Effect of exposure to stress conditions on propidium monoazide (PMA) - qPCR based enumeration of *Campylobacter* in broiler carcass rinses

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Introduction

The reference ISO culture-based method for the enumeration of *Campylobacter* in broiler carcass meat uses a selective medium which contains substances potentially harmful to cells that are frequently sublethally injured due to the different conditions that food undergoes until it reaches the final consumer. As consequence a *Campylobacter* underestimation can happen. In contrast to the culture-based methods, *Campylobacter* quantification by qPCR is unable to quantify just viable cells. To overcome this disadvantage reagents like propidium monoazide (PMA) have been used. In the present study a PMA-qPCR for the enumeration of *Campylobacter* was developed, evaluated and compared to the reference culture-based method for both artificially and naturally contaminated broiler carcass rinses. Additionally an evaluation of the method on stressed cells was performed. Five different stress conditions normally encountered during the broiler slaughter process and/or storage, were inflicted to *Campylobacter jejuni* (*C. jejuni*) or *Campylobacter coli* (*C. coli*) artificially contaminated broiler carcass rinses.

Methods

Quantification:
- Microbiological (CFA and mCCDA)
- qPCR
- PMA-qPCR

Stresses:
- Heat: 55ºC, 5 min
- Cold: 4ºC, 10 days
- Freezing: - 21ºC, 3, 7, 10 and 13 days
- Oxidation: 750µM, 4ºC, 24h
- Acid: pH 4.3, 37ºC, 1h

Conditions:
- *C. jejuni* or *C. coli*
- Initial concentration = 10^6 CFU/ml
- Broiler rinse dilution = 1:5
- Volume of sample extracted = 2mL
- Volume of DNA elution buffer = 100µL

Results

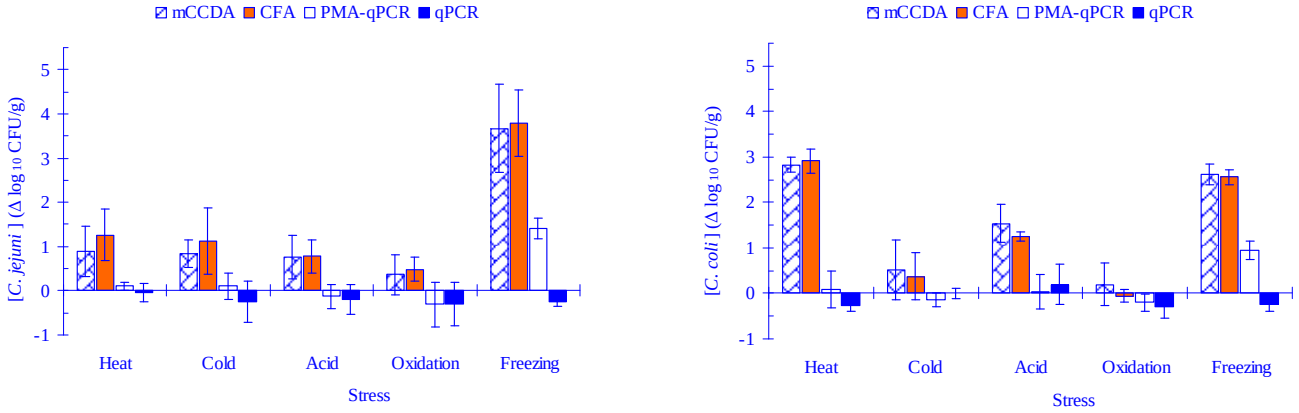

Figure 1: Quantification of the effect caused by the heat, cold, acid, oxidation and freezing stresses induced to broiler rinse artificially contaminated with *C. jejuni* or *C. coli* expressed as Δ log $_{10}$ CFU/g (difference between the enumeration of the non-stressed and stressed samples).

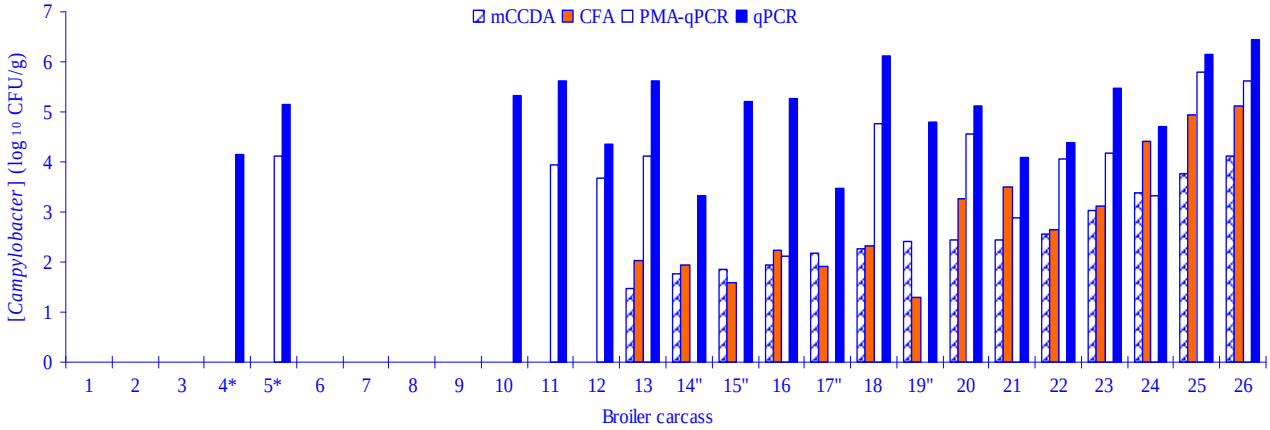

Figure 2: Enumeration of *Campylobacter* in 26 naturally contaminated broiler carcasses meat by the reference method with mCCDA and CFA media (LOQ at 10 CFU/g or 1 log $_{10}$ CFU/g) and by the qPCR method with and without PMA treatment (LOQ at 50 CFU/g or 1,7 log $_{10}$ CFU/g). The samples with asterisk (*) were frozen before analysis and for the samples with (") the PMA-qPCR enumeration was under the method LOQ.

Conclusions

- Only the freezing stress was able to reduce significantly the number of both *Campylobacter* for all used methods.
- Exposure to oxidative stress did not affect the *Campylobacter* counts in any of the used methods.
- For heat and acid a significant higher reduction on the enumerated bacteria using the culture-based methods was observed in *C. coli* as compared to *C. jejuni* although this was not confirmed by the PMA-qPCR method.
- A better correlation between the reference method and the qPCR enumeration was obtained when PMA was used.
- No significant differences were observed between both media for the reference method.
- A potential underestimation might be associated with the culture-based enumeration methods under stress situations that can be overcome by the PMA-qPCR enumeration.

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