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Preliminary study of the microbial spectrum of the digestive tract in broilers fed diets with and without antibiotic supplementation

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ABSTRACT

The predominant bacteria in the small intestine of broilers were *Lactobacillus* spp., *Escherichia coli* and *Streptococcus* spp. Compared with the antibiotic-supplemented group, experimental groups receiving mannan oligosaccharide, lactic acid bacteria, and organic acids contained more *Enterococcus* spp., *Escherichia coli, Streptococcus* spp. in the intestinal content. The predominant bacteria in the caecum were *Escherichia coli*, *Lactobacillus* spp. and *Enterococcus* spp. Unlike the other groups, no *Clostridium* spp. bacteria were found in the caecum of birds receiving antibiotic supplements. There was no effect of the type of acid (fumaric vs formic) in feed on the composition of intestinal microflora of chickens.

KEY WORDS: broiler chicken, ileum, caecum, antibiotic, microbial spectrum

INTRODUCTION

The composition of intestinal microflora is influenced by the presence of bacteria, moulds, and yeast in feed and by the feed's particle size and physical form (Engberg et al., 2002; Knarrenborg et al., 2002). The planned ban on the therapeutic use of antibiotics in poultry nutrition has led researchers to look for substances that protect the digestive tract of birds against pathogenic bacteria (Verstegen and Williams, 2002).

The objective of this study was to determine the effects of mannan oligosaccharide, lactic acid bacteria, fumaric acid and formic acid given in feed on the microflora composition of the small intestine and caecum of broiler chickens.

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MATERIAL AND METHODS

The composition of intestinal microflora was compared in 5-week-old ROSS broiler chickens fed diets with (ANT) and without (CON) antibiotic supplementation, and with supplemental mannan oligosaccharide (MO), lactic acid bacteria (LAB) and feed acidifiers in the form of fumaric acid (FUM) or formic acid (FOR). The broilers were fed a maize, wheat and soyabean meal diet supplemented with 0.5% premixes (BASF Kutno, Poland). The antibiotic feed contained flavofosfolipol (5 mg/kg), while the experimental feed contained mannan oligosaccharide (1 g/kg), lactic acid bacteria and fumaric acid (9.7 g/kg) or formic acid (11.8 g/kg). The bacteria originated from the Institute of Agricultue and Biotechnology in Warsaw. These were *Lactobacillus paracasei* KKP 824, *Lactobacillus rhamnosus* KKP 825, and *Lactobacillus rhamnosus* KKP 826 with an activity of 6.7×10^8 c.f.u./g, mixed at a 1:2:2 ratio, 3 million bacterial cells/bird/day. Intestinal microflora was studied in 5 birds randomly selected from 160 birds of each group. Samples from the small intestine and caecum were taken for analysis under anaerobic conditions into sterile tubes and immediately brought to a laboratory. Analyses were performed at the Department of Clinical Microbiology of the Jagiellonian University Collegium Medicum Children's Hospital, following internal procedures.

RESULTS

The predominant bacteria in the small intestine were *Lactobacillus* spp. in all groups of birds (Table 1). *Escherichia coli* and *Streptococcus* spp. were 10 times less common. Traces of bacteria (*Clostridium* spp., *Staphylococcus* spp., *Enterococcus* spp. and *Bacillus* spp.) were found in the small intestine. The experimental groups contained more *Enterococcus* spp., *Escherichia coli*, *Streptococcus* spp. in the intestinal content. In chickens of both experimental groups, there were traces of *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumonia*. The dominant bacteria in the caecum were *Escherichia coli*, *Lactobacillus* spp. and *Enterococcus* spp. (Table 2). The concentration of *Escherichia coli* in the caecum was 100-fold higher on average than in the small intestine, and that of *Lactobacillus* spp. 10 times lower than in the small intestine. In the caecum, *Enterococcus* spp. bacteria were 10 times more numerous than in the small intestine. In all groups of birds, traces of *Bacillus* spp. were found in the intestines. The dietary antibiotic supplement eliminated *Clostridium* spp., including *Clostridium perfringens*, from the caecum. Acid type (fumaric vs formic) had no effect on the microflora composition of the chickens.

DISCUSSION

Table 1. The intestinal flora in chickens aged 5 weeks

Species of microflora	Without antibiotic (CON)	With antibiotic (ANT)	MO plus LAB	
			fumaric acid (FUM)	formic acid (FOR)
Lactobacillus spp.	+++ 5/5	+++ 5/5	+++ 5/5	+++ 5/5
Clostridium spp.	+ 1/5	negative	negative	negative
Clostridium perfringens	negative	negative	negative	+ 1/5
Enterococcus spp.	+ 2/5	+ 1/5	+ 3/5	+ 3/5
Escherichia coli	+ 3/5	+ 4/5	+ 5/5	++ 4/5
Escherichia fergussoni	negative	negative	+ 1/5	negative
Bacillus spp.	negative	+ 1/5	+ 2/5	+ 1/5
Streptococcus spp.	+ 4/5	+ 2/5	++ 2/5	+ 4/5
Staphylococcus spp.	+ 1/5	+ 2/5	negative	+ 2/5
Staphylococcus aureus	+ 1/5	negative	+ 1/5	+ 1/5
Staphylococcus epidermis	negative	negative	negative	negative
Campylobacter spp.	negative	++ 4/5	negative	negative
Salmonella spp.	negative	negative	negative	negative
Shigella spp.	negative	negative	negative	negative
Corynebacterium spp.	negative	negative	negative	negative
Proteus mirabilis	negative	negative	+ 1/5	+ 1/5
Klebsiella pneumonia	+ 1/5	negative	+ 1/5	negative
Klebsiella oxytoca	+ 1/5	negative	negative	negative

MO mannan oligosaccharide
LAB lactic acid bacteria
+ to 100 cfu
++ to 1000 cfu
+++ more than 1000 cfu
1/5-5/5 present in this number of birds/total number of birds tested

Table 2. The caecum flora in chickens aged 5 weeks

Species of microflora	Without antibiotic (CON)	With antibiotic (ANT)	MO plus LAB[1]	
			fumaric acid (FUM)	formic acid (FOR)
Lactobacillus spp.	++ 5/5	++ 5/5	++ 5/5	++ 5/5
Clostridium spp.	negative	negative	+ 3/5	negative
Clostridium perfringens	negative	negative	+ 3/5	+ 3/5
Enterococcus spp.	+ 3/5	+ 4/5	++ 5/5	+ 4/5
Escherichia coli	+++ 5/5	+++ 5/5	+++ 5/5	+++ 5/5
Bacillus spp.	+ 1/5	+ 2/5	+ 1/5	+ 1/5
Streptococcus spp.	+ 4/5	+ 4/5	negative	negative
Staphylococcus epidermis	+ 2/5	+ 1/5	negative	+ 2/5
Campylobacter spp.	negative	negative	negative	negative
Salmonella spp.	negative	negative	negative	negative
Shigella spp.	negative	negative	negative	negative
Corynebacterium spp.	+ 1/5	negative	negative	negative

[1] for abbreviations see Table 1

The present results indicate that *Lactobacillus* spp. bacteria are predominant in the small intestine of broilers aged 5 weeks, while the type of supplement (including antibiotic) or its absence does not influence the presence of these bacteria. Withdrawal of the antibiotic from chicken diets increases the count of *Streptococcus* spp., *Staphylococcus* spp., and *Proteus mirabilis* in the small intestine. The content of *Lactobacillus* spp. in the caecum is the same regardless of the type of supplement (including the type of organic acid) used as a feed acidifier. *Clostridium* spp., including *Cl. perfringens* bacteria were found in the caecum of chickens receiving mannan oligosaccharide, lactic acid bacteria and organic acids, unlike in chickens receiving the feed antibiotic.

CONCLUSIONS

The replacement of an antibiotic with mannan oligosaccharide and lactic acid bacteria, and acidification of feed with fumaric or formic acid does not reduce *Lactobacillus* spp. and *Escherichia coli* in the digestive tract of broiler chickens. Intestinal microflora of the chickens is not differentiated by fumaric and formic acid used to acidify feeds in the presence of mannan oligosaccharide and lactic acid bacteria.

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STRESZCZENIE

Wstępne badania obrazu mikrobiologicznego przewodu pokarmowego kurcząt brojlerów otrzymujących dietę bez i z antybiotykiem

W jelicie cienkim kurcząt brojlerów dominowały bakterie *Lactobacillus* spp., *Escherichia coli* i *Streptococcus* spp. W treści jelitowej kurcząt grup doświadczalnych (otrzyjmujących mannan, bakterie kwasu mlekowego lub kwasy organiczne) więcej było bakterii *Enterococcus* spp., *Escherichia coli, Streptococcus* spp. niż u kurcząt otrzymujących antybiotyk. W jelicie ślepym przeważały bakterie *Escherichia coli*, *Lactobacillus* spp. i *Enterococcus* spp. W jelicie ślepym ptaków otrzymujących dodatek antybiotyku w diecie, w przeciwieństwie do pozostałych grup, nie stwierdzono bakterii *Clostridium* spp., w tym *Clostridium perfringens.* Rodzaj kwasu (fumarowy vs mrówkowy) nie miał wpływu na skład mikroflory jelitowej kurcząt.