INFLUENCE OF PASTURE REARING ON THE CECAL BACTERIAL MICROBIOTA IN BROILER CHICKENS*

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Differences in quantity of cecal microbiota in broiler chickens from conventional and pasture rearing were investigated by cultivation. Rearing on pasture brings stress reduction and increases comfort and bird welfare, which leads to products with better taste and flavour compared to conventionally produced broiler chickens. A difference in cecal settlement of general anaerobes, coliforms, lactic acid bacteria, and campylobacters and salmonellas in the two different rearing systems was addressed. Whereas numbers of total anaerobes and lactic acid bacteria were not affected, those of coliforms were significantly reduced in pasture rearing. Campylobacters were found only in pasture-reared chickens (in 28% of animals). Salmonellas were not detected in any of the systems.

cecal microbiota; poultry; pasture; coliforms; anaerobes; lactic acid bacteria

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INTRODUCTION

The organic broiler production system operates according to specific and precise standards of production, and this farming technique is becoming increasingly popular. Although conventional rearing systems for poultry products have been commonly used in the animal industry and still represent a decent majority, for instance in the United States it is over 95% of overall poultry production (MacDonald, 2008), demands of consumers have changed from quantity to quality (Webb, O'Neill, 2008). Consumer demand for organic and natural poultry products continues to increase because of an ongoing perception that organic or natural products are better than their conventional counterparts in terms of safety, taste, and increased health benefits (Harper, Makatouni, 2002). The word 'conventional' in the poultry industry essentially refers to commercial broiler chickens that have high feed conversion rates and are raised in housing units for up to 6–8 weeks to achieve an average market weight (2.9–3.9 kg; Fanatico et al., 2008). Pasturing systems, as an opposite system, include the entire group of ways, conditions, and factors of rearing. Birds have free access to grazing crops, foraging, feed selection, and activity, which theoretically improves their welfare (Ponte et al., 2008). Free grazing also brings about positive content of fatty acids and antioxidants, which is reflected in the poultry meat and eggs from free range (Skřivan, Englmaierová, 2014). Product quality and health of chickens can be positively influenced e.g. by essential oils from a number of monoterpenes (Cross et al., 2007), flavonoids, and phenolic compounds (Lahucky et al., 2010). Systems of free-range chickens are based on slow or moderate growth vital genotypes with good health resistance, which are adapted for breeding outside the hall. But also the 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).

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Table 1. Composition of starter (7-BR1), grower (7-BR2), and finisher (7-BR3) feed

Component	7-BR1 (%)	7-BR2 (%)	7-BR3 (%)
Soybean meal	36.00	24.80	21.50
Maize	27.75	21.00	21.00
Wheat	29.00	42.00	48.67
Wheat middlings	-	5.00	3.96
Rapeseed oil	3.00	3.00	1.80
Natrium chloride	0.30	0.30.	0.30
Monocalcium phosphate	1.30	1.10	0.75
Ground limestone	1.70	1.85	1.25
Aminovitan BR1-BR3	0.50	0.50	0.50
L-Lysine hydrochloride	0.13	0.21	0.10
DL-Methionine	0.29	0.21	0.17
L-Threonine	0.03	0.03	-

For the growth and health of reared poultry the composition of intestinal microbiota is essential. The intestinal bacteria play an important role in the pathogenesis of intestinal diseases since they influence the development of gut immunity and thus may prevent colonization of pathogens in the intestine (M e a d , 2000). The prevailing bacteria in the adult cecum are obligate anaerobes (L u et al., 2003). The composition of gut microbiota varies in relation to the type of food intake and rearing management practices (E n g b e r g et al., 2004). In the first days of chickens' life the gut is colonized mainly by coliform bacteria belonging to class *Enterobacteriaceae*, mainly represented by *Escherichia coli*. In the coming weeks, its share decreases in favour of Firmicutes bacteria strain, which includes e.g. *Bacillus* spp. or *Clostridium* spp., and anaerobic *Bacteroidetes* spp. (V i d e n s k a et al., 2014). The ceca are considered the primary site of focus because they not only contain one of the most diverse and abundant bacterial communities in the chicken including strict anaerobes such as methanogens, but also may harbour pathogens such as *S. enterica* and *C. jejuni* where these organisms can be the most numerous (F o l e y et al., 2011). However, these bacteria can cause disease in humans by ingestion of contaminated poultry products that might have been contaminated during slaughter or processing.

The objective of this study was to inquire differences in counts of cecal bacteria between pastured and conventionally reared chickens using culture based methods.

MATERIAL AND METHODS

For the experiment lasting from July 17th till September 10th 2014, 225 one-day-old slowly growing Hubbard JA757 male broilers were used. For the first 28 days the broilers were kept on wood shavings in a penned poultry house with a 16-hour lighting program and ventilation provided by a temperature-controlled fan. Each pen was equipped with nipple drinkers and pan feeders. Feed and water were provided *ad libitum*. On day 29 ninety broilers remained in the poultry house making a control group. The second and the third group comprising 45 and 90 individuals, respectively, were relocated to floorless portable pens (described in S k ř i v a n et al. 2015) on pasture until the end of the experiment at 56 days of age. These two groups differed in stocking density only (8.3 vs 4.15 chick/m^2). The field part of the 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). The dominant species of the existing temporary grassland were *Lolium perenne* ('Merlinda') and *Festuca pratensis* ('Kolumbus'), with 20% being *Trifolium pratense* ('Violetta' and the intergeneric hybrid 'Felina'). The portable pens were moved twice daily, once during the morning feeding at 8:00 h and again at 18:00 h. Each pen contained hat drinkers connected to a water basin. Feed was provided in trough feeders (length of 100 cm) with chicken access on both sides. The chickens were fed mixed feed starter (7-BR1) in days 0–28 of age, grower feed (7-BR2) in days 29–42 of age, and finisher feed (7-BR3) in days 43–56 of age (supplied by Biopharma Chotouň, Czech Republic) (Table 1). The chickens' health status was monitored daily. By 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, 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 (SR 117) + 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

end of the experiment 54 birds (18 from each group) were randomly selected and slaughtered. The contents of ceca were subjected to a microbiological analysis.

One gram of the cecal content was made up to 10 ml with sterile peptone water (NaCl 7.5 g/l, peptone 5 g/l) and diluted decimally to 10^{-8}. Fifty microliters of homogenized suspension from dilution 10^{-4}–10^{-8} was plated on 60 mm Petri dishes containing respective agar medium and cultivated (Table 2). Plates requiring anaerobic or microaerophilic conditions were cultivated in 2.5 l AnaeroJars containing the respective gas developer (AnaeroGen for anaerobes, CampyGen for campylobacters; all supplied by Oxoid, Brno, Czech Republic). Questionable colonies were examined by Gram staining and microscopy. Counts of coliforms, general anaerobes, lactic acid bacteria, salmonellas, and campylobacters were evaluated and subjected to statistical analysis (one-way ANOVA with multiple comparisons using Scheffé's method). All computations were performed with SAS 9.3 software (Statistical Analysis System, Version 9.3, 2011).

RESULTS

Five defined groups of bacteria occurring in chicken gastrointestinal tract were evaluated: coliform bacteria, general anaerobes, lactic acid fermentation bacteria, salmonellas, and campylobacters. At the end of the experiment (at 56 days of age), the content of coliforms was 10^7–10^8 per g of sample, of general anaerobes ca. 10^8–10^9, and of lactic acid bacteria (bifidobacteria and lactobacilli) 10^8 per g of sample (Fig. 1). A significant difference was observed in coliforms. In comparison with counts of coliforms in ceca of conventionally farmed chickens, those in both pastured chicken groups were significantly lowered ($P = 0.0001$). There were no statistically significant differences between tested groups in general anaerobes and lactic acid fermentation bacteria ($P = 0.2989$ and $P = 0.2108$, respectively). Campylobacters were found in 28% of pastured chickens only (5 of 18 samples from each group), ranging 10^4–10^5 per g of cecal content in both groups (data not shown). No salmonellas were detected. Live weights of the conventionally reared chickens on day 53 were significantly higher than of those pastured ($P < 0.01$; data not shown). The density of chicken population on pasture was found insignificant for all tested bacterial groups.

DISCUSSION

In this study, the effect of pasture rearing on the counts of cecal bacteria in chickens was observed. The only significant difference was detected in coliforms; their lowering was confirmed in the ceca of pastured chickens contrary to conventionally reared. In accordance with our results, Losa, Köhler (2001) and Tucker (2002) noticed a reduction of coliforms in chickens fed various essential oils, which might be present in grazed vegetation. On the contrary, Cross et al. (2007) came to a different finding, that higher number of coliforms could be explained by the presence of some antibacterial substances in grazed vegetation selectively affecting Gram-positive bacteria. In general, lower counts of anaerobes in pastured chickens might coincide with lower income of less nutritionally rich material which serves as a substrate for intestinal microbiota (Bjerrum et al., 2006). Tucker (2002) also found out coincidence of essential oils intake in alimentation with the increase of lactobacilli up to

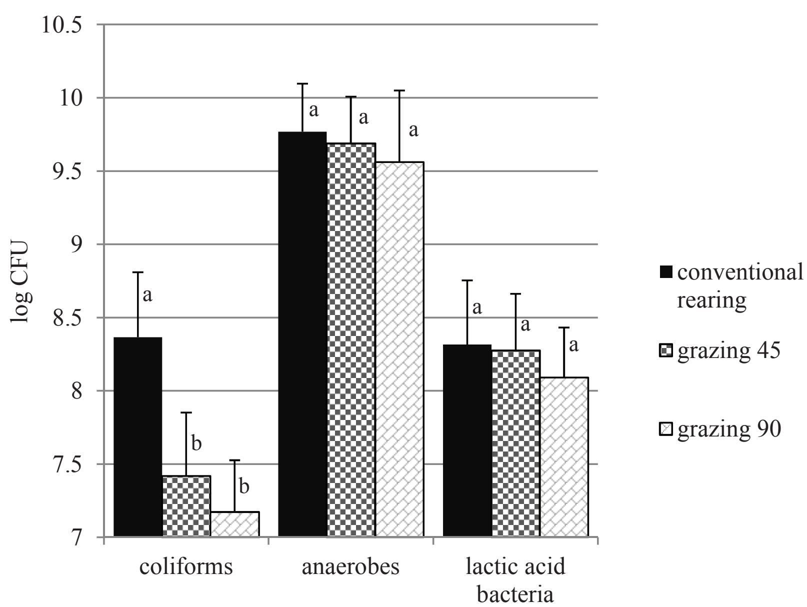

Fig. 1. Effect of mode of rearing on quantity (log CFU) of cultivated cecal bacteria. Different indices (a, b) show statistical significance, whiskers represent standard deviations.

10^9 CFU/g. Performing biochemical characterization of enteric microbiota, Casagrande Proietti et al. (2009) did not identify large differences in organic and conventional chickens.

It has been proposed that dietary fibre can be used preferentially by Bifidobacterium and Lactobacillus species, leading to the production of lactic acid and short-chain fatty acids, both of which being inhibitory to Salmonella (Kaplan, Hutkins, 2000). Furthermore, the presence of fibre can lead to the maintenance of a normal microbial population in the bird gastrointestinal tract (Woodward et al., 2005; Dunkley et al., 2007).

Because Campylobacter species are fairly common commensal microorganisms in chickens, most studies have reported Campylobacter presence in poultry regardless of whether they originated from conventional, organic, or pasture flock poultry (Han et al., 2009; Hanning et al., 2010). In our study, campylobacters were detected only in pasture reared chickens. Possible reasons could be that birds reared under free range or cage free conditions might be more likely to come in contact with wild birds which are known sources of Campylobacter.

Eventually, since it has been reported that at least 80% of the bacteria in a given niche are estimated to be missed by traditional culture techniques (Schabereiter-Gurtner et al., 2001), the use of modern molecular-biological methods for intestinal microbial diversity assessment would be appropriate.

CONCLUSION

This study has shown a significant reduction of coliform bacteria in broiler ceca of pastured contrary to conventionally reared chicken. No differences in numbers of general anaerobes or lactic acid bacteria were discovered. No salmonellas were detected, whereas campylobacters were found only in pastured chickens. No significant difference between the two pastured groups in mobile pens differing in population density was observed.

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