Efficacy of feed additives against Campylobacter in live broilers during the entire rearing period

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ABSTRACT Poultry meat is the major source of human campylobacteriosis, the most frequently reported zoonosis in the EU. The prevalence of Campylobacter colonization in European broiler flocks is 71%. Despite considerable efforts, there is still no effective strategy available to prevent or reduce Campylobacter colonization in broilers.

This study tested a wide variety of feed additives to reduce Campylobacter shedding in primary poultry production. Twelve additives containing organic or fatty acids, monoglycerides, plant extracts, prebiotics, or probiotics were tested. For each additive, broilers contaminated with Campylobacter jejuni were fed with an additive free diet (control group) or with a supplemented diet (treated group) and Campylobacter loads compared at three sampling times.

No treatment was able to prevent broiler colonization by Campylobacter, and there was a high degree of variation in contamination among the birds. At 14 d of age, eight treatments significantly decreased the colonization level compared to the control group by a maximum of 2 log10 CFU/g. At 35 d of age, three of these treatments still had a significant effect with a maximum reduction of 1.88 log10 CFU/g for a probiotic.

At 42 d of age, only one short-chain fatty acid was still significantly efficient with a mean reduction over 2 log10 CFU/g. In addition, a probiotic and a prebiotic-like compound significantly decreased the contamination by a maximum of 3 log10 CFU/g, only at the 42-d sampling period.

This study gives promising results regarding the use of feed additives to reduce Campylobacter infection in flocks. Nevertheless, a global approach, combining intervention measures at the different steps of the broiler meat production chain could have a greater impact on the reduction of public health risk.

Key words: Campylobacter jejuni, broiler, control measure, feed additive

INTRODUCTION Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. In the European Union (EU), it is the most commonly reported gastrointestinal bacterial pathogen since 2005, with more than 214,000 human cases reported in 2013 (EFSA and ECDC, 2015). Campylobacteriosis is usually a self-limiting disease; however, in rare cases some patients develop late-onset complications, including neuropathies such as Guillain-Barré syndrome (GBS) or Miller-Fischer syndrome (MFS) (Nachamkin et al., 1998).

These bacteria can be isolated from the intestinal tract of a variety of wild and domestic animals, but it is estimated that 50% to 80% of human campylobacteriosis may be attributed to the chicken reservoir (EFSA, 2010b).

The prevalence of Campylobacter at the flock level is very high and is maintained along the food chain; the mean count of Campylobacter in the intestinal tract of birds is about 8 log10 CFU/g (Hue et al., 2010); with such a high level the meat products become contaminated during slaughter and carcass processing. The European Union baseline survey in the broiler meat chain carried out in 2008 revealed that the mean prevalence of Campylobacter is respectively 71.2% and 75.8% for broiler batches (cecal content) and carcasses (EFSA, 2010a), although it varies widely from 2% to 100% between the Member States (EFSA, 2010a). In France, an additional survey conducted to assess the prevalence
Experimental Design

of Campylobacter contamination at the slaughterhouse, revealed that Campylobacter spp. is isolated from 77.2% of ceca samples with a mean count of Campylobacter of $8.0 \pm 1.0 \log_{10} CFU/g$ and from 87.5% of carcasses with a mean count of $2.4 \pm 0.8 \log_{10} CFU/g$ (Hue et al., 2010; Hue et al., 2011). Another monitoring plan carried out in 2009 revealed that 76% of chicken meat products in French retail outlets are contaminated with Campylobacter with a mean count of $1.7 \pm 0.9 \log_{10} CFU/g$ (Guyard-Nicodème et al., 2015). As a consequence, the contaminated broiler meat products may serve as a source for cross-contamination to other foodstuffs and surfaces during meal preparation in the consumer’s kitchen (Luber et al., 2006; Fravalo et al., 2009; Guyard-Nicodème et al., 2013).

Therefore, implementation of Campylobacter control measures at the primary production level is needed to reduce human exposure. Reducing the numbers of Campylobacter in the intestines at slaughter by $3 \log_{10}$ CFU, would reduce the public health risk by at least 90% (Romero-Barrios et al., 2013). However, there is still no effective, reliable and practical strategy available to prevent or to reduce Campylobacter colonization in broilers.

Numerous studies have tested the effect of different products added to feed or drinking water against Campylobacter in broilers at the flock level. However, the different studies were conducted using different experimental designs, so it is difficult to compare the results.

The objective of the study was to screen the effect of 24 feed additives (available commercially or still under development) on reducing cecal colonization of Campylobacter in broilers during the entire rearing period until slaughter age. Twelve feed additives were tested in the present study, and 12 other ones were tested in a companion paper (Gracia et al., 2015). The tested additives belonged to different families of compounds: organic acids including short-chain fatty acids (SCFA) and monoglycerides, plant extracts, probiotics, and a prebiotic-like product.

**MATERIALS AND METHODS**

**Experimental Design**

The trials were carried out between February and July 2014 at the Animal Biosafety Level 2 (ABL2) facilities of the ANSES Laboratory of Ploufragan, northwest France. Before the beginning of the trial, experimental facilities (room, feeding and drinking systems) were cleaned and disinfected. A total of 688 day-of-hatch Ross PM3 broiler chicks (male and female) were purchased from a commercial hatchery where they had been vaccinated against infectious bronchitis. No other vaccine was administered throughout the study. Trials lasted 42 d from the reception of the chicks until the last sampling procedure. The birds were kept in $3.42 \, \text{m}^2 \,(1.85 \times 1.85 \, \text{m})$ floor pens with unused wood shavings as bedding material. The building was supplied with programmable electrical lights, automated electric heating and forced ventilation. The environmental temperature was gradually reduced from 32°C (d 1) to 19°C (d 42) in line with common practice at the ANSES experimental station.

Each of the 12 products was tested once. Three independent trials were necessary to test the 12 products. In each trial, a positive control group composed of animals infected by *C. jejuni* and not receiving the product was used. Up to two different groups were housed in the same facility but birds from the two groups were distributed into two separate pens and plastic sheeting from floor to ceiling was placed between the two pens. However, positive control group and probiotic treated groups were always housed alone. Chicks were randomly assigned to the different experimental treatments ($n = 45$ to 49 chicks per group) and to the positive control group at d 1. All the birds were orally challenged with *Campylobacter* at 11 d of age.

The experimental diets were formulated and manufactured by the Department of Poultry Experimentation. They were fairly standard diets for broilers. The starter diet was offered to birds from 1 d old until 10 d of age, grower diet from 11 to 29 d and finisher diet from d 30 to 42 d. Feed was presented as mash.

Products were purchased from their different suppliers and were added to feed at the recommended doses (Table 1). Diets for each treatment were stored in sealed bags. According to suppliers’ recommendations the products were added to the feed throughout the trial. Feed was weighed and manually distributed. Feed and water were available ad libitum.

On d 1, 14, 35, and 42, each animal was individually weighed and feed intake per pen was recorded.

The trials were conducted in an approved establishment for animal experimentation (n° C-22-745-1) in accordance with the principles and specific guidelines presented in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010; http://www.fass.org/).

**Broiler Inoculation**

Animals were individually inoculated orally with 100 $\mu$L of a bacterial suspension of *Campylobacter jejuni* C97Anses640 diluted at $10^5$ CFU/mL in tryptone salt broth.

The strain *C. jejuni* C97Anses640 was isolated from a poultry product and was characterized by multilocus sequence typing (MLST); it belonged to the ST-45 complex. The strain was stored at $-70°C$ in peptone broth containing 20% (v/v) glycerol. Five days before inoculation, the strain was recovered from frozen stock after plating on mCCDA (selective modified Charcoal Cefoperazone Deoxycholate Agar) at 41.5°C for 48 h under a microaerophilic atmosphere (85% $N_2$, 10% $CO_2$ and 5% $O_2$). One colony was used to inoculate 10 mL
Brucella broth. After 24 h at 41.5°C in a microaerophilic atmosphere the bacterial suspension was diluted to 10⁵ CFU/mL in tryptone salt broth.

**Sampling and Microbiological Analysis**

Environmental swabbing from facilities and transport crates were carried out and 5 chicks randomly selected were humanely euthanized (electronarcosis followed by bleeding) upon arrival in order to check the absence of *C. jejuni* colonization. Analyses were carried out according to the ISO 10272 standard (Anonymous, 2006). On d 14, 35, and 42 of age (corresponding to 3, 24, and 31 d postchallenge respectively), 15 birds from each batch were euthanized for cecal sampling. *Campylobacter* was recovered from ceca using direct plating and enumeration. Cecal contents were weighed, and diluted 1:10 (wt:vol) in tryptone salt broth. After homogenization, enumeration was carried out by serial dilution in tryptone salt broth in order to assess *Campylobacter* count on mCCDA plates after 44 ± 4 h of incubation at 41.5 ± 1°C in a microaerophilic atmosphere (85% N₂, 10% CO₂ and 5% O₂). The detection limit for enumeration of the *Campylobacter* was 1.10² CFU/g (2 log₁₀ CFU/g) of cecal content.

**Statistical Analysis**

In each of the three trials, a positive control group was used. However, a significant variation was observed for the control groups across the three trials at d 42. As a consequence the values obtained for the control groups of the three trials were pooled for each sampling date. This way of taking into account the overall variability of the control group across the three trials makes the comparison between the dietary treatments and the control group stricter than the comparison to the control group from each trial. To determine if the sampling date has a significant effect on the colonization by *Campylobacter* in the control group (pooled values of the three trials were used), the non-parametric Kruskal-Wallis and Mann-Whitney tests were used as the criteria of normality and homogeneity of the variances were not validated.

At each sampling period, tests were conducted to compare the means and standard deviations obtained for each dietary treatment with the mean and standard deviation obtained for the control group (pooled values of the three trials were used). The parametric test of Student was used when the criteria of normality and homogeneity of the variances were validated. On the other hand, the nonparametric Mann-Whitney test was used when the criteria of normality and homogeneity of the variances were not validated. Statistical analysis was carried out using the R software (R Core Team, 2013), available at http://www.r-project.org/.

**RESULTS**

**Effect on Colonization**

Results of the dietary treatments on *Campylobacter* counts are presented in Figure 1 and Table 2. To determine the mean of *Campylobacter* counts for the positive control group, the values obtained from the three trials were pooled together for each sampling time. Within the positive control group, mean counts of *Campylobacter* significantly (*P = 0.000*) decreased during the trial, but conversely, variability increased: 3 d after inoculation of the broilers (14 d of age) with a solution containing 5 log₁₀ CFU/mL, the mean counts were 8.23 ± 0.61 log₁₀ CFU/g, they were 7.50 ± 1.31 log₁₀ CFU/g at 35 d of age and 6.29 ± 2.2 log₁₀ CFU/g at 42 d of age (Table 2).

All the dietary treatments were provided from the beginning and throughout the duration of the trial,
Figure 1. Effect of dietary treatment on Campylobacter counts (log_{10} CFU/g) in the caeca of broilers at 14, 35, and 42 d of age (3, 24, and 31 d post-inoculation). Treatments giving significant reduction (P < 0.05) in Campylobacter counts compared to the control groups are marked with an asterisk. Letters (a,b,c) indicated a significant difference (P < 0.05) between the control group at each sampling date.

Table 2. Effect of dietary treatment on Campylobacter counts (log_{10} CFU/g) in the caeca of broilers at 14, 35, and 42 d of age (3, 24, and 31 d post-inoculation).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 14</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>log_{10} CFU/g (Mean ± SD)</td>
<td>log reduction p-value</td>
</tr>
<tr>
<td>Control</td>
<td>45</td>
<td>8.23 ± 0.61 (Mean ± SD)</td>
<td>-</td>
</tr>
<tr>
<td>LACTO-BUTYRIN</td>
<td>15</td>
<td>7.50 ± 0.44 (Mean ± SD)</td>
<td>0.73*</td>
</tr>
<tr>
<td>Biotronic® Top3</td>
<td>15</td>
<td>7.17 ± 2.08 (Mean ± SD)</td>
<td>1.06*</td>
</tr>
<tr>
<td>Campylostat</td>
<td>15</td>
<td>6.20 ± 1.51 (Mean ± SD)</td>
<td>2.02*</td>
</tr>
<tr>
<td>Admix® Precision</td>
<td>15</td>
<td>7.11 ± 1.74 (Mean ± SD)</td>
<td>1.12*</td>
</tr>
<tr>
<td>Excential Butycocat</td>
<td>15</td>
<td>7.78 ± 0.65 (Mean ± SD)</td>
<td>0.45*</td>
</tr>
<tr>
<td>Power Protection®</td>
<td>15</td>
<td>7.79 ± 1.40 (Mean ± SD)</td>
<td>0.44</td>
</tr>
<tr>
<td>Excential Alliin Plus</td>
<td>15</td>
<td>7.27 ± 0.85 (Mean ± SD)</td>
<td>0.96*</td>
</tr>
<tr>
<td>Anta® Phyto</td>
<td>15</td>
<td>7.09 ± 2.13 (Mean ± SD)</td>
<td>1.14*</td>
</tr>
<tr>
<td>Calsporin®</td>
<td>15</td>
<td>7.98 ± 0.48 (Mean ± SD)</td>
<td>0.25</td>
</tr>
<tr>
<td>Ecobiotic®</td>
<td>15</td>
<td>8.43 ± 0.25 (Mean ± SD)</td>
<td>-0.20</td>
</tr>
<tr>
<td>PoultryStart®</td>
<td>15</td>
<td>7.69 ± 0.69 (Mean ± SD)</td>
<td>0.54*</td>
</tr>
<tr>
<td>Original XPCTM™</td>
<td>15</td>
<td>8.18 ± 0.48 (Mean ± SD)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Significant log reduction is represented with an asterisk.
but no treatment was able to prevent contamination by *Campylobacter* 3 d post-inoculation. At 14 d of age, eight dietary treatments (Lactobutyrin BRC, Biotronic® Top3, PoultryStar®, Excential Butycot, Adimix® Precision, Anta® Phytophthora and Campylostat) significantly decreased the colonization by *Campylobacter* compared to the control group (Figure 1 and Table 2). The observed mean reduction was from 0.44 for the treatment with Power Protexion® extract to more than 2 log10 CFU/g for the treatment with Campylostat (Table 2).

It is important to note that the four dietary treatments giving a mean reduction over 1 log10 CFU/g (Biotronic® Top3, Adimix® Precision, Anta® Phytophthora and Campylostat) also presented a higher variability in *Campylobacter* counts (over 1.5 log10 CFU/g) (Table 2).

At 35 d of age (24 d postinoculation), three of these dietary treatments (LACTO-BUTYRIN, PoultryStar® and Adimix® Precision) were still having a significant effect on *Campylobacter* counts (Figure 1 and Table 2). The observed mean reduction varied between 0.85 for the treatment with LACTO-BUTYRIN and 1.88 log10 CFU/g for the treatment with PoultryStar®.

Efficient treatments with PoultryStar® and Adimix® Precision also showed a high variability (over 2 log10 CFU/g) in cell counts (Table 2). At 42 d of age (31 d post-inoculation), Adimix® Precision remained significantly efficient and showed a mean reduction of more than 2 log10 CFU/g (Figure 1 and Table 2). This treatment is the only one for which *Campylobacter* counts were significantly reduced compared to the control group for the three sampling times.

At 42 d of age, two other treatments, Calsporin® and Original XPC™, also showed a significant mean reduction on *Campylobacter* counts of 1.70 and more than 3 log10 CFU/g respectively (Figure 1 and Table 2).

At this sampling time, as observed for the control group, a high variability (over 1 log10 CFU/g) was observed irrespective of the dietary treatment (Table 2). The treatments giving the highest mean reduction, Adimix® Precision and Original XPC™, also showed a high standard deviation of 2.67 and 2.87 log10 CFU/g respectively (Table 2).

### Effect on Growth Performance

Results on growth performance are presented in Table 3. On d 14 the group treated with Campylostat showed a significantly lower weight in comparison with the control group, while the PoultryStar® group showed a significantly higher weight. For all the other groups no significant difference was observed between their mean weight and that of the control group. On d 35 and on d 42 the group treated with Campylostat still showed a significantly lower weight in comparison to the control group. The lower weight in the Campylostat group was linked to lower feed consumption throughout the study.

### DISCUSSION

Many different studies have tested the effect of different feed additives against *Campylobacter* in broilers. However, these studies were conducted using different experimental designs. For example, some of them, studying the effect of the tested product over a short period of time in trials lasting for example 10, 15, or 16 d (Solis de los Santos et al., 2008; Ghareeb et al., 2012; Robyn et al., 2013b) which does not reflect the period, until slaughter age.

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### Table 3. Technical performances by group (the control group was compared to each tested group, the tested groups were not compared one to another).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N d 14</th>
<th>BW (g) d 14 ± SD</th>
<th>N d 35</th>
<th>BW (g) d 35 ± SD</th>
<th>N d 42</th>
<th>BW (g) d 42 ± SD</th>
<th>ADWG (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139</td>
<td>405.7 ± 65.6</td>
<td>93</td>
<td>1958.3 ± 307.4</td>
<td>48</td>
<td>2769.4 ± 441.5</td>
<td>65.2</td>
</tr>
<tr>
<td>LACTO-BUTYRIN</td>
<td>44</td>
<td>466.1 ± 63.2</td>
<td>28</td>
<td>2038.6 ± 263.6</td>
<td>14</td>
<td>2771.9 ± 247.1</td>
<td>65.1</td>
</tr>
<tr>
<td>Biotronic® Top3</td>
<td>46</td>
<td>366.5 ± 108.6</td>
<td>30</td>
<td>1904.7 ± 543.7</td>
<td>16</td>
<td>2525.9 ± 637.2</td>
<td>59.2</td>
</tr>
<tr>
<td>Campylostat</td>
<td>45</td>
<td>*357.1 ± 41.7</td>
<td>29</td>
<td><em>1651.4 ± 196.8</em></td>
<td>14</td>
<td><em>2349.6 ± 280.0</em></td>
<td>54.9</td>
</tr>
<tr>
<td>Adimix® Precision</td>
<td>45</td>
<td>421.1 ± 49.5</td>
<td>30</td>
<td>2096.4 ± 243.8</td>
<td>15</td>
<td>2726.9 ± 454.7</td>
<td>63.9</td>
</tr>
<tr>
<td>Excential Butycot</td>
<td>48</td>
<td>413.8 ± 57.2</td>
<td>33</td>
<td>1890.5 ± 263.9</td>
<td>18</td>
<td>2557.3 ± 395.6</td>
<td>60.0</td>
</tr>
<tr>
<td>Power Protexion®</td>
<td>45</td>
<td>395.7 ± 54.2</td>
<td>29</td>
<td>2064.0 ± 268.1</td>
<td>15</td>
<td>2732.3 ± 274.0</td>
<td>63.7</td>
</tr>
<tr>
<td>Excential Allin Plus</td>
<td>49</td>
<td>398.2 ± 59.9</td>
<td>34</td>
<td>1886.0 ± 253.7</td>
<td>19</td>
<td>2665.2 ± 407.4</td>
<td>61.2</td>
</tr>
<tr>
<td>Anta® Phytophthora</td>
<td>45</td>
<td>387.8 ± 62.1</td>
<td>31</td>
<td>1946.7 ± 261.1</td>
<td>17</td>
<td>2856.3 ± 355.0</td>
<td>66.9</td>
</tr>
<tr>
<td>Calsporin®</td>
<td>47</td>
<td>439.0 ± 58.7</td>
<td>32</td>
<td>2091.0 ± 281.2</td>
<td>17</td>
<td>2919.3 ± 421.3</td>
<td>68.6</td>
</tr>
<tr>
<td>Ecolbrid®</td>
<td>47</td>
<td>382.9 ± 89.6</td>
<td>33</td>
<td>1843.8 ± 367.5</td>
<td>18</td>
<td>2607.4 ± 506.9</td>
<td>61.2</td>
</tr>
<tr>
<td>Poultrystar®</td>
<td>43</td>
<td>b466.0 ± 68.9</td>
<td>30</td>
<td>2052.5 ± 173.1</td>
<td>15</td>
<td>2915.3 ± 250.6</td>
<td>68.5</td>
</tr>
<tr>
<td>Original XPC™</td>
<td>30</td>
<td>391.5 ± 56.3</td>
<td>30</td>
<td>1872.7 ± 206.9</td>
<td>15</td>
<td>2827.5 ± 221.0</td>
<td>66.2</td>
</tr>
</tbody>
</table>

*average weight significantly lower than in the control group.

baverage weight significantly higher than in the control group.

1Average Daily Weight Gain is calculated as follows: (mean body weight d 42 – mean body weight d 1)/42. There is one ADWG per group.
Additive tested in this work were chosen according to several criteria. They had to be available commercial products or products under development, and had to contain compounds belonging to the four families of products usually tested against Campylobacter: organic acids, plant extracts, probiotics and prebiotics (Robyn et al., 2015).

According to the suppliers’ recommendations, a preventive supplementation from day-of-hatch was performed in this study: birds were fed with the additive before the challenge with Campylobacter until the end of the trial. However, no treatment was able to completely prevent the broilers from becoming colonized with Campylobacter at 14 d of age. Eight products gave a significant mean reduction at 14 d of age, but only three of these dietary treatments were significantly efficient against Campylobacter at 35 d of age. The five remaining products could be short-acting products to be used therapeutically 2 to 3 d before slaughter as recommended in other studies (Hilmarsson et al., 2006; Molatova et al., 2011). Some products were able to decrease Campylobacter contamination until 35 d of age, and some other products required 42 d to become efficient. These products are both interesting, as the age of the broilers at slaughter may differ from one Member State to another.

Many studies have tested the effect of several organic acids, as short chain fatty acids (SCFA), medium chain fatty acids (MCFA) or their monoglycerides (Robyn et al., 2015). In this study we tested six SCFA or monoglycerides based products. The monoglyceride mixture based product (LACTO-BUTYRIN) led to a significant reduction of less than 1 log at 14 and 35 d of age, but was not significantly efficient at 42 d of age. It was previously found that a treatment with monocaprin in water and in feed was efficient particularly during the first 2 d of treatment (Hilmarsson et al., 2006).

SCFA are known for their antibacterial activity due to their ability to diffuse across the bacterial membrane in the undissociated form (Sun, O’Riordan, 2013). Two different products containing a mixture of SCFA, Biotronic® Top3 and Campylostat, were tested in this study and led to a significant reduction of about 1 log10 and 2 log10 respectively at 14 d of age, but with no effect later. Campylostat, the most efficient product at d 14, contains a mixture of formic acid and sorbate; Skanseng et al. (2010) have already shown that combinations of these SCFA could reduce or prevent C. jejuni colonization in chicken.

In this study the two coated butyrate-based products, Adimix® Precision® and Excential Butycoat also gave significant reductions at 14 d. Among these products, Adimix® Precision® gave the higher reduction (over 1 log10), and it was still significantly efficient at 35 and 42 d, but the dose used for this product was three times higher than Excential Butycoat. It was also higher than the dose used by Van Deun et al. (2008) who observed that butyrate coated micro-beads was unable to reduce C. jejuni cecal colonization in 2-wk-old broiler chicks.

A product containing butyrate and a tannin extract (Power Protexion®) was also tested but no significant reduction was observed. A bactericidal effect of tannin extract has already been reported on C. jejuni in vitro (Anderson et al., 2012).

Two other products containing plant extract were tested: Excential Alliin Plus and Anta®Phyt.

Excential Alliin Plus is a mixture of freeze-dried garlic and cinnamon. Separately, allicin and cinnamon oil have proved to be effective in reducing Campylobacter numbers in vitro, but they failed to reduce colonization in broilers (Hermans et al., 2011; Robyn et al., 2013b). Under the test conditions applied in this study, Excential Alliin Plus reduced the contamination of about 1 log10 CFU/g 3 d post-inoculation but was not efficient at the slaughter age (35 or 42 d). The same results were obtained with Anta®Phyt, based on a combination of natural plant extracts, essential oils and a prebiotic complex.

The antimicrobial properties of different plant-derived compounds against several food pathogens, including C. jejuni, have been demonstrated in vitro (Friedman et al., 2002; Kollanoor-Johny et al., 2010). However, among the few studies reporting the results of in vivo trials, plant-derived compounds mostly failed to have a noticeable impact in reducing Campylobacter colonization in broilers (Hermans et al., 2011; Robyn et al., 2013b; Arsi et al., 2014; Kurecki et al., 2014). It has been suggested that these compounds could be absorbed or degraded before they reach the ceca (Hermans et al., 2011; Arsi et al., 2014) or that the ceca could protect Campylobacter from their antimicrobial activity (Arsi et al., 2014).

Three probiotic-based products were evaluated in this study: Calsporin®, Ecobiol® and PoultryStar®. Calsporin® and Ecobiol® are mono-species probiotics based on Bacillus strains, B. subtilis C-3102 and B. amyloliquefaciens CECT5940 respectively. Calsporin® has already been shown to reduce Campylobacter in broilers (Maruta et al., 1996; Fritts et al., 2000) and Ecobiol® has been shown to inhibit the growth of C. jejuni populations in vitro (Mallo, 2014). In this study, for these two probiotics, the greatest reduction in Campylobacter counts (over 1 log) was observed at 42 d, although it was not significant for Ecobiol®, due to high variability. PoultryStar®; on the other hand is a multi-species probiotic containing Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis, Lactobacillus salivarius and Lactobacillus reuteri. In this study, PoultryStar® led to a significant reduction at d 14, and an even more significant reduction (1.88 log) was observed at 35 d of age. This reduction is promising, but is quite lower than that previously reported (Ghareeb et al., 2012). A 6-log reduction was observed with PoultryStar® added to drinking water 8 or 15 d postchallenge; however, the chicks were challenged at day-of-hatch and the trial was stopped after 15 d (Ghareeb et al., 2012). Other studies have reported the beneficial effect of different probiotics on
Campylobacter reduction in broilers (Santini et al., 2010; Neal-McKinney et al., 2012; Nishiyama et al., 2014) while others did not observe any effect (Stern et al., 2008; Robyn et al., 2013a).

Prebiotics are indigestible food components that can serve as substrate for the gut microbiome leading to a beneficial effect for the host. Original XPC™ is a prebiotic-like product, composed of the yeast Saccharomyces cerevisiae and the media on which it was grown. In this study this product led to the highest mean reduction (over 3 log) at 42 d. A previous study showed that Original XPC™ fed broilers had a lower prevalence and load of Campylobacter at market age (McIntyre, 2014).

Most of the tested products are commercial additives so they should have been validated for growth performance parameters. Among the tested products, only one (Campylostat) produced animals with lower body weight and decreased feed consumption; however this product is under development. Reduced body weight was also observed by Skanseng et al. (2010) with a similar product. However, lower feed consumption could have a negative impact on the action of the product.

Although this study gives promising results concerning the use feed additives to reduce Campylobacter colonization in flocks, we have to consider the high individual variation observed among the broilers regarding Campylobacter contamination, which was observed in the control group (over 2 log at 42 d). This variability could be due, at least in part, to the large variation in the microbiota composition of chickens, which can occur even under carefully controlled conditions (Stanley et al., 2013), as microbial taxa may increase the likelihood of colonization by C. jejuni (Kaakonsh et al., 2014). Moreover, Hansson et al. (2010) previously observed that colonization of individuals is not always homogenous within the same flock, and the observed variation in Campylobacter loads between ceca in a positive flock can be as high as 6.3 log CFU/g of cecal content (Hansson et al., 2010).

According to Romero-Barrios et al. (2013), reducing colonization in cecal contents of brocks by 2 log or 3 log is estimated to reduce human campylobacteriosis cases attributable to broiler meat by at least 76% or 90% respectively. In this study, at the slaughter age of 42 d, two products (Adimix™ Precision and Original XPC™) were able to reduce the mean colonization of 2 or 3 log, but the observed reduction was not homogenous among the birds; some of them remained contaminated at over 7 log CFU/g. These highly contaminated broilers could be sources of contamination during the slaughtering process. Furthermore, these trials were carried out in experimental facilities, and do not realistically reflect field conditions, which include numerous on-farm sources of Campylobacter (Agunos et al., 2014; Allain et al., 2014) leading to possible recontamination of the flock during the rearing period. So the action of these products should therefore be validated during on-farm trials.

Further research studying the effect of other commercial products or of a combination of different types of products, acting together synergistically to improve the reduction of cecal colonization of Campylobacter in broilers, would be worthwhile. Nevertheless, a global approach, combining intervention measures at the different steps of the broiler meat production chain, at preharvest, harvest, and postharvest could have a greater impact on the reduction of public health risk.

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REFERENCES


EFSA (European Food Safety Authority). 2010b. EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA J. 8:1437.


Gracia, et al. 2015. XXXX, 00:000–000.


