

Effect of diets with different fibrous contents on broiler gut microflora and short-chain fatty acid (SCFA) production.

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Fibers are dietary indigestible carbohydrates supplements which play an important role in avian digestive physiology and thus in production performances. In this study, we examined effects of diets on intestinal and cecal microflora and on production of SCFA in 21-day-old broilers. Diets differed in fibrous content (3, 4.5 or 6% of crude fiber, e.g. T1, T3, and T5, respectively) or in nature of fiber (T6 and T7). Five birds per diet were sacrificed at day 21 and microflora of their intestinal and cecal contents were analyzed using PCR-DGGE method with universal eubacterial 16S rDNA primers. In intestines, lactobacilli were the major bacterial population. However, several supplementary high intensity bands were found in the T5 diet. In ceca, profiles were rather constant, but some new bands appeared in the three experimental group diets T5, T6 and T7. Variations in SCFA were more significant in ceca samples. A significant increase in butyrate was observed in broilers from diets T5, T6 and T7. In contrast, acetate and lactate were the major SCFA observed for broilers on diet T1 and T3. In conclusion, these results provide insights into effect of fiber on the modulation of the broiler gut microbiota and on the SCFA production.

Keywords: broiler; fiber; gut microflora; short-chain fatty acid; PCR-DGGE.

Introduction

The importance of understanding the dynamics of intestinal microbial ecology has been recognized for a long time (Savage, 1977). Since the ban of using antibiotics as growth-promoters in the animal feed, the concept of digestive flora and the management of bacterial balance of digestive tract have become more important. Digestive disorders have increased in parallel to this withdrawal (Van Immerseel *et al.*, 2004) and are often sources of under performances due to healthy problem as necrotic enteritis or coccidiosis (Williams, 2005).

Thus, nutritional balance of feeds takes great significance. Among nutrients constituting poultries' feeds, the fibrous fraction is very low. Some studies have nevertheless indicated that increasing levels of fiber may enhance performance in chickens and turkeys hens (Ricke *et al.*, 1982; Sklan *et al.*, 2003). Jorgensen *et al.* (1995) showed that the source of fibers has an impact on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Intestinal microflora can ferment fibers to SCFA and gas, however, the effect of fiber compounds on gut microflora modulation is poorly studied and understood.

Therefore, in the present study we evaluate the effects of increasing fiber levels and fiber source on SCFA production and on bacterial community of broiler gut using a DNA-based analytical method independent of cultivation. Among the different molecular methods, 16S rDNA-based PCR-DGGE, providing band profiles corresponding to the major bacterial strains and permitting the identification from the excised bands, was used.

Material and Methods

Growth trial. A total of 240 male broilers of commercial strain (ROSS PM3) were randomly allocated to 120 cages of 2 animals. They were given a starter diet from 0 to 8 days, and then received 5 different diets (24 cages of 2 animals per group) from 8 to 28 days. Five experimental diets were formulated with different crude fiber levels. The table 1 presents the fiber composition (list of incorporated fibrous raw materials) and the table 2 presents the diets nutritional analyses (% as fed basis).

Table 1: Diets composition (list of fibrous raw materials in decreasing order)

T1	T3	T5	T6	T7
Corn	Corn	Wheat	Wheat	Corn
Soybean meal	Wheat	Corn	Soybean meal	Soybean meal
Wheat	Soybean meal	Soybean meal	Corn	Wheat
	Sunflower meal	Sunflower meal	Sunflower meal	Sunflower meal
	Soybean hulls	Soybean hulls	Wheat Bran	Alfalfa
	Wheat bran	Wheat bran	Soybean hulls	Soybean hulls
			Sugar beat pulp	Wheat bran

Table 2 : Diets nutritional analyses (% as fed basis)

	T1	T3	T5	T6	T7
ME (kcal/kg)	2900	2900	2900	2900	2900
Analysed Crude Fiber (%)	3.10	4.70	5.40	5.70	4.40
Analysed Crude Fat (%)	3.40	4.80	6.80	6.40	5.30
Analysed Crude protein (%)	18.80	18.90	19.40	19.10	19.20

Samples collections. Five birds per diet were killed on day 21. The small intestine was rapidly excised from Meckel's diverticulum to the ileocecal junction (ileum). The ileal content was collected and pooled per diet. The two ceca were removed from each bird and the contents pooled per treatment. Samples were collected in sterile tubes, immediately frozen and stored at -20°C until microflora profile analysis.

Eubacterial DNA extraction. Samples DNA extraction (25 mg) was realized using QIAamp DNA mini kit (Qiagen, California, USA). DNA extracts were quantified with a spectrophotometer (Victor plate reader, (Perkin-Elmer, Massachusetts, USA).

DNA amplification and DGGE. A nested PCR on 16S rDNA genes was realized using eubacterial universal primer set (PU1 and PU2) for the first step and universal primer set targeted to V2-V3 region of 16S rDNA (HDA1 and HDA2-GC) for the second step. PCR-products were separated by DGGE with a 30 to 55% denaturing gradient and revealed by ethidium bromide staining. Bands of interest were cut with sterile scalpel blades and frozen at -20°C .

Sequencing and bacterial identification. After re-amplification, bands of interest were sequenced and sequence results were compared with the RDPII 16S rDNA database (Cole *et al.*, 2007). The bacterial identification is thus deduced from the best similarity percentage and best S_{ab} score.

Analysis of the SCFAs concentrations. The method of Morales *et al.* (1998) was used with some adaptations. Intestinal contents were diluted at 300 mg.mL⁻¹ in a solution of H₂SO₄ 5mM. The 3-methylvaleric acid was used as internal standard. Samples were HPLC analyzed in an Aminex HPX-87H column using a 215 nm detector. The results are expressed in mg of SCFA/g of sample.

Results and discussion

Figure 1 represents DGGE results. In intestines, lactobacilli were the major bacterial populations. *Lb. salivarius* and *Lb. crispatus* were the most represented species. Supplementary high intensity bands (13, 14) founded in the T5 diet corresponds to variants of *Lb. salivarius* 16S rDNA sequence. In

diets T1, T3 and T7 *Lb. reuteri*, *Lb. mucosae* and *Lb. antri* are most represented that in diets T5 and T6. In T5 diet, which corresponds to the highest level of crude fiber, *C. perfringens* was retrieved (Band 15).

In ceca, profiles were constant, but some specific bands appeared principally in T5, T6 and T7 diets. It's interesting to note that these treatments correspond to the highest levels of crude fiber and differed by the fiber's quality (raw material profiles). This is in agreement with previous study which showed that fiber's quality (in diet with high levels of fiber) influence bacterial profile (Dunkley *et al.*, 2007). Moreover, Green (1988) postulated that intestinal bacteria may be responsible of degradation of some non-cellulose fraction of the crude fibers in broilers. It may influence fecal fermentations too.

Variations in SCFA were greater in cecal samples. An increase in the butyrate was observed in broilers' ceca from diets T5, T6 and T7. In contrast, the acetate and lactate were the major bacterial SCFA observed for broilers on diet T1 and T3 (Fig. 2). Quantitatively, T7 diet produces the most SCFAs (51 mg/g caeca) and diets T1 and T3 the lowest quantity (21.5 and 22 mg/g of cecal samples). In other animal species (rabbit), Jehl and Gidenne (1996) demonstrated that the incorporation of high levels of pectin in the feed (beet pulp for example) induces an elevation of SCFA. In our study, we observed quantitative and qualitative variations in SCFAs in relation with fiber diets. The greater production of SCFA in the ceca, particularly in some fiber diets are very interesting, since SCFA production was linked with a bacteriostatic effect to some enteric bacteria without effect on *Lactobacillus* (Van der Wielen *et al.*, 2000). Therefore, we demonstrate that microflora variations depend of fiber nature and concentration principally in the intestinal compartment. Interesting, in T5 diet, *Cl. perfringens* was present, fact that may indicate that fiber nature may modulate their growth.

In conclusion, these results provide insights in understanding the effect of fiber on the gut microbiota of broilers. Nevertheless further studies will be necessary to understand the possible effect of crude fiber on gut microflora and its impact on broiler growth and health status.

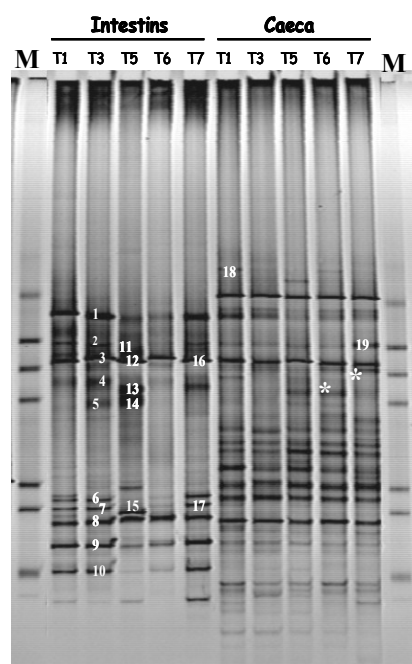


Figure 1. DGGE gel of eubacterial 16S rDNA gene amplifications. M: Marker. Bands: 1, 2, 3 (*Lactobacillus crispatus*); 4 (*Lb. salivarius chimera*); 5 (*Enterococcus cecorum chimera*); 6, 8, 9 (*Lb. reuteri*); 7 (*Lb. antri*); 10 (*Lb. mucosae*); 11, 12, 13, 14, 16 (*Lb. salivarius*); 15 (*Clostridium perfringens*); 17 (SFB); 18 (uncultured bacteria); 19 (*Clostridium sp*); * (identification in process).

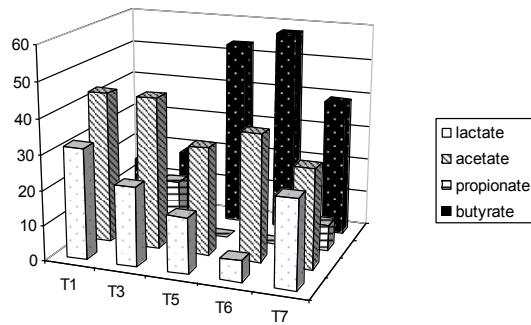


Figure 2. Relative concentration (%) of SCFA in cecal samples from the different diets (T1 to T7)

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