Individual variability in the digestive flora of the broiler chicken analysed by molecular fingerprint

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With the withdrawal of antibiotic growth promotants, a better knowledge and control of the microflora of the digestive tract is essential for animal feeding. The digestive bacterial community varies between individuals. This variability may be lower in broilers due to the increased homogeneity in animals by genetic selection.
Individual variability was studied at various ages according to diet. Broilers were fed with a diet composed of wheat either ground (G) or as whole grains (W) given as free choice feeding. Caecal contents of 6 birds per diet were sampled weekly (from 16 to 44 d) to study the predominant bacterial population using a molecular fingerprinting method. Similarity coefficients (Pearson correlation method) were calculated for each pair of profiles, and were compared between diets with Student's t-test ($p \leq 0.05$).
Gel profiles showed inter-individual differences. At some ages, this variability differed according to diet. At 23 d of age, inter-individual differences appeared higher with W diet than with G diet which may be due to inter-individual variability of whole wheat intake with W diet. However, at 37 and 44 d, W diet led to higher microflora homogeneity between birds than G diet.
The digestive microflora of broilers showed an inter-individual variability that can be modified by diet. This variability must be taken into account when studying factors influencing microflora.

Keywords: Digestive tract; Microflora; Individual variability; Molecular fingerprint

Introduction

Until recently, the digestive flora of farm animals was controlled by the use of antibiotic growth promotants (AGP). Following their ban, a better knowledge of this flora became necessary to allow the development of alternatives. Most of the studies on the digestive flora, particularly in poultry, involved conventional culture methods. However, it is estimated that up to 90 % of bacteria is not cultivable (Lan et al., 2002). Therefore, standard microbiological methods only partially reflect the digestive ecosystem. To solve this problem, molecular techniques, independent from conditions of bacteria viability, were developed. These methods showed that there is an individual variability of the digestive flora in man (Zoetendal et al., 1998) as well as in farm animals such as the pig (Simpson et al., 2000). This variability could lead to a different effect according to the individuals of the alternatives to AGP. In poultry, with the genetic pressure, microflora might be less variable, in particular in broiler chickens. The objective of the present work was to study this individual variability at various ages. Moreover the effect of two different feed types was studied.

Materiels and methods

Broiler chickens (males, Ross) were raised in wire-floored cages in thermostatically controlled heated batteries. At their arrival, 430 chicks (4 to 5 animals / cage) were fed a starter diet composed of ground ingredients (G), wheat (40%), soybean meal, maize, methionine and a vitamin and mineral mixture, without AGP and coccidiostat. It was presented as meal. Birds of extreme weights were discarded (32 %) at 8 d. The remaining birds were separated into two groups of similar body weights (3 birds / cage). One group continued to be fed with the starter G diet, and the other received a starter diet similar to the G diet but with a part of the wheat (50%) given separately as whole grains (W). Chickens of extreme weights in each group were discarded at 15 d of age (30%). The remaining birds (2 birds / cage) were fed with grower diets G and W. For the W diet, 75% of the wheat was presented as whole grains in free choice. In each dietary group, chickens were discarded at 22 d of age (20%): in the G group, the birds of extreme weight; in the W group, the birds not able to eat whole wheat. Chickens (1 by cage) were fed finisher G and W diets until the end of the experiment (44 days). For the W diet, 100% of the wheat was presented as whole grains. The weight and the feed intake of animals were measured every week, from 8 to 43 days. Every week, from 16 to 44 days, 6 representative animals of each dietary treatment were selected, according to their weight. They were killed by intracardiac injection of sodium pentobarbital. The cecal contents were sampled, frozen in liquid nitrogen and stored at –70°C until analysis.

To study digestive microflora, the DNA was extracted from the cecal contents using the QIAamp DNA Stool Mini Kit (Qiagen) with an additional step of lysozyme treatment. Primers Bact 968-GC-f and Bact 1401-r were used to amplify the V6-V8 region of bacterial 16S rRNA genes (Nubel et al., 1996). PCR products were separated by Temporal Temperature Gradient gel Electrophoresis (TTGE) using the Dcode Universal Mutation Detection System (Biorad). The 6 samples from the two dietary treatments obtained at the same age, were analysed on the same gel. A marker consisting of a PCR amplicon mix of 7 cloned rDNAs from different bacterial species was used to normalize the profiles (Suau et al., 1999). Gels were stained in a solution of SYBR green and the fluorescence was read with a UV camera (Gel DOC XR, Biorad). The TTGE profiles obtained on the same gel were compared using the GelCompar II software (Applied Maths, Belgium). They were normalized by means of TTGE marker. The comparisons were based on the Pearson similarity coefficient (SCp) and the un-weighted pair group method using arithmetic averages (UPGMA) for clustering. A positive similarity threshold taking into account variations due to the extraction, the amplification of DNA, and the load on gel was determined; it was 97.2 %.

Data were analysed using the Statview ® software programme version 5. The SCp calculated for every pair of profile of 6 individuals of a given group (the same diet, the same age) were compared between diets for a given age, with the test of Student.

Results and discussion

For each age group, the profiles differed between individuals raised in the same conditions (among the same diet) (Figure 1). Although certain bands were common to several individuals, every animal had a different profile in term of position and intensity of bands. This showed that every individual has a unique digestive flora, as this was previously observed in man (Zoetendal et al., 1998) as well as in farm animals such as pigs (Simpson et al., 2000) and chickens (Zhu et al., 2002).

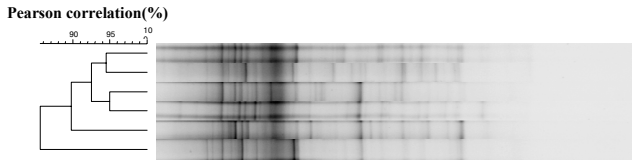

Figure 1 Inter-individual variability of the microflora of caecal content of chickens (ground wheat diet, 30 days)

The comparison of the SCp between individuals' profiles of the same age raised in the same conditions showed that the individual variability of the flora can be modified by the diet. Whereas at 16 d, the SCp were not different between W and G diet (84.9 ± 2.19 % and 87.4 ± 0.99 % respectively, p=0.32), at 23 d, the similarity between individuals seemed lower with the W diet than with the G diet, SCp = 94.5 ± 0.49 % and 96.4 ± 0.25 % ($p<0.01$) respectively. This difference could be due to the variable proportions of whole grains ingested according to the individuals in the case of the W diet (from 12 to 31 %), and thus a more variable composition of the digestive contents, the substrate for the flora. At 37 d, the opposite was observed: a higher similarity between individuals with the W diet compared to the G diet, SCp = 96.3 ± 0.42 % and 94.4 ± 0.56 % respectively ($p<0.01$), while the variation of whole grains consumption by the various individuals was always large (32 to 54 %). At 44 d, the strongest similarity between the animals of the W diet compared to the G diet remained, SCp = 94.0 ± 0.56 % and 90.9 ± 0.86 % respectively ($p<0.01$), the proportion of whole grain intake for W diet also presenting a variation (42 to 56 %). The wheat presented in the form of whole grains would lead to a higher homogeneity of the flora between individuals at the end of 4 weeks of consumption, in spite of the variation of proportions of grains consumed by animals. The physiological modifications due to this mode of feeding, such as the acidification of the gizzard content (Engberg et al., 2004) could explain this regulating effect on the flora. Thus, besides the effect of the feed on the digestive flora (Knarreborg et al., 2002; Gabriel et al., 2003; Engberg et al., 2004), the feed can have an effect on the inter-individual variability of the digestive flora.

This variability could be responsible for a different effect, according to the individuals, of the factors able to modify the digestive flora, as the alternatives to AGP. For example, after the consumption of a probiotic, the cecal fermentations differed according to the individuals (Ohashi et al., 2004). This individual variability can also prevent showing differences in the flora, due to the modification of the feed for example. Thus, while it was shown by conventional culture methods that the use of whole grains in the feed has effects on the digestive flora (Gabriel et al., 2003; Engberg et al., 2004), with an approach of molecular fingerprint with individual samples, no difference was observed at any age. Indeed, the analysis of the dendrogram showed that it was not possible to clearly group together the various individuals of each of both diets (Figure 2).

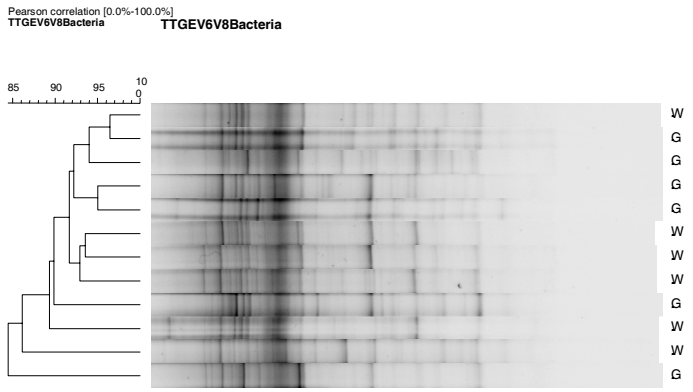

Figure 2 Dendrogram representing the relations between the bacteria profiles of the caecale flora of chickens (30 days) fed a diet containing either ground wheat (G) or whole wheat (W)

To limit the inter-individual variability, when studying the modifications of the flora by molecular fingerprint, certain authors suggested to pool the digestive contents of several individuals, and to use several pools of the studied treatments to keep an image of this variability. Thus by pooling individuals, it was possible to show differences in the flora between segments of the digestive tract as the ileum and the ceca, or between farms (Pissavin et al., 2006). Other authors suggested to eliminate this inter-individual variability by pooling the digestive contents of several individuals, and by choosing, for each studied treatment, a representative pool. The number of individuals in a pool must be big enough to eliminate the inter-individual variability within the treatment, without being too excessive in limiting the number of samples. Thus pools from 6 to 30 individuals were proposed.

Considering the differences in the flora persisting between pools of a few number of individuals (for example 6 samples, Pissavin et al., 2006), it seems that a more important number is necessary. Thus with pools of 16 individuals, Knarreborg et al (2002) showed similar profiles for a given treatment. With pools containing a more important number of animals (30 individuals), differences in digestive flora due to the use of alternatives to AGP were observed (Massias et al., 2006).

In conclusion, in spite of the increased homogeneity of animals by the genetic selection, the technique of molecular fingerprint used in this study showed that the digestive flora of the broiler presents an inter-individual variability, which can be modified by the feed. This variability must be taken into account when studying the factors of variation of the digestive flora.

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