



Campylobacter, Helicobacter & Related Organisms (CHRO)

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Delegate Handbook

O124

Optimization of air chilling process to control *Campylobacter* contamination on broiler legs

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Most cases of campylobacteriosis are associated with eating raw or undercooked poultry meat or cross-contaminated foods by these items. Several risk assessment studies concluded that reducing *Campylobacter* load on meat would reduce significantly the number of campylobacteriosis associated with broiler meat. This work aims to define optimal air chilling conditions to reduce *Campylobacter* levels on poultry carcasses. This study was set up to investigate four major parameters in the chilling process (temperature, duration, air velocity and initial concentration of *Campylobacter*) individually and in interaction on the behaviour of *Campylobacter* using the Doehlert shell design. Three experimental designs were performed using a chilling prototype and a ST-45 strain isolated from poultry. Moreover, several different strains (ST, virulence) were tested according to an optimal combination of these four parameters. The maximum contamination reduction reached a rate of 63% (reduction of 1.5 log CFU/g). Duration of chilling ($p=0.04$), initial concentration ($p=0.03$) and an interaction between temperature and initial concentration ($p=0.01$) had significant effects. When initial concentration was fixed (10^3 CFU/g), temperature effect ($p=0.0045$) was confirmed. Moreover, the interaction between temperature and air velocity ($p=0.007$) was also significant on *Campylobacter* contamination. First results have shown no significant difference between the different strains tested. The most important result is that carcasses presenting more than 10^3 CFU/g of *Campylobacter* would not be significantly decontaminated during the chilling process. Moreover this work shows that a chilling process with low temperature can significantly reduce the bacterial load on chicken carcasses presenting not more than 10^3 CFU/g.

O125

Effect of feed presentation (mash vs pellets) and whole wheat addition on cecal morphology and *Campylobacter jejuni* colonization of broilers orally infected

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An experiment was conducted within project CAMPYBRO for evaluating the effect of feed presentation (FP) and whole wheat (WW) addition on cecal morphology and *Campylobacter jejuni* colonization. There were six treatments factorially arranged with two FP (Mash [M] vs Pellets [P]), and three levels of WW from 0-21/21-42d: 0/0, 7.5/15%, 15/30%. A total of 216 Ross 308 broilers were used (3 birds/cage, 12 cages/treatment). At 14 d, broilers were orally gavaged with 100 μ l (10^5 cfu/ml of ST-45 *C. jejuni*). On days 21, 35 and 42, caeca from 12 birds/treatment were collected and *Campylobacter* counts determined (ISO 10272). At 42d, weight and pH at caeca were taken. Data were analysed by GLM procedure of SPSS. At 21d of age, M tended to show lower *C. jejuni* values than P (7.85 vs 8.27 log₁₀ cfu/g, $P=0.091$). Also, the 7.5/15% inclusion rate showed less contamination than the higher level ($P=0.048$). Also, at 21d M with 7.5% of WW showed less *C. jejuni* population than P + 15% of WW ($P=0.006$). No effects on *C. jejuni* counts were detected at 35 or 42d. There was an interaction FP*WW for caeca (%BW): caeca of birds fed the 15/30%WW were the biggest in M, and 7.5/15%WW in P diets ($P=0.070$); and for cecal pH: WW increased the pH in M but not in P diets ($P=0.016$). It is concluded that M and WW at 7.5/15% showed less *C. jejuni* population than P at 21, and that WW caused different physiological effects depending on FP.

Control strategies for *Campylobacter* - 3

Thursday 5th November

1315-1405

O142

Effect of feeding a combination of a yeast product and a probiotic, alone or in combination with a blend of mono-glycerides, on *Campylobacter* colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a yeast extract (XPC) with a multispecies probiotic (PS) alone or together with a blend of mono-glycerides (MG) added to the feed on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + XPC at 1,250 g/t + PS at 1,000 g/t and T3: T2 + MG at 8,000 g/t. A total of 126 one-day-old Ross 308 broilers were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1×10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as \log_{10} CFU/g caeca content analysed by the nonparametric test of Kruskal-Wallis, followed by the Dunn's test (SPSS v.19.0). No significant differences in the *Campylobacter* counts were observed between the two products tested and the control treatment at 21 and 35 d of age. At the end of trial, both combinations significantly reduced *Campylobacter* colonization when compared with non-treated broilers (8.39^a, 6.86^b and 7.51^b \log_{10} CFU/g, for T1, T2 and T3, respectively). It is concluded that the combination of a multispecies probiotic with a prebiotic is effective and reduces *Campylobacter jejuni* at cecal level.

O143

Picking up *Campylobacter* with the weekly shop: A United Kingdom study to establish the levels of *campylobacter* on the packaging of fresh raw whole chicken at retail sale over a 12 month period

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Background: Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne transmission route for *Campylobacter* infection. Handling chicken in the kitchen has been linked to infection, but the role of packaging of meat at retail sale has received limited attention as a potential source of infection.

Objective: To establish the risk of exposure to *Campylobacter* species to consumers from handling packaged fresh whole raw chicken at retail sale.

Methods: The outer packaging fresh whole retail chickens (n=4005) were swabbed to establish the levels of *Campylobacter* species present. Samples were collected in sterile plastic bags, based on market share data, which allocated the majority of samples to the major supermarkets in the UK although a variety of retailers were represented. Packaging included plastic bags used by butchers shops through to modified atmosphere packaging in plastic film by the supermarket brands. On receipt in the laboratory, the packing was swabbed and analysed to determine carriage of *Campylobacter* on the external surface.

Results: Overall 6.8 % of chicken packaging was contaminated with *Campylobacter*. The majority, 5.2% (n=209), were contaminated with between 10–99 cfu per swab but 1.4% (n=58) had between 100–1000 cfu per swab. At the higher level of contamination, 0.1% (n=5) of chicken packaging tested had >1000 cfu (up to 4500) per swab. *Campylobacter jejuni* and *C. coli* were detected.

Conclusions: This work shows that handling fresh whole raw chicken at retail sale presents some risk to consumers, despite the pre-packaged approach many retailers employ.

P119

Heterogeneity in the infection biology of *Campylobacter jejuni* isolates in three infection models reveals an invasive and virulent phenotype in a ST21 isolate from poultry.

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Background: Our understanding of the infection biology of *Campylobacter jejuni* is based on relatively few isolates. Our recent work has shown that there can be considerable variation in the infection ecology between isolates in the chicken. Understanding phenotypic variation in infection is important in understanding risk and developing controls.

Objectives: To understand the degree of heterogeneity of infection biology of *C. jejuni* in relevant models.

Methods: A panel of 5 *C. jejuni* isolates (M1, 13126, 12662, DBM1 and NCTC 11168H) was tested in three infection models to determine their infection phenotype: 1. Oral infection of broiler chickens, 2. Invasion of human intestinal epithelial cells, and 3, Virulence in the *Galleria melonella* insect model.

Results: All isolates tested colonized the caeca of broiler chickens to similar levels. Extra-intestinal spread to the liver varied; ranging from 9% of birds infected with DBM1 to 60% infected with 13126. All isolates invaded CaCo2 intestinal epithelial cells at a level of <0.01% of the inoculum, except 13126 that invaded to levels around 0.2% of the inoculum. *Galleria* mortality rates at 48 h ranged from 3% for 12262, 17% M1, 33% for NCTC 11168H to 47% for 13126.47%.

Conclusions: The infection biology of *C. jejuni* varies and assumptions based on 'commonly used isolates may not accurately reflect the biology of field isolates such as 13126 that show increased invasion and pathogenicity across a range of infection models. Such isolate may pose an increased risk of spread to the edible tissues of poultry and in causing disease.

P120

Effect of oat hulls addition and whole wheat addition on cecal morphology and *Campylobacter jejuni* colonization of broilers orally infected

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An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of whole wheat (WW) and oat hulls (OH) addition on cecal morphology and *Campylobacter jejuni* colonization in orally infected broilers. There were three treatments: a mash diet (T1), T1+ WW (7.5/15% from 0-21/21-42d, respectively), and T3: T2+5%OH. A total of 108 one-day-old Ross 308 broilers were divided into floor pens (36 birds/pen) and experimental treatments (36 birds/treatment). At 14 days of age, 3 broilers per pen were orally gavaged with 100 µl of a solution containing 1 x 10⁵ cfu/ml of ST-45 *C. jejuni* strain. On days 21 and 42, caeca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). There were not significant differences between treatments at 21d. However, the diet with WW at 7.5% from 0-21d and 15% from 21 to 42d, and 5% OH, showed a 1.4 log₁₀ cfu/g reduction in cecal *C. jejuni* counts with respect to Control diet (9.48 vs 8.10 log₁₀ cfu/g for Control diet vs Control+whole wheat+oat hulls, respectively; P=0.001). The WW alone decreased 0.48 log₁₀ cfu/g with respect to Control diet, but differences were not significant. It is concluded that WW at 7.5/15% plus 5% OH showed less *C. jejuni* population than control diet at 42d.
