

# Dynamics of microbial cecal lumen ecosystem of broiler chickens with age measured by Terminal Restriction Fragment Length Polymorphism (t-RFLP)



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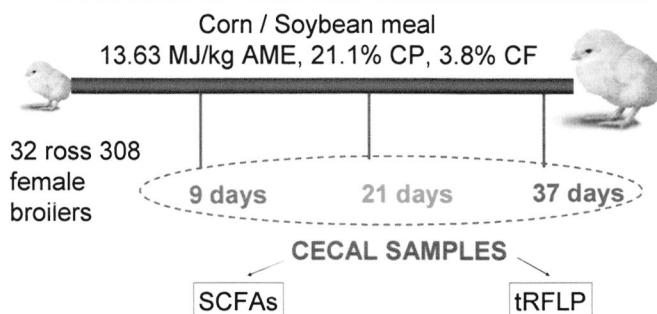
## INTRODUCTION

Nowadays, it is known that intestinal microflora of broiler chickens plays an important role for growth performance and health. However, there is still a limited knowledge about the process of colonization of the chick gut.

## OBJECTIVES

The objective of the present work was to study how the indigenous microbiota is established in the young chick using molecular fingerprinting techniques and analyzing the fermentation end-products (short chain fatty acids, SCFAs).

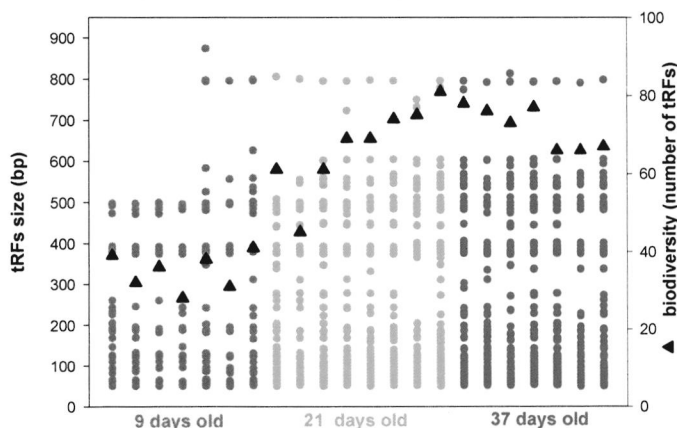
## MATERIALS AND METHODS



Microbiota dynamics in caecum was assessed by t-RFLP using fluorescently labelled 16S rRNA gene forward primer (FAM0008) and restriction enzyme *HhaI*. SCFAs were analyzed by gas chromatography.

## RESULTS

**Figure 1: Cecal biodiversity analysis.** This graph presents size distribution and number of terminal fragments (tRFs) extracted from cecal samples. Each vertical dot-line represents one animal.



The number of tRFs was significantly ( $p < 0.001$ ) affected by the age of the animals increasing from day 9 to 37 (mean values 35.0, 66.9 and 71.8 respectively).

**Table 1: Compatibility analysis of major tRFs found in cecal samples.** (area >2%)

tRFs (bp)	Compatible bacteria *	Number of animals that presents the compatible bacteria (in the same line, each box represent one animal)		
		9 days old	21 days old	37 days old
82-109	<i>Cytophaga, Flexibacter, Bacteroides, Flavobacterium, Campylobacter</i>	█	█	█
140-147	<i>Fibrobacter succinogenes</i>	█	█	█
184-194	<i>Clostridium clostridioforme, Ruminococcus, Eubacterium</i>	█	█	█
223-225	<i>Lactobacillus delbrueckii</i>	█	█	█
373-381	<i>Escherichia coli, Eubacterium</i>	█	█	█
385-394	<i>Lactobacillus aviarius</i>	█	█	█
546-557	<i>Clostridium butyricum</i>	█	█	█

\* Compatible bacteria from TAP-TRFLP (Ribosomal Database Project)

**Table 2. Short chain fatty acid production.** This table summarizes results from total SCFA production, the profile of the main fatty acids analyzed and the concentration of lactic acid in cecal samples

Age of the animals	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Valeric acid (%)	Branched acids (%)	Total SCFA ( $\mu\text{mol/g}$ )	Lactic acid ( $\mu\text{mol/g}$ )
9 days old	65.53 <sup>b</sup>	13.18 <sup>a</sup>	17.43	2.38	1.47 <sup>a</sup>	63.84	0.21
21 days old	75.21 <sup>a</sup>	8.28 <sup>b</sup>	15.37	2.06	1.29 <sup>a</sup>	51.09	0.15
37 days old	70.95 <sup>ab</sup>	11.12 <sup>ab</sup>	13.24	2.20	2.51 <sup>b</sup>	68.57	0.25
SEM	6.09	3.10	4.50	0.62	0.72	15.57	0.087
p-value	0.034	0.033	0.251	0.644	0.008	0.114	0.124

<sup>a,b</sup> Different superscripts in the same column denotes significant difference between the age of the animals studied ( $P < 0.05$ ).

## CONCLUSION

Results obtained show that in chickens the establishment of an adult microbiota is associated with an increase in the complexity of the ecosystem. Total SCFA does not seem to be affected by the age of the animals. Specific changes in acetic, propionic and branched acids were detected but were not associated with the maturation of the gut microflora. Some variations in specific bacterial groups (*Clostridium clostridioforme*, *Ruminococcus*, *Eubacterium*, *Clostridium butyricum*, *Fibrobacter succinogenes*) could also be detected related to age.

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