Effect of feeding polyphenols on Campylobacter counts in broilers

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Two experiments were conducted to evaluate the effect of a blend of polyphenols (P; 90% gallic acid, catechin and epicatechin) on Campylobacter counts in broilers. There were three treatments (T1: Non infected, T2: Infected and T3: Infected + P). Polyphenols were applied either to the whole experimental period (1-42 d, P at 238 ppm; Exp. 1) or exclusively in the finisher diets (21-41 d, P at 159 ppm; Exp. 2). Birds from T2 and T3 were infected at 14 d with 100 µl of a solution containing 10^5 CFU/ml of C. jejuni strains (ST-21 and ST-45). Ten broilers per treatment were euthanized and caecum removed for Campylobacter enumeration at 7, 21 and 28 d after inoculation. In Exp. 1, P supplementation significantly decreased log CFU/g of Campylobacter at 7 d (7.13 vs 7.84 log10; P = 0.0391), 21 d (7.25 vs 8.11 log10; P = 0.0316) and 28 d post-infection (6.80 vs 7.6 log10; P = 0.0353). However in Exp. 2, P supplementation only a numerical decrease of Campylobacter infection at 35 and 42 d was observed, but differences were not significant. It is concluded that supplementation of broiler diets with a blend of polyphenols decrease Campylobacter colonization in broilers. However, more research is needed regarding dosage and supplementing period.

Keywords: campylobacter; polyphenols; broiler

Introduction

Campylobacteriosis is a foodborne disease associated with the infection of humans by thermophilic Campylobacter spp., mainly C. jejuni and C. coli (LASTOVICA AND SKIRROW, 2000). The number of confirmed cases of campylobacteriosis in the European Union has followed a significant increasing trend in the last four years, along with a clear seasonal trend. In 2011, the number of notified cases of thermotolerant Campylobacter in the EU increased by 2.2 % compared with 2010 and it was the most commonly reported zoonosis with 220,209 confirmed human cases (EFSA Journal, 2013).

Broiler meat is considered to be a major source of human campylobacteriosis, as a result of undercooking and cross-contamination of RTE foods, as well as through direct hand-to-mouth transfer during food preparation. The EFSA’s Panel on Biological Hazards (BIOHAZ) concluded in its scientific opinion (EFSA Journal, 2011) that handling, preparation and consumption of broiler meat may account for 20 % to 30 % of human campylobacteriosis cases in the EU, while 50 % to 80 % may be attributed to the chicken reservoir as a whole. In 2011, fresh broiler and other poultry meat were again the foodstuffs in which Campylobacter was most frequently reported. Overall, about one third of the samples were reported as positive, although there were large differences between the Member States (MSs).

The public health benefits of controlling Campylobacter in primary broiler production are expected to be greater than the controls later in the chain, as the bacteria may also spread from farms to humans by other pathways than broiler meat. Implementation of strict biosecurity measures in primary production and GMP/HACCP during slaughter may reduce colonization of broilers with Campylobacter, and contamination of carcasses. The reduction of the numbers of Campylobacter in the intestines at slaughter by 3 log 10 -units, would reduce the public health risk by at least 90%.

Campylobacter preventive measures aim at reducing the probability of Campylobacter colonisation in birds (GHAREEB et al., 2013). For this purpose several approaches, as the use of bacteriophages (WAGENAAR et al., 2005), bacteriocins (Line et al., 2008), organic acids (BYRD et al., 2001; CHAVEERACH et al., 2004a; VAN DEUN et al., 2008; SKANSENG et
al., 2010) and their derivatives (HILMARSSON et al., 2006) or medium chain fatty acids (HERMANS et al., 2010) have been proposed over the past few years. So far there is no effective, reliable and practical intervention measure available to reduce colonization of the broiler gut with Campylobacter (LIN, 2009). Research has focused also on applying beneficial microorganisms (probiotics) as microbial antagonists. Few studies in vivo have evidenced a possible role of probiotics in preventing the shedding of C. jejuni, although in vitro several studies reported a strong antimicrobial activity of different probiotic strains (CHAVEERACH et al., 2004b, GHAREEB et al., 2012). Also phytochemicals could be considered as an alternative. These substances have low molecular weight and they are derived from the plant secondary metabolism and include glucosides, alkaloids, essential oils, saponins, mucilage, phenolic compounds, and flavonoids (MARTINS et al., 2000). Grape seeds and skins are good sources of phytochemicals such as gallic acid, catechin, and epicatechin and are suitable raw materials for the production of antioxidative dietary supplements. Therefore, the objective of this study was to evaluate the effects of including a blend of polyphenols on the Campylobacter counts in broilers raised to 42 days of age.

**Materials and methods**

Two experiments were conducted to evaluate the effect of a blend of polyphenols (P, 90% gallic acid, catechin and epicatechin) on Campylobacter counts in broilers. A total of 288 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were three treatments (T1: Non infected, T2: Infected and T3: Infected + P). Polyphenols were applied either to the whole experimental period (1-42 d, P at 238 ppm, Exp. 1) or only in the finisher diets (21-42 d, P at 159 ppm, Exp. 2). Prior to the infection of the animals caecal samples of 10 birds randomly selected were collected (day 14) to verify that chicks were all Campylobacter-free. Birds from T2 and T3 were infected at 14 d with 100 µl of a solution containing 10^5 cfu/ml of two C. jejuni strains (ST-21 and ST-45). The trial was carried out at the Animal Biosafety Level 2 Unit for broilers of Imasde experimental facilities. Broilers were housed in cages in groups of three. The individual bird was the experimental unit. Mash (non-pelleted) feeds were fed ad libitum with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially.

On days 7, 21 and 28 after inoculation (21, 35 and 42 days of age), ten chicks per treatment (T2 and T3) were sacrificed and the caecum removed for Campylobacter enumeration. At each of the sampling days, caecal samples of 2 negative control broilers (T1) were pooled together to form 5 different samples that were analysed for presence/absence of Campylobacter.

The basic statistical technique applied was Analysis of Variance (ANOVA). The data were analysed as a completely randomised design by GLM of SPSS (v. 19.0). Statistical significance is declared at P ≤ 0.05, with 0.05 < P ≤ 0.10 considered as a near-significant trend.

**Results and discussion**

In Experiment 1 (Table 1), Campylobacter counts were significantly reduced by P supplementation at 7 d (7.13 vs 7.84 log_{10}; P = 0.0391), 21 d (7.25 vs 8.11 log_{10}; P = 0.0316) and 28 d post infection (6.80 vs 7.6 log_{10}; P = 0.0353). In Experiment 2 (Table 2), P supplementation numerically decreased Campylobacter infection both at 21 and 28 days post-infection, but differences did not reach significance. The stronger effect of P in the first study might have been caused by the higher dose of the product, the longer administration period or a combination of both effects. Flavonoids, as catechin and epicatechin, have been shown to be
able to affect various biological functions: capillary permeability, cellular secretary processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers (RHAMA AND MADHAVAN, 2011). Thousands of phytochemicals, including phenols, have already been identified to be inhibitory towards microorganisms (COWAN, 1999). In fact, RHAMA AND MADHAVAN (2011) proved that several flavonoids had even more in vitro antibacterial activity than tetracycline. Previous studies carried out by GANAN et al. (2009, 2011) also found that some phenolic compounds present in wine and grape residues significantly reduced in vitro the viability of Campylobacter. However, their ability to reduce Campylobacter has not been investigated in vivo and differences between in vitro and in vivo trials are not fully understood. In this sense, it is described that many types of polyphenols can lose their antioxidant capacity and bioavailability in vivo (MANACH et al., 2004). However in the present experiments has been demonstrated that the inhibitory effect of polyphenols in vitro are maintained in vivo in the broiler gut.

Conclusions
Under our experimental conditions, it is concluded that supplementation of broiler diets with a blend of polyphenols could decrease Campylobacter infection in broilers, and thus, could be considered as a priority strategy in the primary poultry production, at a farm stage. However more research is needed regarding dosage and supplementing period.

References


EUROPEAN FOOD SAFETY AUTHORITY PANEL ON BIOLOGICAL HAZARDS, 2011: Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal, 9 (4): 2105.


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polyphenols blend, ppm</th>
<th>Campylobacter infection</th>
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<td></td>
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</tr>
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</tr>
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Table 1. Effect of dietary treatment (T1: Negative Control, T2: Positive Control and T3: Polyphenols blend, ppm Campylobacter infection Days post-infection 7 d 21 d 28 d Absence Absence Absence 7.84 8.11 7.60 7.13 7.25 6.80 0.226 0.264 0.224 0.0391 0.0316 0.0353.
Polyphenols) on *Campylobacter* counts (cfu/g of caecal content expressed as log10) in Experiment 1.

<table>
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<tr>
<th>Treatment</th>
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<th><em>Campylobacter</em> infection</th>
<th>Days post-infection</th>
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Table 2. Effect of dietary treatment (T1: Negative Control, T2: Positive Control and T3: Polyphenols) on *Campylobacter* counts (cfu/g of caecal content expressed as log10) in Experiment 2.