Aviguard / Competitive exclusion product / Caecal microbiota

Lallemand Animal Nutrition

Content

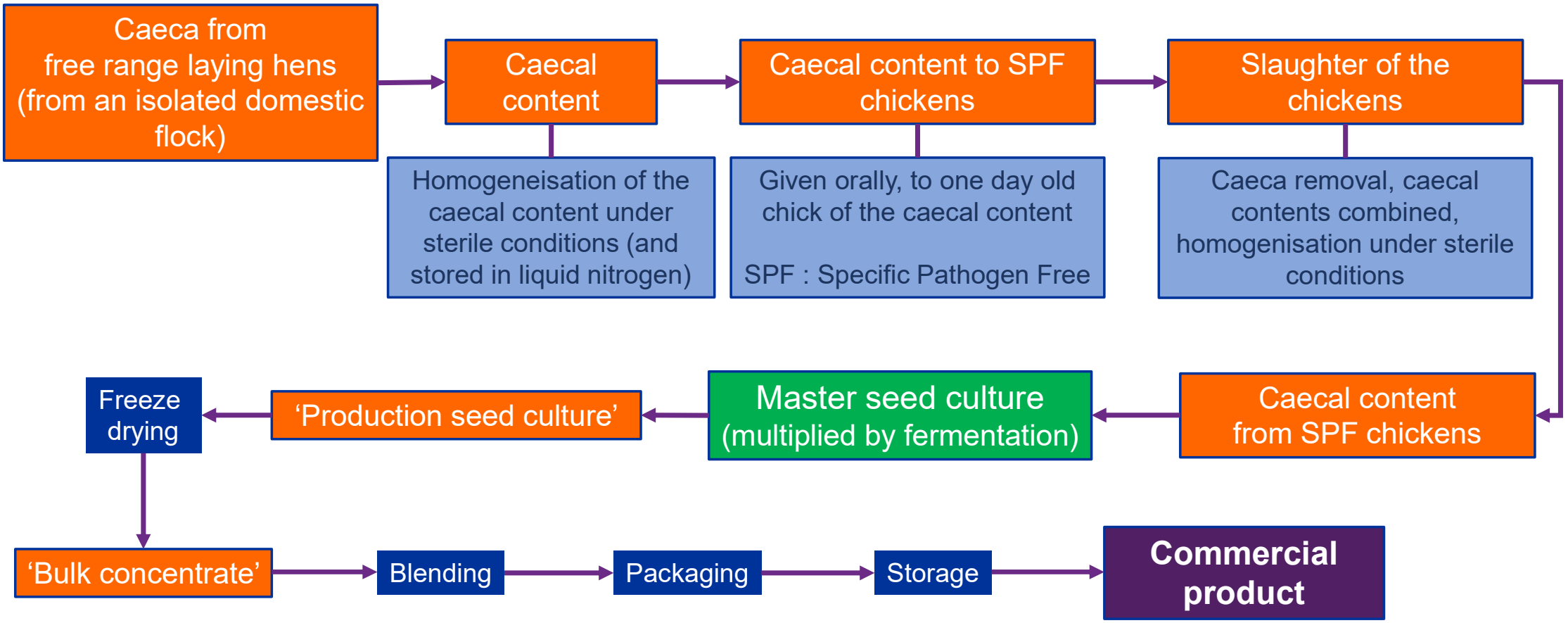
Part I. Aviguard

i. History of the product

History of the product

- 1982: foundation of Microbial Developments Ltd (MDL) (Malvern, UK)
- **1992: Aviguard created**
- 1995: MDL acquired by Bayer AG (until 2003, it was a part of the Animal Health Division)
- 2003: MDL independent once again
- 2012: MDL acquired by Lallemand
  - Silage additives, zootechnical additives and Aviguard
  - Production of a range of complementary feeds and other microbial products

1992… Birth of Aviguard and its industrial production



Part I. Aviguard

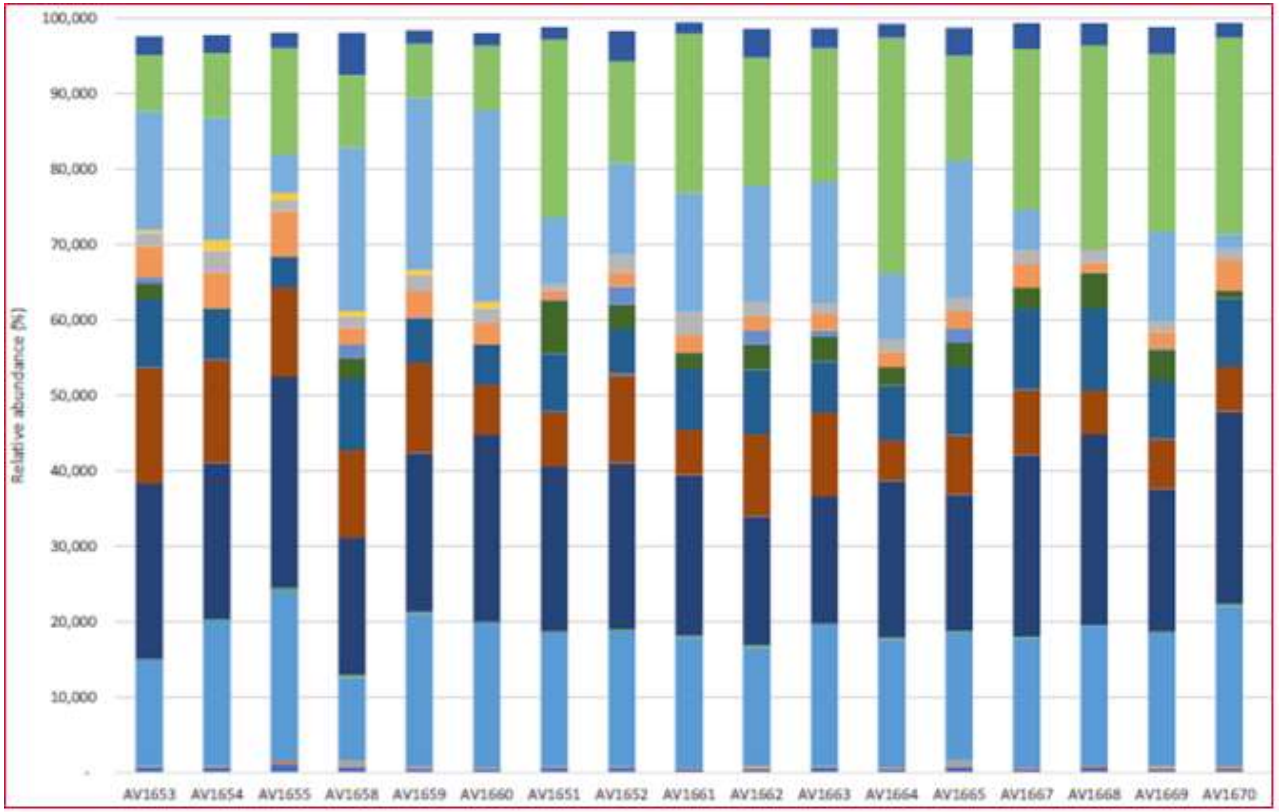
ii. Product composition

Aviguard, a caecal microbiota complex

- Bacteria from five main phyla typically present in poultry microbiota are identified:
  - Actinobacteria
  - Bacteroidota
  - Firmicutes
  - Fusobacteriota
  - Proteobacteria

- Batch-to-batch variation of batches from one seed culture (16s sequencing)

- Several independent studies have reported the composition of Aviguard using amplicon sequencing techniques (for example, Meijerink et al. 2020)



Meijerink N, Kers JG, Velkers FC, van Haarlem DA, Lamot DM, de Oliveira JE, Smidt H, Stegeman JA, Rutten VPMG and Jansen CA (2020) Early Life Inoculation With Adult-Derived Microbiota Accelerates Maturation of Intestinal Microbiota and Enhances NK Cell Activation in Broiler Chickens. Front. Vet. Sci. 7:584561. doi: 10.3389/fvets.2020.584561

Product specifications

- Absence of pathogens
  - Same approach as in the case of human faecal transplants.

- Potency test
  - Based on a world-wide standard for the evaluation of the activity against *Salmonella* colonization (Mead et al. 1989, Journal of Food Protection 52:500-502).
  - Efficacy/potency is not affected by batch-to-batch variability which reflects the gut microbiota dynamic.

Part I. Aviguard

iii. Target species, application and efficacy

Target species

- Poultry
  - Broilers
  - Laying hens/Breeding hens
  - Turkeys
  - Guinea fowl, quails, pigeons, pheasants, partridge, ostrich, ducks etc

Application (main use)

- Spray on day old chicks at hatcheries/in the farm
  - When chicks are day old and in a confined space i.e. in their delivery boxes or in cages.
  - Action relies on chicks preening each other and pecking the Aviguard solution off each other and ingesting it.

Efficacy / benefits

- (re)Establishment of normal/mature healthy gut flora in day-old chicks or adult poultry
- It helps maintain poultry healthy and improve their resilience against opportunistic bacteria (such as *Salmonella spp.*, *E. coli* and/or, *Clostridium perfringens* or *Enterococcus)*
- Healthy animals require less medicinal treatments such as with antibiotics which helps reduce risk of AMR
- Veterinarians recommend using Aviguard after prescribing therapeutic antibiotics to restore gut flora

Gut microbiota establishment and maturation: a natural process

- Early on set of the microbiota is crucial for both maturation of the immune system and colonization resistance against enteric pathogens
  - ➔ This happens in nature through the contact of the chicks with the hens
- In modern poultry farming, random and delayed colonization of the digestive microbiota delays the installation of a mature gut microbiota and consequently their immune repertoire. Hence, animals are more sensitive to intestinal inflammation which is favorable to the development of opportunistic bacteria (e.g. facultative anaerobic bacteria such as Salmonella)

➔ Caecal microbiota products mirror the contact with the hens and accelerate the implantation of normal gut microbiota

Kaspers et al. 2015. DEVELOPMENT OF THE GUT ASSOCIATED IMMUNE SYSTEM. 20th European Symposium on Poultry Nutrition | 24–27 August 2015 | Prague, Czech Republic.

Kubasova et al. (2019) Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLOS One 14(3):e0212446 DOI:10.1371/journal.pone.0212446

Meijerink et al. (2020) Early Life Inoculation With Adult-Derived Microbiota Accelerates Maturation of Intestinal Microbiota and Enhances NK Cell Activation in Broiler Chickens. Front. Vet. Sci. 19:7:584561 https://doi.org/10.3389/fvets.2020.584561

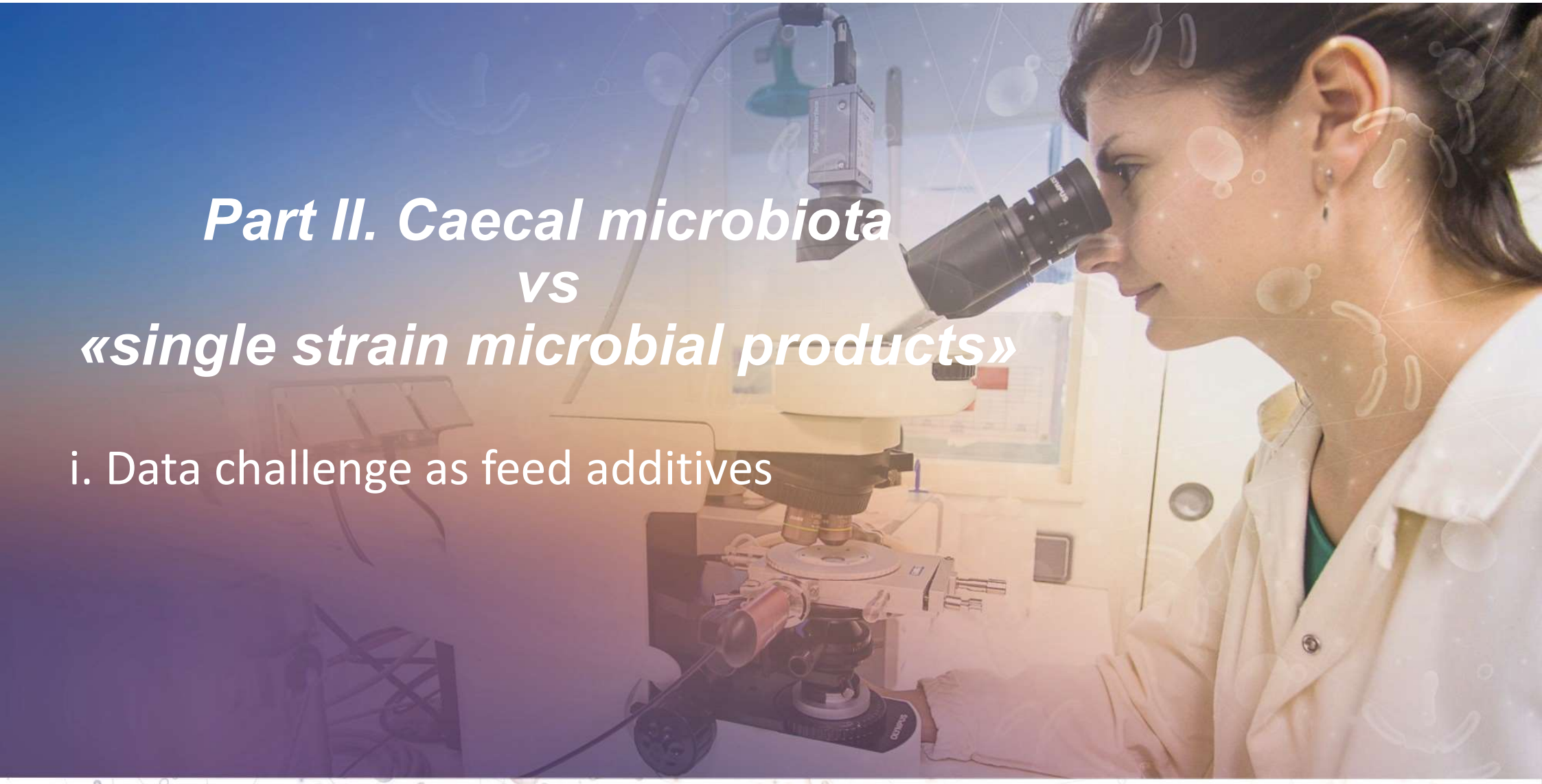
Zenner et al. 2021. Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. mSystems 6:e01300-20. https://doi.org/10.1128/mSystems.01300-20.

Part I. Aviguard

iii. Registrations / Placing on the market WW

EU and third countries

- EU
  - Czech Republic, Hungary, Slovakia: registered as non-medicinal veterinary product
  - Other EU countries (and UK): spray applications, non-registered products
  - As per SCOPAFF decision in 2011 → Product is not a feed additive
- Third countries (main markets)
  - Africa
  - Middle-East
  - Oceania
  - South-East Asia



Part II. Caecal microbiota vs «single strain microbial products»

i. Data challenge as feed additives

Conditions of use of the additive

Regulation 429/2008

2.5.1. *Proposed mode of use in animal nutrition*

- The proposed use, in feed or water shall be defined
- Details of the proposed method of administration and level of inclusion must be provided for premixtures, feedingstuffs or water for drinking

- Main use: spraying the product on day-old chicks
- Caecal microbiota products are not used in compound feeds (and only seldomly in water for drinking)
- Conditions of use are not defined in CFU as for microbial feed additives

Composition of the additive

Regulation 429/2008

- 2.1.3 Qualitative and quantitative composition of the additive
- The active substance(s)/agent(s) and all other components of the additive shall be listed, giving the proportion by weight in the final product. The qualitative and quantitative batch to batch variation of the active substance(s)/agent(s) shall be determined
- For micro-organisms: the number of viable cells or spores expressed as CFU per gram shall be determined.

- Caecal microbiota products are not characterised on the basis of a content in active agent(s)
- Products (their content in active agents) are not described in terms of CFU

Microbial feed additives

Regulation 429/2008

- 2.2. Characterisation of the active substance(s)/agent(s)
- Name and taxonomic classification of each micro-organism shall be provided, according to the latest published information in the International Codes of Nomenclature (ICN)
- Microbial strains shall be deposited in an internationally recognised culture collection (preferably in the European Union) and maintained by the culture collection for the authorised life of the additive.
- A certificate of deposition from the collection, which shall specify the accession number under which the strain is held, must be provided.

- Identification of each microorganism (each species, and the different strains of the same species) within the product ?
- Deposition of each strain in a culture collection : none are isolated, not all can be grown on standard culture medium
- Not possible to have a deposition certificates for the strains in the product

Microbial feed additives

EFSA guidance on microorganisms (2018)

- Requirements for scientific information according to the type of feed additive

	Section	Feed additives containing viable microorganisms	
		Bacteria	Fungi – yeasts
Identification	2.1	✔	✔
Antimicrobial susceptibility	2.2	✔	
Antimicrobial production	2.3	✔	✔
Toxigenicity and pathogenicity	2.4	✔	✔

2.1.1. Use of whole genome sequence for characterisation of microorganisms

- Whole genome sequence analysis (including chromosome(s) and extra-chromosomal genetic elements, e.g. plasmids) is required for bacterial and yeast strains intended for use either as products or production strains

- Current EFSA guidance document not created to address those end points in Caecal microbiota products

2.2. Antimicrobial susceptibility

- Two sets of data should be provided:
  - Phenotypic testing based on determination of a minimum inhibitory concentration (MIC) for a selected group of antimicrobials.
  - A search of the WGS for the presence of known AMR genes

- Current EFSA guidance document not created to address those end points in Caecal microbiota products

Methods of analysis

Regulation 429/2008

2.6. **Methods of analysis and reference samples**

- The methods of analysis shall be submitted in the standard layout as recommended by ISO.
- Detailed characterisation of the qualitative and, where applicable, quantitative analytical method(s) for determining compliance with maximum or minimum proposed levels of the active substance(s)/ agent(s) in the additive, premixtures, feedingstuffs and, when appropriate, water, shall be provided.

- No standardised analytical tool so far available for official controls for such product.

Efficacy

- EC guidelines and EFSA guidance documents are establishing efficacy requirements
- In vivo studies required (mostly related to zootechnical performance)

- What functional group would have to be targeted?
  - Gut flora stabilisers
  - Other zootechnical additives ?
- Efficacy requirements not adapted to caecal microbiota products (moslty due to conditions of use and end-points)
- Are challenge tests permitted for feed additives?

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