



**Concept: Microbial  
Community Profiling  
and Characterisation  
(MCPC) — A comparison  
with other methods for the diagnosis of  
bacterial overgrowth in the duodenum of  
broiler chickens —**

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**“Biography”**

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**Since 1999:** Research and development of various DNA based analysis methods with Dr. Van Haeringen Laboratorium b.v.

**1991 - 1999:** Research scientist at Wageningen Agricultural University, Department Molecular Genetics of Industrial Microorganisms. Projects focused on fungal- biotechnology, enzymology, primary metabolism, fermentation and molecular biology.

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# **Concept: Microbial Community Profiling and Characterisation (MCPC) -- A comparison with other methods for the diagnosis of bacterial overgrowth in the duodenum of broiler chickens**

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## **“Abstract”**

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### **Summary and Introduction**

Broilers frequently suffer from dysbacteriosis, characterised by too loose, too thin, voluminous, bad digested and or viscous faeces. The growth is retarded and the litter quality deteriorates. The activity of the birds is slightly reduced.

The objective of this study was to find a correlation between detailed bacterial profiles of the duodenal micro flora of broilers with the results of other methods that are used as a diagnostics for dysbacteriosis. For the comparison animals were selected from one or more houses on different farms in Belgium and The Netherlands. Because of this experimental design the birds formed a highly variable group with respect to age, housing, feed, food supplements and medication.

The birds were initially classified on the basis of the flock faeces score. A score of 0 is indicative for a house free of dysbacteriosis whereas a score of 1 is considered to be affirmative for the clinical diagnosis of dysbacteriosis. The validity of this method was checked by a semi-quantitative test plate test, originally developed by van Saene(1). The plate test is based on serial dilution of a swab sample from the duodenal contents on MacConkey and Mannitol plates, the plate-dilution swab test. The clinical diagnosis of dysbacteriosis was considered to be confirmed by the plate-dilution swab test when more than 25 % of the swabs, obtained from a group of broilers from one house showed bacterial growth on either the MacConkey and Mannitol plates or on both plates. Confirmation of the diagnosis free from dysbacteriosis was defined as no growth on the plates.

A selection of samples obtained in the course of this study was submitted to Dr. Van Haeringen laboratory for the determination of bacterial profiles using the MCPC method. MCPC is a PCR based method by which a detailed profile of a complex microbial community is generated. In addition the MCPC method can be used to get an indication of the identity of the species present in the sample.

After normalisation and grouping of the data we discovered one specific peak that showed a clear correlation with the flock faeces score and with growth on either or both of the plates used in the swab-dilution test. This peak was not found in 13 control samples that were free of dysbacteriosis.

In addition some of the samples showed a pattern that is indicative for the presence of high amounts of at least four different Lactobacillus strains. Another group of samples showed only a (large) number of very small peaks, which may be indicative for the presence of antibiotic substances in the feed or water.

This was a remarkable finding since, at first glance, the resulting MCPC profiles were highly variable, especially between samples obtained from different farms, which is consistent with the high degree of variation in the origin of the samples.

## **Materials and methods**

### *Selection of the broilers.*

Farms with clinical dysbacteriosis in one or more houses were selected. The clinical diagnosis was based on the flock faeces score. In addition, four houses from farms with a history of being free from dysbacteriosis were selected as a control group. From each house five individuals were selected at random.

### *Determination of the flock faeces score*

The clinical selection criterion for dysbacteriosis was the faecal score of a house. For this diagnosis faecal droppings are collected on absorbing paper placed in a specially designed litter box. A dropping is considered humid when it shows a ring of humidity with a diameter that is at least twice the diameter of the dropping. When at least 3 out of 10 droppings are humid the flock faeces score is 1, and this is considered to be affirmative for the clinical diagnosis of

dysbacteriosis. When less than 3 out of 10 droppings are humid the faeces score is 0 and the flock is considered to be clinically free of dysbacteriosis.

#### *Additional criterion for the samples in the control group*

For the samples in the control group, birds were selected that produced a dry dropping at sacrifice. Effectively this means that each individual from this group can be considered free from dysbacteriosis.

#### *Sampling of the duodenum with swabs*

For the sampling sterile cervical swabs (rigid nylon brush on a metal wire) were used.

The birds were sacrificed by applying Electro-voltage, a barbiturate or by cervical dislocation. The duodenum was removed by cutting the duodenal loop from the gizzard until the caudal part of the pancreas. The surface was disinfected by local heating (hot scalpel moving over the intestinal outside, with the speed of cutting cheese). Subsequently the duodenum was opened with a sterile scalpel and a swab sample was taken by moving the swab up and down over a length of 3 cm, starting 1 cm behind the exit of the gizzard, whilst applying mild pressure from the outside with two fingers. In this way both the contents and the superficial mucosa were sampled.

#### *The plate-dilution swab test*

The swabs were semi-quantitative cultured on two different media, MacConkey agar (bio Trading, code K039) and Mannitol (bio Trading, code K040). The media were inoculated, using a four-quadrant dilution method. Bacterial growth in only quadrant 1 was given the score 1, growth in quadrant 1 and quadrant 2, was given score 2, a positive finding up to quarter 3, was recorded as score 3 and up to quarter 4, score 4.

#### *Definition of positive swab scores*

No growth was defined as negative. Growth on the MacConkey and / or the Mannitol plate was considered to be positive for dysbacteriosis. If more than 25 % (in this case two or more out of five) of the swabs originating from animals from one house are positive, the clinical diagnosis dysbacteriosis is confirmed.

## **MCPC**

The basic technique that is used to establish an MCPC profile is generally referred to as 16S rRNA T-RFLP or 16S ribosomal RNA Terminal Restriction Fragment Length Polymorphism (2,3). This technique is based on the selective amplification of the 16S rRNA gene from bacteria by PCR (Polymerase Chain Reaction). The 16S rRNA genes from a large number of different bacteria can be amplified in a single PCR reaction. The length of the resulting PCR product is variable and strain dependant. However for a large number of strains the same length of PCR product will be found. Therefore the PCR products are cut into smaller pieces using restriction enzymes. These enzymes will cut at different positions in the PCR products from different strains. The resulting fragments exhibit a much larger variation in length and will thus result in a higher resolution. A product of a specific length is indicative for a very limited

number of micro organisms. The final products of the reaction are separated on an Applied Bio systems ABI Prism 3100 Genetic Analyser that allows very accurate determination the length of the products.

Using the fact that a large number of 16s RNA sequences are publicly available we have generated a database containing the theoretical length of the products for a large number of known micro organisms. With this database we are able to get an indication of the species that are present in a sample.

## Results and Discussion

MCPC profiles were established for 130 samples that were obtained from a total of 26 flocks, reared at 20 different farms. The MCPC profiles were highly variable, especially between different flocks. The profiles of the control flocks showed a small number of large peaks. Based on our database these peaks are likely to represent four to five different *Lactobacillus* species. Even in the other samples, where these peaks are less pronounced the same group of peaks is often dominant. The latter is not true though for a group of 53 samples that exhibited an extremely low total peak height. In most of the samples from this group only a few peaks were present. Fifty of these samples were obtained from a group 14 flocks originating from 8 different farms. These observations indicate that these flocks may have received antibiotic treatment or supplements with antibiotic activity. For only two farms this could actually be confirmed.

Superficially there is no specific profile that is correlated with either the flock faeces score or the plate-dilution swab tests. The data were therefore analysed in more detail by converting the peak patterns into a table. In the table each peak is represented by its height. The data were normalised by dividing the height of each peak in a sample by the sum of the peak heights of that sample. Furthermore the samples that exhibited an extremely low total peak height were excluded from the analysis and are not taken into the consideration in the next section.

The normalised data were then grouped based on different experimental parameters. Based on this normalised partial data set we found that the presence of a peak with a length of approximately 473 nucleotides shows a positive correlation with a positive flock faeces score, and a positive score on the either or both of the plates used in the plate-dilution swab test. The strongest correlation is found for those samples where both the flock faeces score and the mannitol plate test are positive. This peak with a length of 473 nucleotides was not found in any of the samples that combined a negative flock faeces score with negative plat-dilution swab test. These results corroborate our findings in an earlier research project (3), which also indicated that the microorganism(s) responsible for a peak with a length of approximately 473 nucleotides might be associated with the clinical diagnosis of dysbacteriosis. According to our database there are only two possible microorganisms that can be responsible for a peak of this length, *Clostridium paradoxum* and *Clostridium thermoalcaliphilum*. Whether this indication is correct and whether they play a role in the development of dysbacteriosis in will need to be established by further research.

## Conclusions

Using the MCPC technique we found a clear correlation between a peak with a length of 473 nucleotides in the bacterial profile and the combination of a positive flock faeces score and a positive on either or both plates in the plate-dilution swab test. For those samples where a positive flock faeces score was combined with a negative mannitol plate in the plate-dilution swab test this peak was found in only three out of nine samples.

Further analysis of the different bacterial profiles in correlation with feed and farm factors will in the future improve the understanding and solution of dysbacteriosis.

## References

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