Xenobiotics

Soil, Food and Human Health Interactions

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Contents

Introduction 7

Chapter 1 Soil contamination and food quality 9

Contents of selected risky elements in agricultural soil from various environmentally loaded areas in Slovakia 11

Effect of different doses digested sludge from agricultural by-products on microbial biomass carbon and dynamics of carbon mineralization in Haplic Luvisols 18

Trace elements content of organic and conventional juices. Some plant and soil issues..... 33

Chapter 2 Food processing and its quality 49

Occurrence of plant protection product residues in apples in 2008-2010.......................... 51

Effect of order of sheep entry into milking parlour on udder health and milk composition 61

Fusarium spp. occurrence and mycotoxins content in grain of non-transgenic maize cultivars and their genetically modified (Bt) counterparts in the years 2005 and 2007 in different parts of Poland 70

Effect of sanitation on microbiological safety of milk products.. 88

Storage effect on physicochemical and antioxidant properties of honey from the Southern Poland 100

Examination of storage life as a function of heat treatment methods. 112

Examination of grinding operation in the food chain 123

Chapter 3 New methods in healthy food production 133

Linuron contaminated water detoxification by ozonolysis and Fenton reaction................. 135

Remediation of imidacloprid contaminated soil - comparison of two different reactors for the ozone treatment... 147

Oxidative stability of meat chickens Ross 308 after application of propolis extract in their nutrition.. 159

Lactobacillus genus identification isolated from gastrointestinal tract of chickens after application of propolis using FISH and RTQ PCR methods 169

Meat performance of chickens after application of bee pollen in their diets 181

Enterococcus species identification by RTQ PCR isolated from gastrointestinal tract of chickens after bee pollen application to feed mixtures 191

Effect of bee pollen used in the broiler feed mixtures to the selected quality indicators of cooked ham..202

Can be improve the immune response with apple cider vinegar in chicken? 211

Mycological profile of new crossbred wheat and its mill fractions....................................217

Effect of feeding grains naturally contaminated with *Fusarium* mycotoxins with and without an organic mycotoxin adsorbent on blood biochemistry of broiler chickens 226

Utilization of lutein and lycopene from fortified egg yolk powder in mice 235

Chapter 4 Food and human health 243

Encapsulated forms of biologically active substances – possible risks of their use 245

Agave fructans`s and/or calcium influence on the changes of selected biomechanical properties of the shaft right femur in laboratory rats, depending on their level in the experimental diet of animals tested..255

Microbiological, antimicrobial and physicochemical properties of commercial honeys 267

Antioxidants in therapy and prevention of diabetes mellitus 276

Benefits and risks of cereal products enriched with functional ingredients 292

Benefits and risks of apple juices enriched with herbal extracts 304

Benefits and risks of raw materials and fermented beverages containing functional compounds..313

Potential production of roquefortine C and PR toxin by *Penicillium roqueforti* strains isolated from blue type cheese 323

The content of biogenic metals in healthy tissues of esophagus, stomach small intestine and large intestine of human..333

Superoxide dismutase activity and essential elements levels in breast milk in early stages of lactation...341

In vitro antibacterial activity of thyme essential oil against enterococci 350

Author index 359

Enterococcus species identification by RTQ PCR isolated from gastrointestinal tract of chickens after bee pollen application to feed mixtures

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Abstract

The aim of this study was to examine the effect of pollen on the Enterococci colonization of chickens. Pollen was administered to both feed mixtures in various amounts except of the control group. The first experimental group was with the addition of 400 mg pollen to 1 kg of compound, the second experimental group was with the addition of 800 mg pollen to 1 kg of compound. Quantitative counts of Enterococci in ceca of 49-day-old chicken (Ross 308) using classical method and qualitative identification using RTQ PCR method were investigated. Counts of Enterococci on Slanetz-Bartley agar were monitored. Counts of Enterococci in CFU were compared in experimental and control treatments, respectively. The highest count of feacal Enterococci was found in the group in which 400 mg of pollen to 1 kg was added. The lowest count of faecal Enterococci was found in the control group. Statistical significant differences were not found among groups. Using Real-time PCR method was identified the species range of the genera *Enterococcus* in the intestinal tract of broilers. Detected species from the genus *Enterococcus* in the gastrointestinal tract of chickens were: *E. avium, E. casseliflavus, E cecorum, E. faecalis, E. faecium, E. gallinarum, E. hirae* and *E. malodoratus*. In the experimental groups (caecal samples) were found the most frequent species of *E. avium E. faecium* and *E. gallinarum*.

Keywords: Enterococci, GIT, chickens, bee pollen, RTQ PCR

Introduction

Studies on chicken caecal microflora, by both culture-based (Wise and Siragusa, 2007) and culture-independent (Gong et al., 2002; Zhu et al., 2002; Lu et al., 2003) methods, have indicated that this environment is dominated by obligate anaerobes, but a diverse range of species have been detected. The traditional culture-based methods of assessing mammalian gastrointestinal tract community

structure are extremely laborious, and it has been estimated that only 10–60% of total bacteria from this environment are able to be cultured (Wise and Siragusa, 2007). Nonculture methods for assessing gut microbial ecology (Zoetendal et al., 2004), such as the construction and analysis of 16S rDNA clone libraries (Gong et al., 2002; Zhu et al., 2002; Lu et al., 2003), for example, have been instrumental in the discovery of new intestinal bacterial groups. Molecular indices of diversity, such as the community fingerprinting tools DGGE (Knarreborg et al., 2002; Van der Wielen et al., 2002), T-RFLP (Gong et al. 2002) and %G + C profiling (Apajalahti et al., 2001), have also provided insight into chicken gut microbial ecology. Although these procedures have proved useful for detecting community structure shifts, with the exception of fluorescent *in situ* hybridization-based studies (Zhu and Joerger, 2003), they have the drawback that they are typically not quantitative. Real-time PCR, on the contrary, can be quantitative as the number of target gene copies in DNA directly extracted from an environmental sample can be determined. Using group-specific primer sets, the abundance of a particular gene marker for a defined group in the community can be estimated by comparison to a standard curve.

The history of the enterococci began when Thiercelin (1899) first used the term to indicate the intestina origin of a Gram-positive diplococcus. The new genus *Enterococcus* was proposed by Thiercelin and Jouhaud (1903). Later on, Andrewes and Horder (1906) renamed Thiercelin's "entérocoque'' as *Streptococcus faecalis*. It was assumed that the strain, isolated from a patient with endocarditis, originated from the human intestine.

Organisms belonging to the genus *Enterococcus* are ubiquitous in nature, being found in very diverse environmental habitats as well as animal, bird, and invertebrate hosts. Currently five groupings, comprising sixteen different species are recognized. Clinically, the majority of human infections associated with enterococci are due to *E. faecalis* and to a lesser extent *E. faecium*. Other enterococcal species are less frequently, if at all, isolated clinically (McMurphy et al., 2010).

Pollen is a fine, powder-like material produced by flowering plants and gathered by bees considered as a valuable special food with varied enhancing effects on health. This beehive product also has several useful pharmacological properties, such as antibiotic, antineoplastic, antidiarrhoeatic and with nutritional composition, antioxidant and antiradical activity. Pollen contains nutritional compounds such as carbohydrates, proteins, amino acids, lipids, vitamins, minerals and traces of micronutrients. In addition, pollen contains significant amounts of polyphenolic substances, mainly flavonoids (Kolesárová et al., 2011).

The purpose of this study was to compare the *Enterococcus* species in gut microflora of broiler chickens in control group against experimental groups with the additions of pollen. In this study for enumeration and identification of enterococci were used classical method and real time polymerase chain reaction.

Material and Methods

In this experiment, quantitative counts of individual groups of microorganisms in caecum of 49-day-old chicken were investigated. The trial was carried out on an experimental basis at the Department of Poultry and Small Farm Animals at the Slovak Agricultural University in Nitra. The experiment was realized in three-etage cage from the company SALMET. Cage technology was divided into 3 parts: each cage (11 pcs of chickens), i. e. one group of experiments (3 cages), i.e. a total of 33 chickens. Each cage had parameters 70x100 cm.

Monitoring of the impact of extract pollen applied as a feed additive into the feed mixture was realized in half-operating conditions in the experimental operation. Fattening itself went from 1 to 49 days of chicken age. One-day-old chickens of Ross 308 breed were randomly distributed in 3 groups. Chickens were fed *ad libitum* with standard mixture in two phases of feeding:

HYD-01 starter (powder mixture) Norm-type within 21 days of feeding
HYD-02 growth (powder mixture) Norm-type from 21st day of feeding to the end of feeding (42 days)
Bee pollen was extracted with ethanol (80%), under reflux condenser at 80°C during one hour. After chilling the mixture was centrifuged and supernatant was evaporated in the vacuum rotary evaporator at temperatures 40-45°C. The evaporation residue was dissolved. Residue of bee pollen was applied to feed mixture.

Dosing of feed additives

Control group: the feed mixture without the addition of pollen.

1st Experimental group: feed mixture with the addition of 400 mg pollen to 1 kg of compound,

2nd Experimental group: feed mixture with the addition of 800 mg pollen to 1 kg of compound.

Plate diluting method

Determination of CFU counts: Plate diluting method was applied for quantitative CFU counts determination of respective groups of microorganisms in 1 g of substrate.

Gelatinous nutritive substrate in Petri dishes was inoculated with 1 ml of chyme samples by plate method on surface (faecal Enterococci) in three replications. Homogenized samples of faecal chyme (chyme was taken to sterile Petri dishes) were prepared in advance by sequential diluting based on decimal dilution system application. Counts of Enterococci on Slanetz-Bartley agar were monitored. Isolated species, genera and groups of microorganisms and their fundamental identification signs [Holt et al, 1994] were observed.

Bacterial Strains and DNA Extraction

For isolation of DNA growth colonies of bacteria were used our obtained isolates of individual samples in pure culture. Before DNA isolation of Gram-positive bacteria was prepared in peptone water the following composition: peptone 10 g, NaCl 5.0 g, distilled water 1000.0 ml. Peptone and NaCl in hot water were dissolved, filtered and pH adjusted to 7.2 to 7.8, as appropriate and then sterilized in an autoclave at 0.1 MPa for 20 minutes. For isolation GenElue TM Bacterial Genomic DNA Kit (Sigma Aldrich, St. Louis, USA) were used:

G^+ bacteria: 1.5 ml of 24 hours bacterial culture was centrifuged 2 min/12.000 to 16.000 g. The supernatants were removed, the pellet was dissolved in 200 μl lysis solution and 30 min / 37 °C were incubated, 20 μl proteinase K were added and 30 min/55° C were incubated, then 200 μl of lysis solution C was added, about 15 s vortex mixed and at 55° C incubated for 10 min. We added 500 μl Column Prep. Solution to each GenMiniprep Bindinb Colum, about 12000 g centrifuged for 1 min 200 μl of ethanol (95-100%) were added in the lysate and vortex mixed 5-10 sec. then about 6500 g centrifuged for 1 min. The eluates were removed, 500 μl washing buffer was added, then centrifuged at maximum speed untill drying of membrane and then transferred to a new Eppendorf tube, 200 μl of elution solution directly to the center of the membrane was added, then centrifuged for 1 min. at 6500 g.

The types of primers used were designed by Desay et al. (2000), species identification of enterococci used is showed in the Table 1.

Table 1. Characteristics of primers used for enterococci identification

species	primer	sequence (5'- 3')
E. avium	AV1	GCTGCGATTGAAAATATCCG
	AV2	AAGCCAATGATCGGTGTTTT
E. caseliflavus	CA1	TCCTGAATTAGGTGAAAAAC
	CA2	GCTAGTTTACCGTCTTTAACG
E. cecorum	CE1	AAACATCATAAACCTATTTA
	CE2	AATGGTGAATCTTGGTTCGCA
E. gallinarum	GA1	TTACTTGCTGATTTGATTCG
	GA2	TGAATTCTTCTTTGAATCAG
E. faecium	FL1	TAGAGACATTGAATATGCC
	FL2	TCGAAATGTGCTACAATC
E. faecalis	FM1	ATCAAGTACAGTTAGTTCT
	FM2	ACGATTCAAGCTAACTG
E. hirae	HI1	CTTCTGATATGGATGCTGTC
	HI2	TAATTCTTCCTTAAATGTTG
E. malodoratus	MA1	GTAACGAACTTGAATGAAGT
	MA2	TTGATCGCACCTGTTGGTTT

Real-time PCR

Data were collected during each elongation step. PCR products were detected by monitoring the increase in fluorescence of the reporter dye at each PCR cycle. Applied Biosystems software plots the normalized reporter signal, ΔRn, (reporter signal minus background) against the number of amplification cycles and also determines the threshold cycle (Ct) value i.e. the PCR cycle number at which fluorescence increases above a defined threshold level were used. Components of PCR reactions are showed in the Table 2.

Table 2. Components of PCR reaction

Components	Quantity [20 μl]
Master mix	10.00
Primer F	0.20
Primer R	0.20
Sample	2.00
PCR water	7.60

The basic statistical values and P value, were evaluated by STATGRAPHIC software.

Results and Discussion

The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining of animal health. Disease and stress induced changes in the physicochemical environment in the gastrointestinal tract, or simple changes in feed management practices can significantly affect the microbial populations and their effects on animal performance and health. In the last five decades, increased knowledge of the factors that influence the activities of microorganisms in the alimentary tract has helped to define the critical role of these symbiotic organisms (Kačániová et al., 2006).

The application of bee pollen affected faecal Enterococci of chickens, see the table 3. In the trial with chickens after application of bee pollen, no statistically significant differences were found. The number of enterococci count in the control group ranged from 6.78 to 6.95 $CFU.g^{-1}$. In the first group with 400 mg per 1 kg of feed mixtures enterococci number ranged from 7.69 to 7.97 $CFU.g^{-1}$ and in the second group with 800 mg pre 1 kg of feed mixtures enterococci number ranged from 7.56 to 7.97 $CFU.g^{-1}$. The highest count of feacal Enterococci was found in the group where 400 mg of pollen to 1 kg was added to feed mixture. The lower count of faecal Enterococci was found in the control group.

SB agar (Slanetz and Bartley, 1957) also known as M-enterococcus agar has been widely used for the isolation, cultivation and enumeration of enterococci from water, sewage and faeces, in combination with the membrane filter method. Samples can be directly plated onto the medium in order to detect and enumerace faecal streptococci (Anonymous, 1992; Atlas, 1995).

Table 3. Summary statistical values for *Enterococcus* spp.

Values/Groups	K	P1	P2
Average	6.87	7.85	7.74
Standard deviation	0.09	0.14	0.21
Coefficient of variation (%)	1.24	1.84	2.72
Minimum	6.78	7.69	7.56
Maximum	6.95	7.97	7.97

Using Real-time PCR method, we identified the species range of the genera *Enterococcus* in the intestinal tract of broilers. Detected species from the genus *Enterococcus* were: *E. avium, E. casseliflavus, E cecorum, E. faecalis, E. faecium, E. gallinarum, E. hirae* and *E. malodoratus*. In the experimental groups (caecal samples) were the most frequent species of *E. avium E. faecium* and *E. gallinarum*.

In the study of King et al. (2009) *Enterococcus faecalis* were the most frequently observed protease-secreting bacterial species isolated from 28 of 82 chickens (34%), followed by *Enterococcus gallinarum* 26 of 82 (32%), and *Proteus mirabilis* 20 of 82 (24%).

Enterococci are commonly found in poultry environments and are considered as normal microflora of the intestinal tract of poultry [Wages, 2003].

In the study of Nováková et al. (2010) the major species isolates of *Enterococcus* genus in various GIT segments of broilers were observed. Four enterococcal species: *E. faecium, E. faecalis, E. gallinarum* and *E. cecorum* were isolated from broilers at the end of feeding. The PCR method using *sodA* gene which catalyzes the dismutation of superoxide showed that in some cases distinct bands were not evident for *E. cecorum* and *E. gallinarum*. On the other hand, a distinct DNA band for *E. faecium and E. faecalis* was observed. These organisms covered 100% of the total isolated enterococci. *E. gallinarum* was the most frequently identified *Enterococcus* spp. (87.5%) especially in ceaces. They found similar isolated species as was described in our study.

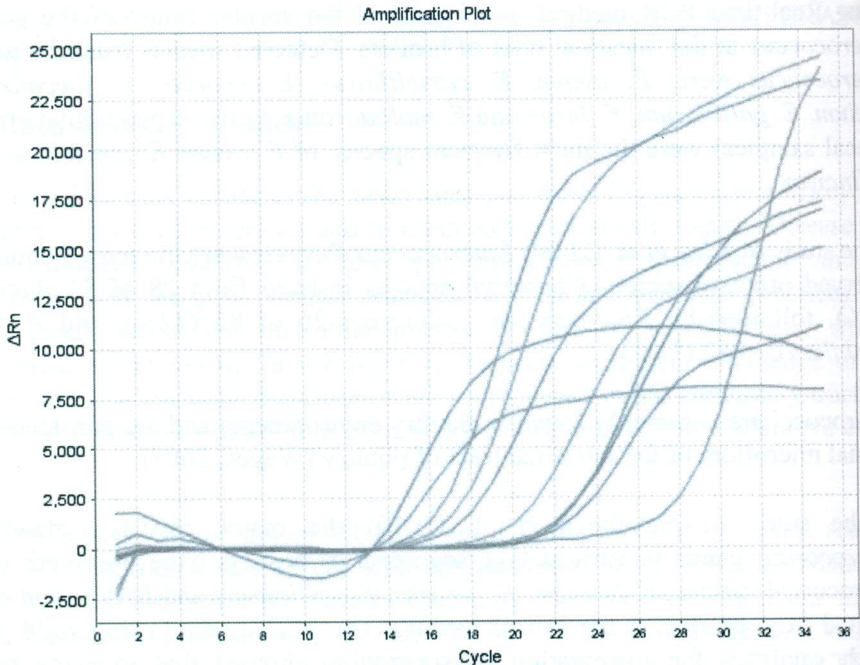

Figure 1. Evaluation of RT-PCR in cells of *Enterococcus faecium*

We monitored in the individual isolates representation of species *E. avium,* which captured RTQ PCR sensitivity ranged from 1 to 10^5 CFU.ml^{-1} and identification of the species *E. casseliflavus* in each of isolates by RTQ PCR method, we found that the presence of these bacteria were in the range of 1 to 10^4 CFU.ml^{-1}.

Enterococcus faecium was detected in all tested samples (Figure 1). The threshold value was 1282.62 by *E. faecium* samples. The (Ct) value of positive samples was on average 33.44 whereby the lowest value of positive samples was found at 17.39 and the highest value was at 39.32. In our samples we could detect strain of *E. gallinarum* in seven out of ten samples. The threshold value was 822.72 by *E. gallinarum* samples. The (Ct) value of positive samples was on average 53.53 whereby the lowest value of positive samples was found at 29.16 and the highest value was at 57.61. In our samples we could detect strain of *E. cecorum* in six out of ten samples. The threshold value was 11.76 by *E. cecorum* samples. The (Ct) value of positive samples was on average 18.35 whereby the highest value was at 24.25 and the lowest value of positive samples was found at 11.16.

Thus, the PCR-based detection of bacteria depends on the efficiency of DNA extraction procedure used to prepare the template DNA. We could detect strain of *E. faecalis* in all tested samples. The threshold value was 1325 by *E. faecalis* samples. The (Ct) value of positive samples was on average 18.73 whereby the lowest value of positive samples was found at 13.83 and the highest value was at 27.68.

Several previous molecular-based studies on the poultry microbiota environment have employed the clone survey approach. In this method, universal or domain Bacteria-specific primers are used with directly extracted DNA to amplify sequences coding for small subunit rRNA genes. Products are then cloned and sequenced to construct an inventory of 16S rDNAs that can be compared with extensive databases. Clone surveys have substantially increased our knowledge of the gut microbiota of the chicken by identifying the presence of not-yet-cultured groups of bacteria; however, because of known biases regarding primer annealing to mixed templates (Suzuki and Giovannoni 1996), these data cannot be strictly considered quantitatively. The clone survey approach, does identify the major sequence types present in this environment. Indeed data from these earlier studies provided us with the knowledge on which to base our group-specific primer choices.

Conclusion

Today, a variety of methods is used for the examination of enterococci in diverse materials. However, enterococcal contaminants cannot be distinguished from the *Enterococcus* strains using culture methods. For this purpose, further examination based on the application of phenotypic and genotypic methods are necessary. The highest count of feacal Enterococci was found in the group where 400 mg of pollen to 1 kg was added to feed mixture. Using Real-time PCR method, we identified the species range of the genera *Enterococcus* in the intestinal tract of broilers. Detected species from the genus *Enterococcus* in the gastrointestinal tract of chickens were: *E. avium, E. casseliflavus, E cecorum, E. faecalis, E. faecium, E. gallinarum, E. hirae* and *E. malodoratus*. In the experimental groups (caecal samples) were found the most frequent species of *E. avium E. faecium* and *E. gallinarum*. In this experiment we did not find any statistical significant differences between experimental (with pollen addition) and control group.

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References

Andrewes F.W., Horder T.J., 1906. A study of the streptococci pathogenic for man. *Lancet*, 2: 708-713.

Anonymous, 1992. *Enterococcus group*. In Greenberg, A.E., Clesceri, L.S., Eaton, A.D., Franson, M.A.H. (Eds.), Standard methods for the examination of water and wastewater. 18th Edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, USA, pp. 69-73.

Apajalahti J.H., Kettunen 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.

Atlas R.M., 1995. *Handbook of Microbiological Media for the Examination of Food*. CRC Press, Boca Raton, FL, USA.

Desai P.J., Pandit D., Mathur M., Gogate A., 2001. Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. *Ind. J. Med. Microbiol.,* 19(3): 132-137.

Gong J., Forster R.J., Yu H., Chambers J.R., Sabour P.M., Wheatcroft R., Chen S., 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken caeca and comparison with bacteria in the caecal lumen. *FEMS Microbiol. Lett.*, 208: 1-7.

Holt G.J., Krieg N.R., Sneath P.H.A., 1994. *Bergey 's Manual of Determinative Bacteriology*. Baltimore: Williams and Wilkins, 787. ISBN 0-683-00603-7.

Kačániová M., Kmeť V., Čuboň J., 2006. Effect of *Enterococcus faecium* the Digestive Tract of Poultry as a Probiotic. *Turk. J. Vet. Anim. Sci.*, 30: 291-298.

King M.D., Guentzel M.N., Arulanandam B.P., Lupiani B., Chambers J.P., 2009. Proteolytic bacteria in the lower digestive tract of poultry may affect avian influenza virus pathogenicity. *Poult. Sci.* 88: 1388-1393.

Knarreborg A., Simon M.A., Engberg R.M., Jensen B.B., Tannock G.W. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. *Appl. Environ. Microbiol.,* 68; 5918-5924.

Kolesarova A, Capcarova M, Bakova Z, Galik B, Juracek M, Simko M, Sirotkin A.V. The effect of bee pollen on secretion activity, markers of proliferation and apoptosis of porcine ovarian granulosa cells *in vitro*. *J. Environ. Sci. Health B,* 63(3): 207-212.

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

McMurphy M.A., Applegate R.D., Crupper S.S., 2010. *Enterococcus* Species Isolated from Northern Bobwhite *{Colinus virginianus)* Chicks: Species Distribution and Antibiotic Resistance. *Int. J. Poul. Scie.*, 9(1): 43-47.

Nováková I., Kačániová M., 2010. Identification of *Enterococcus* sp. in GIT of Broiler Chickens after Application of Biological Preparations. *Anim. Scie. Biotechnol.,* 43(1): 304-306.

Suzuki M.T., Giovannoni S.J., 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol*., 62: 625-630.

Thiercelin M.E., 1899. Sur un diplocoque saprophyte de l'intestin susceptible de devenir pathogene. C.R. *Seances Soc. Biol*., 5: 269-271.

Thiercelin M.E., Jouhaud L., 1903. Reproduction de l'enterocoque; taches centrales; granulations peripheriques et microblastes. *Seances Soc. Biol*., 55: 686-688.

Van Der Wielen P.W., Keuzenkamp D.A., Lipman L.J., Van Knapen F., Biesterveld S., 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microb. Ecol.,* 44: 286-293.

Wages D.P., 2003. *Enterococcosis. In Diseases of Poultry*. Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald and D. Swayne, (eds.). Iowa State Press, Ames., pp. 802-812.

Wise M.G., Siragusa G.R., 2007. Quantitative analysis of the intestinal bacterial community in one- to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. *J. Appl. Microbiol.,* 102(4): 1138–1149.

Zhu X.Y., Joerger R.D., 2003. Composition of microbiota in content and mucus from cecae of broiler chickens as measured by fluorescent in situ hybridization with group-specific, 16S rRNA-targeted oligonucleotide probes. *Poult. Sci.,* 82: 1242-1249.

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

Zoetendal E.G., Collier C.T., Koike S., Mackie R.I., Gaskins H.R., 2004. Molecular ecological analysis of the gastrointestinal microbiota: a review. *J Nutr*., 134: 465-472.