HIGHLIGHTING OF SOME COMMENSAL BACTERIA, POTENTIALLY PATHOGENIC, FROM BROILER CHICKEN CECUM IN WHICH FEED WERE USED FEED ADDITIVES

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Abstract

Potentially pathogenic commensal bacteria in the digestive tract of chickens can promote chicken discomfort leading ultimately to reduce economic efficiency and possible contamination of those carcasses. To influence the micro flora composition at this level, we used feed additive as pre/probiotics, acidifiers and herbal extracts. Analyses were performed by classical methods according to SR EN ISO 4833-2003 for the establishment of NTG, SR EN ISO 16649-2007 for the number of E. coli colonies, SR EN ISO 6549/AC/2006 to detect colonies from Salmonella spp. and SR EN ISO/TS 10272-2-2007 for isolation and confirmation of Campylobacter spp. colonies. The results of the chicken cecum micro flora from the experience showed very large values of variations depending on the feed additive used, the lowest values for NTG, the number of E. coli colonies Salmonella and Campylobacter spp. existence were found in the experimental group in which feed were used an feed additive based on plant extract.

Key words: broiler, Salmonella spp., Campylobacter spp.

INTRODUCTION

In the current economic crisis and population extension planned for the third millennium, most categories of people are looking for quality products at a low price, in these social circumstances poultry acquires new dimensions because products derived from this species of animals are most appropriate.

While chicken has nutrient specifications desired by consumers, a special importance should be given to microorganisms which can cause severe food poisoning.

Gastrointestinal tract is the principal organ of digestion and absorbtion and plays a crucial role in raising broilers. A diverse micro flora may be on the entire length of the digestive tract but it is more comprehensive in the cecum. Digestive tract micro flora has an important role in nutrition by detoxification of certain compounds, growth performance and protection against pathogenic bacteria [1].

The first segment of the digestive tract of birds where are found common bacteria is the small intestine, in which segment are found only lactobacilli and streptococci, the latter in a small number, if other types of bacteria found here this is considerate abnormal. Barnes's researches from 1979 showed that only one chicken from 51 had other types of bacteria than those normally in the digestive tract.

Newly hatched chicken's digestive tract is usually sterile, after the first contact with the mother and the environment within 3-6 hours after hatching, a large number of anaerobic bacteria invading chickens cecum. During the first 2-4 days after hatching, streptococci and enterobacteria colonize the cecum and small intestine. After de first week in the small intestine of chicken predominant lactobacillus and cecum mainly colonized by anaerobic bacteria (*E. coli* and *Bacteroides spp*.) and a smaller number of facultative aerobic bacteria. A typical micro flora in the intestine of young birds is installing in the first 2 weeks of life and cecum micro flora stabilizes in about 30 days

old when the predominant bacteria is bifidobacterium and bacteroides [1, 2].

The segment in which we find most of micro flora, over 40 different types of cocci and anaerobic Gram negative and Gram positive bacilli exceeding value 10^9 is cecum were normally is located about 10^{11} bacteria/g ceca materials[2].

By feeding birds we can influence microbiological load from their digestive tract, feed additives used in this experience acts directly on this element.

Pathogenic bacteria from the digestive tract may encourage a discomfort to birds eventually leading to a reduction in economic efficiency by decreasing daily average or increasing mortality, but some of them can cause contamination of carcasses without in any way effect the health of birds.

MATERIAL AND METHOD

To achieve the experience of this work have been used 200 chickens belonging to Cobb 500 which were divided into four groups of 50 chickens each. The technology followed were super-intensive, chickens were bought at the age of one day and was distributed in BP4 batteries in a controlled environment guide to the specifications of the hybrid under study. To ensure the health of the chickens during the experiments they was vaccinate as Romanian legislation (anti-avian pseudopesta, anti-avian infectious bursitis, anti-avian infectious bronchitis).

The four proposed groups were distributed according to the feed used in a control group (LC) who received a mere feed and three experimental groups that received feed that incorporate different feed additives, such for first experimental group (LE1) was used a synergistic combination of organic and inorganic acids (formic acid, propionic acid, lactic acid, citric acid, ascorbic acid), his salts and plant extract (Biotronic Se Forte, 1‰); for second experimental group (LE2) was used a product made from a combination of ingredients: probiotic "*Enterococcus faecium*", prebiotic "Fructo-oligosaccharides - Inulin", fragments of cell walls and seaweed extracts (Imbo 1,5‰ first 14 days, 1‰ between 15 and 25 days and 0,5‰ from 25 days until slaughter) and for the third experimental group (LE3) was used a mixture of essential oils from plants (thymol, carvacrol, anethole, limon), prebiotic substances Fructo-oligosaccharides – Inulin and extract of plants (oregano, fennel, chicory) (PEP 125, 0.125‰).

Were made four recipes that were given to all groups as planned: starter I from 0 to 7 days (3% of total feed), starter II from 8 to 14 days (7% of total feed), grower from 15 to 25 days (34% from total feed), finisher from 26 to 42 days (56% from total feed).

Intestinal bacteria on which we stopped were *Escherichia coli*, bacteria from genus *Salmonella* and *Campylobacter* spp. To determine the number of germs were collected a total of 40 samples respectively 10 samples from each group of broiler at slaughter age (42 days). The samples were represented in the caecal contents of chickens, each sample removed from a single chicken. They are agreed that the working methods used be the standards SR EN ISO 4833 – 2003 for the determination of NTG; SR EN ISO 16649 – 2007 for determining *E. coli*; SR EN ISO 6579/AC/2006 for determining bacteria of the genus *Salmonella* and SR EN ISO/TS 10272-2-2007 for determining *Campylobacter spp*.

RESULTS AND DISCUSSION

E. coli is a Gram negative bacillus sometimes showing filamentous forms. E. coli is a germ well adapted to its environmental life. It is increase on simple culture medium were glucose is the only organic constituent.

It is an aerobic, facultative anaerobic that may have both fermentative or respiratory metabolism [3]. E. coli is a permanent constituent of normal micro flora from the large intestine of warm-blooded animals, serves as an sanitary microbial indicator of environment faecal pollution, play an important role in the physiology of the colon and like the antagonist of transient micro flora. In certain circumstances this bacterium can become pathogenic for the body that normally inhabit it causing enteritis in respect of various species of animals or humans [1].

It was shown that Streptococcus faecalis and Escherichia coli bacteria are predominant in the colon chicken in the first two weeks of life [4].

The digestive segment that contained the majority of micro flora is cecum. Korver and Yegani from the University of Alberta, Canada were considered for a bird's digestive NTG more than 10^{12} colonies/g with a total number of species of bacteria between 400 and 500. Pop determined values of the cecum NTG between 10^{11} and 10^{20} NTG/g. As can be seen in the digestive tract NTG value of chicken can vary within very large limits [6, 8]. In experiments conducted for this study, at the age of 42 days (6 weeks) found a number of colonies of *E. coli* between $3.2x10^{10}$ and $2.1x10^{12}$, representing a rate between 15.2 and 85.3 from NTG in the cecum of chickens (tab. 1).

Table 1 Medium values for NTG and *Escherichia coli* from broiler chicken cecum

Group	Number of samples	Medium values of NTG/g sample	Medium values of *E. coli*/g sample	V% of *E. coli*	% *E. coli* from NTG
LC	10	$4,2 \times 10^{11}$	$2,5 \times 10^{11}$	111,90	59,5
LE 1	10	$1,5 \times 10^{12}$	$1,3 \times 10^{12}$	100,53	85,3
LE 2	10	$1,5 \times 10^{12}$	$5,6 \times 10^{11}$	56,44	37,3
LE 3	10	$3,3 \times 10^{11}$	$5,0 \times 10^{10}$	84,85	15,2

LE1 = LC + Biotronic Se Forte; LE2 = LC + Imbo; LE3 = LC + PEP 125

For the group studied can be observed less colonization with E. coli in chicken cecum in the group that was used PEP 125 additive, LE3, respectively 15.2% to 85.3% in group were has been used pro/prebiotic additive, LE2, value was higher than that obtained in LC group, respectively 59.5%, in group which was used acidifier LE1, the proportion of colonies of E. coli in NTG was also lower than LC group, 37.3%. The values obtained for colonies of E. coli in groups studied showed a high variability which confirms the influence that these additives present on ceca micro flora.

Genus *Salmonella* belongs to the family *Enterobacteriaceae* and comprises a single species, *Salmonella enterica*. This species includes over 2300 differentiated serotypes. The main habitat of Salmonella is the intestinal tract of humans and animals. The most important animal reservoirs are: chicken, turkeys, pigs and cows. Because of their ability to survive in eggs, egg powder, raw meat and incompletely cooked animal products; animal products are the most important vehicle of transmission, the most common food poisoning salmonelosis [3]. Incidence of *Salmonella* colonies on broiler carcasses chilled in a unit from Iasi in 2007 was 4.68% [5].

Morphologically the genus *Campylobacter* comprises Gram negative spiral-shaped, or curved like letter S, fitted with a single polar flagellum at one or both ends, which gives a specific movement fly flight. It requires special conditions of cultivation: microaerophilic, special media enriched and a growth temperature of 42-43^0C with an incubation of 48-72 hours. Germs of the genus *Campylobacter* are commensal in the intestine of animals, usually birds: chicken, turkeys, ducks, gulls (C. lari) and mammals: cattle, sheep, dogs and cats [3].

Campylobacter jejuni is recognised as one of the main causes of food poisoning in many developed countries, followed to a lesser extent by *Campylobacter coli*.

In USA and other developed countries, the most common way to obtaining these types of food poisoning (up to 70% of cases) is ingestion of contaminated poultry meat that has not been sufficiently cooked. Some studies indicate that in the USA, diarrheal illness caused by Campylobacter spp. is more common that that caused by Salmonella and Shigella with infections occurring during the year, but there is a peak incidence in summer and early autumn, as if Salmonella infections [7].

Table 2 The number of samples which detected *Salmonella* and *Campylobacter*

Group	Number of samples	Numbers of samples with *Salmonella*		Numbers of samples with *Campylobacter*	
		Number	% from all samples	Number	% from all samples
LC	10	5	50	9	90
LE 1	10	3	30	7	70
LE 2	10	4	40	9	90
LE 3	10	2	20	5	50

LE1 = LC + Biotronic Se Forte; LE2 = LC + Imbo; LE3 = LC + PEP 125

In the detection of Campylobacter colonies groups with the largest number of samples that have been detected this type of colonies (9 of 10) were the control group and experimental group two, a smaller number of samples which was detected this bacteria is met at first experimental group, 7, and the lowest number of Campylobacter detected came from experimental group three with 5 positive samples.

Salmonella was detected in all groups of chicken from our experience in the largest proportion, 50% in group LC followed by the group LE2, 40%, group LE1 with 30% and group LE2 with 20%. In the detection of Campylobacter colonies group with the highest number of samples that were detected this type of colonies (9 of 10) were LC and LE2 groups, if a group LE1 presence of this bacteria was detected in a number of 7 samples, the smallest number of samples detected with Campylobacter were in case of group LE3 with 5 positive samples.

CONCLUSIONS

❖ Microorganisms in the digestive tract is the main source of contamination of broiler chicken carcass.

❖ In all chicken groups studied were detected colonies of the genus Salmonella and Campylobacter in their cecums.

❖ Given the fact that the health of the offspring of the groups studied was good, Salmonella and Campylobacter colonies detected shown no virulence to the host organism.

❖ We observe that the number of colonies for the NTG and E. coli and the number of samples detected with Salmonella and Campylobacter have a smaller values for group in that has been used additive from plant extract, LE3, so this has a strong bactericidal effect.

BIBLIOGRAPHY

Journal article:

[1] Amit-Romach E., Sklan D., Uni Z., 2004 – „Microflora ecology of the chicken intestine using 16S ribosomal DNA primers", Poultry Science Associaion;

[2] Barnes Ella M., 1979 – „The intestinal microflora of poultry and game bird during life and after storage", The Society for Applied Bacteriology, 1979;

[5] Crețu Carmen, Carp-Cărare M., Isan Elena, Brădățan Gh., 2008 – „Researches regarding *Salmonella spp*. incidence in poultry carcasses destined to human consume", Buletin USAMV Veterinary Medicine, 65(2)/2008;

[4] Mead G. C., Adams B. W., 1975 – „Some observation on the caecal microflora of the chick during the first two weeks of life", British Poultry Sciences 16, 169-176;

Book:

[3] Toma Săcărea Felicia, 2006 – „Bacteriologie medicală", University Press Târgu Mureș;

Other sources:

[6] Pop I. M., 1995 – „Cercetări cu privire la posibilitățile de utilizare a unor produse obținute prin biotehnologie, în alimentația broilerului de găină crescut intensiv", teză de doctorat, Iași;

[7] FAO and WHO, 2009 – *Salmonella* and *Campylobacter* in chicken meat, meeting report, Microbiological risk assessment series, 19;

[8] Yegani M., Korver D. – „Manipulation of poultry gut microflora with probiotics", http://www.wattagen.com/PrintPage.aspx?id=16103, Jun 07, 2010;

- SR EN ISO 4833 – 2003 pentru determinarea numărului total de germeni;
- SR EN ISO 1664 – 2007 pentru determinarea *Escherichia coli*;
- SR EN ISO 6579/AC/2006 pentru determinarea bacteriilor din genul *Salmonella*;
- SR EN ISO/TS 10272-2-2007 pentru determinarea *Campylobacter spp.*