

## EFFECT OF CHAMOMILE SUPPLEMENTS TO FEEDING DOSES ON ANTIMICROBIAL PARAMETERS IN POULTRY

Zuzana Jakubcova, Ladislav Zeman, Petr Mares, Jiri Mlcek, Tunde Jurikova, Lenka Dostalova, Eva Mrazkova, Eva Mrkvicova, Stefan Balla, Jiri Sochor

### ABSTRACT

Due to a ban of use of antibiotic growth promoters in the poultry industry it is necessary to look for alternative solutions. The use of some herbs showing antimicrobial effects can be one of such alternatives. In this experiment, effects of three different concentrations of chamomile (*Matricaria chamomilla*) extract, (0.3%; 0.6% and 1.2%) in feeding doses on the microbial population in the gastrointestinal tract of growing broiler chickens were studied. The main attention was paid to the population of *Clostridium perfringens* and to numbers of coliform microbes. Clostridia were cultivated under anaerobic conditions at 46 °C on the Tryptone Sulfite Neomycin (TSN) agar for a period of 24 hours. Coliform microbes were grown on the violet red bile lactose (VRBL) agar at 37 °C for a period of 24 hours. The experiment lasted 39 days and involved 80 chicks that were slaughtered in the course of their growth period at the age of 18, 25, 32 and 39 days; there were 5 chicks in each group. The obtained results indicated that increasing doses of chamomile in the feeding ration decreased numbers of coliform microbes in the digestive tract of chicks and also reduced the population of *C. perfringens*.

**Keywords:** poultry; chamomile; *Clostridium perfringens*; *Escherichia coli*

### INTRODUCTION

*C. perfringens* is one of the most frequent etiological agents in both animals and humans (Allaart, et al., 2013). In poultry industry, *C. perfringens* pathogens of the type A and/or C play an important role (Shanmugavelu, et al., 2006). Microbes of the species *C. perfringens* are gram-positive, not-moving and spores producing bacteria that are present in soil, feedstuffs, litter and intestinal tract of both ill and healthy birds. Temperatures and pH values required for the growth and propagation of these bacteria range from 12 °C to 50 °C and from 6.0 to 7.0, respectively. Under optimum temperatures (i.e. 43 °C - 47 °C) bacteria of the species *C. perfringens* propagate extremely quickly: the generation interval is 8 - 10 min, and the growth is associated with gas production. Spores of these bacteria are very resistant against heat, desiccation, acids and many of chemical disinfectants (Hafez, 2011).

While the acute form of this disease causes an increase in mortality, the symptoms of its subclinical (i.e. latent) form involve inhibited digestion and absorption of nutrients, lower weight gains and less efficient conversion of feedstuffs. As far as the poultry is concerned, bacteria of the species *C. perfringens* are considered to be a common part of their intestinal microflora. The manifestation of the necrotic enteritis requires the existence of several pre-disposing factors. The coccidiosis infection is one of the most important factors of this type (Yegani, et al., 2008). Coccidia can damage intestines, cause inflammations and intensify the production of mucus. In this way favourable conditions for the propagation of *C. perfringens* bacteria

can be created and these thereafter damage intestines more and more (Cvikova, et al., 2006).

*Escherichia coli* is a commensal species belonging to the group of facultatively anaerobic microflora living in the large intestine. The pathogenic species *E. coli* is an important infective agent causing extraintestinal infections. Some pathotypes of *E. coli* cause infections in humans and some other in animals (Kaper, 2005). In layers and broilers, avian pathogenic *E. coli* (APEC) cause above all infections of their reproductive organs. In chickens, the APEC infections develop probably independently on other microbes occurring in the intestinal reservoir. Although the inflammatory diseases of the oviduct are mostly of chronic nature, an acute sepsis may occur in 28% of infections (Pires-dos-Santos, et al., 2014).

APEC stems cause colibacillosis in both domestic and wild birds. This infection plays an important role in the world poultry industry. Although the colibacillosis may be influenced by antimicrobial compounds, the most important problem represent residues of antibiotics in foodstuffs. Chances that we will find some successful alternative solutions are dependent on an exact characterisation of individual groups of pathogens (especially those that are infective also for humans) and also of possibilities and mechanisms of these infections (Mora, et al., 2013). Plant extract, volatile oils as well as pollen are complex mixtures of many natural components in inexact proportions that have different structure and show different effects. The basic characteristics of plant additives and essential oils is their wide antimicrobial activity (Opletal, et al., 2010, Omer et al., 2013). After the ban of application of antibiotics as

growth promoters in poultry the use of essential oils is more and more frequent. Basing on results of a literary survey it was possible to identify four different mechanisms of essential oils functioning of in the following categories: perception (i.e. interpretation of sensory information), metabolism, antioxidant activity and antimicrobial efficiency (Brenes, et al., 2010, Liptaiová, et al., 2010).

The aim of this study was to assess effects of chamomile extract on two bacteria species (*C. perfringens* and *E. coli*) occurring in the digestive tract of growing broiler chickens.

## MATERIAL AND METHODOLOGY

### Experimental design

The experiment involved 80 male chicks of the hybrid combination Ross 308. All chicks were seven days old. There were altogether 4 groups of these birds, viz. control and three experimental groups receiving chamomile extract supplements in concentrations of 0.3%, 0.6% and 1.2%. In the course of the growing period of chicks, always five birds of each group were killed on Days 18, 25, 32 and 39 of age.

### Birds and experimental conditions

Prior to the beginning of the experimental period, chicks were weighed, identified with wing tags, assorted into four groups and placed into metabolic cages. All birds received water and feed mixture *ad libitum*. The feed mixture consisted of following components: wheat (25%), maize (37%), soybean meal (28%), sunflower oil (6%), mineral-vitamin mixture without anticoccidial drugs (3%), monocalcium phosphate (0.8%) and finely ground limestone (0.2%). Chamomile extract was added into the feed mixture in concentrations of 0.3%; 0.6% and 1.2%.

The light regime was 6 hours of darkness and 18 hours of light. On the 7<sup>th</sup> day of age chicks were kept at the ambient temperature of 29.9 °C and relative humidity of 50%.

### Preparation of samples

Diluted samples of excrements were homogenised in a Biosan (Latvia) multi-vortex. Samples of 1 ml were transferred by an automatic pipette into sterile Petri dishes and overflowed with the corresponding agar (manufacturer the company Biokar, France). The violet red bile (VRBL) agar contained in 1 litre of medium: 7 g of peptic meat digest; 3 g of yeast extract; 10 g of lactose; 1.5 g of bile salts; 5 g of sodium chloride; 30 mg of neutral red; 2 mg of crystal violet and 12 g of bacteriological agar. The tryptone sulphite neomycin (TSN) agar contained in 1 litre of medium: 15 g of tryptone; 10 g of yeast extract; 1 g of sodium sulphite; 0.5 g of ferric ammonium citrate; 50 mg of neomycin sulphate, 20 mg of polymixin B sulphate and 13.5 g of bacteriological agar. Samples were prepared in two dilutions (*C. perfringens* 10<sup>-1</sup> and 10<sup>-2</sup> and of *E. coli* 10<sup>-7</sup>, 10<sup>-8</sup>).

### Microbiological analysis

Microorganisms were assessed as follows: Petri dishes with coliform microbes inoculated on the violet red bile (VRBL) agar were placed into a thermostat and cultivated at the temperature of 37 °C for a period of 24 hours. Petri

dishes inoculated with *C. perfringens* were placed at first into an anaerobic system (manufacturer the company Merc, Germany) with a generator of the anaerobic environment Anaerocult A (Merc, Germany). The anaerobiosis system was thereafter closed and placed into a thermostat and the sample was cultivated at 46 °C also for a period of 24 hours. Numbers of bacteria in 1 ml of sample were assessed on the base of characteristic colonies growing in Petri dishes and expressed as CFU (Colony Forming Units).

### Statistical processing

Obtained results were analysed using the programme Microsoft Excel 2010 and the software Statistica 10 CZ.

## RESULTS AND DISCUSSION

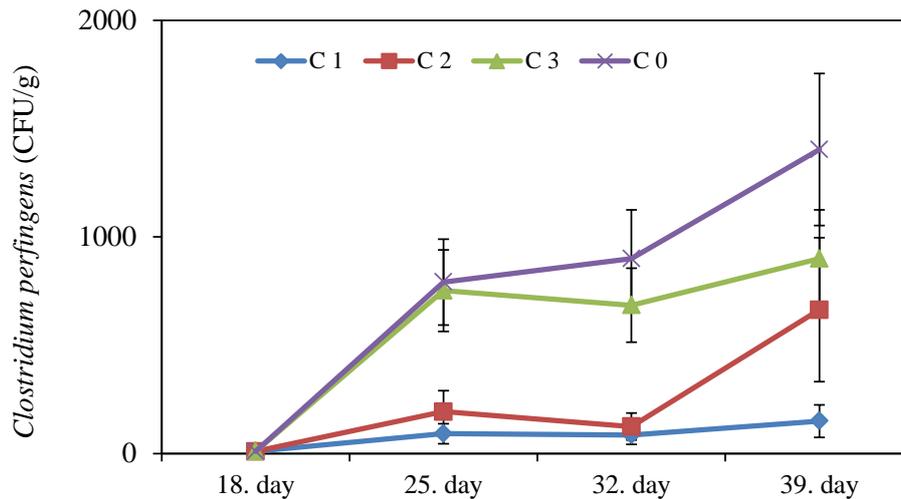
Chamomile (*Matricaria chamomilla* L.) belongs to a large group of cultivated medicinal plants. Chamomile plants contains a great number of therapeutically interesting active compounds. In general, sesquiterpens, flavonoids, coumarins and polyacetylenes are considered as the most important components of the chamomile drug. Chamomile is used above all because of its antiphlogistic and antiseptic effects (Singh, et al., 2011).

The aim of this study was to assess effects of different concentrations of chamomile extracts on the microbial population living in the small intestine and their propagation in the course of the growing period of broiler chickens of hybrid combination Ross 308. Attention was paid to *C. perfringens* and *E. coli* and the results were expressed in CFU.g<sup>-1</sup>. Samples were taken from the small intestine in weekly intervals so that it was possible to monitor either the increase or the decrease in numbers of colonies in the small intestine in the course of the growing period of broiler chickens.

The highest numbers of clostridia were recorded in the control group (Fig 1). The maximum increase took place between Days 18 and 25 of age of birds (P <0.05). The minimum increase was recorded in the group receiving chamomile extract in the concentration of 0.3%; in this group, there was only a slight increase in numbers of clostridia (Fig. 1).

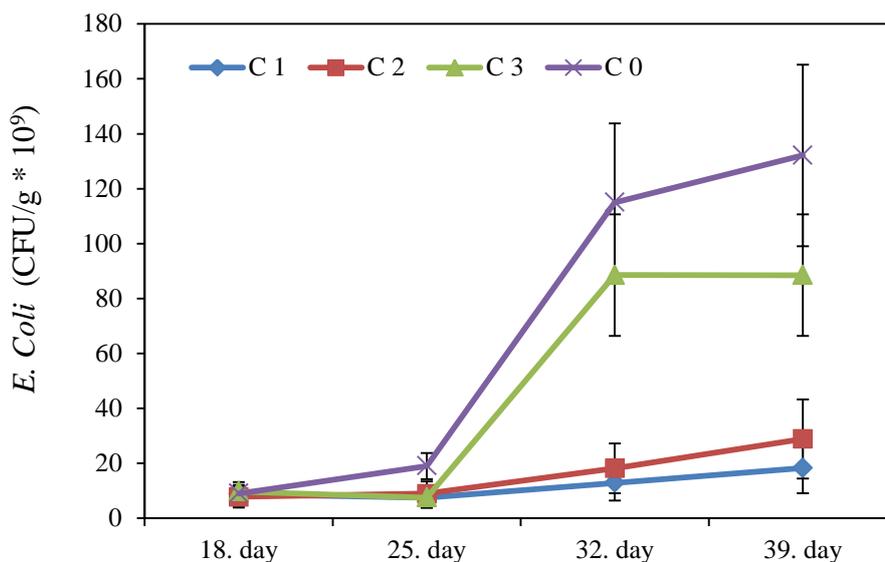
The highest number of coliform bacteria were found out in the control group while the lowest one in the group C1 (i.e. with the concentration of 0.3% of chamomile extract) (Fig. 2). The observed increase in CFU was insignificant in all groups under study. To the end of the experimental period (i.e. between Days 25 and 32), the highest increase numbers of coliform bacteria was recorded in the control group C 0 (with the zero concentration of chamomile extract) and in the group C 3 (with the concentration of 1.2% of chamomile extract) (Fig. 2).

Abdoul-Latif, et al., (2011) demonstrated a high antimicrobial activity of *M. chamomilla* methanol extract and essential oil. Their results indicated that *M. chamomilla* could be used as a natural antimicrobial substance suitable either for the treatment of human infections or as a food preservative. An extended application of natural antimicrobial substances could also reduce negative environmental impacts of synthetic chemicals and drugs.



**Figure 1** Changes in average numbers of *C. perfringens*.

C 1 - concentration 0.3% of chamomile extract, C 2 - concentration 0.6% of chamomile extract, C 3 - concentration 1.2% of chamomile extract, C 0 - concentration 0% of chamomile extract



**Figure 2** Changes in average numbers of *E. coli*.

C 1 - concentration 0.3% of chamomile extract, C 2 - concentration 0.6% of chamomile extract, C 3 - concentration 1.2% of chamomile extract, C 0 - concentration 0% of chamomile extract.

The effect of the addition of chamomile ethanol extract into drinking water on stress symptoms was already tested in broilers. It was found out that (as compared with the control group) the applied extract contributed to both a decrease in the level of cholesterol and to an increase in the level of the immunoglobulin complex in blood of experimental birds. The chamomile extract also contributed to an increase in the live body weight of broilers (Skomorucha, et al., 2013). Marques et al., (2010) tested the effect of *M. chamomilla* on stress and growth of Japanese quails. In this experiment, tested concentrations of *M. chamomilla* did not show any effects

on the growth, behaviour and physiological parameters of reared Japanese quail chicks.

Mitsch et al., (2004) demonstrated that specific blends of essential oils components can control the proliferation of *Clostridium perfringens* in the broiler intestine. In this study essential oils significantly reduced the number of *Clostridium perfringens* in the intestine and feces of broilers and therefore may have reduced the risk of necrotic enteritis. The fact that some essential oils have good potential in the control of *Clostridium perfringens* demonstrated in his study also Si et al., (2008).

## CONCLUSION

The obtained results indicate that increasing concentrations of chamomile extract showed a positive effect on the reduction of the number of coliform microbes in the digestive tract of experimental chickens and, above all, on microbes of the species *C. perfringens*. The highest and the most intensive increase in numbers of clostridia were observed in controls between Days 18 and 25 of the age ( $P < 0.05$ ). The highest numbers of coliform bacteria were recorded also in the control group C 0 while the lowest one in the group C1 (i.e. with the concentration of 0.3% of chamomile extract). The increase in CFU was insignificant in all groups under study. The most pronounced inhibiting effect of chamomile extract of bacteria species *E. coli* and *C. perfringens* was observed in the group C1 with the supplement of 0.3% of chamomile extract into the feed mixture.

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### Contact address:

Zuzana Jakubcova, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: zuzanajakubcova@seznam.cz.

Ladislav Zeman, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: zeman@mendelu.cz.

Petr Mares, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: maresp@mendelu.cz.

Jiri Mlcek, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T. G. Masaryka 275, 762 72 Zlin, Czech Republic, E-mail: mlcek@ft.utb.cz.

Tunde Jurikova, Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institut for Teacher Training, Drazovska 4, 949 74 Nitra, Slovakia, E-mail: tjurikova@ukf.sk.

Lenka Dostalova, Mendel University in Brno, Faculty of Agronomy, Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: lenka.dostalova@mendelu.cz.

Eva Mrazkova, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: eva.mrazkova@mendelu.cz.

Eva Mrkvicova, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: eva.mrkvicova@mendelu.cz.

Stefan Balla, Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institut for Teacher Training, Drazovska 4, 949 74 Nitra, Slovakia, E-mail: sballa@ukf.sk.

Jiri Sochor, Mendel University in Brno, Faculty of Horticulturae, Department of viticulture and enology, Valtická 337, 691 44 Lednice, Czech Republic, E-mail: sochor.jirik@seznam.cz.