Bacterial counts and characterization of intestinal flora in organic and conventional chickens.

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The aim of this study was to count, isolate and identify by traditional methods the major bacteria colonizing the intestinal tract of organic and conventional chickens. The study was performed in 2 conventional and 2 organic chicken farms. Intestinal samples (24 from ileum and 24 from caecum) were collected from organic reared chickens during the production cycle (40 days of age) and at slaughter (80 days of age). Twenty four intestinal samples were also collected from conventional chickens at slaughter (40 days of age).

Facultative anaerobic bacteria (enterobacteria, enterococci, staphylococci, lactobacilli) and anaerobic obligate bacteria colonizing the ileum and the caecum were counted, isolated, identified and expressed as n. x 10^6.

Results showed that at 40 days the aerobic count was higher in the caecum than in the ileum in both conventional and organic chickens (respectively 296.4 and 307.2 for the caecum vs 46.7 and 57.5 for the ileum). The anaerobic flora was higher in the caecum than in the ileum of both conventional and organic chickens ($P<0.05$).

Data related to organic intestinal samples (ileum and cecum), collected at 40 and 80 days, were characterized by the values of aerobic population (more represented in cecum than in ileum) lower than the values of anaerobic organisms at 80 days .

The biochemical characterization of enteric flora did not identify large differences in organic and conventional chickens. Further investigations are necessary to better assess the role and the effect of the enteric flora on the productive performance and on the health status of reared chickens.

Keywords: organic chickens; intestinal microbial flora; bacterial count

Introduction

Intestinal bacteria play an important role in pathogenesis of intestinal diseases since they are believed to protect against colonization of the intestine by pathogens and to stimulate the immune response of chickens (Mead, 2000).

Extensive studies of the culturable bacterial flora of chickens have been performed in animals intensively reared (Barnes et al., 1972; Barnes and Impey, 1972; Mead and Adams, 1975; Barnes, 1979; Mead, 1989; Rolfe, 2000; Gong et al., 2002; Jiangrang et al., 2003). The main bacteria in cecum are obligate anaerobes (Barnes et al., 1972; Barnes and Impey, 1972; Salanitro et al., 1974ab; Jiangrang et al., 2003), while *Lactobacillus, Enterococcus* and *Streptococcus* are prevalent in ileum (Salanitro et al, 1978; Jiangrang Lu et al. 2003).

Metabolic stress associated with diet composition, environment and management practices can affect the delicate balance among the microbiological components of the gut, impairing growth, feed conversion and health.

The data reported in literature are referred only to chicken intensively reared while studies related to the intestinal microbiota of organic chicken are not available.

The aim of this study was to count and characterise the intestinal bacterial flora in organic and conventional chickens by traditional methods.

Material and Methods

The investigations were performed in 2 conventional and 2 organic chicken farms. In the conventional farms diets, housing system and genetic strain (Ross 305) were standard. Organic farms, according to the EU rules (EC Council Regulation No 1804/99), has a specific rearing protocol which provides older age at slaughter (81d), organic ingredient in the diets, the ban of pharmacological treatments and the presence of external paddock (4 m^2 bird).

Twenty-four intestinal samples (ileum and caecum) were collected in organic farms during the production cycle (40 days) and immediately before slaughter (80 days), the same number of samples were collected from conventional chickens only at 40 days of age. The caecum and the ileum (from the duodenum and Merkel's diverticulum) of each bird, was accurately removed and 6 samples of the intestines of single bird were pooled to obtain 4 samples of each intestinal region (ileum and caecum).

One gram of intestine content was first put with a sterile stick in a sterile measuring tube together with 2 mL 0.9% sterile saline solution. The stool was pressed and mixed in this solution and volume was completed to 10 mL with 0.9% sterile saline solution. Each pool (0,1 mL) was diluted serially via 10-fold dilutions (from 10^{-1} to 10^{-10}).

MacConkey agar, Violet red bile agar and KF streptococcus agar were respectively used for the isolation and enumeration of *Enterobacteriaceae, Streptococcaceae* and *Enterococcaceae*.

Baird Parker agar and Mannitol salt agar were used for isolation and enumeration of *Staphylococcaceae*. All the plates were incubated at 37 °C, aerobically, for 24h-48h. The number of grown colonies was determined and the colonies obtained were examined (oxidase test for *Enterobacteriaceae,* catalase test for *Streptococcaceae* and *Staphylococcaceae*), Gram stained and subcultured to obtain pure cultures. All bacteria were identified using biochemical tests (API 20E, API Strep, API Staph, Bio Merieux,). For the isolation and enumeration of anaerobic bacteria Schaedler agar, enriched with 5% sheep blood and 1 mg/mL K1 vitamin, was used as anaerobe blood agar. The anaerobe bood agar supplemented with 7.5 mg/mL vancomycin and 100 mg/mL kamamycin was used as kanamycin vancomycin blood agar.

For the enumeration of anerobic bacteria Reinforced Clostridial agar was also used. Anaerobic incubation was made in anaerobic jars (Oxoid) for a minimum of 7 days before initial examination. Anaerobic conditions were obtained with Anaerogen (Oxoid) and were controlled by methyl blue strips as oxidation reduction indicator. After incubation of 7 days at 37 °C, primary anaerobic plates were examined and all types of colonies grown on anaerobe blood agar were described and subcultured. The colony description included the size, shape, edge, profile, colour, opacity, hemolysis, fluorescence, pigment and pitting characteristic. One colony of each type described was Gram stained and subcultured to:

1) two chocolate agars of which the first incubated on 37 °C and the second on CO^2 area to verify the aerobic or aerotollerant character of the colony;

2) anaerobe blood agar to obtain pure culture of each colony. All bacteria were identified using API20 A Kit. (BioMerieux).

For the enumeration of lactobacilli Rogosa agar was used. The plates were incubated for 3 days at 35 °C under microaerophilic condition.

Statistical analysis - Data were expressed as n. x 10^6 and analysed by linear models (STATA, 2005) comprising the effect of farming systems (conventional *vs.* organic). The effect of age (40 *vs.* 80) was analysed only for organic birds. Significance of differences was assessed by the t-test.

Results and discussion

Data related to intestinal bacterial flora count of both conventional and organic chickens at 40 d are shown in *Table 1*. The total aerobic bacteria were higher in the caecum than in ileum in both conventional and organic chickens (respectively 296.4 and 307.2 for the caecum vs. 46.7 and 57.5 for the ileum).

In particular, the values of some facultative anaerobic bacteria, such as lactobacilli and enterococci, usually more represented in ileum (Salanitro et al., 1978; Jiangrang et al., 2003), were higher in the caecum of the both chicken groups.

The enterobacteria were higher in caecum than the ileum. The value was slightly higher in conventional than in organic chickens (respectively, 18.3 *vs.* 7.0 for ileum and 70.8 *vs.* 59.5 for caecum).

The anaerobic bacteria values confirmed previous findings (Barnes et al., 1972; Barnes et Impey 1972; Salanitro et al., 1974ab; Jiangrang et al., 2003); indeed they are higher in the final intestinal tract. The numerical value was also significantly higher in conventional caecal samples.

Table 1. Bacterial counts from the intestinal tract of organic and conventional chickens at 40 days (n. x 10^6)

	Conventional		Organic		SEM
	Ileum	**Caecum**	**Ileum**	**Caecum**	
Total aerobic bacteria	46.7a	296.4b	57.5a	307.2b	44.8
Total anaerobic bacteria	242.5b	631.7c	0.1°	293.1b	132.5
Enterobacteria	18.3a	70.8b	7.0a	59.5b	9.6
Staphylococci	7.6b	19.1c	0.1°	6.7b	3.3
Enterococci	16.4a	101.4b	35.8°	120.8b	33.8
Lactobacilli	3.9a	104.5b	19.4°	120.0b	22.5

a..c: P< 0.05.

The comparison between the samples collected at 40 and 80 days in organic chickens are reported in *Figure 1a-1b*. In the ileum, the total anaerobia bacteria and the enterobacteria greatly increased with the age whereas lactobacilli, enterococci and the total aerobic bacteria decreased (*Figure 1a*).

In the caecum the bacteria were 10 fold more concentrated than in ileum (*Figure 1b*) as reported in literature (Apajalahti et al., 2002)

Results related to biochemical characterisation are showed in Tables 2, 3, 4 and in total confirmed data reported in literature (Barnes et al., 1972; Barnes and Impey, 1972; Salanitro et al, 1974; Mead and Adams, 1975; Barnes, 1979). It has to be underlined the presence in the caecum of several species of anaerobic bacteria not detected in the ileum (*Table 2* and *3*). In the organic system the birds showed more bacterial species at 80 d (+90%).

However, on the basis of biochemical bacteria characterization it was not possible to make any discrimination between organic and conventional birds though there were marked differences in the two rearing systems.

Figure 1a. Bacterial distribution in ileum of organic chickens at different ages (95% confidence interval).

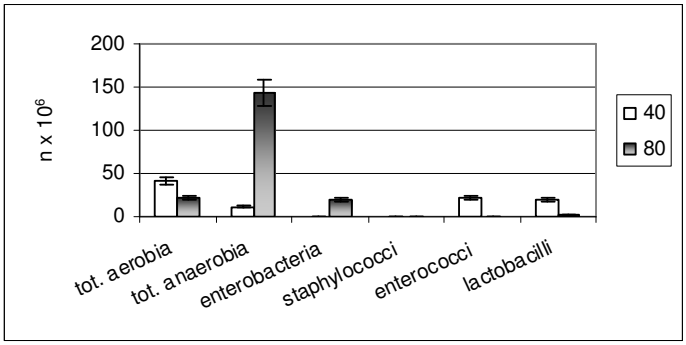
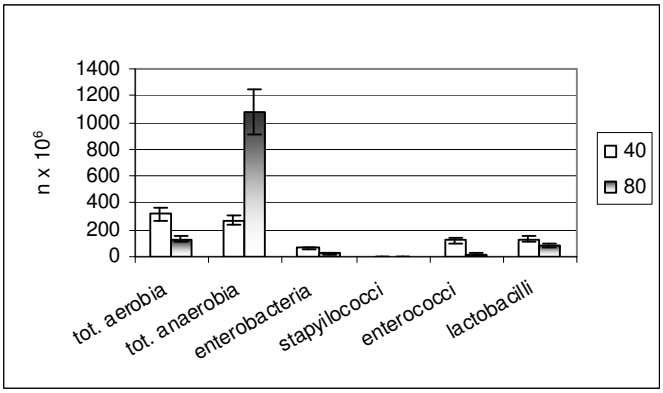


Figure 1b. Bacterial distribution in caecum of organic chickens at different ages (95% confidence interval).



In conclusion, the present study showed that the caecum of both conventional and organic chickens had a major number of bacteria than the ileum; however, the differences between the two groups, detected in bacterial count at the same age, are not sufficient to discriminate rearing systems.

It is possible to hypothesize that farming system can affect the equilibrium among the different classes but it does not influence particular bacteria species.

It should be underlined that biochemical methods have a limited reliability to show the "total" microflora. Thus, further investigations with molecular techniques are required to confirm and increase our knowledge on the gut microflora.

Table 2 Main bacteria isolated from the ileum of organic chickens

		40 days	80 days
Group	**Class**	**Genus and species**	**Genus and species**
Gram -	*Enterobacteriaceae*	*Escherichia coli*	*Escherichia coli*
		Kluyvera spp.	*Klebsiella oxytoca*
	Bacteroidaceae	*Bacteroides ovatus*	*Bacteroides distasonis*
Gram +	*Enterococcaceae*	*Enterococcus casseliflavus*	*Emterococcus durans*
		Enterococcus faecium	*Enterococcus faecium*
		Enterococcus faecalis	*Enterococcus faecalis*
	Staphylococcaceae	*Staphylococcus xylosus*	*Staphylococcus xylosus*
		Staphylococcus aureus	*Staphylococcus sciuri*
			Staphylococcus simulans
			Staphylococcus lentus
	Clostridiaceae	*Clostridium beijerinckii*	*Clostridium beijerinckii*
			Clostridium perfringens
	Peptostreptococcacaceae		*Peptostreptococcus spp.*

Table 3. Main bacteria isolated from the caecum of organic chickens

		40 days	80 days
Group	**Class**	**Genus and species**	**Genus and species**
Gram -	*Enterobacteriaceae*	*Escherichia coli*	*Escherichia coli*
			Serratia odorifera
		Kluyvera spp.	*Citrobacter youngae*
	Bacteroidaceae	*Bacteroides distasonis*	*Bacteroides distasonis*
			Bacteroide fragilis
		Bacteroides ovatus	*Bacteroides ovatus*
	Prevotellaceae	*Prevotella spp.*	*Prevotella spp.*
	Fusobacteriaceae		*Fusobacterium mortiferum*
Gram +	*Enterococcaceae*	*Enterococcus faecium*	*Enterococcus faecium*
		Enterococcus faecalis	*Leuconostoc spp.*
			Enterococcus avium
	Staphylococcaceae	*Staphylococcus xylosus*	*Staphylococcus xylosus*
		Staphylococcus aureus	*Staphylococcus aureus*
			Staphylococcus lentus
			Staphylococcus haemolyticus
	Clostridiaceae	*Clostridium perfringens*	*Clostridium beijerinckii*
			Clostridium perfringens
	Peptostreptococcaceae		*Peptostreptococcus spp.*
	Lactobacillaceae		*Lactobacillus fermentum*
	Propionibacteriaceae		*Propionibacterium fermentum*
	Actnomycetaceae	*Actinomyces naeslundii*	

Table 4. Main bacteria isolated from ileum and cecum of conventional chickens (40 d)

		Ileum	Caecum
Group	**Class**	**Genus and species**	
Gram –	*Enterobacteriaceae*	*Escherichia coli* *Escherichia fergusonii* *Citrobacter koseri* *Kluyvera spp.*	*Escherichia coli* *Enterobacter cloacae*
	Bacteroidaceae	*Bacteroides ovatus*	*Bacteroides ovatus* *Bacteroides fragilis*
Gram +	*Enterococcaceae*	*Enterococcus faecium* *Enterococcus faecalis*	*Enterococcus faecium* *Enterococcus faecalis* *Enterococcus durans*
	Streptococcacee	*Streptococcus gallolyticus*	
	Staphylococcaceae	*Staphylococcus lentus* *Staphylococcus xylosus*	*Staphylococcus lentus* *Staphylococcus xylosus* *Staphylococcus aureus*
	Propionibacteriaceae		*Propionibaterium propionicum*

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