**Enterobacteriaceae Genera Representation of Chicken’s Gastrointestinal Tract after Feed Additives Application**

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**Abstract**

The aim of this study was to examine the effect of different feed additives on the *Enterobacteriaceae* genera colonization of chicken (Coob 500) gastrointestinal tract. The Agolin Poultry, Agolin Tannin Plus, Biostrong 510+FortiBac and Agolin acid were administered to forth feed mixtures and Biocitro to drinking water in various amounts except of the control group. The addition of 1.5 g Agolin Poultry to 15 kg of feed was included in the first experimental group, the addition of 7.5 g Agolin Tannin Plus to 15 kg of feed in the second experimental group, the addition of 15 g Biostrong510+FortiBac to 15 kg of feed the third experimental group, 15 g in the fourth experimental group of Agolin acid to 15 kg of feed and 10 ml fifth experimental group of drinking water. The highest count of *Enterobacteriaceae* genera counts was found in the control group. The lower count of *Enterobacteriaceae* genera counts was found in the second experimental group where 7.5 g of Agolin Tannin Plus to 15 kg was added to feed mixture.

**Keywords**: feed additives, gastrointestinal tract colonization, *Enterobacteriaceae* genera

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**1. Introduction**

In Europe-wide increase of resistance to all antibiotic classes under surveillance for the most common Gram-negative bacteria responsible for bacteraemia and urinary tract infections, *Escherichia coli*. The growing threat of multidrug resistance which is being observed more frequently in some Gram-negative bacteria such as *Escherichia coli*, *Salmonella* sp., *Shigella* spp., *Campylobacter* sp. and *Yersinia* sp., continues to cause concern [1]. The family *Enterobacteriaceae* consists of many genera and strains that colonize the small and large intestine, and includes members of the non-pathogenic autochthonous (commensal) microbiota as well as pathogens. Several pathogenic *Enterobacteriaceae*, specially *Escherichia coli* strains, cause diarrhoea, urinary tract infections, mastitis, arthritis and meningitis in both humans and animals [2-4]. Over the last decade, the importance of gastrointestinal tract health in broiler chicken has been increasingly recognized due to its contribution to their overall health and performance [5-7]. The use of antibiotics at subtherapeutic levels has been a cornerstone of the poultry industry for the control of subclinical diseases, maintenance of gut health

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and growth promotion for a number of decades. As new antibiotic-resistant strains of pathogens emerge, the routine use of antibiotics in animal feed has become less common. In Europe, subtherapeutic use of antibiotics in poultry rearing has been phased out since 2006. In accordance to these restrictions, alternative means for preventing bacterial infections common to poultry and enhancing growth performance are required [8]. The aim of this study was to examine the effect of different feed additives on the Enterobacteriaceae genera colonization of chicken (Coob 500) gastrointestinal tract.

2. Materials and methods

Animals
Caecums of 42 day old chickens (n = 30) were used for the quantitative determination of the selected individual groups of microorganisms. Gastrointestinal tracts of COBB 500 hybrid chicken broilers were used for the microbiological analysis. The animals were kept in a thermoneutral hall (33 °C at the beginning, 19 °C at the end). The fattening was performed from day 1 to day 42 of the experiment. One day old broilers were randomly divided into six groups and fed with a complete KKZ feed mixture. Feed and water were provided ad libitum. The chickens were healthy; the conditions of animal care, manipulation and use corresponded to the instructions of the Ethical Commission. Care and use of the animals as well as experimental devices met the requirements established in the Certificate of Authorization to Experiment on Living Animals (State Veterinary and Food Institute of Slovak Republic, No. SK PC 30008).

Dosage of feed additives
The animals were divided into the following groups: the control group (C)-feed mixture without feed additives, the first experimental group (P1) with the addition of 1.5 g of AgolinPoultry per 15 kg of feed mixture, followed by the second experimental group (P2)-7.5 g of AgolinTannin Plus 15 kg-1, the third (P3)-Biostrong 510+FortiBac 15 g.15 kg-1, the fourth (P4) - 15 g. 15 kg-1 and the fifth experimental group (P5)-Biocitrol 10 ml.10l of water.

Characteristics of feed additives
AGOLIN® POULTRY is plant extracts are known to stimulate digestive enzymes production, control the growth of bacteria and for their antioxidant properties. AGOLIN POULTRY is a blend of high quality plant active ingredients from e.g. lavender, clove and pepper, designed to optimize the animal’s feed intake and digestive functions. AGOLIN® TANNIN is a unique, coated chestnut extract, which contains hydrolysable tannins. The product is designed to use special properties of chestnut extracts on the digestive system, like astringent or antimicrobial while maintaining feed intake and animal performance. BIOSRONG510 is a phytogenic feed additive consisting of a standardized combination of microencapsulated essential oils and high valued herbal substance aligned to the needs of fattening poultry. The main effect of BIOSTRONG510 are the improved palatability of feed and the improved barn climate and reduced odor and ammonia. AGOLIN® ACID is plant extracts and acids are known to control the growth of bacteria, whereas certain plant ingredients stimulate the digestive enzymes production and have antioxidant properties. AGOLIN ACID is a blend of high quality essential oil compounds from e.g. bergamot, clove, thyme, pepper and preservatives like fumaric and citric acids. AGOLIN ACID is designed to optimize feed intake and digestive functions for poultry. BIOCITRO is a product with antioxidant (free radical scavenger) and prolonged anti-microbial properties which has been developed by Quinabra as a state of the art environmentally friendly organic ingredient. Spectrum of Biocitro Activity: Biocitro is effective against numerous gram+ and gram- pathogenic bacteria as well as fungi. It also possesses antioxidant properties.

Plate diluting method
The plate diluting method was applied for the quantitative CFU counts determination of respective groups of microorganisms in 1 g of substrate. Gelatinous nutritive substrate was inoculated in Petri dishes with 1 mL of chyme samples per plate method in three repetitions. Homogenized samples of faecal chyme (chyme was taken to sterile Petri dishes) were prepared in advance using sequential diluting based on the decimal dilution system. Coliform bacteria were
counted on the MacConkey agar (Biolife, Italy) and incubated at 37 °C for 24–48 h.

**Biochemical identification of the Enterobacteriacea genera**

Triple sugar iron agar (Biolife, Italy) was used for the basic biochemical identification of Enterobacteriacea genera. Furthermore, the ENTEROtest 24 (Pliva-Lachema, Czech Republic), including TNW Lite 7.0 identification software (Pliva-Lachema, Czech Republic) were applied for a more detailed biochemical identification. Preparation of the ENTEROtest 24 identification plates was done inside the Laminaire box (ADS Laminaire, Le Pre-Saint Gervais) to ensure a higher sterility, a lower risk of contamination from the air and precise results. The working scheme of the ENTEROtest 24 is described in the manual provided by the manufacturer.

### 3. Results and discussion

The productivity of broilers was increased significantly via genetical improvement but, on the other hand, they became more susceptible to various pathogens, especially to enteropathic microbes such as *E. coli*, *Salmonella* spp., *Clostridium perfringens* and *Campylobacter* spp. Wide use of antimicrobial growth promoters in feed, applying therapeutic and subtherapeutic dosages, was introduced in attempt to control these infections. There is a delicate balance of beneficial and pathogenic bacteria in the gastrointestinal tract (GIT) influenced by many symbiotic and competitive interactions [9].

#### Table 1. Summary statistical values of Enterobacteriaceae genera number in GIT (log cfu/g)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Coefficient of variation %</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>6.92</td>
<td>0.47</td>
<td>6.77</td>
<td>6.32</td>
<td>7.30</td>
</tr>
<tr>
<td>P2</td>
<td>6.07</td>
<td>0.46</td>
<td>7.54</td>
<td>5.60</td>
<td>6.66</td>
</tr>
<tr>
<td>P3</td>
<td>6.36</td>
<td>1.13</td>
<td>17.76</td>
<td>5.00</td>
<td>7.31</td>
</tr>
<tr>
<td>P4</td>
<td>6.78</td>
<td>0.26</td>
<td>3.31</td>
<td>3.31</td>
<td>7.11</td>
</tr>
<tr>
<td>P5</td>
<td>6.66</td>
<td>1.00</td>
<td>15.07</td>
<td>5.30</td>
<td>7.53</td>
</tr>
<tr>
<td>C</td>
<td>7.27</td>
<td>0.17</td>
<td>2.36</td>
<td>7.04</td>
<td>7.44</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6.86</strong></td>
<td><strong>0.81</strong></td>
<td><strong>11.81</strong></td>
<td><strong>5.00</strong></td>
<td><strong>8.11</strong></td>
</tr>
</tbody>
</table>

control group C, P1 with the addition of 1.5 g of AgolinPoultry per 15 kg of feed mixture, P2-7.5 g of AgolinTannin Plus 15 kg⁻¹, P3- Biostrong 510+FortiBac 15 g.15 kg⁻¹, P4 - 15 g. 15 kg⁻¹, P5-Biocitrol 10 ml.10l of water

Several mechanisms are described by which the normal population of bacteria in the GIT protects the host and enhances productivity: production of certain substances such as volatile fatty acids which inhibit multiplication of non-indigenous microorganisms, competition with nonindigenous organisms for nutrients available in limited supply, and competition for available tissue attachment sites in the GIT [10].

The number of Enterobacteriaceae genera after feed additives application in chicken gastrointestinal tract is shown Table 1. There were found statistically significant differences (P < 0.05) between second and fourth experimental group in the trial with chickens after application of feed additives. The number bacteria of Enterobacteriaceae genera ranged from 7.04 to 7.44 log CFU/g in the control group, from 8.15 to 8.52 log CFU/g in the first one, from 6.32 to 7.30 log CFU/g in the second group, from 5.00 to 7.31 log CFU/g in the control group.
log CFU/g in the third group and from 6.55 to 7.11 log CFU/g in the fourth group and from 5.30 to 7.53 log CFU/g in the fifth one. The highest count of *Enterobacteriaceae* genera was found in the control group. The lower count of *Enterobacteriaceae* genera was found in the second experimental group where was 7.5 g of AgolinTannin Plus added to 15 kg of feed mixture. Many publications include data showing an important role of gut microflora in maintaining the immune homeostasis of the GIT and protecting it from inflammation. The composition of the feed greatly influences the characteristics of intestinal microflora [11]. The microbial community of the gastrointestinal tract ultimately reflects the coevolution of microorganisms with their animal host and the diet adopted by the host. Changes in the composition of the animal's microflora can have beneficial or detrimental effects on health, growth, and maturation of the animal host, as is evident from the beneficial effects of rearing food animals on feeds containing antibiotics. With concerns over agricultural use of these growth-promoting antibiotics and the emergence of antibiotic resistance in human or zoonotic pathogens, there is increasing pressure to eliminate this practice from animal husbandry [12]. Unfortunately, it is unknown how these antibiotics influence the intestinal microflora and ultimately affect feed conversion, growth, and health of the food animal. Intestinal bacteria play an important role in health through their effects on gut morphology, nutrition, pathogenesis of intestinal disease, and immune responses. The microbial flora is also believed to protect against colonization of the intestines by pathogens and to stimulate the immune response [13]. Intestinal bacteria are primarily responsible for degrading the copious amounts of mucus produced by goblet cells in the intestine [14]. However, many factors can affect the composition of the avian bacterial community, such as diet [15], age [15-17], antibiotic administration, and infection with pathogenic organisms [15].

4. Conclusions

Results of our investigation in general indicate that there are good future possibilities for alternative growth promoting feed additives such as AgolinPoultry, AgolinTannin Plus, Biostrong 510+FortiBac and Biocitrol in successfully replacing the antimicrobial growth promoters. Future scientific investigations can also develop phytogenic feed additives allowing to compose antibiotic-free feed ratios and keeping the same level of productivity.

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References