Evaluation of allicin as antibacterial agent against *Campylobacter jejuni* in *in vitro* experiments and in a broiler seeder experiment

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Introduction & Aim

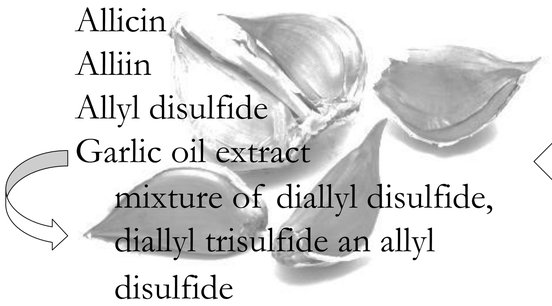
Human campylobacteriosis is mainly caused by the consumption of *Campylobacter jejuni* contaminated poultry meat. Lowering the *C. jejuni* excretion and external contamination of broilers prior to slaughter by 1, 2 or 3 log colony forming units, could lead to an average reduction of human campylobacteriosis 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 allicin precursor alliin and allicin decomposition prducts like allyl disulfide and garlic oil extract were also tested for their anti-*C. jejuni* activity.

Reference: Messens, *et al.* Quantitative risk assessment of human campylobacteriosis through the consumption of chicken meat in Belgium. In *XVIII European symposium on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs and Egg Products*, 2007. Prague, Tsjechië, 167-168.

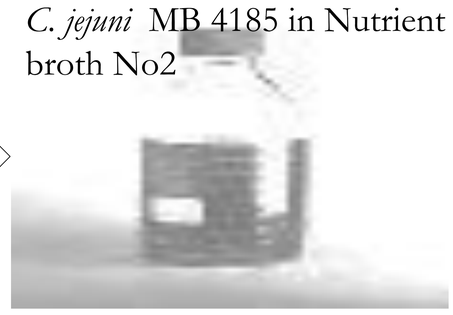
Materials & Methods

A. *In vitro* testing of garlic derived phytochemicals

Allicin
Alliin
Allyl disulfide
Garlic oil extract
mixture of diallyl disulfide, diallyl trisulfide an allyl disulfide



C. jejuni MB 4185 in Nutrient broth No2



1. Allicin concentrations tested: 500, 250, 125, 62, 31, 15 and 7,5 ppm
2. Allicin 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. Alliin concentrations tested: 50 ppm

1. Microaerobic incubation for 48h at 41.5°C.
2. Tenfold dilutions of samples taken at 0 h, 24 h and 48 h
3. Plated out on mCCDA. Microaerobic incubation for 24 to 48h at 41,5°C.

B. Batch fermentation studies

Campylobacter jejuni MB 4185 ⇕ **Allicin concentrations**

Batch Fermentation

- New Brunswick Scientific BioFlo110 fermentor
- Nutriënt broth n°2 + 0,1% mucine from porcine stomach
- 41.5°C, pH 6.5, 150 rpm agitation, 5% O_2 - 10% CO_2 - 85% N_2

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 fermentor 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 10^4 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

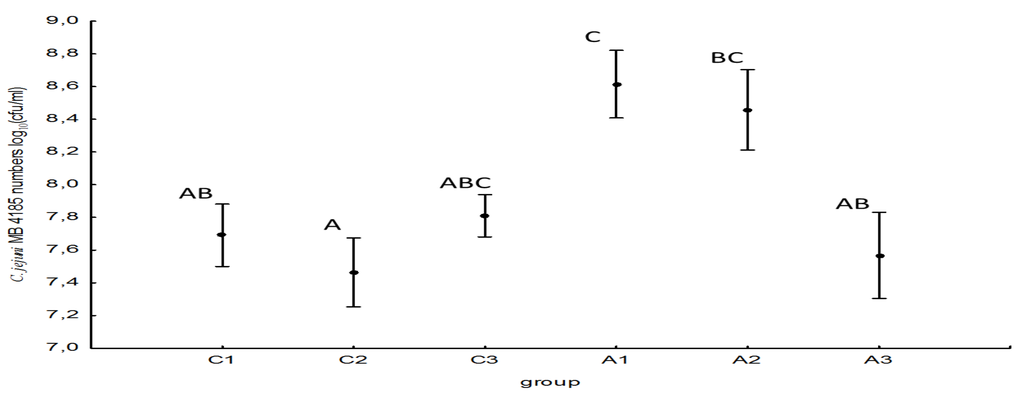
Results

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.
- Alliin: no influence. Garlic oil extract and allyl disulfide: both tested concentrations bactericidal after 24 h

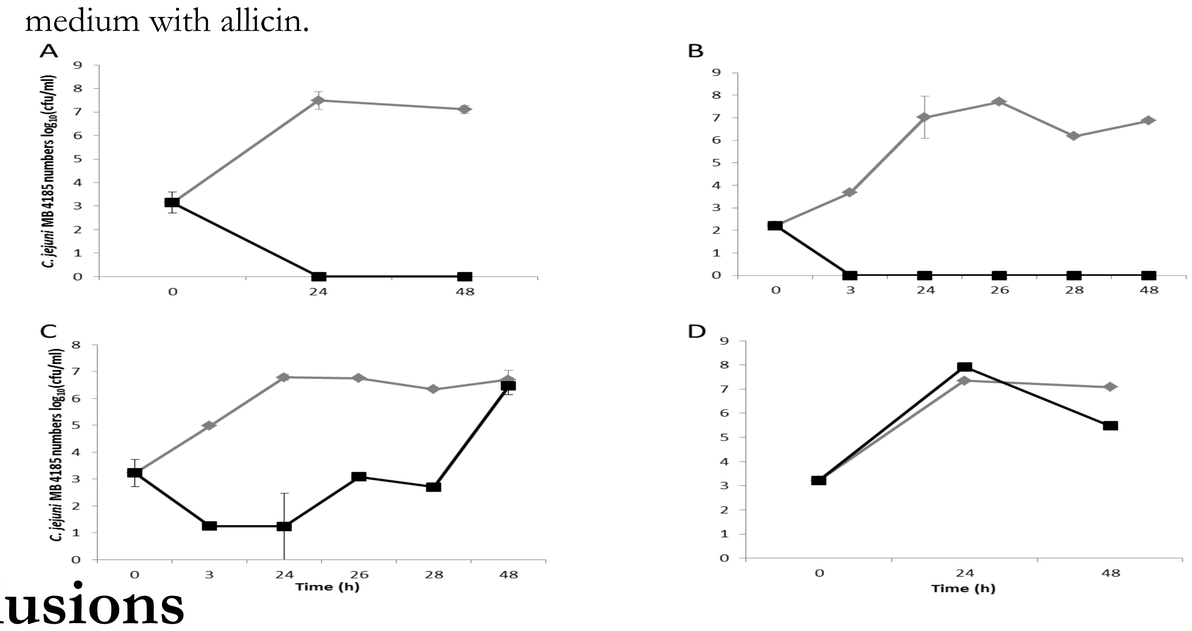
C. *In vivo* experiments

- 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.



B. Batch fermentation studies

- Therapeutic influence of A) 125, B) 50, C) 25 and D) 10 ppm of allicin on *C. jejuni* MB 4185 numbers**.** No *C. jejuni* found after 24h incubation in presence of 125 and 50 ppm allicin. No influence of 10 ppm allicin. Allicin concentrations of 25 ppm inhibit *C. jejuni* growth in the first 24h, but growth resumes after 48h. Results are comparable in protective design. ◊: positive control. □: *C. jejuni* MB 4185 in growth medium with allicin.



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 derivates 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 allcin concentration was unable to reduce cecal *Campylobacter* colonization. *C. jejuni* might be protected by cecal mucus, as mucus contains a lot of cystein 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.

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