



Effect of caprylic acid on *Campylobacter* concentration in broiler caeca

MAF Technical Paper No: 2011/57

Final Report on a Contract Research Project Conducted by
Massey University, Palmerston North, NEW ZEALAND

ISBN: 978-0-478-38452-9 (online)
ISSN: 2230-2794 (online)

March 2011



Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere



RESTRICTED DISTRIBUTION OF REPORTS

This report contains information that is proprietary to NZ Food Safety Authority, Wellington (Now part of the Ministry of Agriculture and Forestry (MAF)). The purpose is to report the results of a broiler chicken study conducted for NZ Food Safety Authority. The contents of this report may be disclosed to study personnel and to the Ethics Committee of Massey University. The contents of this report may not be disclosed to any other parties without the written permission from NZ Food Safety Authority.

Any supplement that may be added to this report is likewise proprietary to NZ Food Safety Authority and the distribution thereof is restricted as stated above.

Requests for further copies should be directed to:

Publication Adviser
MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at
www.foodsafety.govt.nz/elibrary

© Crown Copyright, 2011 - Ministry of Agriculture and Forestry



**Effect of caprylic acid on *Campylobacter* concentration
in broiler caeca**

**Final Report on a Contract Research Project Conducted by
Massey University,
Palmerston North
NEW ZEALAND**

For

**NZ Food Safety Authority
Wellington**

March 2011

RESTRICTED DISTRIBUTION OF REPORTS

This report contains information that is proprietary to NZ Food Safety Authority, Wellington. The purpose is to report the results of a broiler chicken study conducted for NZ Food Safety Authority. The contents of this report may be disclosed to study personnel and to the Ethics Committee of Massey University. The contents of this report may not be disclosed to any other parties without the written permission from NZ Food Safety Authority.

Any supplement that may be added to this report is likewise proprietary to NZ Food Safety Authority and the distribution thereof is restricted as stated above.

Critical Dates and Responsible Staff

Trial dates

Feed manufacture: 07 May 2010

Trial start date: 17 May 2010

First day of treatment: 07 June 2010

Completion in-life phase: 29 June 2010

Research site contact/study director	Professor V. Ravindran
Research site diet preparation	Don Thomas
Research site Animal supervisor	Don Thomas and Colin Naftel
Research site Statistician	Dr Patrick Morel
Research site Veterinarian	Dr. N. Christensen
Campylobacter analysis	Professor Nigel French and his staff
Report preparation	Professors V. Ravindran and Nigel French

Study Sponsor

NZ Food Safety Authority, Wellington.

Experimental Farm

Poultry Research Unit, Massey University, Palmerston North.

Objectives

- To investigate the effects of caprylic acid on *Campylobacter* concentration in the caeca of broiler chickens reared under commercial conditions.
- To investigate the changes in *Campylobacter* concentration in caeca as the birds age.
- To examine possible competition between *Campylobacter* genotypes (ST474 and ST45)

Ethics approval

The experimental procedures were approved by the Massey University Animal Ethics Committee and, complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes (Approval No. MUAEC 10/25).

Product

Caprylic acid was supplied by the sponsor.

Agreed Protocol – Animal Trial

Male broiler (Ross 308) chicks were obtained as day-olds from a commercial hatchery and randomly assigned to 8 floor pens on wood shavings in an environmentally controlled room. Each treatment was then randomly allocated to four pens; the number of birds in each pen was 22, giving a total of 88 birds in total per treatment group.

The temperature was maintained at 31 °C at the first week, then gradually reduced to 22 °C at 35 days of age and maintained at this temperature until the termination of the trial. The birds received 20 hours of fluorescent illumination per day and, allowed free access to the diets and water. General bird management was broadly comparable to that practiced commercially in New Zealand.

1. There was a separate control group and an intervention group (fed diets containing 0.7% caprylic acid).
2. On day 21, all birds were orally gavaged with MLST types: P179a (ST 474) and P197b (ST 45) at infectious dose of 1×10^6 cfu (supplied by Professor Nigel French). Both are STs commonly found in poultry, but ST 474 is more poultry-associated than ST-45, which is a more ubiquitous strain found in poultry, livestock and wildlife. The two STs have allelic profiles that differ at all alleles.
3. Both the treated group and the untreated group received untreated starter and grower rations. The rations were changed from starter to grower on day 12.
4. The treated group received an untreated finisher ration from day 26 to day 29 (including). Then the treated group received 0.7% caprylic acid in their finisher ration (30 – 35 days) and their withdrawal ration (35 days onwards).
5. The control group received unsupplemented, standard ration throughout the experimental period.

6. At the ages of 33, 39 and 43, seven (7) birds per replicate pen were killed by cervical dislocation and, caecal samples were collected and sent to laboratory for *Campylobacter* analysis (Professor Nigel French). This gave a total of 56 samples at each age (7 birds/ replicate x 4 replicates/ treatment x 2 treatments) and grand total of 168 samples.
7. Group body weights and feed intake were recorded on days 21, 33, 39 and 43. Mortality was recorded daily. Feed conversion ratio will be calculated.
8. Farm and laboratory staff were 'blind' to which groups (treated or not) the birds belong.
9. Six (6) hour feed withholding time was observed.
10. The birds were not used for human or animal consumption afterwards, but were incinerated.
11. The experimental procedures were approved by the Animal Ethics Committee of Massey University.

Protocol deviations

The study was conducted as per agreed protocol (with NZFSA) and there were no protocol deviations.

Methodology – Campylobacter analysis

Challenge

Cultures of *Campylobacter jejuni*, P179a (ST 474) and P197b (ST 45) were revived from glycerol cultures stored at -80 °C and grown on Columbia Horse Blood Agar (BA) (Fort Richard Laboratories, Auckland, New Zealand) in a microaerobic incubator (VA500, Don Whitley Scientific, Yorkshire, UK) at 42°C. Growth was scraped from a two day old culture and resuspended in 10 ml of Phosphate Buffered Saline (PBS), pH 7.3 to a density equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 cells/ml). Two mls of the PBS suspension was added to 198 ml of PBS to give a suspension of approximately 1×10^6 cells/ml and the two 200 ml suspensions were immediately dispatched for use as the challenge.

Serial ten-fold dilutions of the 10 ml cell suspensions were made in peptone diluent (Fort Richard Laboratories, Auckland, New Zealand) and 100 µl volumes were spiral plated (Don Whitley Scientific, Yorkshire, UK) onto BA with microaerobic incubation for 48 hours to ascertain the viable cell counts.

After challenge, the remaining challenge suspension was returned to the laboratory, serial ten-fold dilutions were made in peptone diluent and 100 µl volumes were spiral plated onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Fort Richard Laboratories, Auckland, New Zealand) to ascertain the post-challenge viable cell counts.

Isolation

Sections of chicken caeca were received in the laboratory in individual test-tubes, on ice, as soon as practicable after dissection. 1.0 +/- 0.2 gram aliquots were added to 9.0 ml volumes of peptone diluent and vortexed well to suspend the caecal contents evenly. Sterile glass beads were added when required to aid homogenisation. Hundred-fold dilutions in peptone diluent were made from the primary dilution and 100 µl volumes of the 10⁻¹, 10⁻³ and 10⁻⁵ dilutions were spiral plated onto mCCDA followed by microaerobic incubation at 42 °C for 48 hours. A 1 ml aliquot of the primary 10⁻¹ dilution was added to 20 ml of Boltons *Campylobacter* enrichment broth and this was also incubated microaerobically at 42 °C for 48 hours.

After incubation colonies resembling *Campylobacter* were counted and Boltons broth was subcultured to mCCDA if no *Campylobacter*-like colonies were seen. At least one colony from every caecum was subcultured to BA and frozen in glycerol broth at -80 °C

PCR and MLST

Three colonies from each of 12 caeca were subcultured to BA and after microaerobic incubation at 42 °C for 48 hours DNA preparations were made by suspending 1-4 colonies in 2% Chelex^(TM) in water and heating at 100 °C for 10 minutes. The supernatant was used as a DNA preparation for PCR, using the *mapA* primer to confirm the bacteria as *C. jejuni*. Multi Locus Sequence Typing (MLST) was performed by amplifying the *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkl* and *uncA* genes and sequencing the resulting products. MLST types were assigned using the Oxford MLST database.

Cultures of *C. jejuni*, P179a (ST 474) and P197b (ST 45) were grown on Columbia Horse Blood Agar (BA) (Fort Richard Laboratories, Auckland, New Zealand) microaerobically at 42°C. Growth was resuspended in 200 ml of Phosphate Buffered Saline (PBS), pH 7.3 to give a suspension of approximately 1 x 10⁶ cells/ml and the two suspensions were immediately dispatched for use as the challenge. 100 µl volumes of appropriately diluted challenge cultures were spiral plated (Don Whitley Scientific, Yorkshire, UK) before and after use to ascertain the viable cell counts.

PCR and MLST were performed on three colonies from each of 12 caeca as described by Müllner et al. (2010).

REFERENCE

Müllner, P., Collins-Emerson, J., Midwinter, A., Carter, P., Spencer S., Van derLogt, P., Hathaway, S. & French, N. P. (2010). "Molecular epidemiology of *Campylobacter jejuni* in a geographically isolated country with a uniquely structured poultry industry." *Applied and Environmental Microbiology*, **76**(7), 2145-2154.

RESULTS

The results are presented in two parts – Part A presenting the performance data, and Part B the campylobacter data.

PART A

PERFORMANCE DATA

For performance data, pen means were used as the experimental unit. The data were analysed by one-way analysis of variance using the General Linear Model procedure of SAS (1997). Differences were considered to be significant at $P < 0.05$.

Mortality during the 43-day trial was low – 2.8 (5 out of the 176 birds). There were also 7 cases of ascites and samples were not collected from these 7 birds. Thus a total of 164 caecal samples were collected – 56 on days 33, 56 on day 39 and 52 on day 43.

The influence of caprylic acid on the performance of broiler chickens at days 33, 39 and 43 are summarised in Tables 1, 2 and 3, respectively. At all three ages, the body weight, feed intake and feed conversion ratio of broiler chickens were not affected ($P > 0.05$) by the dietary treatments imposed.

Table 1. Influence of caprylic acid supplementation on the body weight, feed intake and feed conversion ratio (FCR) of broiler chickens – Day 33¹

	Body weight, g/bird	Feed intake, g/bird	FCR, g feed/ g gain ²
Control diet	2530	3872	1.58
Caprylic acid diet	2627	3950	1.53
Pooled SEM ³	49.9	95.2	0.022
Probability, $P \leq$	0.21 ^{NS}	0.58 ^{NS}	0.20 ^{NS}

¹ Each mean represents values from four replicates (22 birds/replicate).

² Corrected for mortality.

³ Pooled standard error of mean.

Table 2. Influence of caprylic acid supplementation on the body weight, feed intake and feed conversion ratio (FCR) of broiler chickens – Day 39¹

	Body weight, g/bird	Feed intake, g/bird	FCR, g feed/ g gain ²
Control diet	3314	4784	1.53
Caprylic acid diet	3312	4885	1.54
Pooled SEM ³	93.3	115.0	0.017
Probability, P≤	0.99 ^{NS}	0.62 ^{NS}	0.70 ^{NS}

¹ Each mean represents values from four replicates (15 birds/replicate).

² Corrected for mortality.

³ Pooled standard error of mean.

Table 3. Influence of caprylic acid supplementation on the body weight, feed intake and feed conversion ratio (FCR) of broiler chickens – Day 43¹

	Body weight, g/bird	Feed intake, g/bird	FCR, g feed/ g gain ²
Control diet	3808	5672	1.58
Caprylic acid diet	3804	5781	1.58
Pooled SEM ³	220.6	146.3	0.019
Probability, P≤	0.99 ^{NS}	0.62 ^{NS}	0.70 ^{NS}

¹ Each mean represents values from four replicates (6-8 birds/replicate).

² Corrected for mortality.

³ Pooled standard error of mean.

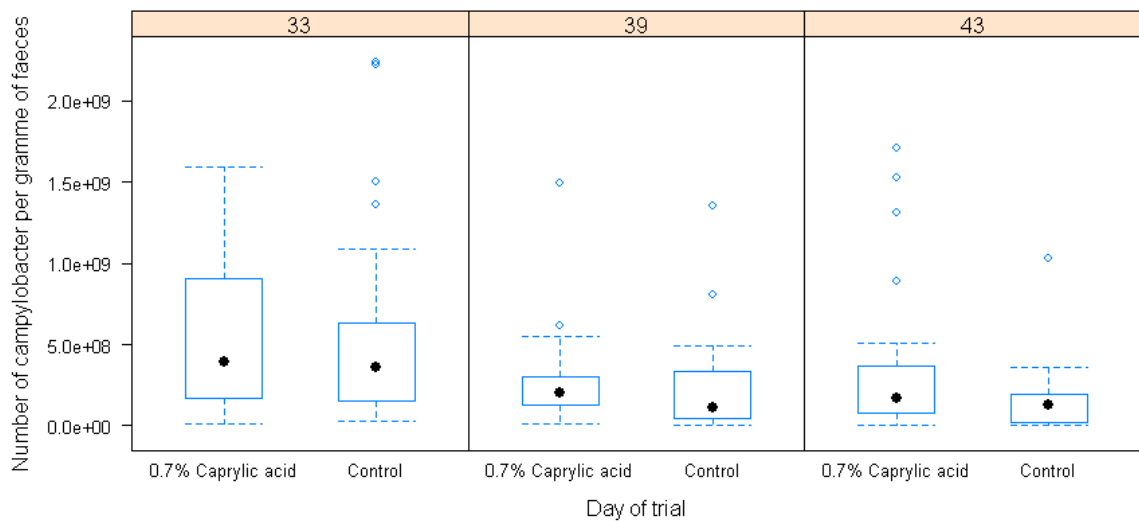
CONCLUSION

Supplementation of broiler diets with 0.7% caprylic acid had no effect on broiler performance.

PART B

CAMPYLOBACTER DATA

Figure 1 shows box plots and a tabular summary of the estimated counts per gram of caecal contents for treatment and control groups on each day of the trial. There is evidence of a decline in counts after day 33 and considerable overlap between the distributions. There is little difference between the values for the treatment and control groups, with the median values being slightly higher for the treatment group in all time points.



	Day 33		Day 39		Day 45	
Summary	Control	Treatment	Control	Treatment	Control	Treatment
Minimum	2.51E+07	1.20E+07	1.64E+06	1.20E+07	6.55E+05	1.22E+04
1st Quartile	1.56E+08	1.76E+08	4.53E+07	1.29E+08	2.51E+07	8.66E+07
Median	3.64E+08	3.96E+08	1.12E+08	2.02E+08	1.25E+08	1.72E+08
Mean	5.64E+08	5.32E+08	2.34E+08	2.67E+08	1.59E+08	3.41E+08
3rd Quartile	6.00E+08	8.88E+08	3.24E+08	2.96E+08	1.91E+08	3.55E+08
Maximum	2.24E+09	1.59E+09	1.35E+09	1.49E+09	1.03E+09	1.71E+09

Figure 1. Box plots and tabular summary of the counts of *Campylobacter* per gram of caecal contents in each treatment and control group at each time point. The black dots represent the median values, the box captures the interquartile range, the dashed lines capture the 2.5% and 97.5% interval and the open blue circles are outliers.

We conducted a formal analysis of the relationship between treatment and caecal counts using a linear mixed effects model, taking into account the day of the trial (fixed effect) and pen group (random effect). The model was:

$$Y_{i,j} \sim \text{Norm}(\mu_{i,j})$$

$$\mu_{i,j} = \beta_0 + \beta_1 \text{Tmnt}_{i,j} + \beta_2 \text{Day}_{i,j} + U_j$$

$$U_j \sim \text{Norm}(0, \sigma_j)$$

Where $Y_{i,j}$ is the transformed outcome variable for bird i in group j (see below), and this is assumed to be normally distributed with mean μ_j . $\beta_{0,2}$ represent the regression coefficients for the two variables of interest, and U_j the random effect for pen group j .

The residuals for models considering both untransformed and log transformed counts showed strong departure from normality (see left plot in Figure 2). Hence alternative transformations were examined. Dividing the counts by a large number (10^7) and then adding 1 resulted in more normally distributed residuals (see right plot in Figure 2).

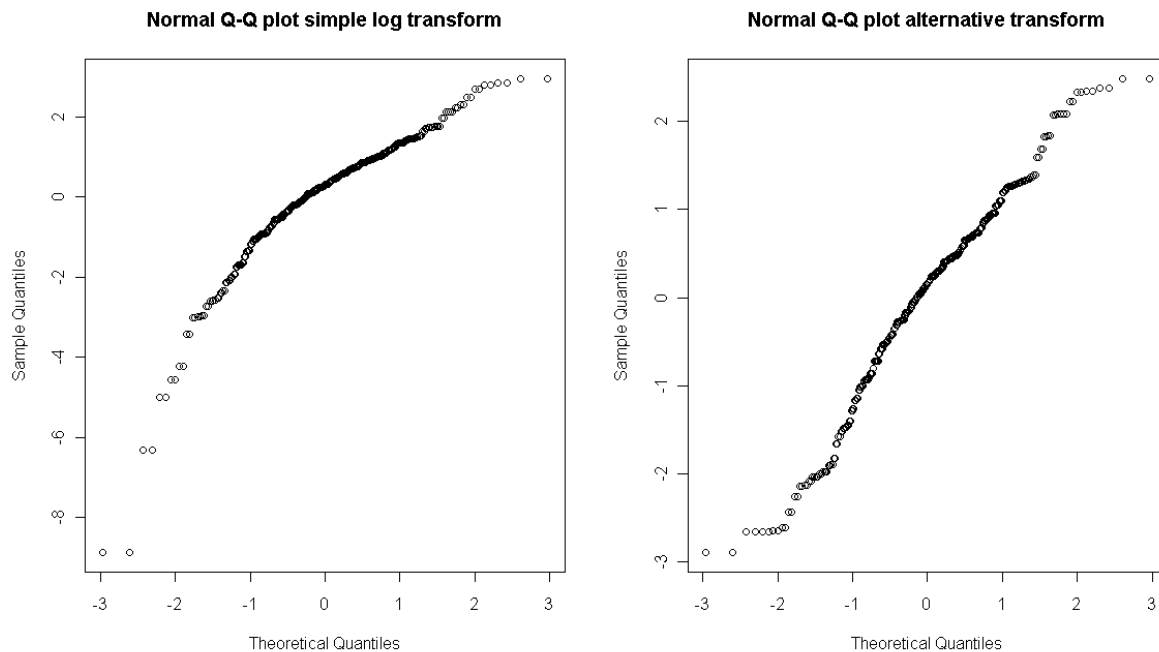


Figure 2. Normal Q-Q plots for the model residuals.

The coefficients, standard errors and P values for a model with simple log transformation and alternative transformation are shown in Table 4. Both models show a significant reduction in counts after day 33, but no significant treatment effect. The positive coefficients for Treatment compared to Control are consistent with the slightly higher values seen in Figure 1.

Table 4. Two models of the relationship between the caecal counts of *Campylobacter* and the day of the trial and treatment group. Standard errors were adjusted for the effect of pen group by the inclusion of a random effect term in the model.

	Log transformed counts				Alternative transformed counts			
	Coefficient	Std.Error	t-value	p-value	Coefficient	Std.Error	t-value	p-value
Intercept	3.33	0.18	18.12	<0.0001	19.39	0.26	74.75	<0.0001
Day 39	-0.76	0.22	-3.36	0.001	-0.85	0.32	-2.68	0.0081
Day 43	-1.02	0.23	-4.45	<0.0001	-1.44	0.32	-4.45	<0.0001
Treatment vs control	0.35	0.19	1.86	0.112	0.34	0.26	1.29	0.2458

CONCLUSIONS

Caecal counts declined in both treatment and control groups after day 33, but there was no significant effect of treatment. Although the birds were inoculated with an equal mixture of ST 474 and ST 45, only ST 474 was recovered from samples later in the trial. This indicates that ST 474 rapidly out-competed ST 45 in both treatment and control groups.

Disclaimer

Massey University has taken every care to ensure that the contents of this report provide a correct reflection of its current understanding of these results and that the information presented is accurate. Massey University cannot, however, accept responsibility for any inadvertent errors in the information presented. Similarly, no responsibility is accepted for any interpretations made from the information provided.

Professors V Ravindran and N. French
Massey University
10 March 2011