Evaluation of intestinal bacterial flora of conventional and organic broilers using culture-based methods

Patrizia Casagrande Proietti, Alessandro Dal Bosco, Friedrike Hilbert, Maria Pia Franciosini & Cesare Castellini

To cite this article: Patrizia Casagrande Proietti, Alessandro Dal Bosco, Friedrike Hilbert, Maria Pia Franciosini & Cesare Castellini (2009) Evaluation of intestinal bacterial flora of conventional and organic broilers using culture-based methods, Italian Journal of Animal Science, 8:1, 51-63, DOI: 10.4081/ijas.2009.51

To link to this article: https://doi.org/10.4081/ijas.2009.51

Copyright 2009 Taylor & Francis Group LLC

Published online: 01 Mar 2016.

Submit your article to this journal

Article views: 93

View related articles
Evaluation of intestinal bacterial flora of conventional and organic broilers using culture-based methods

Patrizia Casagrande Proietti¹, Alessandro Dal Bosco², Friedrike Hilbert³, Maria Pia Franciosini¹, Cesare Castellini²

¹Dipartimento di Scienze Biopatologiche ed Igiene delle Produzioni Animali ed Alimentari. Università di Perugia, Italy
²Dipartimento di Biologia Applicata. Università di Perugia, Italy
³Institute for Meat Hygiene, Meat Technology and Food Science. University of Wien, Austria

Corresponding author: Dr. Alessandro Dal Bosco. Dipartimento di Biologia Applicata. Facoltà di Agraria, Università di Perugia. Borgo XX Giugno 74, 06100 Perugia, Italy - Tel. +39 075 5857110 Fax: +39 075 5857122 - Email: dalbosco@unipg.it

Paper received April 1, 2008; accepted July 5, 2008

ABSTRACT

The major bacteria colonizing the intestinal tract (ileum and caecum) of organic (O) and conventional (C) chickens were counted, isolated and identified by conventional methods. Chickens were obtained from 7 conventional and 7 organic chicken farms (n=203). Intestinal sampling was performed at different ages, every 10 days, starting at 20 days until 40 and from 20 days to 80 days of age, respectively, for conventional and organic birds. Statistical analysis was performed on two separate data sets (40 days of age and all ages). The comparison of C vs O systems was analyzed with univariate and multivariate procedures. There were large differences in bacterial counts in relation to the portion of intestine, the rearing system and the farms. In the ileum of conventional birds Enterobacteria were higher than in organic birds (7.03 vs 6.09 CFUxlog/g; P<0.05), whereas the contrary was observed for Lactobacilli (6.75 vs 7.07 CFUxlog/g; P<0.05). With respect to the other microflora, the effect of farm probably masked possible differences. The effect of rearing system was more visible in the caecum than in the ileum: Enterobacteria levels were higher in C than in O chickens (7.42 vs 7.05 CFUxlog/g; P<0.01), whereas Enterococci (7.65 vs 6.55 CFUxlog/g; P<0.05), Lactobacilli (7.85 vs 7.31 CFUxlog/g; P<0.05) and total aerobia (8.12 vs 7.66 CFUxlog/g; P<0.01) counts were higher in organic chickens. Multivariate analysis of caecum microflora showed the possibility of discriminating the rearing system. In the ileum of conventional birds Enterobacteria and total aerobia increased with age, while Lactobacilli decreased. In the O system, Enterobacteria, Lactobacilli and total anaerobia showed a similar trend, whereas total aerobia and Enterococci showed the opposite trend. A similar situation was observed in the caecum. Further investigations are necessary to better assess the role and effect of the enteric flora on the productive performance and on the health status of reared chickens.

Key words: Conventional chickens, Organic chickens, Intestinal microflora, Culture-based methods.
RIASSUNTO

VALUTAZIONE DELLA FLORA BATTERICA INTESTINALE IN POLLI CONVENZIONALI E BIOLOGICI MEDIANTE L’UTILIZZO DI METODI MICROBIOLOGICI

Lo scopo di questa ricerca è stato quello di effettuare una valutazione della microflora intestinale di polli allevati con diversi sistemi di allevamento (convenzionale, C vs biologico, B). Lo studio è stato condotto in 7 allevamenti convenzionali ed in 7 biologici (n= 203). Il campionamento è stato effettuato a livello dell’ileo e del cieco a differenti età, ogni 10 giorni a partire da 20 giorni fino a 40 e 80 giorni di età rispettivamente per i polli convenzionali e quelli biologici. L’analisi statistica dei dati è stata effettuata con procedura univariata e multivariata su 2 data set differenti: il primo con animali di 40 giorni e l’altro per tutte le età considerate. I risultati ottenuti mostrano differenze in relazione al tratto di intestino, al sistema di allevamento ed alla singola azienda considerata. Nell’ileo dei polli convenzionali gli Enterobatteri hanno mostrato livelli più alti rispetto a quelli biologici (7,03 vs 6,09 CFUxlog/g; P<0,05) al contrario dei Lattobacilli (6,75 vs 7,07 CFUxlog/g; P<0,05). Per ciò che concerne le altre popolazioni microbiche è probabile che l’effetto dell’allevamento abbia mascherato le potenziali differenze. Nel cieco l’effetto del sistema di allevamento è stato più rilevante: infatti gli Enterobatteri erano più rappresentati nei polli convenzionali rispetto ai biologici (7,42 vs 7,05 CFUxlog/g; P<0,01) mentre gli Enterococci (7,65 vs 6,55 CFUxlog/g; P<0,01), i Lattobacilli (7,85 vs 7,31 CFUxlog/g; P<0,05) e gli aerobi totali (8,12 vs 7,66 CFUxlog/g; P<0,01) avevano un trend opposto. L’analisi multivariata della microflora ciecale è stata in grado di discriminare efficacemente il sistema di allevamento. Nell’ileo dei polli convenzionali Enterobatteri e aerobi totali sono aumentati con l’età, mentre i Lattobacilli sono diminuiti. Nel gruppo biologico solo Enterobatteri, Lattobacilli ed anaerobi totali hanno mostrato un andamento simile, mentre gli aerobi totali hanno mostrato un trend opposto. Nel cieco è stata osservata una situazione sovrapponibile. Ulteriori ricerche sono necessarie al fine di meglio definire il ruolo e gli effetti della flora intestinale sulle performance e sullo stato di salute dei polli allevati.

Parole chiave: Polli convenzionali, Polli biologici, Flora batterica intestinale, Metodi microbiologici.

Introduction

In poultry the intestinal bacteria play an important role in the pathogenesis of intestinal diseases since they influence the development of gut immunity and thus may prevent colonization of pathogens in the intestine (Mead, 2000).

Populations of digestive bacteria change according to the age of chickens and can be influenced by diet (Netherwood et al., 1999; Binek et al., 2000) as well as by many environmental factors.

Consequently, metabolic stress associated with composition of the diet and environmental and management stressors can affect the balance among the microbiological components of the gut, impairing the growth, feed conversion, health and welfare of chickens.

The main bacteria in the caecum are obligate anaerobes (Barnes et al., 1972; Salanitro et al., 1974; Lu et al., 2003), while Lactobacillus, Enterococcus and Streptococcus are prevalent in the ileum (Lu et al., 2003).

Extensive studies on the cultivable bacterial flora of chickens have been performed in intensively reared animals (Barnes and Impey, 1972; Mead and Adams, 1975; Barnes, 1979; Mead, 1989; Rolfe, 2000; Gong et al., 2002), whereas the bacterial profile of free-range animals has not been extensively investigated.

In the organic system, many aspects of rearing, strictly controlled by EC Council Regulation No. 1804/99 (feeding, housing system, animal density and drug administration), could influence the intestinal microbiota, as already described by Bjerrum et al. (2006).
The main objective of this study was to show possible differences in the intestinal bacterial counts between organically and conventionally reared chickens using culture based methods. Furthermore, multivariate analysis was used to check for discrimination of microflora in the different rearing systems.

Material and methods

Animals, rearing systems and diets

The study was carried out on 14 poultry farms: 7 conventional (C) and 7 organic (O), with comparable management practices for each rearing system (number of animals, feed, genetic strain).

Conventional farms used standard feeding protocols (NRC, 1994), prophylactic drugs and fast-growing genetic strains (Ross 308). Chickens were housed in buildings (0.12 m²/bird) at 17.5±2.7°C, relative humidity 65-75% and a photoperiod of 16 hours light/24 hours. Chicks were vaccinated against Marek and Newcastle diseases. Slaughtering of birds was performed at 42±5 days.

On organic farms the slow growing chicks were housed in indoor pens (0.12 m²/bird) with access to a grass paddock (4 m²/bird) according to EC Regulation No. 1804/1999. Chicks were fed an organic diet ad libitum and, as required by the EC Regulation, more than 80% of ingredients were organic ingredients certified by a national agency. In addition, the organic poultry farms followed the specific rearing protocol requiring an older age at slaughter (81d) and the ban of any pharmacological treatments.

Sampling

Twenty-four caecum and ileum samplings were collected from all chickens, every 10 days, starting from 20 to 80 days of age in organic groups and from 20 to 40 days for conventional groups in order to evaluate the effect of age and farming system on intestinal microflora. Since 40 days is approximately the slaughter age of conventionally reared chickens, a higher number of samples was collected at this age.

The timing and number of the samples are reported in Table 1.

Analytical procedures

The caecum and ileum (from the duodenum and Merkel's diverticulum) of each bird were carefully removed and intestinal samples of six individual birds were pooled to obtain 4 samples of each intestinal region (ileum and caecum). A sterile stick was used to put 1 g of intestinal contents into a sterile test tube together with 2 mL 0.9% sterile saline solution. The stool was pressed and

| Days | 20 | 30 | 40 | 50 | 60 | 70 | 80 | Total |
|------|----|----|----|----|----|----|----|-------|
| Group: |    |    |    |    |    |    |    |       |
| Conventional | n | 14 | 14 | 42 |    |    |    | 70    |
| Organic | " | 14 | 14 | 42 | 14 | 14 | 14 | 21 | 133 |
| Total | " | 28 | 28 | 84 | 14 | 14 | 14 | 21 | 203 |
| Data set | | Data set 1 | | Data set 2 | | | | |

Table 1. Sampling schedule of trial (samples for each intestinal tract: ileum and caecum).
mixed in this solution and the tube was brought to volume (10 mL) with 0.9% sterile saline solution. Each pooled sample (0.1 mL) was serially diluted via 10-fold dilutions (from $10^{-1}$ to $10^{-10}$). MacConkey agar, Violet red bile agar and KF streptococcus agar were used for the enumeration of *Enterobacteriaceae*, *Streptococci* and *Enterococci*, respectively. Baird Parker agar and Mannitol salt agar were used for enumeration of *Staphylococci*.

All the plates were incubated at 37°C, aerobically, for 24-48h and the number of colonies was counted.

For the 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 blood agar supplemented with 7.5 mg/mL vancomycin and 100 mg/mL kamamycin was used as kanamycin/vancomycin blood agar. Anaerobic incubation was carried out in anaerobic jars (Oxoid) at 37°C for 48h. Anaerobic conditions were obtained using Anaerogen (Oxoid) and were checked using methyl blue strips as oxidation reduction indicator.

For the enumeration of anaerobic bacteria, Reinforced Clostridial agar was also used. The plates were incubated in anaerobic jars (Oxoid) with Anaerogen (Oxoid) at 37°C for 48h.

For the enumeration of *Lactobacilli*, Rogosa agar (Oxoid) was used. The plates were incubated for 3 days at 35°C under microaerophilic condition.

The total aerobic count was determined using Standard plate count agar medium (OXOID). The plates were incubated at 30°C, aerobically, for 24-48h.

All the data are expressed as CFUxlog/g

*Statistical analyses*

Statistical analysis was performed on two separate data sets. In the first data set only the samples from 40-day-old birds were collected (n=84 intestinal samples), whereas in the second data set, samples from all ages were analyzed (from 10 to 80 d; n=203).

Univariate and multivariate procedures (Statacorp, 2005) were used to compare C vs O systems

Univariate analysis was done with linear models taking into consideration the main effects of rearing system (fixed) and farm/flock (random). Significance of differences was assessed by the t-test.

Since the caecum showed less variability and seemed more affected by the rearing system, further multivariate analysis was applied only to the caecum.

Multivariate procedures were performed to determine the relationship among different microflora classes and to discriminate rearing systems (procedures factor and discriminant, respectively).

The effect of age on intestinal microflora was assessed in a second data set and linear regression curves were fitted and compared for each intestinal site and rearing system.

**Results and discussion**

Colony counts of bacterial classes showed differences due to the rearing system and to the farm/flock (Table 2); in the case of ileum the farm always had a significant effect.

Data related to the bacterial count from the caecum and ileum of organic and conventional chickens at 40 days are shown in Tables 3 and 4.

As expected, the different intestinal compartments showed different microfloral patterns; significant differences among single bacteria classes were due to the rearing system.

In the ileum of conventional birds *Enterobacteria* were higher than in organic birds, whereas the contrary was observed for *Lactobacilli*. For the other microflora, the effect of farm probably contributed to
masking potential differences.

In the caecum the effect of rearing system was relevant; as previously reported (Salanitro et al., 1974), Enterobacteria were more numerous in organic than in conventional chickens whereas Enterococci, Lactobacilli and total aerobia were higher in organic compared to conventional chickens.

Factor analysis of caecal samples retained 2 main factors which explain about 98% of the variability (Table 5). The first factor mainly represented the total aerobia, while the other variables had more or less quite the same weight; in the second factor, Enterobacteria (0.31) and Enterococci (-0.33) showed extremes.

Factor 1 accounted for about 89% variability while factor 2 accounted for only about 9% (data not shown). However, the uniqueness of variances was mainly represented by factor 2 (Lactobacilli, total anaerobia, Staphylococci and Enterobacteria).

The discriminant scores of conventional and organic farms are reported in Figure 1. Multivariate analyses showed that it is possible to discriminate rearing system using the microflora profile of the caecum. Correct predictions were about 96% with high sensitivity and specificity (96%). However, there were some errors (e.g. organic farms no. 23 and no. 24 were classified as conventional farms and conventional farms no. 6 were classified as organic).

Thus, the farm confirmed its importance since the same farms plotted very close. It could also be speculated from the results of genotypic characterization with T-RFLP (data not shown), that the farms have a characteristic caecal microflora sometimes not strictly dependent on the housing system.

### Table 2. Significance of effect on bacterial counts of organic and conventional reared chickens (data set 1).

| Rearing system | Farm | R²  |
|----------------|------|-----|
| Ileum:         |      |     |
| Total anaerobia| ns   | **  | 0.34 |
| Total aerobia  | ns   | *   | 0.66 |
| Enterobacteria | *    | **  | 0.62 |
| Staphylococci  | ns   | **  | 0.64 |
| Enterococci    | ns   | **  | 0.67 |
| Lactobacilli   | *    | **  | 0.44 |
| Caecum:        |      |     |
| Total anaerobia| ns   | **  | 0.35 |
| Total aerobia  | **   | ns  | 0.52 |
| Enterobacteria | **   | ns  | 0.49 |
| Staphylococci  | *    | **  | 0.60 |
| Enterococci    | *    | **  | 0.67 |
| Lactobacilli   | *    | **  | 0.60 |

N=84 observations for each group; *: P<0.05; **: P<0.01; ns: not significant.
Table 3. Bacterial counts from the ileum intestinal tracts of organic and conventional reared chickens (CFUxlog/g).

| Housing system | Conventional | Organic | SEM |
|----------------|--------------|---------|-----|
| Total anaerobia| 7.68         | 7.36    | 0.44|
| Total aerobia  | 7.51         | 7.43    | 0.50|
| Enterobacteria | 7.03<sup>a</sup> | 6.09<sup>a</sup> | 0.58|
| Staphylococci | 5.11         | 4.77    | 0.32|
| Enterococci    | 6.98         | 6.98    | 0.48|
| Lactobacilli   | 6.75<sup>a</sup> | 7.07<sup>b</sup> | 0.40|

N=84 observations for each group; a, b: P<0.05.

Table 4. Bacterial counts from the caecum intestinal tracts of organic and conventional reared chickens (CFUxlog/g).

| Housing system | Conventional | Organic | SEM |
|----------------|--------------|---------|-----|
| Total anaerobia| 8.22         | 8.26    | 0.26|
| Total aerobia  | 7.66<sup>A</sup> | 8.12<sup>B</sup> | 0.24|
| Enterobacteria | 7.42<sup>B</sup> | 7.05<sup>A</sup> | 0.12|
| Staphylococci | 5.78         | 5.52    | 0.34|
| Enterococci    | 6.55<sup>a</sup> | 7.65<sup>b</sup> | 0.41|
| Lactobacilli   | 7.31<sup>a</sup> | 7.85<sup>b</sup> | 0.28|

N=84 observations for each group; a, b: P<0.05; A, B: P<0.01.

Table 5. Factor loadings and uniqueness of variances explained.

| Variables      | Factor1 | Factor2 | Uniqueness |
|----------------|---------|---------|------------|
| Total anaerobia| 0.49    | 0.16    | 0.68       |
| Total aerobia  | 0.93    | -0.01   | 0.12       |
| Enterobacteria | 0.55    | 0.31    | 0.57       |
| Staphylococci | 0.55    | 0.05    | 0.64       |
| Enterococci    | 0.60    | -0.33   | 0.50       |
| Lactobacilli   | 0.48    | -0.14   | 0.68       |
Intestinal microflora of chickens

The discriminant coefficients of the microflora component (Table 6) confirmed that the main factors of discrimination were the total aerobic count, Enterobacteria and Enterococci with a good robustness of estimation ($R^2=0.75$, Canonical Correlation=0.80 $X^2=65.53**$ data not shown).

Data related to the bacterial count from the ileum and caecum of organic and conventional chickens at different ages are shown in Figures 2 and 3.

In the ileum of conventional birds, Enterobacteria, Enterococci and total aerobia increased with age, while Lactobacilli decreased; age did not affect the trend of Staphylococci and total anaerobia (Table 7).

In the organic group, only Enterobacteria, Lactobacilli and total anaerobia showed a trend similar to that observed in the conventional group, whereas total aerobia and Enterococci showed the opposite trend. The age-related trend of Staphylococci and total aerobia was not affected by the age.

In the caecum a similar situation was observed: in conventional birds, Enterobacteria, and total anaerobia increased with age, while Lactobacilli, Enterococci, Staphylococci and total aerobia did not show an age-related trend (Table 7).

In the organic group, the starting values were generally higher and only Enterobacteria and total anaerobia showed a trend similar to that of the conventional group, while Enterococci and Lactobacilli showed an opposite, decreasing trend.

It should be noted that in this trial the bacterial count was assessed using a culture-based method and thus only the main bacterial families were determined. These methods are extremely laborious and a low...
bacterial recovery is obtained, due to the fact that the growth requirements are still not well defined and cannot be reproduced in laboratory conditions. Many authors have reported that at least 80% of the bacteria in a given niche are estimated to be missed by traditional culture techniques (Torsvik et al., 1990; Ward et al., 1990; Schabereiter-Gurtner et al., 2001). Molecular techniques, less selective than culture methods, have been used to profile complex microbial communities (Ward et al., 1990; Muyzer et al., 1993; Apajalahti et al., 1998, 2001; Holben et al., 1998; Favier et al., 2002; Zhu et al., 2002). However, molecular methods for bacterial community analysis are generally qualitative and require the joint use of conventional methods or elaborative molecular methods (e.g. Real-time multiplex PCR).

In our case the main objective of the work was to determine the main bacterial populations in organic and conventionally reared chickens, considering only some bacterial groups, easy to cultivate. The literature available on the intestinal bacteria of organic chickens is poor and thus, it is very

| Variable           | Enterobacteria | Staphylococci | Enterococci | Lactobacilli | Total aerobia | Total anaerobia | Constant |
|--------------------|----------------|---------------|-------------|--------------|---------------|----------------|----------|
| Enterobacteria     | 5.53           | 1.30          | -1.52       | -0.86        | -6.41         | 0.67           | 5.14     |

Table 6. Discriminant function coefficients of caecal microflora.

Table 7. Coefficient of determination ($R^2$) of linear regression and age effect on bacterial counts of organic and conventional reared chickens (data set 2).

|                | Conventional | Organic | Age | $R^2$ | Age | $R^2$ |
|----------------|--------------|---------|-----|-------|-----|-------|
| Ileum:         |              |         | Age |       |     |       |
| Total anaerobia| ns           | 0.02    | *   | 0.15  | ns  | 0.07  |
| Total aerobia  | *            | 0.12    | ns  | 0.09  | ns  | 0.07  |
| Enterobacteria | *            | 0.12    | **  | 0.19  |     |       |
| Staphylococci  | ns           | 0.09    |     | 0.10  |     |       |
| Enterococci    | *            | 0.12    | **  | 0.24  |     |       |
| Lactobacilli   | *            | 0.11    | **  | 0.37  |     |       |
| Caecum:        |              |         |     |       |     |       |
| Total anaerobia| **           | 0.25    | **  | 0.20  |     |       |
| Total aerobia  | ns           | 0.06    | *   | 0.11  |     |       |
| Enterobacteria | **           | 0.15    | *   | 0.12  |     |       |
| Staphylococci  | ns           | 0.06    | ns  | 0.03  |     |       |
| Enterococci    | ns           | 0.02    | *   | 0.12  |     |       |
| Lactobacilli   | ns           | 0.02    | **  | 0.14  |     |       |

$N = 203$ observations for each group; *: $P<0.05$; **: $P<0.01$; ns: not significant.
Figure 2. Age-related trend of ileal microflora in conventional (C) and organic (O) chickens (fitted values and 95% confidence interval; n = 203).
Figure 3. Age-related trend of caecal microflora in conventional (C) and organic (O) chickens (fitted values and 95% confidence interval; n= 203).
difficult to make comparisons and formulate conclusive results.

In conventional farms the aerobic flora counts were lower than the anaerobic flora counts, above all in the caecum, as already reported by other authors (Barnes and Impey 1972; Barnes et al., 1972; Lu et al., 2003). Despite values of some facultative anaerobic bacteria, such as Lactobacilli and Enterococci, usually more common in the ileum, they were higher in the caecum of both organic and conventional chickens.

Counts of anaerobic bacteria confirmed previous findings (Salanitro et al., 1974); indeed they were higher in the final intestinal tracts of both groups.

Surprisingly, the results showed that the number of the total aerobic bacteria in the caecum of organic chickens were higher than that detected in the ileum; generally aerobic bacteria are more represented in the ileum of younger chickens (Lu et al., 2003). This value decreased with age to the benefit of the anaerobic population. The same trend was observed for Enterobacteria and Enterococci.

The counts of Lactobacilli in both intestinal tracts of organic chickens were higher than the conventional chickens. It is known that Lactobacilli are among the bacterial species that might play an important role in the digestive process of the host, particularly in stimulating the immune response in the gut and thus protecting against infectious intestinal diseases (Meslin et al., 1999; Mead, 2000).

The diet and the environment are known to be factors influencing the microbial status of the gastrointestinal tract; indeed, dirty litter can affect the microbial composition of the chickens’ intestinal tract, both directly by providing a continuous source and indirectly by influencing the immune defense of the birds (Beasley et al., 2004).

In the case of organic farming, it could be argued that open space and the bannning of drugs is beneficial to the immune system of the animals and might play a role in determining the composition of the intestinal microflora.

It should be noted that the difficulties in collecting and comparing data were numerous, due to the fact that the investigations were performed directly on the farm where rearing conditions could vary. Nevertheless, our results may provide new aspects of organic farming considering the intestinal flora that may be beneficial for new strategies in both organic and conventional production systems.

Conclusions

Although our data are not sufficient to draw conclusive results, some main points can be underlined:

1. the rearing system affects the microfloral profile of the intestines of chickens and the caecum seems more sensitive to such modifications;
2. within the same system the farm largely influences the microfloral profile;
3. discrimination of rearing system is possible only on the basis of a multivariate index which estimates not a single bacteria but a microflora combination;
4. the age-related trend of the microflora is distinct for organic and conventional birds.

Further investigations are needed to elucidate the effects of environmental factors and the role of such changes on the health status of chickens. The use of a molecular approach in association with culture-based methods would permit further characterization of the intestinal microbiota of organically reared chickens.

Research funded by PoutryFlorGut project task 4.6.
REFERENCES

Apajalahti, J.H.A., Ketunen, A., Bedford, M.R., Holben., W.E., 2001. Percent G+C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. Appl. Environ. Microbiol. 67:5656-5667.

Apajalahti, J.H.A., Sarkilahti, L.K., Maki, B.R.E., Heikkinen, J.P., Nurminen, P.H., Holben, W.E., 1998. Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. Appl. Environ. Microbiol. 64:4084-4088.

Barnes, E.M., 1979. The intestinal microflora of poultry and game birds during life and after storage. J. Appl. Bacteriol. 46:407-419.

Barnes, E.M., Impey, C.S., 1972. Some properties of the nonsporing anaerobes from poultry caeca. J. Appl. Bacteriol. 35:241-251.

Barnes, E.M., Mead, G.C., Barnum, D.A., Harry, E.G., 1972. The intestinal flora of the chickens in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Brit. Poultry Sci. 13:311-326.

Beasley, S.S., Takala, T.M., Reunanen, J., Apajalahti, J., Saris, P.E., 2004. Characterization and electrotransformation of Lactobacillus crispatus isolated from chicken crop and intestine. Poultry Sci. 83:45-48.

Binek, M., Borzemska, W., Pisarski, R., Blaszczak, B., Kosowska, G., Malec, H., Karpinska, E., 2000. Evaluation of the efficacy of feed providing on development of gastrointestinal microflora of newly hatched broiler chickens. Arch. Geflugelkd. 64:147-151.

Bjerrum, L., Engberg, R.M., Leser, T.D., Jensen, B.B., Finster, K., Pedersen, K., 2006. Microbial community composition of the ileum and caecum of broiler chickens as revealed by molecular and culture-based techniques. Poultry Sci. 85:1151-1164.

European Commission, 1999. Council Regulation 1804/1999 of July 1999 supplementing Regulation (EEC) 2092/91 on organic production of agricultural products. In: Official Journal, L 222, 24/08/1999, pp 1-28.

Favier, C.F., Vaughn, E.E., De Voe, W.M., Akkermans, D.L., 2002. Molecular monitoring of succession of bacterial communities in human neonates. Appl. Environ. Microbiol. 68:219-226.

Gong, J.R., Forster, H.Y., Chambers, J.R., Sabour, P.M., Wheatcroft, R., Chen, S., 2002. Diversity and phylogenetic analysis of bacteria in the cecal lumen. FEMS Microbiol. Lett. 208:1-7.

Holben, W.E., Noto, K., Sumino, T., Suwa, Y., 1998. Molecular analysis of bacterial communities in a three-compartment granular activated sludge system indicates community-level control by incompatible nitrification processes. Appl. Environ. Microbiol. 64:2528-2532.

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of intestinal bacterial community of the maturing broiler chickens. Appl. Environ. Microbiol. 69:6816-6824.

Mead, G.C., 1989. Microbes of the avian caecum: types present and substrates utilized. J. Exp. Zool. 3:48-54.

Mead, G.C., 2000. Prospects for competitive exclusion treatment to control salmonellas and other foodborne pathogens in poultry. Vet. J. 159:111-123.

Mead, G.C., Adams, B.W., 1975. Some observations on the caecal microflora on the chick during the first two weeks of life. Brit. Poultry Sci. 16:169-176.

Meslin, J.C., Fontaine, H., Andrieux, C., 1999. Variation of mucin distribution in the rat intestine, caecum and colon: effect of the bacterial flora. Comp. Biochem. Physiol. A 123:235-239.

Muyzer, G., De Waal, E.C., Litterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase-chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC, USA.
Intestinal microflora of chickens

Netherwood, T., Gilbert, H.J., Parker, D.S., O’Donnell, A.G., 1999. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Appl. Environ. Microbiol. 65:5134–5138.

Rolfe, R.D., 2000. The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130 (Suppl.):396-402.

Salanitro, J., Fairchilds, P., Zgornicki, G.T., 1974. Isolation, culture characteristics and identification of anaerobic bacteria from the chicken caecum. Appl. Microbiol. 27:678-687.

Schabereiter-Gurtner, C., Maca, S., Rolleke, S., Nigl, K., Lukas, J., Hirschl, A., Lubitz, W., Barisani-Asenbauer, T., 2001. 16S rDNA-based identification of bacteria from conjunctival swabs by PCR and DGGE fingerprinting. Invest. Ophthalmol. Vis. Sci. 42:1164-1171.

StataCorp, 2005. Stata Statistical Software: Release 9.0. College Station, TX, USA.

Torsvik, V., Gokso, J., Daae, F.L., 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56:782-787.

Ward, D.M., Weller, R., Bateson, M.M., 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature. 345:63–65.

Zhu, X.Y., Zhong, T., Pandya, Y., Joeger, R.D., 2002. 16S rRNA-based analysis of microbiota from the caecum of broiler chickens. Appl. Environ. Microbiol. 68:124-137.