffered formalin solution and were then used for morphometric analysis.
  - The morphometric variables examined included: villus height (from the tip of the villi to the villi crypt junction), crypt depth (defined as the depth of the invagination between adjacent villi). Values are means from the best 20 villi and only vertically oriented villi and crypts from each slide were measured.

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Results and discussion: growth performance

	Control (C)	C+AGP	C + *Bacillus subtilis* DSM 17299	C+AGP + *Bacillus subtilis* DSM 17299	SEM
BWG, kg	2.09	2.13	2.15	2.18	0.01
FI, kg	3.65^{a}	3.66^{a}	3.55^{b}	3.60^{a}	0.05
FCR, kg/kg	1.75^{a}	1.73^{ab}	1.66^{b}	1.65^{b}	0.02

*BWG = Body weight gain, FI = Feed intake and FCR = Feed conversion ratio

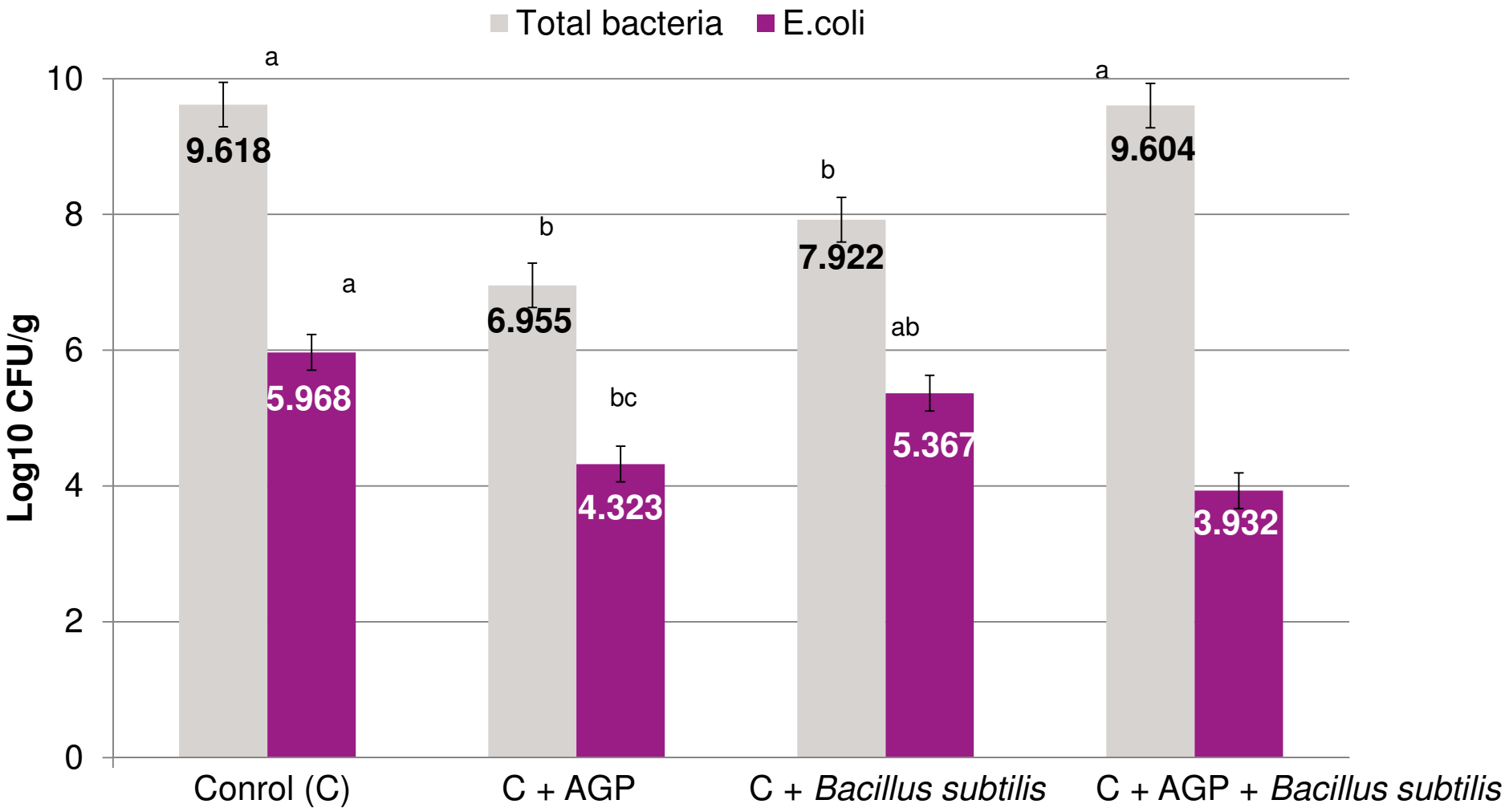
$^{a\text{-}b}$ Values with different superscript letters within a row indicate significant differences ($P < 0.05$).

Results and discussion: fecal microbiology

Microbial count, log_{10}CFU/g	Control (C)	C+AGP	C+ *Bacillus subtilis* DSM 17299	C+AGP + *Bacillus subtilis* DSM 17299	SEM
LAB	6.15^{b}	6.42^{b}	6.89^{a}	6.98^{a}	0.09
ENT	5.73^{a}	5.40^{ab}	4.64^{c}	5.21^{b}	0.08

$^{a-c}$ Means within a row with common superscripts are not significantly different (P > 0.05); LAB=lactic acid bacteria; ENT=*Enterobacteriaceae.*

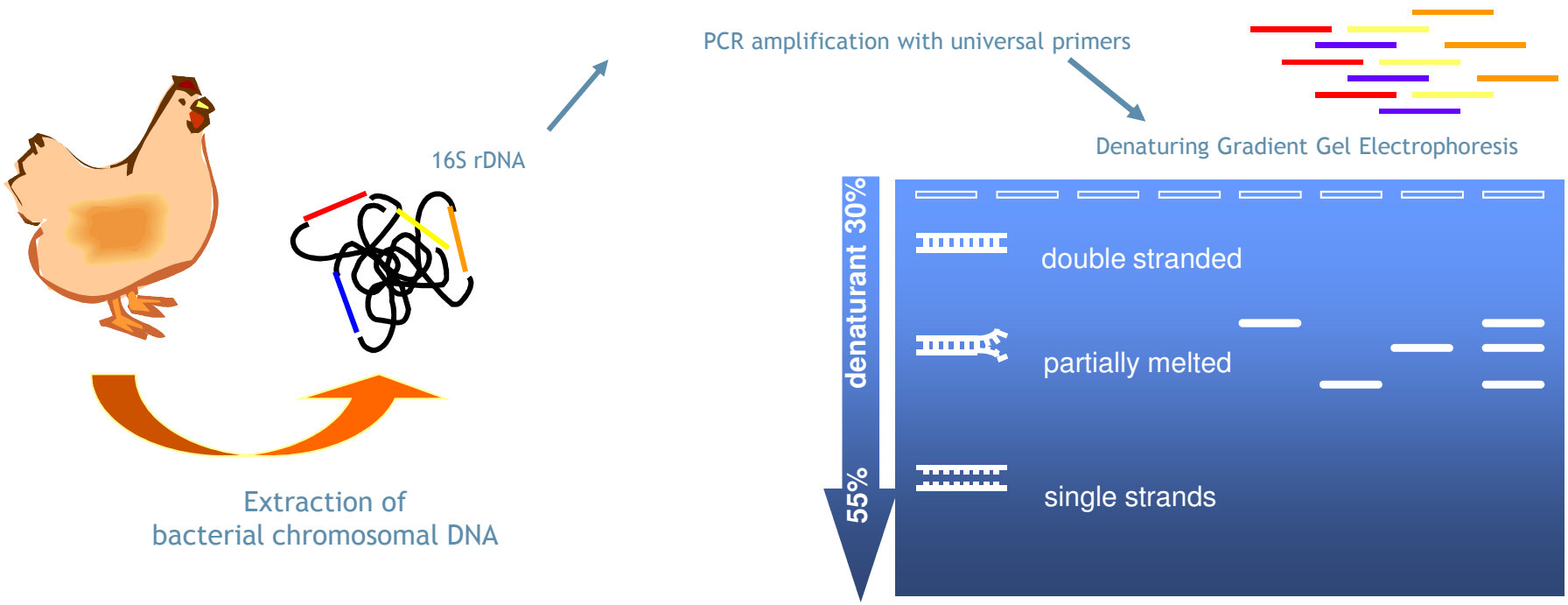
Ileal total bacteria and *E.coli* counts



(Doranalli et al., 2013)

Digestive tract micro flora analysis

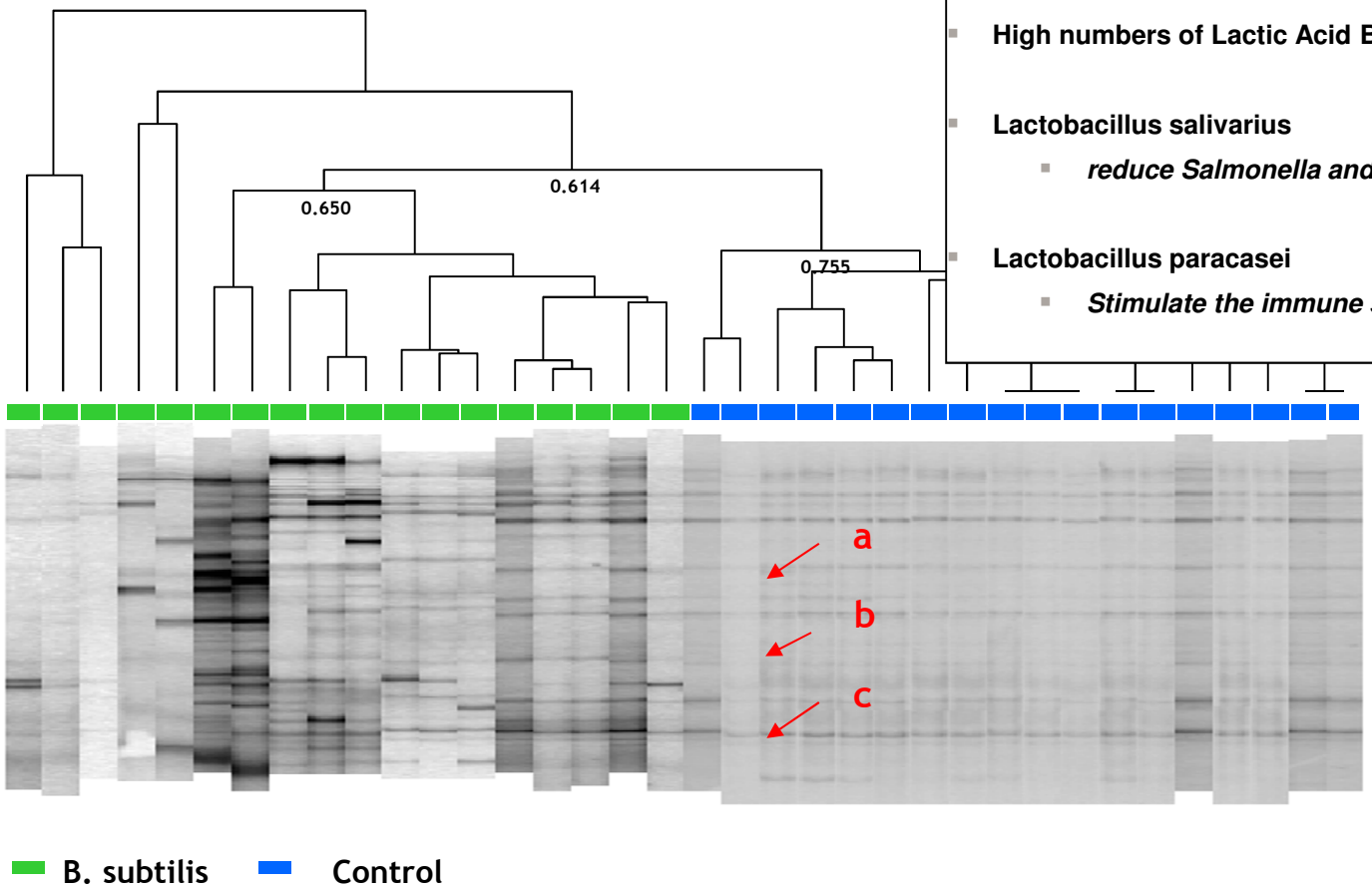
- A total of 3000 day-old male chicks (Ross 308) were housed in 12 pens (250 birds/pen)
- Two dietary treatments: corn-soy based standard feed **-/+ *Bacillus subtilis* DSM 17299**
- At age 35 days of age, 3 birds were randomly selected per pen unit for gut excision and collection of ileal digesta for determination of micro flora profile



Knarreborg et al., 2008 International journal of Probiotics and Prebiotics 3 2 83-88

Increases beneficial bacteria in the intestine

Dice coefficient analysis of the ileal profiles from control birds and birds fed *Bacillus subtilis* DSM 17299



- More diverse and complex bacteria composition in the gut
- High numbers of Lactic Acid Bacteria
- Lactobacillus salivarius
  - *reduce Salmonella and E. coli*
- Lactobacillus paracasei
  - *Stimulate the immune system*

a: ***Lactobacillus salivarius***

b: ***Streptococcus spp.***

c: ***Lactobacillus paracasei***

Knarreborg et al., 2008 International journal of Probiotics and Prebiotics 3 2 83-88

Results and discussion: Intestinal morphology

	Control (C)	C+AGP	C+Bacillus subtilis DSM 17299	C+AGP+Bacillus subtilis DSM 17299	SEM
Villi height, µm					
Duodenum	1682.47^{c}	1756.38^{b}	1752.00^{b}	1871.62^{a}	9.70
Jejunum	1183.38^{c}	1200.43^{c}	1407.74^{a}	1310.50^{b}	9.00
Ileum	738.83^{b}	676.96^{c}	872.35^{a}	843.80^{a}	7.00

$^{a-c}$ Means within a row with common superscripts are not significantly different (P > 0.05); VH=villus height

Conclusions

- It can be concluded that inclusion of *Bacillus subtilis* (DSM 17299) to broiler diets improved growth performance and small intestinal morphology.
- Synergistic effects were observed in combination of *Bacillus subtilis* and AGP for some response variables.
- In addition, *Bacillus subtilis* supplementation showed its effects on modulating microbial populations, which was evidenced by increased amounts of LAB, mostly accounting for beneficial gut microbiota and reduced counts of *Enterobacteriaceae*, the latter group containing gut pathogenic bacteria like *E. coli* and *Salmonella*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