EFFECT OF GENOTYPE ON ILEAL AND CAECAL MICROBIOTA IN PASTURE-REARED DOMINANT COCKERELS*

L. Čermák[1], J. Vlčková[1], E. Skřivanová[1,2], K. Laloučková[1,2], M. Englmaierová[1]

[1]Institute of Animal Science, Prague-Uhříněves, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Prague, Czech Republic

Outdoor access is an important part of organic and free-range poultry production, yet limited information exists on the effect of various housing and production systems on the growth performance and colonization of food-borne pathogens. Therefore, the primary purpose of the current study was to evaluate the influence of different housing systems (particularly fixed versus small, portable houses, with and without outdoor access to pasture) and different broiler genotypes on the gastrointestinal bacteria in broilers. The fundamental factor studied was the presence of any quantitative changes in common gastrointestinal microbiota, including pathogenic genera such as *Campylobacter* sp. and *Salmonella* sp. The results showed differences in intestinal microbiota and confirmed lowered counts of caecal coliforms in pasture-reared broilers.

poultry; coliforms; anaerobes; lactic acid bacteria; campylobacter; salmonella

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INTRODUCTION

The organic poultry production system, including egg production, operates according to specific and precise standards of production, and this farming technique is becoming increasingly popular. Although conventional rearing systems for poultry products are commonly used in the animal industry and still represent a decent majority, quality begins to play a bigger role in customer demands (Webb, O'Neill, 2008). The perception of organic or natural products as 'better' than their conventional counterparts in terms of safety, taste, and increased health benefits is fundamental (Harper, Makatouni, 2002). Pasturing systems include all factors and conditions of rearing. Birds in such systems can freely move and show natural behaviour such as grazing, foraging or feed selection, which theoretically improves their welfare (Ponte et al., 2008). Systems of free-range chickens are based on slow- or moderate-growth-vital genotypes with good health resistance, and these chickens are adapted for breeding outside the hall. However, rapidly growing chicks commonly reared intensively in a limited space in the hall are often chosen for production in free farming (Sirri et al., 2011). Gastrointestinal microbiota has one of the highest cell densities of any ecosystem, and in poultry, this density ranges from 10^7 to 10^{11} bacteria per g of gut content (Apajalahti et al., 2004). The primary function of the gastrointestinal tract is to absorb nutrients from the diet and excrete waste products; it also contains a unique microbial ecosystem affected by dietary nutrients, host secretions, and the systemic re-

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Table 1. Composition and nutrient content in diet and pasture

	Starter	Grower	Finisher	Lyophilized pasture
Wheat (g kg^{-1})	290.0	420.0	486.7	
Maize (g kg^{-1})	277.5	210.0	210.0	
Soybean (g kg^{-1})	360.0	248.0	215.0	
Wheat bran (g kg^{-1})	–	50.0	39.6	
Rapeseed oil (g kg^{-1})	30.0	30.0	18.0	
Monocalcium phosphate (g kg^{-1})	13.0	11.0	7.5	
Sodium chloride (g kg^{-1})	3.0	3.0	3.0	
Limestone (g kg^{-1})	17.0	18.5	12.5	
L-Lysine hydrochloride (g kg^{-1})	1.3	2.1	1.0	
DL-Methionine (g kg^{-1})	2.9	2.1	1.7	
L-Threonine (g kg^{-1})	0.3	0.3	–	
Vitamin-mineral premix[1] (g kg^{-1})	5.0	5.0	5.0	
Analysed composition				
Dry matter (g kg^{-1})	883.7	902.6	890.9	937.9
Crude protein (g kg^{-1})	216.6	183.9	180.5	150.8
Ether extract (g kg^{-1})	59.3	52.5	40.4	43.6
Crude fibre (g kg^{-1})	43.0	44.3	43.1	241.9
AME (by calculation MJ kg^{-1})	11.8	12.0	11.8	5.4
ALA (mg 100 g^{-1})	498	382	178	1270
EPA (mg 100 g^{-1})	6.1	4.5	2.7	3.0
DHA (mg 100 g^{-1})	3.2	2.5	1.8	2.0
SFA (mg 100 g^{-1})	1599	1386	1142	475
MUFA (mg 100 g^{-1})	2913	2258	1630	182
PUFA (mg 100 g^{-1})	2748	2062	1337	1645
Vitamin E (mg 100 g^{-1}))	57.8	31.9	20.2	34.8
Vitamin A (mg 100 g^{-1})	3.0	2.7	1.7	–
Zeaxanthin (mg 100 g^{-1})	0.75	0.69	0.91	162.9
Lutein (mg 100 g^{-1})	1.04	1.00	1.28	187.2

AME = apparent metabolizable energy, ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

[1]vitamin-mineral premix provided per kg of diet: retinyl acetate 3.6 mg, cholecalciferol 13 µg, α-tocopherol acetate 30 mg, menadione 3 mg, thiamine 3 mg, riboflavin 5 mg, pyridoxine 4 mg, cyanocobalamin 40 µg, niacin 25 mg, calcium pantothenate 12 mg, biotin 0.15 mg, folic acid 1.5 mg, choline chloride 250 mg, copper 12 mg, iron 50 mg, iodine 1 mg, manganese 80 mg, zinc 60 mg, selenium 0.3 mg

sponses of the host (Noy, Sklan, 1997; Klasing, 2007; Rehman et al., 2007). The initial microbiota to which chicks are exposed and the nutrient composition of their diet affects commensal gut microbiota and immune system development (Shira et al., 2005, Yin et al., 2010). The composition of the intestinal microbiota is profoundly influenced not only by the environment and housing system (Casagrande Proietti et al., 2009) but also by the genotype of the bred animals (Zhao et al., 2013).

The growth and health of reared poultry crucially depends on the composition of the intestinal microbiota. Intestinal bacteria play an important role in the pathogenesis of intestinal diseases, as they may influence the development of gut immunity and prevent colonization of pathogens in the intestine (Mead, 2000). Dominant bacteria identified within the ileum of chickens are lactobacilli and related genera, while those in the caecum are related predominantly to clostridia (Lu et al., 2003; Gong et al., 2007). The

Table 2. Cultivation conditions and used agar media (supplier Oxoid, Brno, Czech Republic)

Target bacterial group	Agar media + supplements	Plating technique	Cultivation conditions
Coliforms	MacConkey agar No. 3 (CM0115)	spread	aerobic, 24 h 37°C, 24 h
Salmonella spp.	X.L.D. agar (CM0469)	spread	aerobic, 37°C, 24 h
Campylobacter spp.	Campylobacter agar base CM0689 + Preston campylobacter selective supplement (SR0117) + Laked horse blood (SR0048)	spread	microaerophilic, 37°C, 48 h
General anaerobes	Wilkins-Chalgren anaerobe agar (CM0619)	spread	anaerobic, 37°C, 48 h
Lactic acid bacteria	M.R.S. agar (CM0361)	pour	aerobic, 37°C, 48 h

caeca are considered the primary site of focus; they not only contain one of the most diverse and abundant microbial communities in the chicken, including strict anaerobes such as methanogens, but can also harbour pathogens such as *Salmonella enterica* and *Campylobacter jejuni* (Foley et al., 2011). These bacteria can cause disease in humans via ingestion of contaminated poultry products that may have been contaminated during slaughtering.

The goal of this study was to detect differences in counts of ileal and caecal bacteria between different genotypes of pasture-reared broilers using culture-based methods.

MATERIAL AND METHODS

A total of 300 one-day slow-growing Dominant cockerels were used for the experiment. According to the genotype, chickens were divided into three groups of 100 individuals (Dominant Sussex D104, Dominant Brown D102, Dominant Tinted D723). Up to 7 weeks of age, i.e., 49 days, they were housed on wood shavings in a penned poultry house. From 50 days of age, chickens were housed in mobile pens in the Netluky farm area. The chickens were fed mixed feed starter (7-BR1) from days 0 to 28 of age, grower feed (7-BR2) from days 29 to 70 of age, and finisher feed (7-BR3) from days 71 to 77 of age (Table 1). Pasture intake was indirectly assessed using the modified method of Dal Bosco et al. (2014). Pasture samples were collected in each area in a 50 cm × 50 cm square, and were extrapolated to the entire area of each pen. Measurements were taken at 12 locations and evenly distributed throughout the pasture plot to achieve an average difference. The sward was first measured before the placement of the portable pen and again after the portable pen was relocated (Skrivan et al., 2015).

The field experiment was conducted on 0.7 ha of experimental grassland at Netluky village, Czech Republic (50°2′21.344″ N, 14°36′51.075″ E). At 7 and 11 weeks, representing initial and latter stages of grazing, six birds from each group were randomly selected and slaughtered. The numbers of coliform bacteria, total anaerobes, lactic acid bacteria, *Campylobacter* and *Salmonella* in caecum and the proximal part of the ileum were monitored. The ileal and caecal contents from both experiments were promptly transferred on ice and subjected to microbiological analysis. One gram of fresh intestinal content was prepared with 10 ml of sterile peptone water (NaCl 5 g l^{-1}, peptone 10 g l^{-1}) and diluted decimally to 10^{-8}. Fifty microlitres of homogenized suspension from dilution 10^{-4}–10^{-8} was plated on 60-mm Petri dishes containing the respective agar medium and cultivated (Table 2). Questionable colonies were examined by Gram staining and microscopy. Counts of coliforms, general anaerobes, lactic acid bacteria, *Salmonella* and *Campylobacter* were evaluated and subjected to statistical analysis (one-way ANOVA) using SAS software (Statistical Analysis System, Version 9.3, 2011). Scheffé's method was used as a post-hoc test.

RESULTS

There were no significant differences between genotypes in ileum at the initial stage of grazing (age 7 weeks). At the latter stage (age 11 weeks), the Dominant Brown genotype showed one order of magnitude more coliforms than the Dominant Tinted genotype ($P = 0.0423$, Fig. 1), and the numbers of anaerobes and lactic acid bacteria showed no difference between genotypes ($P > 0.05$). Dominant Sussex had one order of magnitude more *Campylobacter* than the other genotypes ($P = 0.0077$). Coliforms at the latter stage of grazing significantly decreased by one order of magnitude for the Dominant tinted genotype when compared to the initial stage ($P = 0.0004$). Comparing the stages of pasturing, anaerobes were significantly reduced in all genotypes: Dominant Brown ($P = 0.0006$), Dominant Sussex ($P = 0.0124$), and Dominant Tinted ($P = 0.0017$). In a similar way, the number of lactic acid bacteria increased by 0.6 orders of magnitude for the Dominant Brown genotype ($P = 0.0003$), by almost one order of magnitude ($P = 0.0001$) for the Dominant

Sussex genotype, and *Campylobacter* counts decreased significantly by almost one order of magnitude for the Dominant Tinted genotype ($P = 0.0020$).

In the caecum at the latter stage of grazing, apart from *Campylobacter*, the number of bacteria did not differ between genotypes ($P > 0.05$, Fig. 2). Prior to grazing, there was a statistically significant difference in the number of *Campylobacter* between the Dominant Tinted and Dominant Brown genotypes ($P = 0.0318$). At the latter stage of grazing compared to the initial one, the number of coliform bacteria for all genotypes was significantly reduced (Dominant Brown $P = 0.0382$, Dominant Sussex $P = 0.0355$, Dominant Tinted $P = 0.0006$). The number of anaerobes for the Dominant Tinted genotype increased significantly with grazing by 0.4 orders of magnitude ($P = 0.0030$); the other genotypes remained unchanged. An increase in the number of lactic acid bacteria by 0.3–0.4 orders of magnitude was observed for the Dominant Sussex ($P = 0.0478$) and Dominant Tinted genotypes ($P = 0.0118$). The number of *Campylobacter* decreased significantly by one order of magnitude between stages for the Dominant Tinted genotype ($P = 0.0020$).

DISCUSSION

Bacterial species differ in their substrate preferences and growth requirements, thus the chemical composition and structure of the digesta largely determine the species distribution of the bacterial community in the gastrointestinal tract (A p a j a l a h t i et al. 2004). In this study, the effect of genotype on the ileal and caecal microbiota of pastured broiler chickens was addressed. We observed a significant difference in the number of coliform bacteria. In concordance with these results and our recently published results (C e r m a k , S k r i v a n o v a , 2016), L o s a , K o h l e r (2001) and

Fig. 1. Effect of genotype and stage of pasturing on quantity (log CFU g^{-1}) of cultivated bacteria in ileum

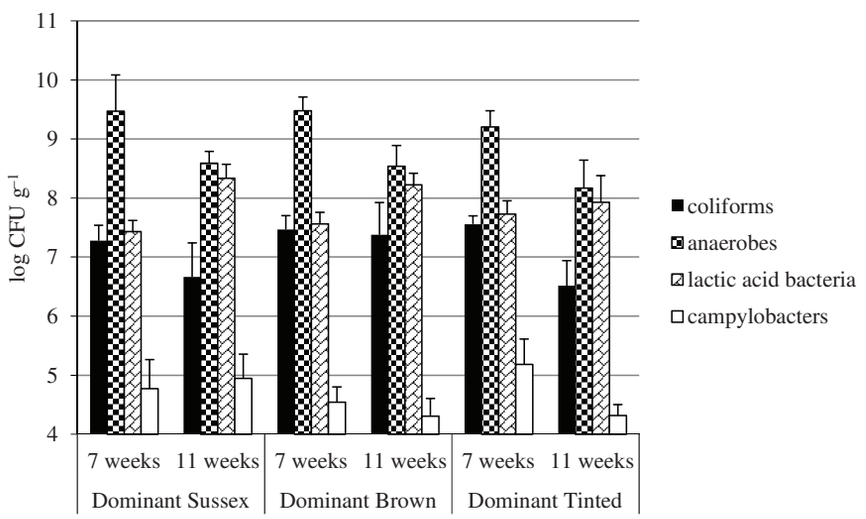
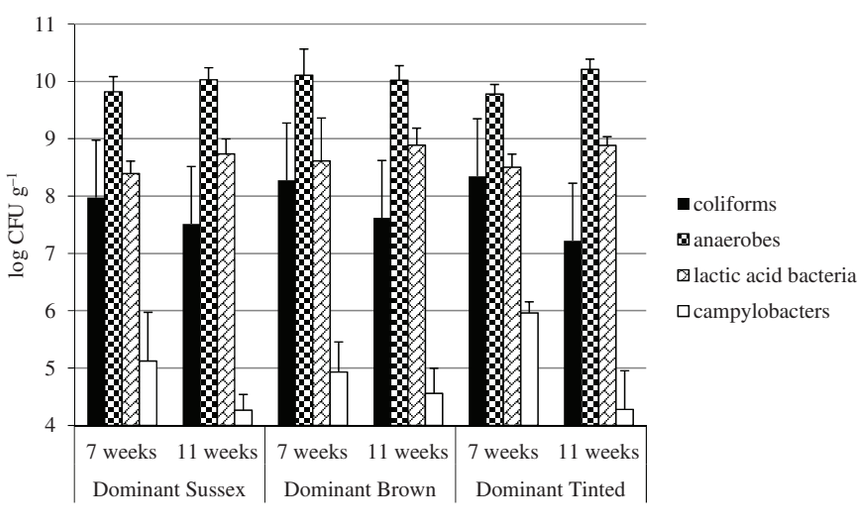


Fig. 2. Effect of genotype and stage of pasturing on quantity (log CFU g^{-1}) of cultivated bacteria in caecum



Tucker (2002) have noticed a decrease in the number of coliform in chickens fed with essential oils that may occur in grazed vegetation. In general, fewer anaerobes in pasture chickens are commonly associated with a lower intake of less nutritious materials that serve as a substrate for intestinal microbiota (Bjerrum et al., 2006). The intake of vegetable essential oils in the diet might increase the number of lactobacilli, up to 10^9 CFU g^{-1} (Tucker, 2002). Casagrande Proietti et al. (2009) performed biochemical characterization of intestinal microbiota and found no major differences between grazing chickens and conventional breeding. Kaplan, Hutkins (2000) theorized that dietary fibre is primarily used by lactobacilli, which leads to the production of lactic acid and short chain fatty acids; these substances inhibit *Salmonella*. Indeed, no salmonellas were found in any gut samples. In addition, the fibre can lead to the maintenance of a normal microbial population in the gastrointestinal tract of birds (Woodward et al., 2005; Dunkley et al., 2007). Since *Campylobacter* are one of the most common commensal microorganisms in chickens, many studies have confirmed the presence of *Campylobacter* in poultry regardless of breed (Han et al., 2009; Hanning et al., 2010).

Some studies (Videnska et al. 2014; Borda-Molina et al. 2018) indicate that the gastrointestinal tract microbiota can be also affected by age of animal. Videnska et al. (2014) defined 4 different stages of caecal microbiota development in laying hens. The first stage lasts for the first week of life and is characterised by a high prevalence of *Enterobacteriaceae* (phylum *Proteobacteria*). The second stage lasts from week 2 to week 4 and is characterised by nearly an absolute dominance of *Lachnospiraceae* and *Ruminococcaceae* (both phylum *Firmicutes*). The third stage lasts from month 2 to month 6 and is characterised by the succession of *Firmicutes* at the expense of *Bacteroidetes*. The fourth stage is typical for adult birds aged 7 months or more and is characterised by a constant ratio of *Bacteroidetes* and *Firmicutes* formed by equal numbers of the representatives of both phyla. The decrease of coliform bacteria in latter stage of grazing in this study may correspond with decline of *Enterobacteriaceae* after the first stage of caecal microbiota development, as stated by Videnska et al. (2014).

In a study comparing organic farms to conventional farms, a higher number of *C. perfringens* was found in ileum and caecum samples of broilers from organic farms (Bjerrum et al., 2006). Moreover, they found lower counts of *Enterobacteriaceae* and higher lactobacilli numbers in the ileal content of the birds raised on the organic farms (Bjerrum et al., 2006). Access to an outdoor range was demonstrated to enrich *Bifidobacterium* in caeca and ileum in Ross broilers (Gong et al., 2008).

Animals may also suffer from heat stress. Although temperature in poultry houses is often controlled, poultry production may decrease because of the unfavourable influence of a hot environment, especially when high ambient temperatures are combined with high relative humidity (Laudadio et al., 2012). When birds experienced stress due to exposure to higher temperatures for 24 h, greater changes were shown to occur in the ileal content compared to caecal samples, indicating that the microbiota in the ileum may be more sensitive to changes than the caecal microbiota (Burkholder et al., 2008).

In conclusion, since at least 80% of bacteria in certain niches are traditionally non-cultivable (Schabereiter-Gurtner et al., 2001), the use of modern molecular biology methods to determine intestinal microbial diversity is desirable and sometimes necessary.

CONCLUSION

This study has shown that the chicken breed genotype may affect intestinal microbiota during pasturing. Our experiment consistently revealed reduction of coliform bacteria in the broiler gut after grazing. The Dominant Tinted genotype responded most to grazing fattening, with significant changes in bacterial counts in both the ileum and caecum. Additionally, the results indicate that grazing may support the growth of beneficial lactic acid bacteria or at least does not reduce their numbers.

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Corresponding Author:

Mgr. Ladislav Čermák, Ph.D., Institute of Animal Science, Přátelství 815, 104 00 Prague 10-Uhříněves, Czech Republic, phone: +420 267 009 717, e-mail: cermak.ladislav@vuzv.cz