Introduction & Aim

Human campylobacteriosis is mainly caused by the consumption of Campylobacter jejuni contaminated poultry meat. Lowering the C. jejuni excretion and external contamination of broilers prior to slaughter by 1, 2 or 3 log colony forming units, could lead to an average reduction of human campylobacteriosis cases in Belgium by 60%, 87% or 96%, respectively (Messens, et al., 2007). The project aims to lower the level of C. jejuni colonization and excretion during primary poultry production by providing drinking water containing allicin (a garlic derived phytochemical) to broiler chickens. Other garlic derived phytochemicals like the allin precursor allicin and allicin decomposition products like allyl disulfide and garlic oil extract were also tested for their anti-C. jejuni activity.


Materials & Methods

A. In vitro testing of garlic derived phytochemicals

![Garlic oil extract](image)

C. jejuni MB 4185 in Nutrient broth No2

1. Allicin concentrations tested: 500, 250, 125, 62, 31, 15 and 7.5 ppm
2. All concentrations tested in absence and presence of C. jejuni growth supplement in Nutrient broth No 2
3. Garlic oil extract and allyl disulfide concentrations tested: 100 & 50 ppm
4. Allicin concentrations tested: 50 ppm

- Microaerobic incubation for 48h at 41.5°C
- Tenfold dilutions of samples taken at 0 h, 24 h and 48 h
- Plated out on mCCDA. Microaerobic incubation for 24 to 48h at 41.5°C

B. Batch fermentation studies

Campylobacter jejuni MB 4185

Aliacin concentrations

- New Brunswick Scientific Bioflo110 fermentor
- Nutrient broth pH 6.5, 150 rpm agitation, 5% O2, 10% CO2 - 85% N2

Experimental design

1. Design 1: inoculation of C. jejuni and addition of allicin at the same time (therapeutic influence)
2. Design 2: addition of allicin, followed 24 hours later by C. jejuni inoculation (protective influence)
3. Filter sterilized allicin concentrations tested: 125, 50, 25 and 10 ppm
4. One fermenter vessel: control → only C. jejuni KC 40 inoculation

- Sample taking: 0 to 48h (design 1 and 2)
- Enumeration on mCCDA → C. jejuni KC 40

C. In vivo experiments

Experimental Design

1. Six groups of ± 10 chickens: 3 control + 3 provided with drinking water containing 25 ppm allicin (tolerated by chicks) from day 1.
2. Seeder model: Day 15: 2 chickens per group: orally inoculated with 1.0 x 10⁶ cfu of C. jejuni MB 4185
3. Day 21: chickens euthanized with T61 injection
4. Aseptically collecting ceca
5. Enumeration of C. jejuni on mCCDA

Results

A. In vitro testing of garlic derived phytochemicals

- All concentrations of allicin are bactericidal against C. jejuni after 24 h. In presence of C. jejuni growth supplement only 500, 250 and 125 ppm bactericidal.
- Allin: no influence. Garlic oil extract and allyl disulfide: both tested concentrations bactericidal after 24 h

C. In vivo experiments

- Six groups: Control (C1-3) and Allicin provided groups (A1-3)
- Two allicin provided groups had statistically significant higher cecal C. jejuni counts than all three control groups and one allicin provided group.

Conclusions

Obtained results suggest that in both in vitro batch fermentation designs (therapeutic and protective) allicin is able to inhibit C. jejuni growth in the first 24 h (concentration = 25 ppm) of incubation or over longer incubation periods (concentration > 25ppm). Allicin derivatives are also bactericidal at a 50 ppm concentration. According to a risk model, this could lead to an average reduction of human campylobacteriosis cases in Belgium by > 96%. The 25 ppm allicin concentration was unable to reduce cecal Campylobacter colonization. C. jejuni might be protected by cecal mucus, as mucus contains a lot of eysite groups which bind the active group of allicin and lower allicin activity. Allicin might also inhibit other cecal /intestinal bacteria neutralizing a part of the possible competitive exclusion by other bacteria.