An encapsulated blend of essential oils and organic acids used as feed additive lowers broiler chicken *Campylobacter* carcass contamination without any beneficial effect in the caecum.

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Abstract

Introduction. *Campylobacter jejuni* colonizes the chicken gut at high numbers: up to 10^9 CFU/g of fecal matter. Elimination or reduction of this bacterial load would lower the risks for consumers to contract campylobacteriosis through consumption and mishandling of chicken meat products. Many studies have tried to achieve this goal using feed additives, but with inconsistent success. Application of universal control measures of *Campylobacter* at the farm is also complicated by the extensive genetic and phenotypic diversity of strains that are able to colonize the chicken gut, making the evaluation of the efficacy of a control method on distinct strains crucial. In this study, an experimental blend of essential oils and organic acids (developed by JEFO Nutrition inc.) was tested as an alimental strategy to control *C.jejuni* chicken gut colonization, using co-inoculation of strains possessing different phenotypic properties.

Materials and Methods. Day-old birds (n = 200) acquired from a local hatchery were distributed in 2 rooms. In each room, 2 groups (n = 50) were constituted: one received the feed additive at 500 ppm throughout the study and the other received the same diet without it. At 14 days of age, chickens were orally inoculated; all chickens in room 1 received strains CRVS-1 and CRSV-2 while those in room 2 were inoculated with strains CRSV-3 and CRSV- 4. These 4 different strains possessed different levels of autoagglutination, chemotaxis and adhesion/invasion of primary chicken caecal cells. Weekly, chickens were euthanized by bleeding after an electronarcosis, scalded, mechanically plucked and manually eviscerated. *Campylobacter* present in the caecum and on the carcasses (400 ml buffered peptone water rinses) were enumerated on CASA agar. Total aerobic bacteria counts were also recorded for the carcasses rinses using Petrifilms.

Results. The feed additive did not prevent chicken gut colonization. In room 1, in the caecum, the feed additive had an effect only at 21 days post-inoculation (PI): counts were higher in the treated chicken by 1.4 log CFU/g. In room 2, caecal counts were lower for the treated birds only at 7 PI by 1.3 log CFU/g. Unexpectedly, for the room 1 treated chicken carcasses, a lower contamination of 1.4 log CFU/carcass was observed at 21 PI. For room 2, the feed additive had no significant effect on the carcasses but a decrease of 0.5 log CFU/carcass for the treated chickens could be observed also at 21 PI. Carcasses total aerobic bacterial counts were not affected by the feed additive.

Conclusions. The effect of the feed additive was strain dependant. The feed additive was successful to lower carcass contamination at slaughter age, in contradiction with its effect on caecum colonization level. Based on our results, we hypothesized that the feed additive affected positively some *C.jejuni* strains but selected for or rendered them more sensitive to the stresses encountered during processing.

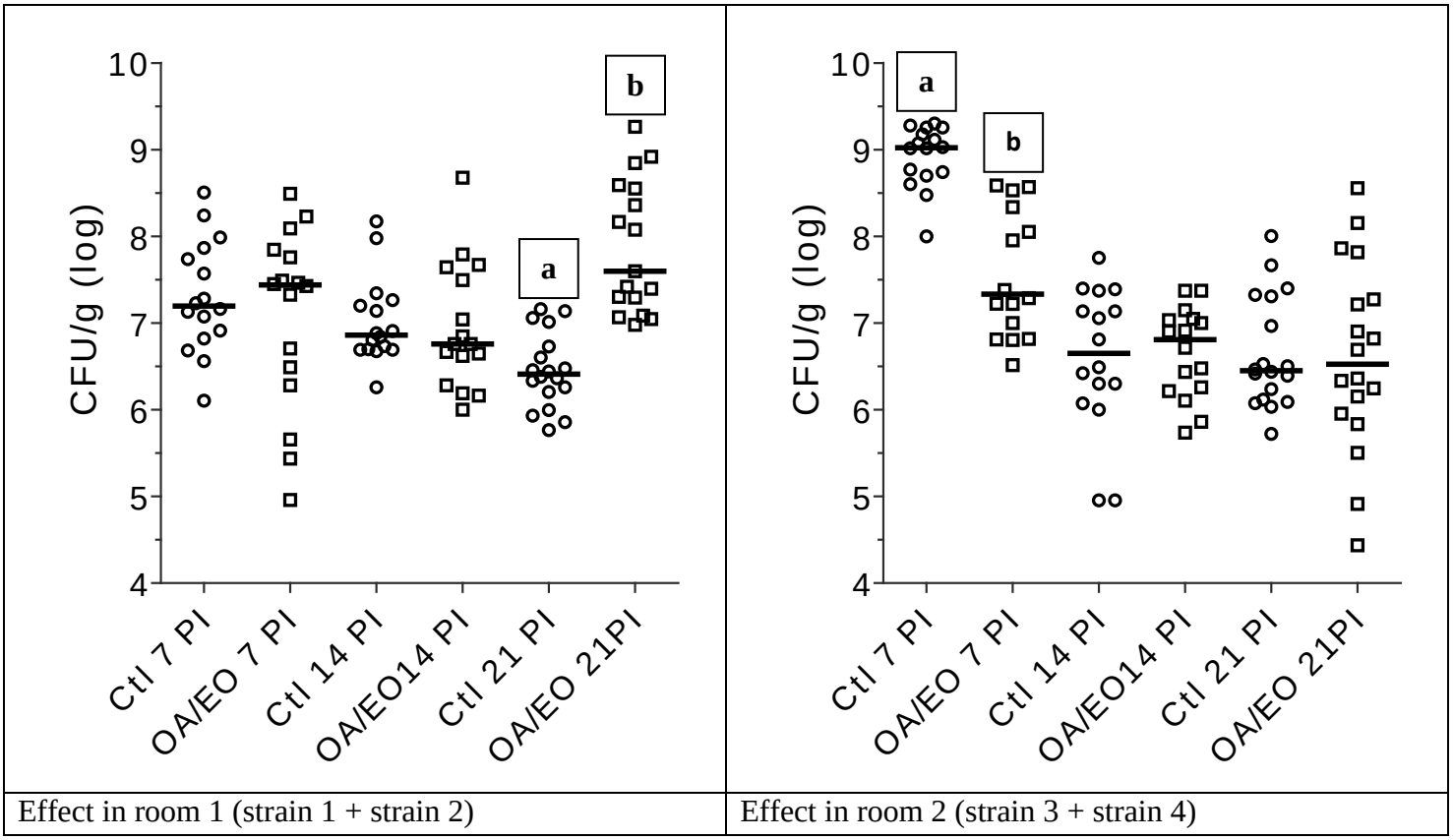
Supplemental material

Table 1: Phenotypical properties of strains inoculated to each chicken group

Strain	Autoagglutination	Chemotaxis	Adhesion	Invasion	Room
Strain 1	65 ± 14	0.49 ± 0.07	0.41 ± 0.03	0.41 ± 0.02	1
Strain 2	78 ± 13	0.48 ± 0.07	0.50 ± 0.17	0.28 ± 0.01	
Strain 3	62 ± 3	0.22 ± 0.02	0.22 ± 0.02	0.25 ± 0.02	2
Strain 4	63 ± 15	0.12 ± 0.06	0.24 ± 0.02	0.21 ± 0.01	

Adhesion, invasion and chemotaxis: -log (recovered bacteria after test/initial bacteria); Autoagglutination (DO 630nm after 3 H at room temperature incubation/DO 630 nm initial bacterial suspension x 100). Strain 1 and 2 were co-administered to all chickens in room one; strain 3 and 4 were co-administered to all chickens in room 2.

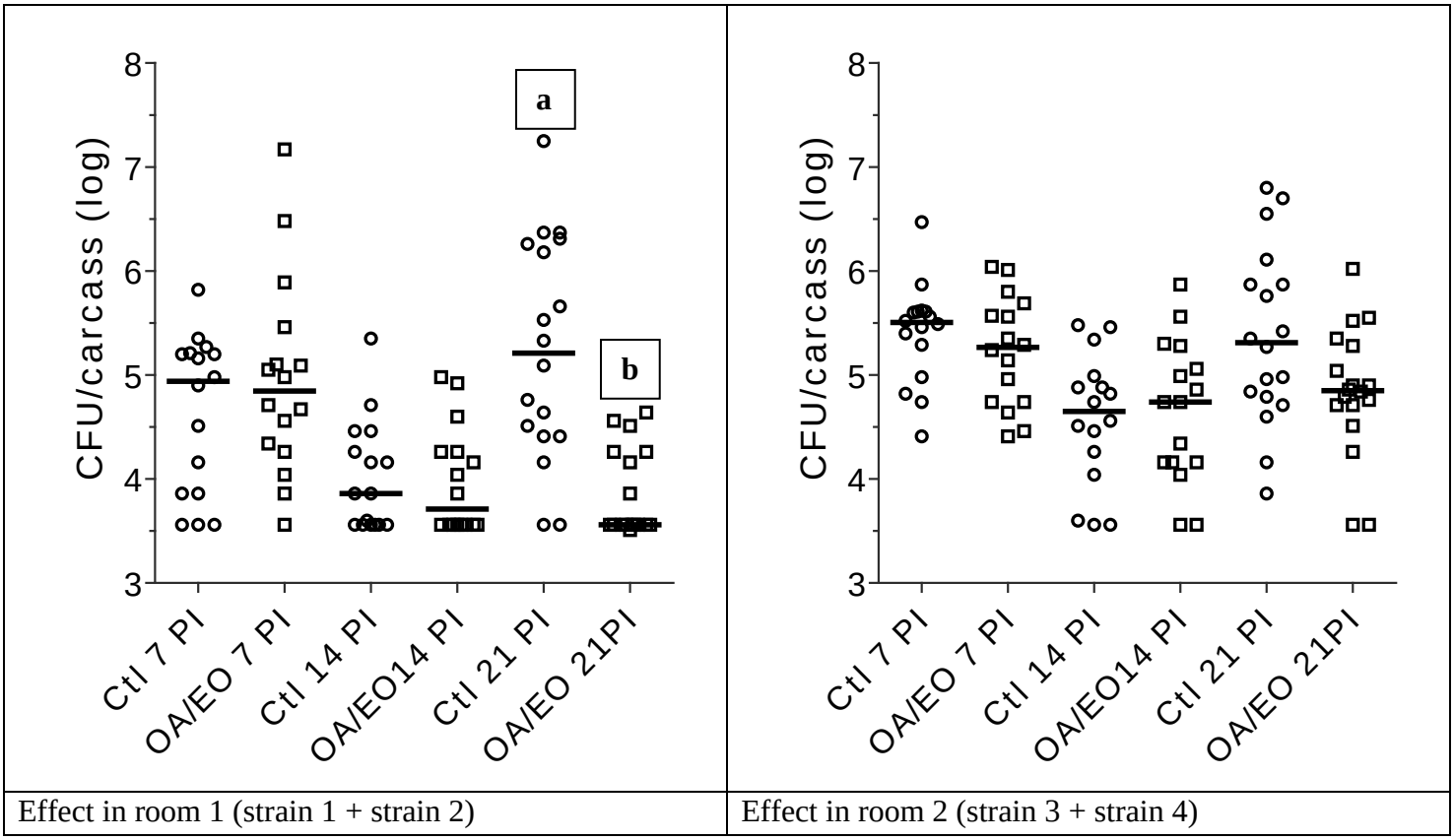
Figure 1: Feed additive effect on caecal *Campylobacter* levels.



Effect in room 1 (strain 1 + strain 2) | Effect in room 2 (strain 3 + strain 4)

PI = days post-inoculation; n = 16 for 7 PI and 14 PI; n = 18 for 21 PI;
In the same room, a different than b; ($p < 0.05$, Mann-Whitney); the horizontal bar represent the median

Figure 2: Feed additive effect on carcass *Campylobacter* levels.



Effect in room 1 (strain 1 + strain 2)	Effect in room 2 (strain 3 + strain 4)

PI = days post-inoculation; n = 16 for 7 PI and 14 PI; n = 18 for 21 PI;

In the same room, a different than b; ($p < 0.05$, Mann-Whitney); the horizontal bar represent the median