A Campylobacter fertőzöttségi szint telepi szintű csökkentésének lehetőségei: A CAMPYBRO project

Control of Campylobacter infection in broiler flocks through two-steps strategy: nutrition and vaccination

-CAMPYBRO- FP7-SME-2013-605835

Dr. Pedro Medel
IMASDE AGROALIMENTARIA SL
www.e-imasde.eu

Budapest, 24/06/2016

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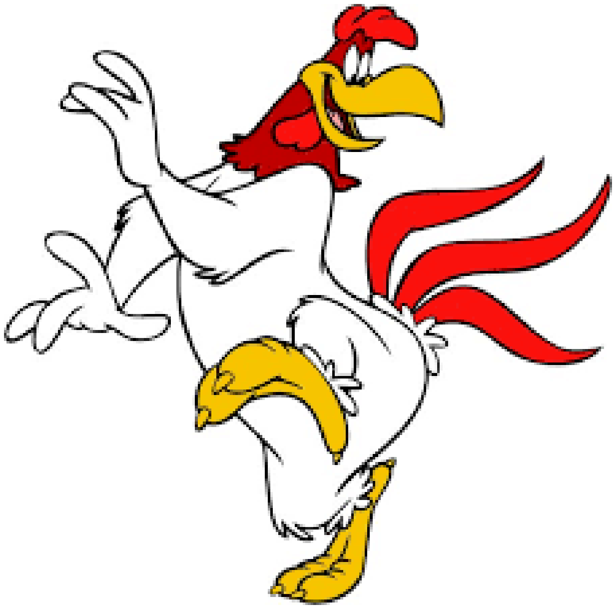
1. Introduction
2. Pre harvest measures
3. Conclusions

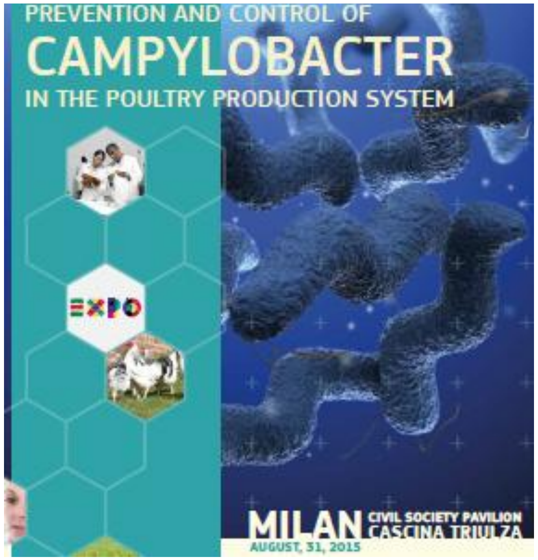


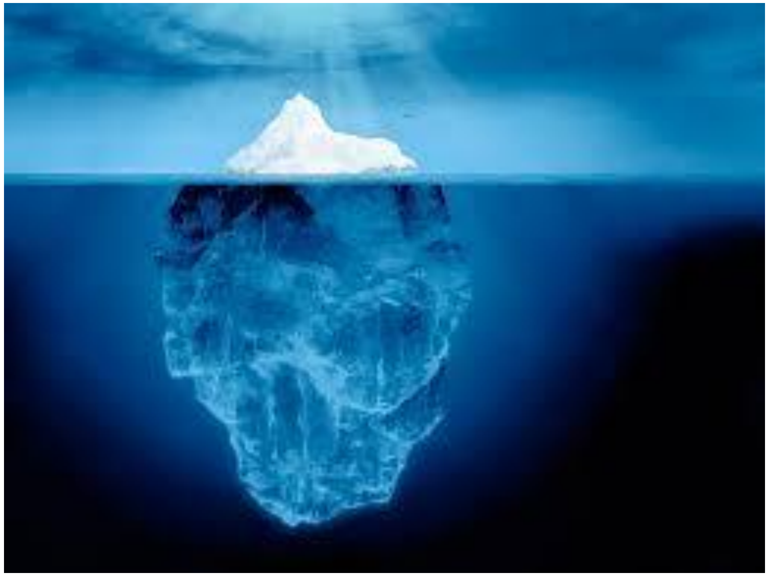
Campylobacterirosis

In order to appraise more realistically the impact of campylobacteriosis in EU/EEA MS we compiled data from three different sources: an ECDC-funded sero-epidemiological study, reported cases in The European Surveillance System (TESSy) database, and data stemming from literature reviews.

In the EU/EEA MS, the annual rate of exposure to *Campylobacter spp.* is estimated to be around 0.83 per person-year, translating in more than 420 million yearly infections. The vast majority of exposed cases do not develop the clinical disease and remain asymptomatic. Based on community studies, the related incidence of campylobacteriosis disease is 475 per 100 000 (CI 95%: 423-524 per 100 000) or 2.4 million cases per year amongst European citizens. Underestimation of the disease, therefore, is considered to be 11 times the notification rate. Moreover, in a recent burden of disease study (BCoDE 2015), ECDC estimated that about 600 deaths are related to campylobacteriosis every year, largely among elderly people. Results from BCoDE 2015 also found that campylobacteriosis is the food and water-borne disease producing the highest number of DALYs.

Cassini, 2015. ECDC





Regulation proposal

EUROPEAN
COMMISSION

COMMISSION REGULATION (EC) No 2073/2005

of 15 November 2005

on microbiological criteria for foodstuffs

(Text with EEA relevance)

(OJ L 338, 22.12.2005, p. 1)

Brussels, XXX
SANTE-2015-12077
[…](2015) XXX draft

COMMISSION REGULATION (EU) No …/..

of XXX

amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcases

(Text with EEA relevance)

Regulation proposal

Annex I to Regulation (EC) No 2073/2005 is amended as follows: In Chapter 2, Row 2.1.9 is added:

Food category	Micro-organisms	Sampling plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies	Action in case of unsatisfactory results
		n	c	m	M			
2.1.8 Meat preparations	*E. coli* (8)	5	2	500 cfu/g or cm^2	5 000 cfu/g or cm^2	ISO 16649-1 or 2	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
2.1.5 Poultry carcases of broilers and turkeys	*Salmonella* spp. (10)	50 (5)	7 (6) From 1.1.2012 c = 5 for broilers From 1.1.2013 c = 5 for turkeys	Absence in 25 g of a pooled sample of neck skin		EN/ISO 6579 (for detection)	Carcases after chilling	Improvement in slaughter hygiene and review of process controls, origin of animals and biosecurity measures in the farms of origin
"2.1.9 Poultry carcases of broilers	**Campylobacter spp.**	**50 (5)**	**10(11) From 1.1.2018 c=7 From 1.1.2020 c=5**	**1000 cfu/g**		**ISO/TS 10272-2**	**Carcases after chilling**	**Improvements in slaughter hygiene and review of process controls, origin of animals and of the biosecurity measures in the farms of origin"**

…neck skins from a minimum of ***15 poultry carcases*** *shall be sampled at random after chilling during each sampling session. A piece consisting of minimum 10 g of neck skin shall be obtained from each poultry carcase…the neck skin samples from three poultry carcases from the same flock of origin shall be pooled in order to form* ***5 x 25 g final samples once per week***

CARCASS-CECAL CONTAMINATION RELATIONSHIP

F. Reich et al. / International Journal of Food Microbiology 127 (2008) 116–120 119

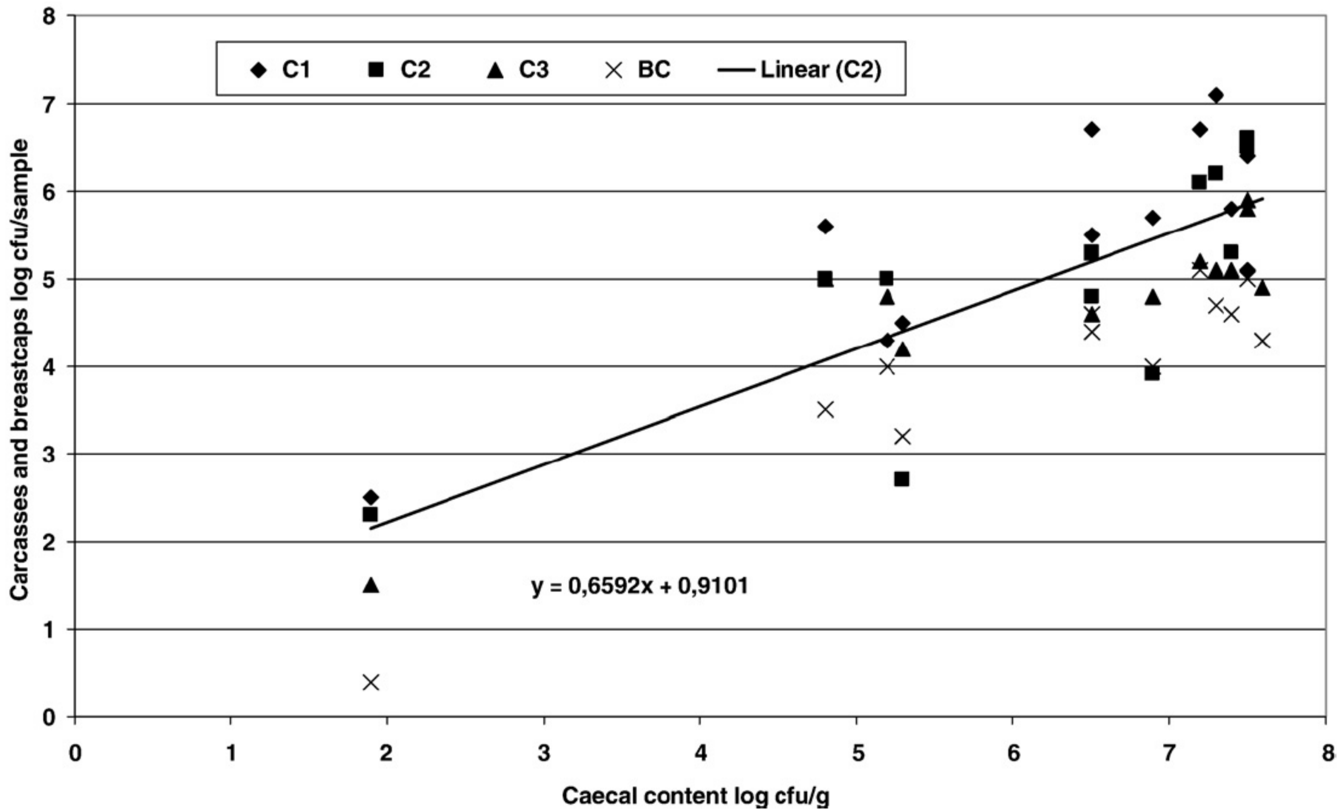
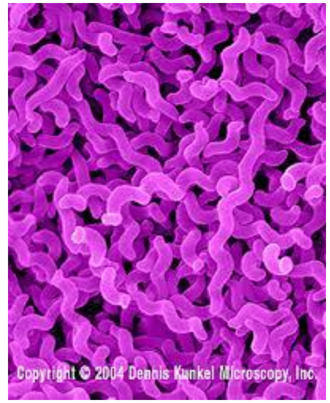


Fig. 2. Correlation of the number of *Campylobacter* in caecal contents of positive flocks with the contamination of broiler carcasses. Each dot represents the mean value of one slaughter day. The *x*-axis is the *Campylobacter* concentration in caecal contents in $\log_{10}$ cfu/g, the *y*-axis carcass/breast caps contamination in $\log_{10}$ cfu/sample. A trend line with an equation. of regression is shown for C2. C1: carcass after scalding/defeathering; C2: carcass after evisceration; C3: carcass after chilling; BC: breast cap.

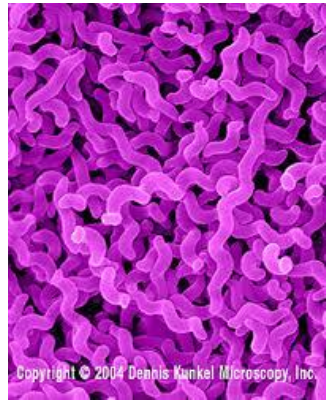
Slope:0.66

Campylobacter control



- **PRE-HARVEST**
  - Biosafety Management
  - Vaccine
  - Nutrition
    - Feed form
    - Phages
    - Additives
- **POST-HARVEST**
  - Transport
  - Slaughterhouse
  - Packaging
  - Retail
  - Consumers

CAMPYBRO PROJECT



PRE-HARVEST
- Biosafety Management
- Vaccine
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  - Feed form
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POST-HARVEST
- Transport
- Slaughterhouse
- Packaging
- Retail
- Consumers

Consortium

ProPollo

CIDEF
COMMITÉ INTERPROFESSIONNEL DE LA DINDE FRANÇAISE

NEPLUVI

IMASDE
AGROALIMENTARIA

CAMPY BRO

BTT
Baromfi Termék Tanács

FIA
Fédération
des Industries Avicoles

anses
French agency for food, environmental
and occupational health & safety

MIKROLAB és SPF Kft.

Redondo

CZV
CZ VETERINARIA

Work Packages

CAMPYBRO	WP	Year 1												Year 2												Year 3											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
WP 1. Efficacy of several compounds against Campylobacter in broilers orally infected looking for synergies	WP1																																				
T1.1. In vivo effectiveness of products based on plant extracts, organic acids, prebiotics, and probiotics against Campylobacter.	T1.1	•	•	•	•	•	•	•	•	•																											
T1.2 In vitro effectiveness of mixtures of products: Synergistic effect	T1.2										•	•	•																								
T1.3. In vivo effectiveness of product mixtures based on plant extracts, organic acids, prebiotics, and probiotics against Campylobacter.	T1.3													•	•	•																					
WP 2. Feed presentation strategies against Campylobacter.	WP2																																				
T2.1. Effect of feed composition, particle size and feed presentation on the prevalence of Campylobacter in broilers orally infected	T2.1	•	•	•	•	•	•	•	•	•																											
T2.2 Effect of whole grain feeding on the prevalence of Campylobacter in broilers orally infected.	T2.2										•	•	•	•	•	•																					
WP 3. Interactions between products and feed presentation against Campylobacter. Synergies.	WP3																																				
T3.2. Interactions between product mixtures and feeding strategies against Campylobacter looking for synergies	T3.1																•	•	•	•	•	•	•	•	•												
T3.2 Studies in the effect of the duration of treatment on the final infection: design of funtional diets	T3.2																•	•	•	•	•	•	•	•	•												
T3.3. Study on the correlation between in vitro and in vivo results. Cost-Benefit analyses.	T3.3																						•	•	•												
WP 4. Application of different nutritional strategies against Campylobacter in experimental farm and field trials.	WP4																																				
T4.1. Effect of different strategies against Campylobacter on performance parameters and level of infection of broilers chickens in experimental farm.	T4.1																									•	•	•	•	•	•						
T4.2. Effect of different strategies against Campylobacter on performance parameters and level of infection of broilers chickens in commercial farms.	T4.2																									•	•	•	•	•	•						
T4.3. Effect of different strategies against Campylobacter on performance parameters and level of infection of turkeys in commercial farms.	T4.3																									•	•	•	•	•	•						
WP 5. Development of a novel vaccine against Campylobacter based on reserve vaccinology	WP5																																				
T5.1. Exhaustive identification of new potential vaccine antigens against Campylobacter using the reverse vaccinology strategy.	T5.1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•																					
T5.2. Development of an in vitro test to visualize the recognition of Campylobacter antigens by antibodies.	T5.2																•	•	•	•	•	•	•	•	•												
T5.3. Determination of an efficient sub-unit vaccination protocol	T5.3	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•												
T5.4. Selection of the Campylobacter proteins that will be evaluated for their protective capacity	T5.4																									•	•	•	•	•	•	•	•	•	•	•	•
T5.5. Assessment of the protective potentials against Campylobacter induced by the selected vaccine candidates.	T5.5																									•	•	•	•	•	•	•	•	•	•	•	•
WP 6. Evaluation of the developed nutritional strategies in different geographical situations.	WP6																																				
T6.1. Evaluation of developed nutritional strategies in South, Central, and East European conditions	T6.1																															•	•	•	•	•	•
WP 7. Project Management	WP7																																				
T7.1. Contractual, legal, Administrative and financial management and overseeing of ethical and gender issues	T7.1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T7.2. Monitoring and coordination of technical activities of the project, and planning, organizing and reporting of Project Coordinating Committee and General Assembly	T7.2	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T7.3. Relationship with the European Commission	T7.3	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
WP 8. Dissemination, training and exploitation	WP8																																				
T8.1. Dissemination of project results	T8.1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T8.2. Training to achieve project results implementation	T8.2													•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
T8.3. Exploitation of project results	T8.3													•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
MILESTONES		MILESTONE 1									MILESTONE 2						MILESTONE 3									MILESTONE 4						MILESTONE 5					

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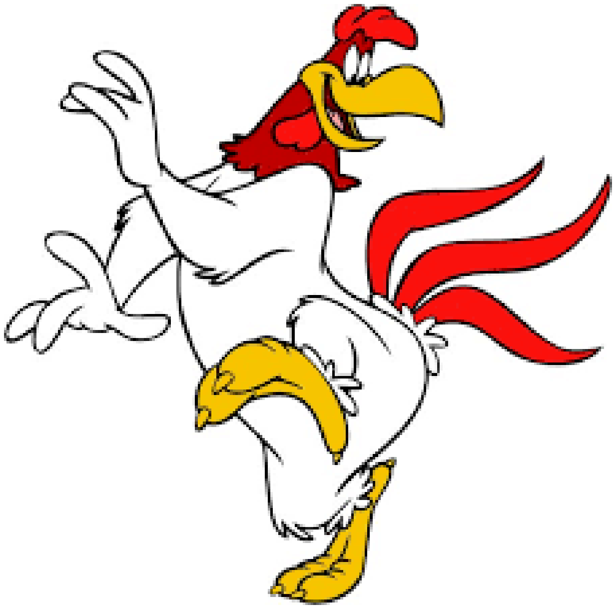


Genetic diversity within a farm

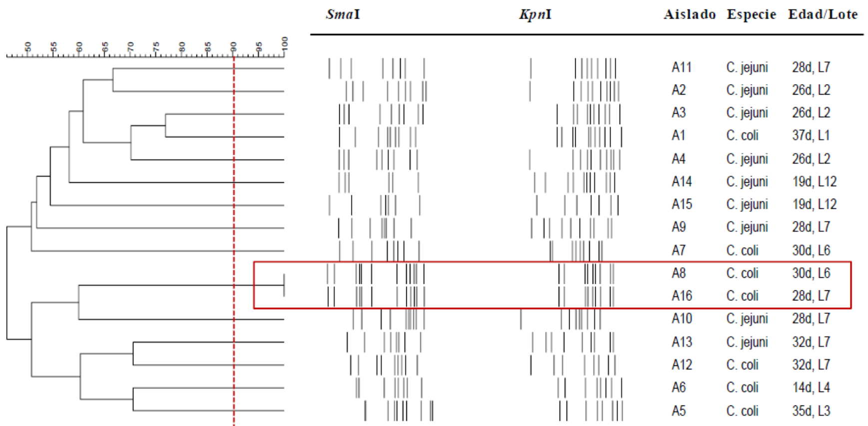


Figura 1. **Dendrograma de PFGE de los aislados de *C. jejuni* y *C.coli* de la granja A. La similitud entre aislados se evaluó mediante el coeficiente de Dice (tolerancia 1%, optimización 1%) y el método UPGMA. Los recuadros señalan los *clusters* que agrupan aislados con un nivel de similitud ≥ 90%.**

Diversidad genética y dinámica de *Campylobacter* termófilos en granjas de pollos de engorde en Cataluña

G. CANTERO* y M. CERDÀ-CUÉLLAR

LII simposio científico de avicultura
MÁLAGA del 28 al 30 de octubre de 2015

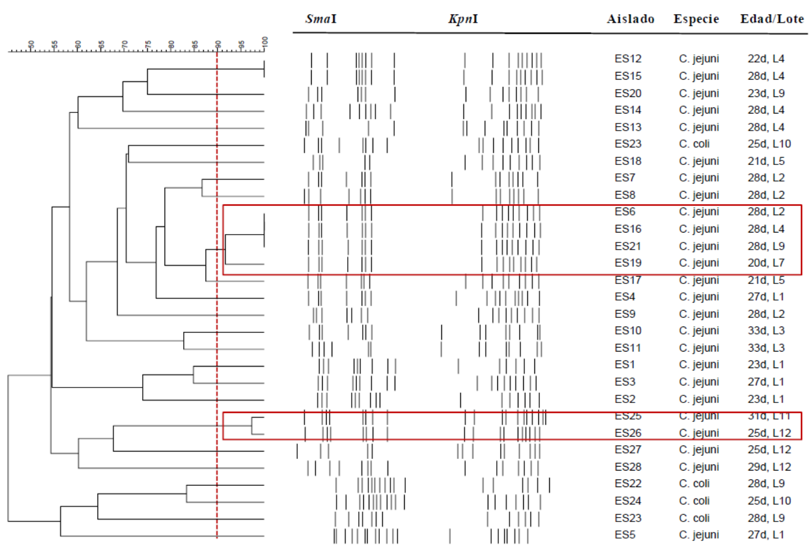


Figura 2. Dendrograma de PFGE de los aislados de *C. jejuni* y *C.coli* de la granja E. La similitud entre aislados se evaluó mediante el coeficiente de Dice (tolerancia 1%, optimización 1%) y el método UPGMA. Los recuadros señalan los *clusters* que agrupan aislados con un nivel de similitud ≥ 90%.

Genetic diversity along the food chain

Table 4. *Campylobacter* genotypes detected in each chicken meat production units.

Production chain	Production unit	Sample	Number of isolates examined	Genotype[a]	
				flaA SVR	MLST
A	Breeding farm	Cloacal swab	5	353, 506, 783, 1211, 1485	**1232**, 6876
	Broiler farm	Cloacal swab	36	18, 22, **57**, 312	45, 354, **574**
		Environment inside the target house[b]	4	18, 22, **57**	45, 354, **574**
		Environment outside the target house[c]	3	22, **57**	45, **574**
	Slaughterhouse	Cloacal swab and cecum	10	18, **22**, 57, 312	**45**, 354, 574
		Transport crate	3	**45**	**2409**
		Environmental sample[d]	5	18, **22**	**45**, 354
		Meat and carcass rinse	15	18, **22**, 57, 177	**45**, 354, 574, 583
B	Breeding farm	Cloacal swab	2	**54**	**464**
	Broiler farm	Cloacal swab	34	**54**, 18	**464**, 354
	Slaughterhouse	Cloacal swab and cecum	4	**54**	**464**
		Environmental sample	11	**54**, 783	**464**
		Meat and carcass rinse	10	**54**	**464**
C	Breeding farm	Cloacal swab	6	30, **34**, 54, 312	460, 574, **6996**
	Broiler farm	Environment inside the target house	1	22	45
		Cloacal swab	2	**629**	**2209**
	Slaughterhouse	Cloacal swab and cecum	4	**68, 629, 1340**	**2209**
		Transport crate	2	783	5213
		Environmental sample	2	783, 1340	5213
		Meat and carcass rinse	17	**18**, 68, 783, 1340	**354**, 2209
D	Breeding farm	Cloacal swab	1	677	2131
	Broiler farm	Cloacal swab	28	48, **783**	**1232**, 2131
		Environment inside the target house	4	**783**	**1232**
	Slaughterhouse	Cloacal swab and cecum	13	**783**	**1232, 5213**
		Environmental sample	5	22, **783**	1075
		Meat and carcass rinse	9	**783**	1232
E	Breeding farm	Cloacal swab	5	21, 54, 45, 402, 48	2131
	Broiler farm	Cloacal swab	52	18, 45, 57, 253, 255, **287**, 854, 1527	1919, **5247**
		Environment inside the target house	2	287, 1239	5247
		Environment outside the target house	3	255, 287, 1397	6995
	Slaughterhouse	Cloacal swab and cecum	4	253, **783**,1527	n/a[e]
		Environmental sample	3	45, 253, 652	n/a
		Meat and carcass rinse	6	45, **287**, 312, 652	**5247**

Distribution and Genetic Profiles of *Campylobacter* in Commercial Broiler Production from Breeder to Slaughter in Thailand

Sakaoporn Prachantasena[1], Petcharatt Charununtakorn[1], Suthida Muangnoicharoen[1], Luck Hankla[1], Natthaporn Techawal[1], Prapansak Chaveerach[3], Pravate Tuitemwong[4], Nipa Chokesajjawatee[5], Nicola Williams[6], Tom Humphrey[7], Taradon Luangtongkum[1,2]*

PLOS ONE | DOI:10.1371/journal.pone.0149585

February 17, 2016

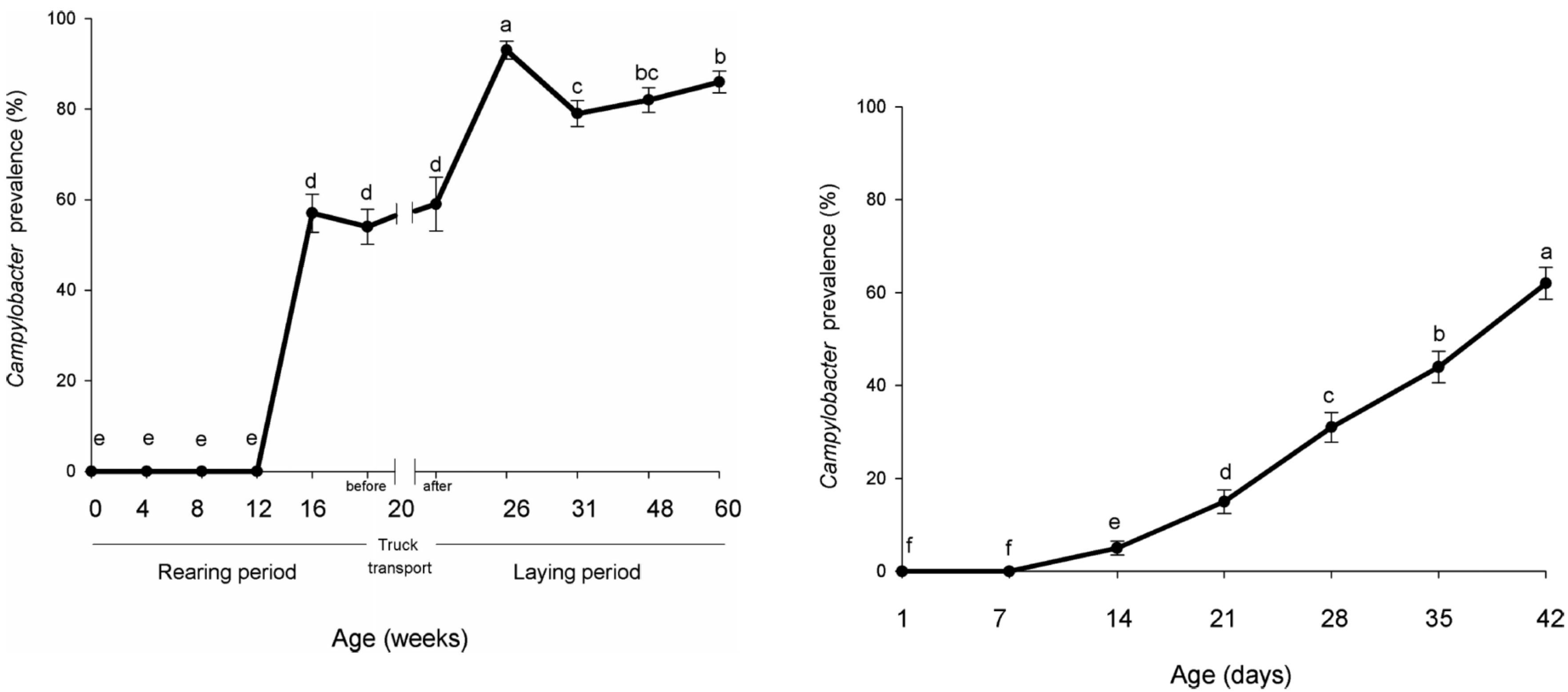
Pre-harvest: No vertical transmission

Campylobacter epidemiology from breeders to their progeny in Eastern Spain

S. Ingresa-Capaccioni,* E. Jiménez-Trigos,* F. Marco-Jiménez,† P. Catalá,‡ S. Vega,* and C. Marin*,[1]

2016 Poultry Science 00:1–8

However, in broiler production all day-old chicks were found negative for *Campylobacter* spp, and the bacteria was first isolated at d 14 of age (5.0%), with a significant increase in detection during the fattening period with 62% of *Campylobacter* positive animals at the end of the production cycle. Moreover, non-positive sample was determined from environmental sources. These results could be explained because



Pre-harvest: Biosecurity

Best Practice
Manual

for production of poultry with reduced
Campylobacter contamination

www.camcon.eu

Pre-harvest: Biosecurity

- Thinning
  - Biosecurity break
  - High risk factor for *Campylobacter* infection
  - *Campylobacter* present in the environment can potentially be carried into the house via boots, clothes, and equipment of the farmer or farm staff or of external staff responsible for flock thinning

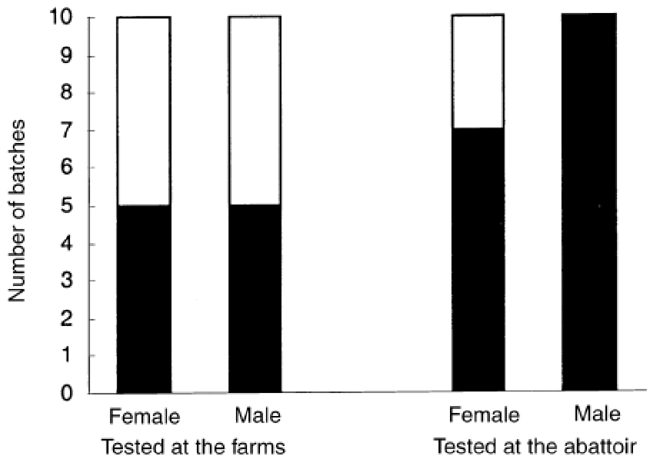


Fig. 1 Campylobacter status of the female and male batches by testing on farms and subsequently at the abattoir. The period elapsed between the two testing rounds was 2–6 days for the female batches and 11–13 days for the male batches. (■), Campylobacter-positive batches; (□), Campylobacter-negative batches

Letters in Applied Microbiology 2001, **32**, 253–256

Role of batch depletion of broiler houses on the occurrence of *Campylobacter* spp. in chicken flocks

B. Hald, E. Rattenborg and M. Madsen
Danish Veterinary Laboratory, Aarhus, Denmark

Pre-harvest: thinning

An investigation of broiler caecal *Campylobacter* counts at first and second thinning

L. Koolman[1,2], P. Whyte[2] and D.J. Bolton[1]

Journal of Applied Microbiology **117**, 876–881 © 2014

Table 1 Mean increase in *Campylobacter* caecal counts (log_{10} CFU g^{-1}) in flocks between first and second thinning (with 4 days between first and second thin)

Flock*	First thin Mean† (log_{10} CFU g^{-1})	First thin Prevalence (%)	Second thin Mean† (log_{10} CFU g^{-1})	Second thin Prevalence (%)	Difference between means (log_{10} CFU g^{-1})	Mean daily increase (log_{10} CFU g^{-1})	Mean generation time‡(h)
Flocks that were *Campylobacter* negative at first thinning							
1	ND	0	6·3 (0·7)	100	6·3	1·6	4·62
2	ND	0	6·2 (0·6)	100	6·2	1·5	4·69
3	ND	0	6·2 (0·6)	100	6·2	1·5	4·69
4	ND	0	5·5 (1·2)	97	5·5	1·4	5·29
5	ND	0	5·8 (0·7)	100	5·8	1·4	5·02
6	ND	0	5·5 (1·3)	100	5·5	1·4	5·29
7	ND	0	6·1 (0·6)	100	6·1	1·5	4·77
8	ND	0	6 (0·4)	100	6	1·5	4·85
9	ND	0	6·1 (0·6)	100	6·1	1·5	4·77
Mean			6		6	1·5	4·89
Flocks that were *Campylobacter* positive at first thinning							
10	1·4 (2·2)	30	6·6 (0·6)	100	5·2	1·3	5·59
11	1·5 (1·6)	53	6·6 (1·3)	97	5·1	1·3	5·7
12	1·8 (0·9)	90	5·7 (0·9)	100	3·9	1	7·46
13	2·5 (1·3)	90	5·9 (0·8)	100	3·4	0·8	8·56
14	3·5 (1·3)	100	5·6 (0·8)	100	2·1	0·5	13·85

ND: not detected.
*Flocks were assigned a flock number based on increasing initial *Campylobacter* counts.
†Figures in parentheses represent the standard deviation of the concentration of *Campylobacter* in each flock.
‡Mean generation time over the period between first and second thinning (4 days).

Diet manipulation

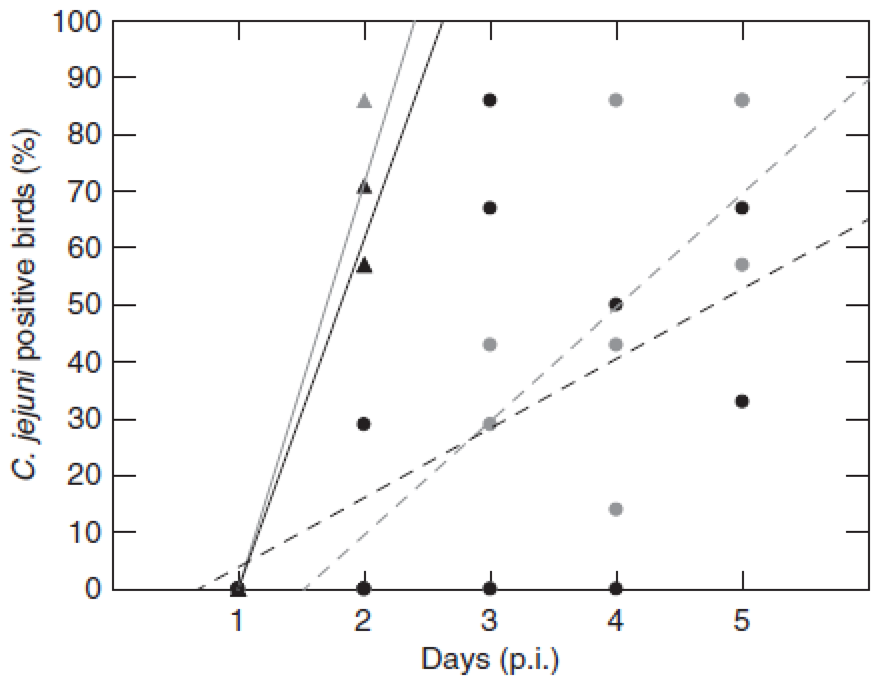
Journal of
Applied Microbiology

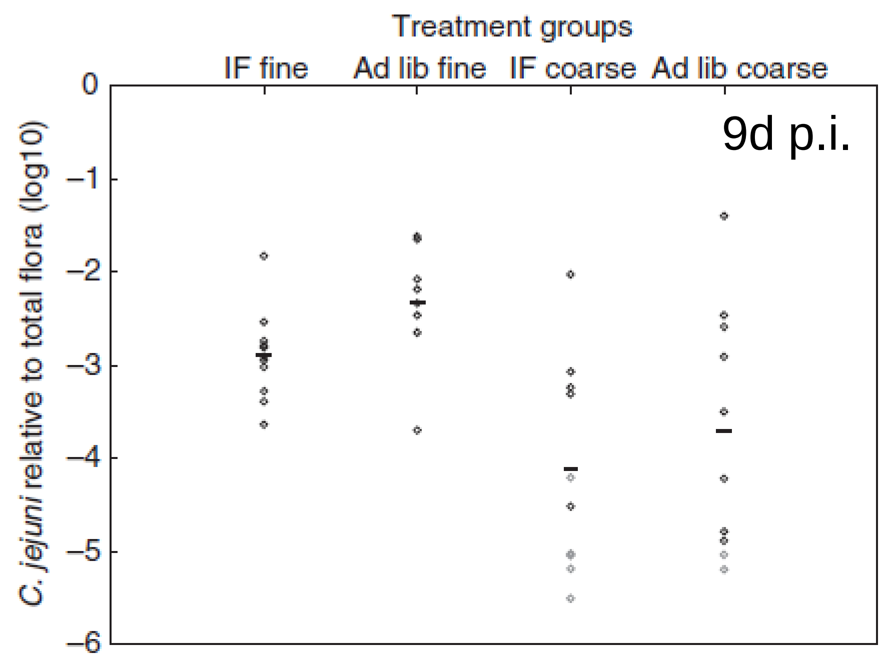
Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

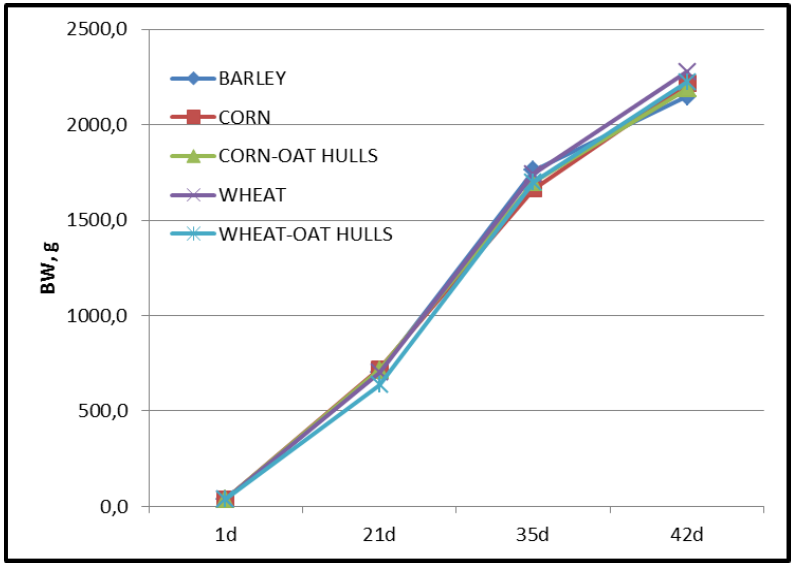
Reduced spread of *Campylobacter jejuni* in broiler chickens by stimulating the bird's natural barriers

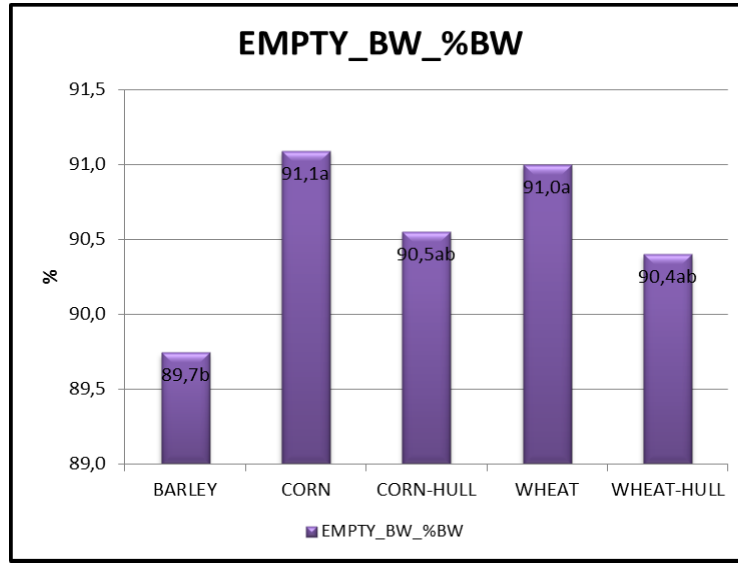
B. Moen[1], K. Rudi[1,2,3], B. Svihus[4] and B. Skånseng[1]

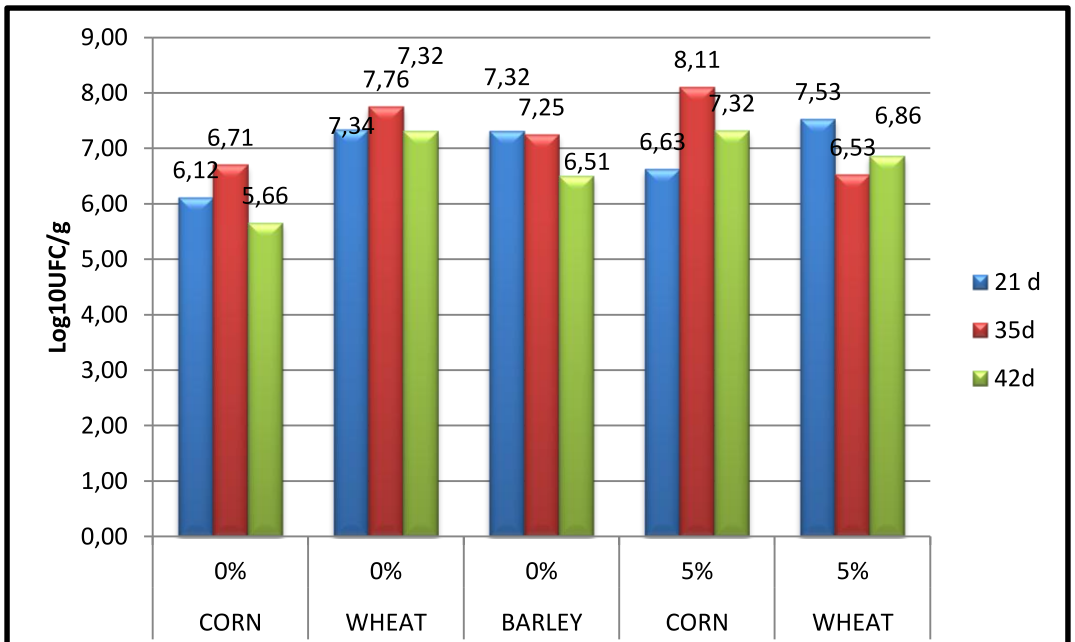




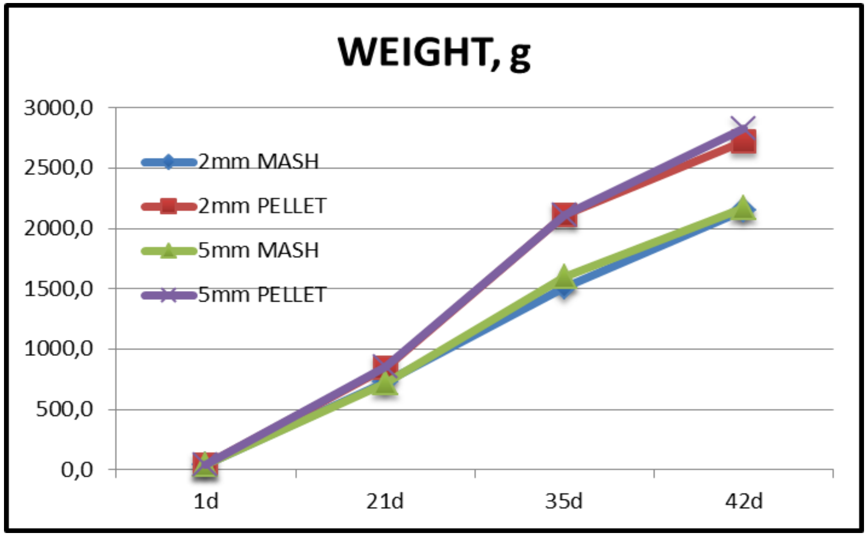
Cereal type & Oat hulls

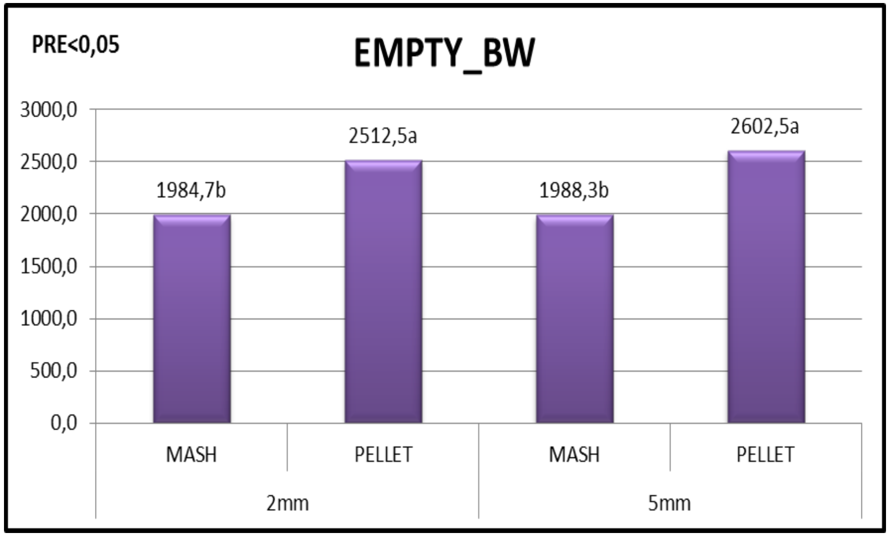


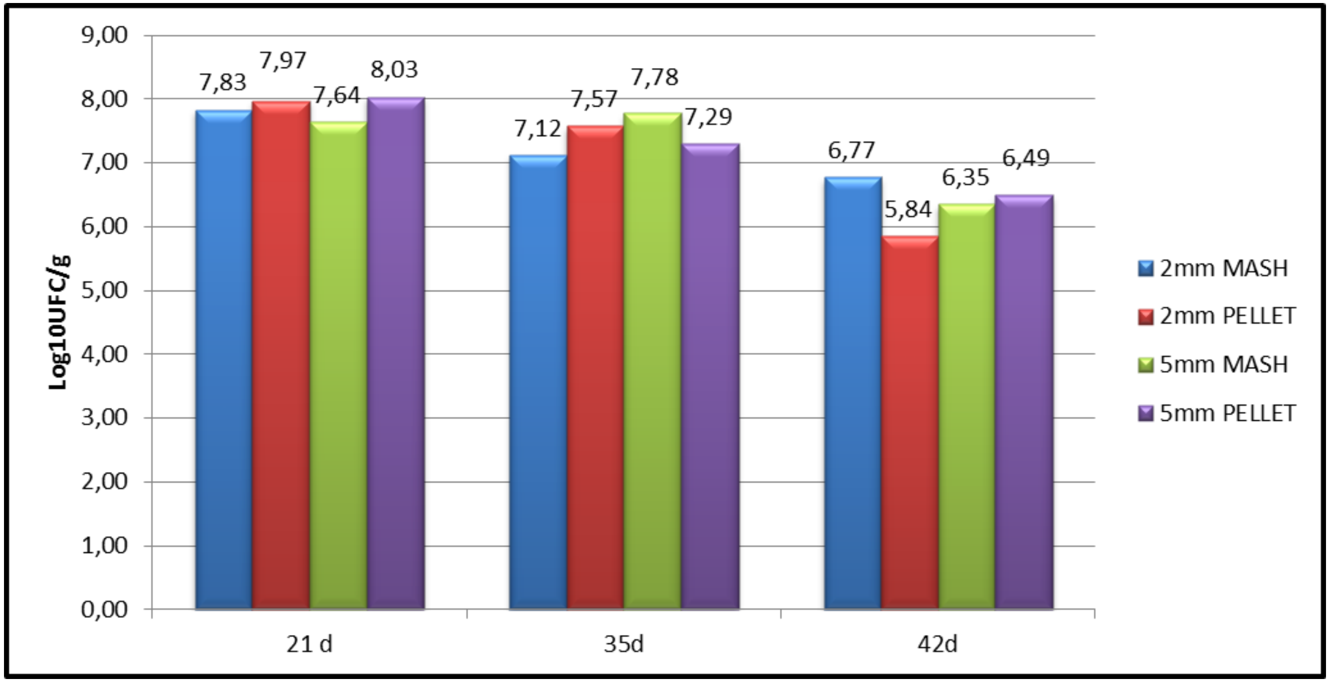




Pelleting and particle size

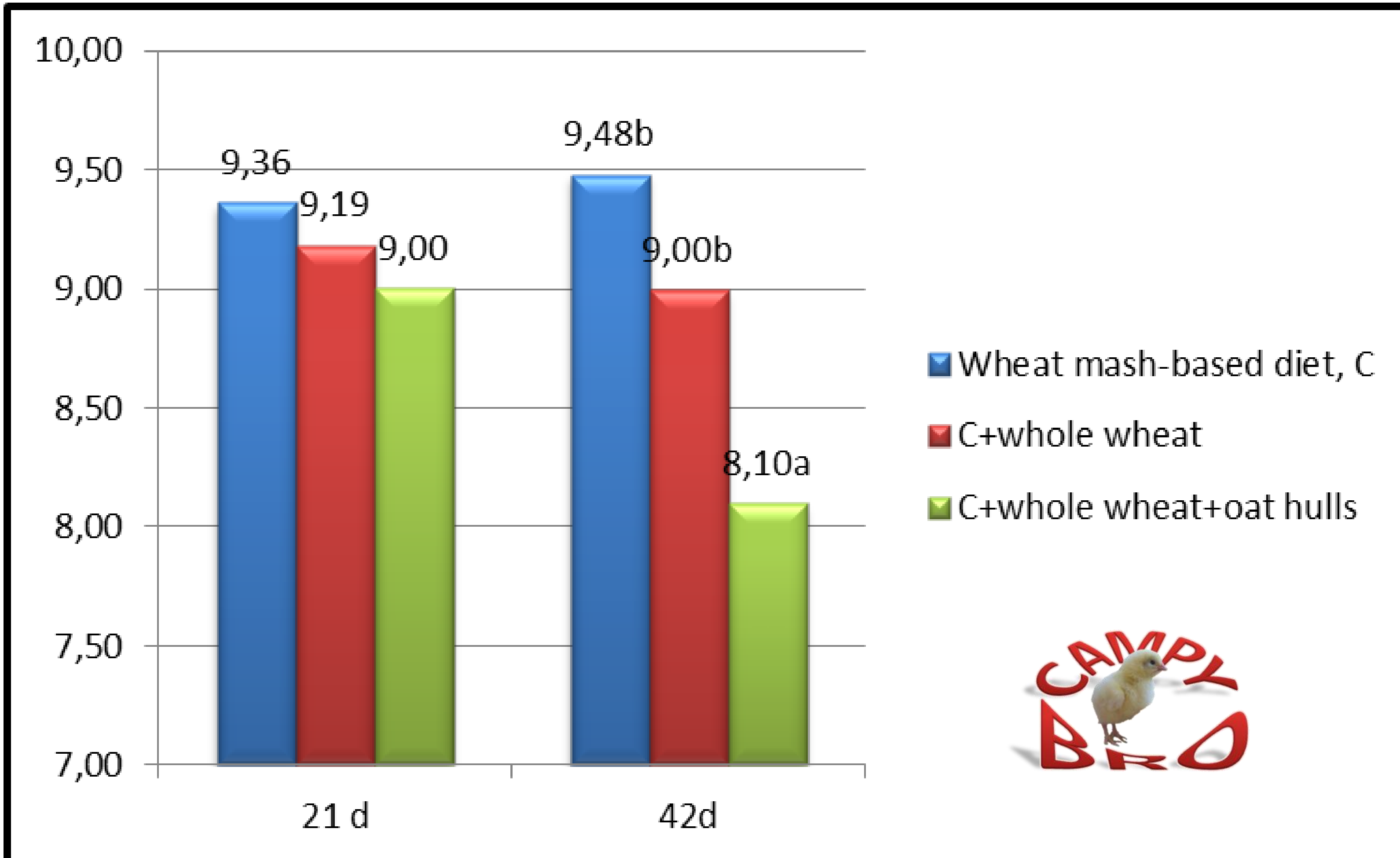






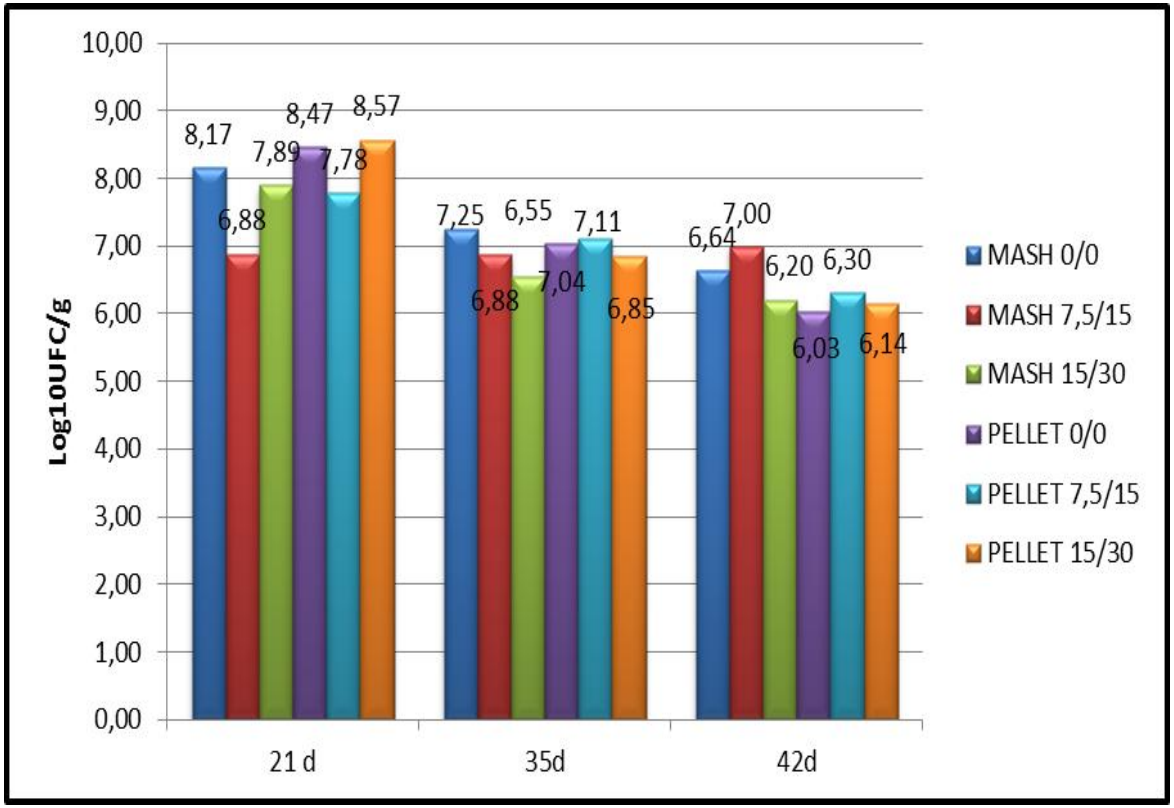
Gracia et al., 2015

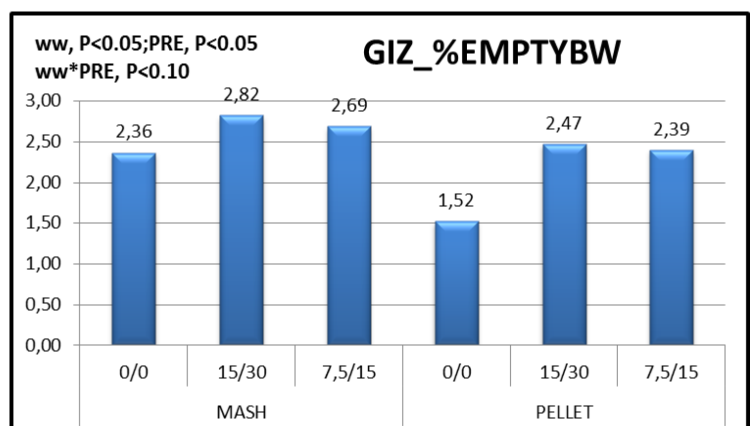
Whole wheat and oat hulls



Gracia et al., 2015

Pelleting and whole wheat





Probiotics

Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens

K. Ghareeb,*† W. A. Awad,‡[1] M. Mohnl,§ R. Porta,# M. Biarnés,# J. Böhm,* and G. Schatzmayr||

2012 Poultry Science 91:1825–1832
http://dx.doi.org/10.3382/ps.2012-02168

Table 1. The effect of administration of a multispecies probiotic product (PoultryStar sol, BIOMIN GmbH, Herzogenburg, Austria) on the cecal colonization of *Campylobacter jejuni* in broiler chickens in 2 in vivo experiments[1]

Item	Treatment: Control (n = 10)	Treatment: PoultryStar sol (2 mg/bird/day) (n = 10)	Treatment: PoultryStar sol (20 mg/bird/day) (n = 10)	SEM	*P*-value
First experiment					
C. jejuni (log cfu/g) (8 d postchallenge)	6.77[a]	3.00[b]	—	0.51	0.001
C. jejuni (log cfu/g) (15 d postchallenge)	8.00[a]	2.50[b]	—	0.23	0.001
Second experiment					
C. jejuni (log cfu/g) (8 d postchallenge)	7.81[a]	<2.00[b]	<2.00[b]	0.52	0.001
C. jejuni (log cfu/g) (15 d postchallenge)	7.85[a]	<2.00[b]	<2.00[b]	0.51	0.001

[a,b]Means within the same row with different superscripts are significantly different (Mann Whitney test was performed for the first experiment, n = 10/treatment and Kruskal Wallis test followed by Mann Whitney test for the second experiment, n = 10/treatment).

[1]Data presented as means of logarithms of 10 cecal samples per group (log cfu/g).

Plant extracts

Is allicin able to reduce *Campylobacter jejuni* colonization in broilers when added to drinking water?

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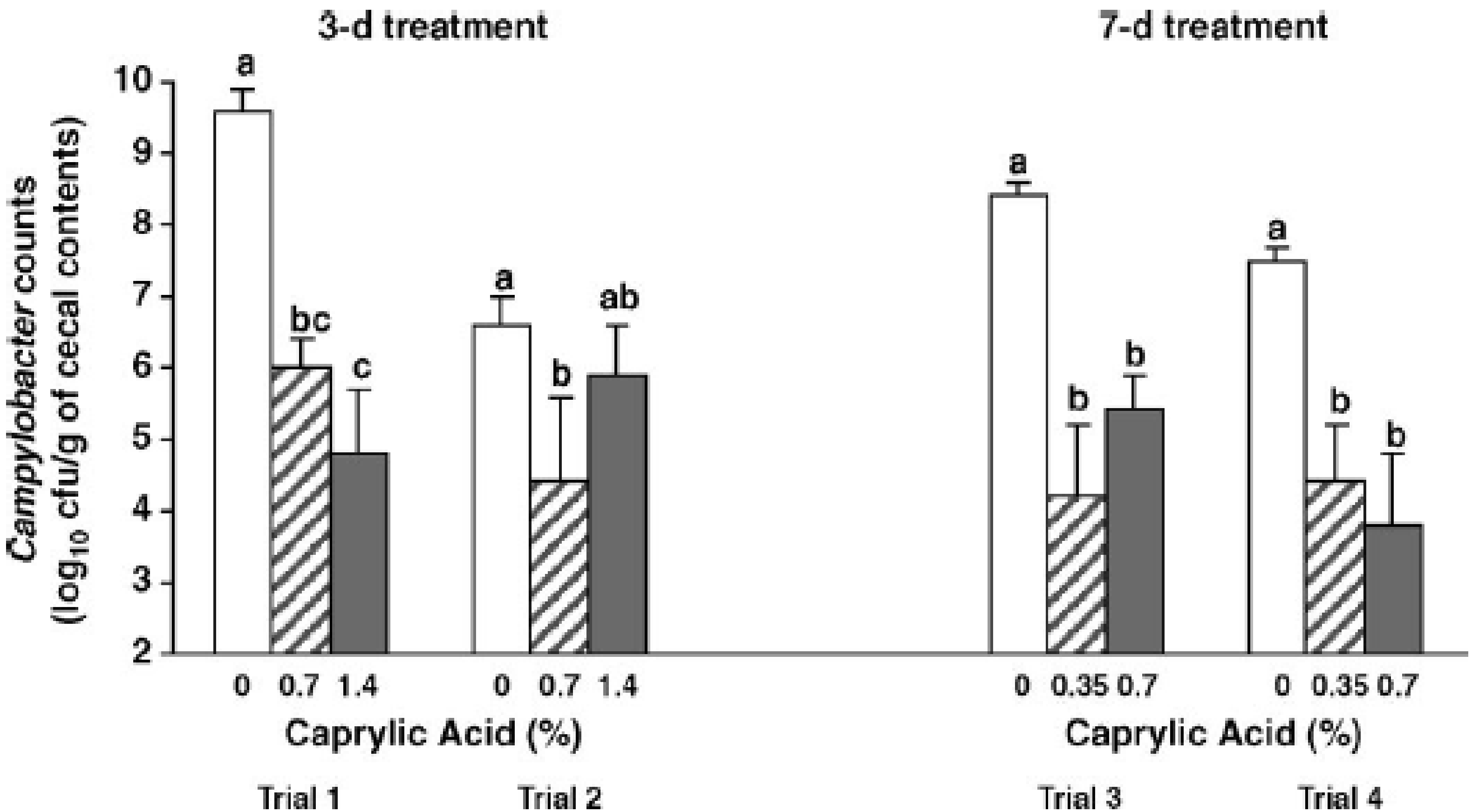
ABSTRACT Reducing *Campylobacter* shedding on the farm could result in a reduction of the number of human campylobacteriosis cases. In this study, we first investigated if allicin, allyl disulfide, and garlic oil extract were able to either prevent *C. jejuni* growth or kill *C. jejuni* in vitro. Allyl disulfide and garlic oil extract reduced *C. jejuni* numbers in vitro below a detectable level at a concentration of 50 mg/kg (no lower concentrations were tested), whereas allicin reduced *C. jejuni* numbers below a detectable level at a concentration as low as 7.5 mg/kg. In further experiments we screened for the anti-*C. jejuni* activity of allicin in a fermentation system closely mimicking the broiler cecal environment using cecal microbiota and mucus isolated from *C. jejuni*-free broilers. During these fermentation experiments, allicin reduced *C. jejuni* numbers below a detectable level after 24 h at a concentration of 50 mg/kg. In contrast, 25 mg/kg of allicin killed *C. jejuni* in the first 28 h of incubation, but anti-*C. jejuni* activity was lost after 48 h of incubation, probably due to the presence of mucin in the growth medium. This had been confirmed in fermentation experiments in the presence of broiler cecal mucus. Based on these results, we performed an in vivo experiment to assess the prevention or reduction of cecal *C. jejuni* colonization in broiler chickens when allicin was added to drinking water. We demonstrated that allicin in drinking water did not have a statistically significant effect on cecal *C. jejuni* colonization in broilers. It was assumed, based on in vitro experiments, that the activity of allicin was thwarted by the presence of mucin-containing mucus. Despite promising in vitro results, allicin was not capable of statistically influencing *C. jejuni* colonization in a broiler flock, although a trend toward lower cecal *C. jejuni* numbers in allicin-treated broilers was observed.

Key words: *Campylobacter jejuni*, allicin, in vivo, broiler, drinking water

2013 Poultry Science 92:1408–1418
http://dx.doi.org/10.3382/ps.2012-02863

Acidification: MCFA



De los Santos et al., 2009

Acidification: MCFA MG

Effect of Glycerol Monocaprate (Monocaprin) on Broiler Chickens: An Attempt at Reducing Intestinal *Campylobacter* Infection

2006 Poultry Science 85:588–592

H. Hilmarsson,* H. Thormar,*[1] J. H. Thráinsson,* and E. Gunnarsson†

Table 1. *Campylobacter* counts in cloacal swabs collected on d 2 to 10 and in cecal specimens collected at euthanasia on d 13 (Experiment A) from groups of 7 chickens infected with *Campylobacter jejuni*[1]

		Viable *Campylobacter* count ($\log_{10}$ cfu/mL)[2]	
Experiment	Day	Treated group	Control group
A	2	4.7 ± 0.5[3]	6.8 ± 0.5
	5	5.3 ± 0.5[4]	6.5 ± 0.3
	10	5.1 ± 0.6[4]	6.5 ± 0.7
	13	6.9 ± 1.9	7.3 ± 1.3
B	2	4.7 ± 1.1[3]	6.4 ± 0.3
	4	3.4 ± 1.1[4]	5.5 ± 0.8
	10	5.9 ± 0.8	6.0 ± 0.9

[1]One group was treated with an emulsion of 5 m*M* (0.12%) monocaprin and 0.02% polysorbate 40 in drinking water and feed for 13 d following contact between 2 infected and 5 uninfected chickens on d 0. A group of 7 untreated chickens was used as control.

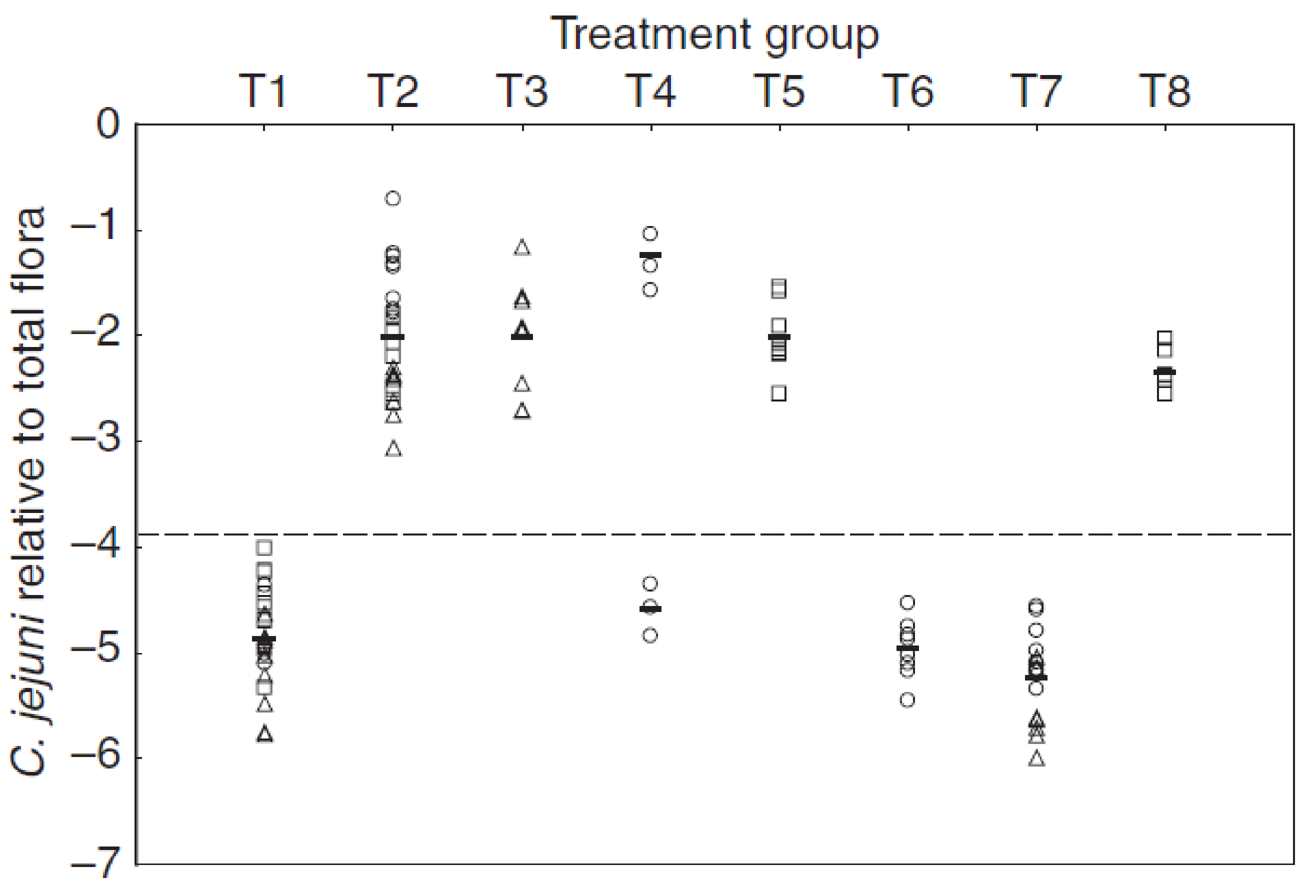
Table 2. *Campylobacter* counts in cloacal swabs of chickens naturally infected with *Campylobacter* and treated for 3 d with 10 m*M* (0.24%) monocaprin and 0.04% polysorbate 40 emulsion added to their drinking water and feed[1]

		Viable *Campylobacter* count ($\log_{10}$ cfu/mL)[2]	
Experiment	Day	Treated group	Control group
A	0	7.2 ± 0.4	6.6 ± 1.0
	3	5.4 ± 1.3[3]	6.9 ± 0.3
B	0	4.7 ± 0.8	5.1 ± 0.2
	3	3.2 ± 0.3[4]	5.4 ± 0.3

Acidification: OA

Prevention of intestinal *Campylobacter jejuni* colonization in broilers by combinations of in-feed organic acids

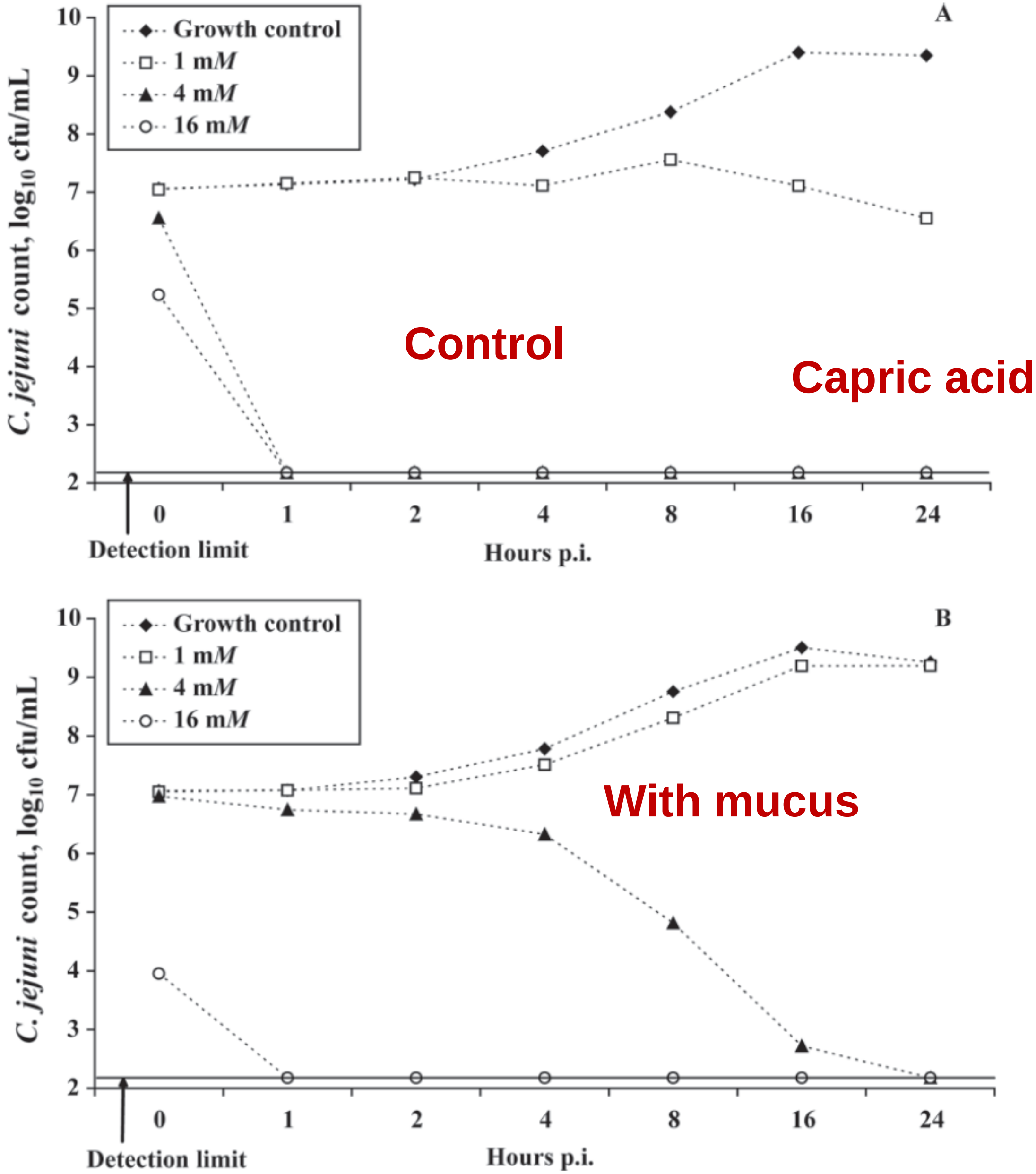
B. Skånseng[1], M. Kaldhusdal[2], B. Moen[1], A.-G. Gjevre[2], G.S. Johannessen[2], M. Sekelja[1,3], P. Trosvik[4] and K. Rudi[1,5]



Treatment groups*
Experiment
1. Negative control‡
2. Positive control¶
3. 1·0% formic acid
4. 2·0% formic acid
5. 1·0% formic acid + 0·1% sorbate
6. 1·5% formic acid + 0·1% sorbate
7. 2·0% formic acid + 0·1% sorbate
8. 0·1% sorbate

Journal of Applied Microbiology **109** (2010) 1265–1273

Acidification: MCFA



Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids

D. Hermans,*[1] A. Martel,* K. Van Deun,* M. Verlinden,* F. Van Immerseel,* A. Garmyn,* W. Messens,† M. Heyndrickx,† F. Haesebrouck,* and F. Pasmans*

Single additives

Efficacy of feed additives against *Campylobacter* in live broilers during the entire rearing period[1]

M. Guyard-Nicodème,*[,2] A. Keita,[†] S. Quesne,* M. Amelot,[†] T. Poezevara,* B. Le Berre,[†] J. Sánchez,[‡] P. Vesseur,[#] Á. Martín,[§] P. Medel,[‡] and Marianne Chemaly*

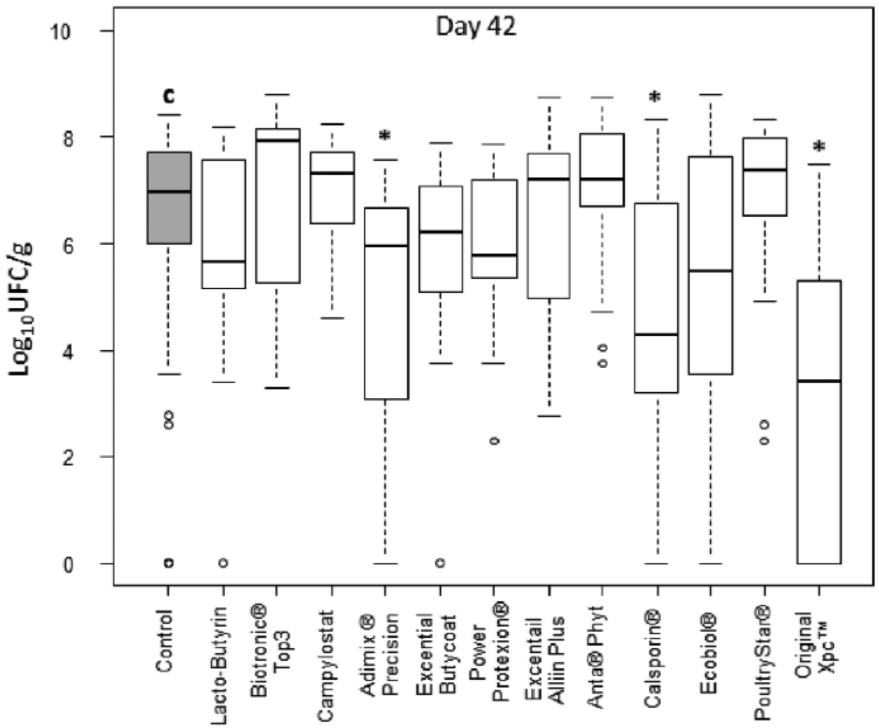


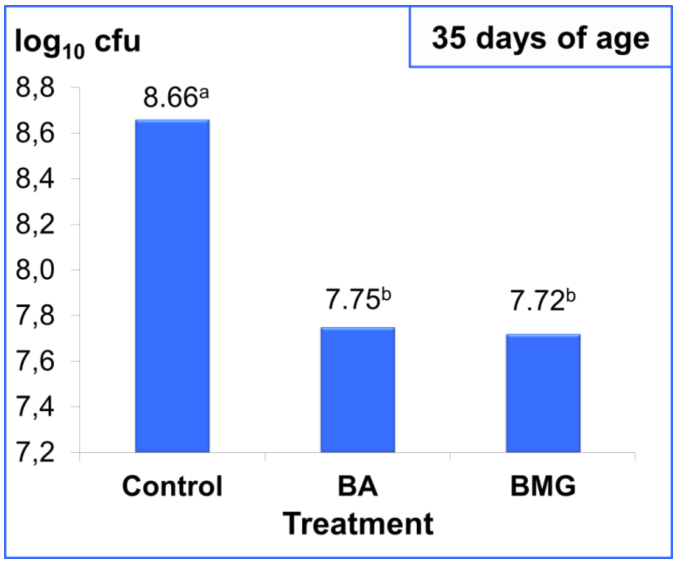
Figure 1. Effect of dietary treatment on *Campylobacter* counts ($\log_{10}$ CFU/g) in the caeca of broilers at 14, 35, and 42 d of age (3, 24, and 31 d postinoculation). Treatments giving significant reduction ($P < 0.05$) in *Campylobacter* counts compared to the control groups are marked with an asterisk. Letters (a,b,c) indicated a significant difference ($P < 0.05$) between the control group at each sampling date.

Poultry Sci. 95 (2016): 298-305

MCFA and Monoglycerides

Efficacy of feed additives against *Campylobacter* in live broilers during the entire rearing period: Part B

M. I. Gracia,[*,1] C. Millán,[*] J. Sánchez,[*] M. Guyard-Nicodème,[†] J. Mayot,[‡] Y. Carre,[§] A. Csorbai,[#] M. Chemaly,[†] and P. Medel[*]



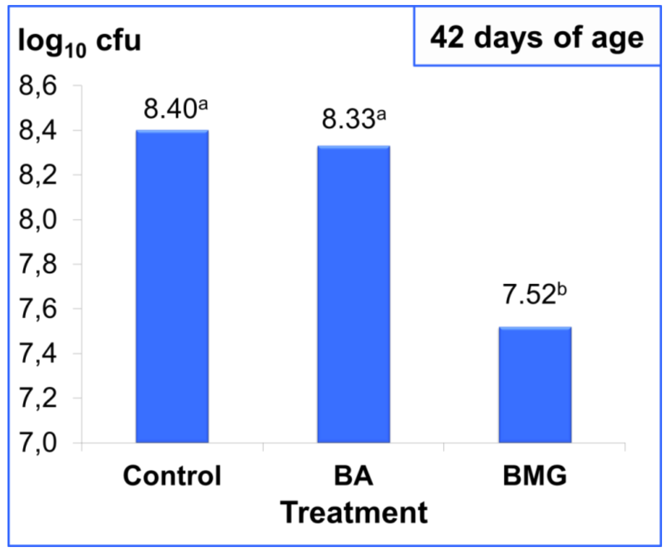
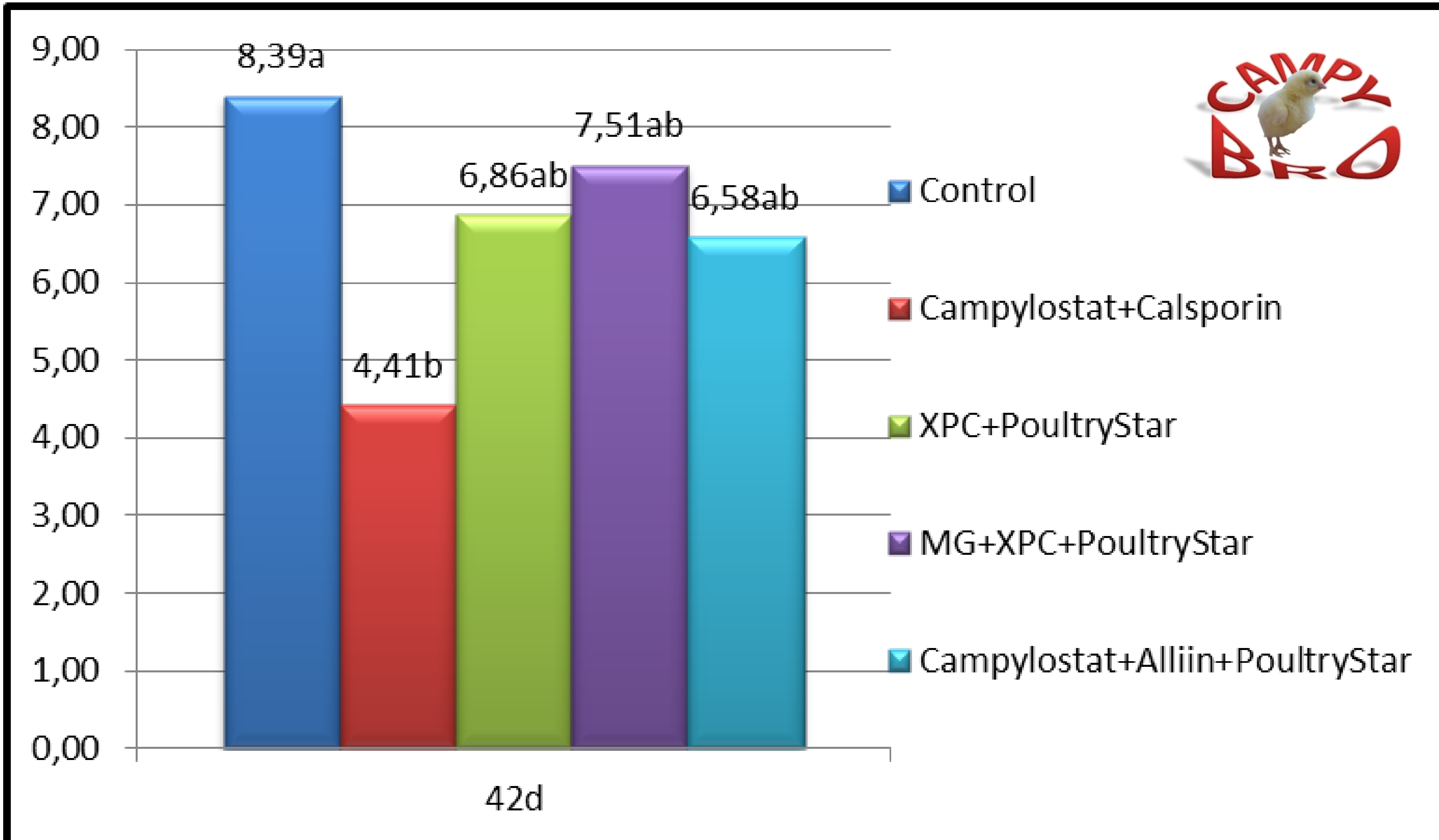


Table 2. Effect of dietary treatment on the cecal colonization of *Campylobacter jejuni* in broiler chickens in Trial 1.[1]

Treatment	7 d post challenge	21 d post challenge	28 d post challenge
1 Control		8.66[a]	8.40[a]
2 C8 and C10	8.68 ± 1.26[2]	7.75[b]	8.33[a]
3 Monoglycerides of C8 and C10		7.72[b]	7.52[b]
SEM	–	0.256	0.206
Probability	–	0.0268	0.0242

Poultry Sci. 95 (2016): 886-892

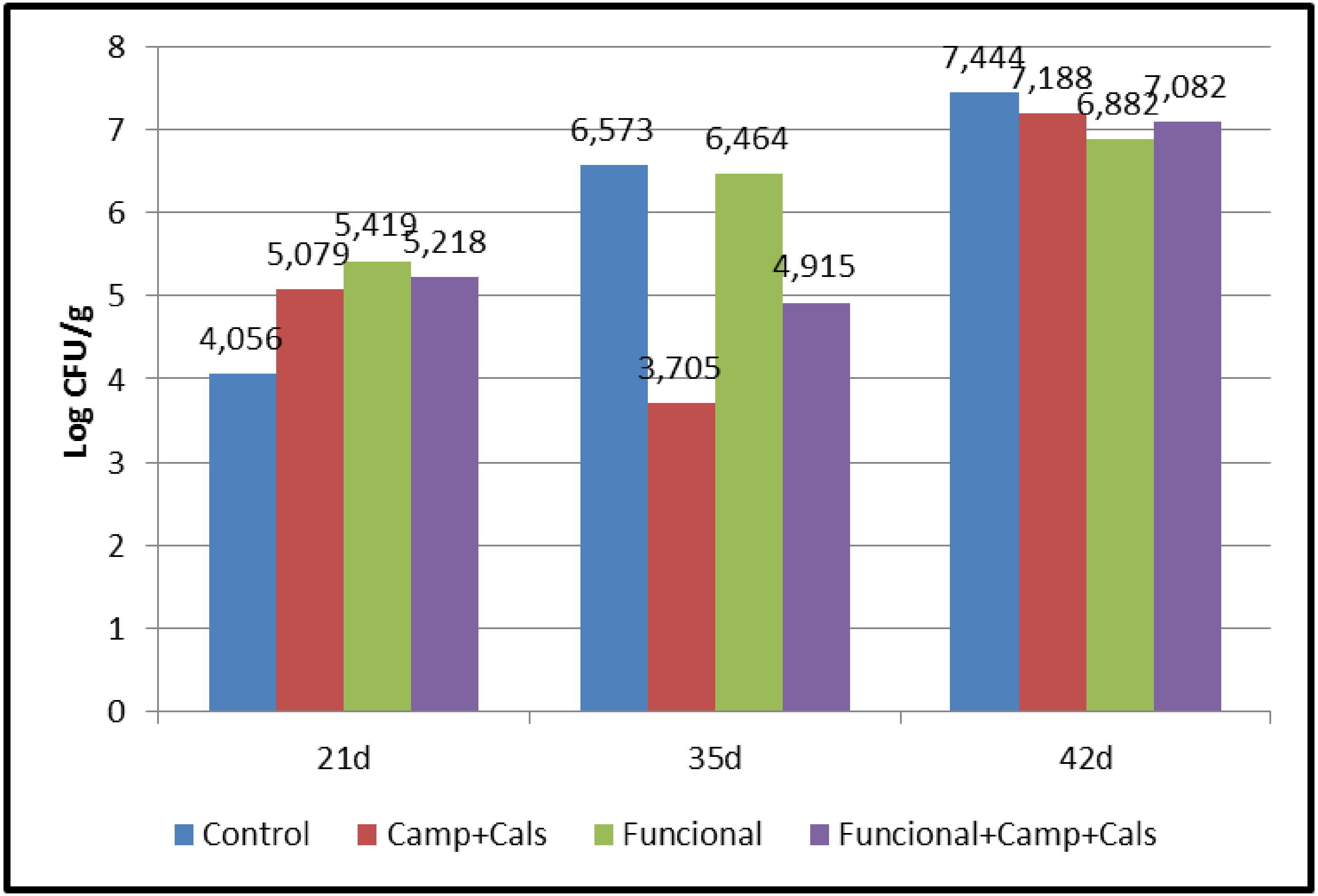
Product combination 42d



Casabuena et al., 2015

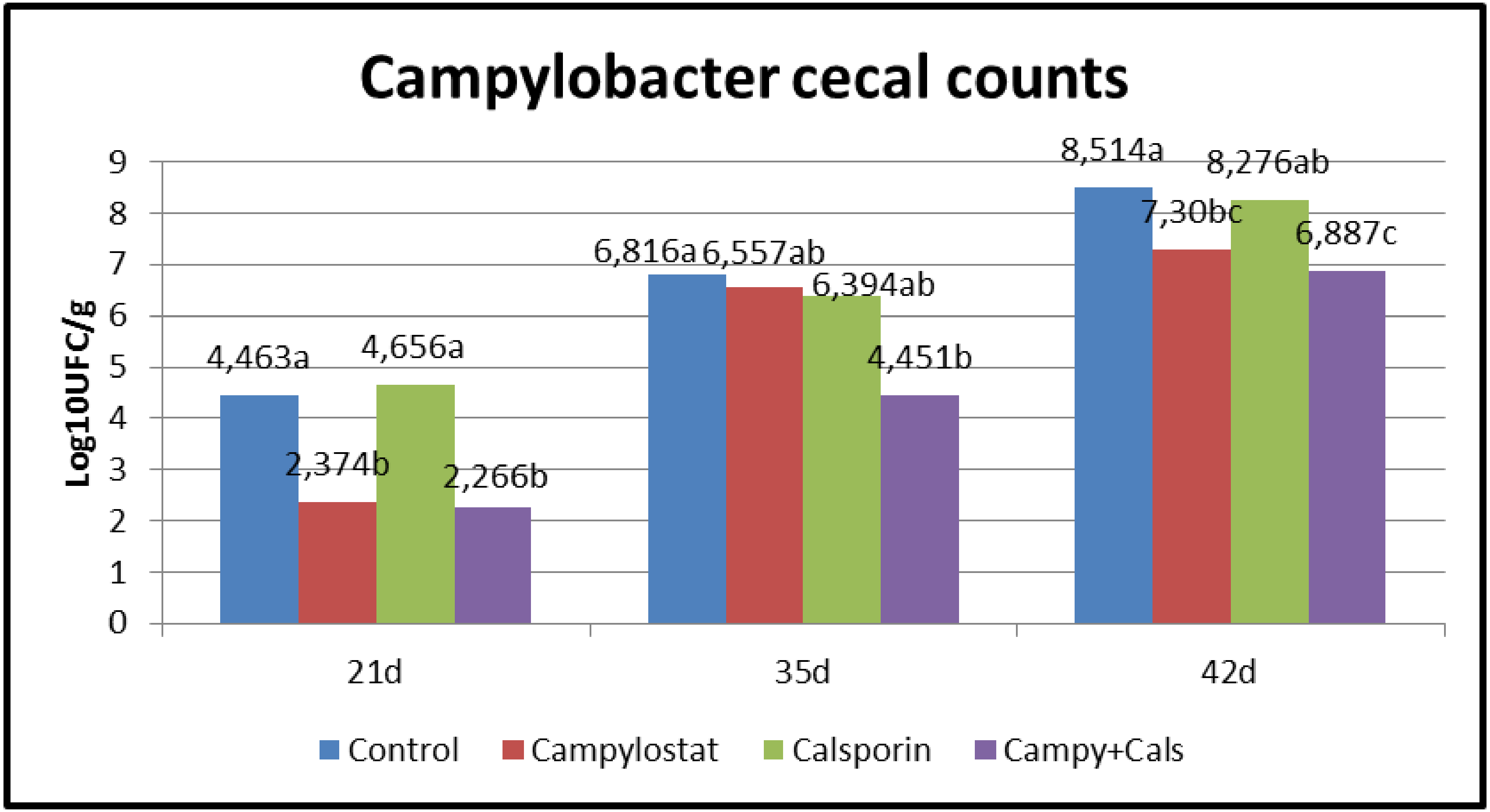
EXP.11. Interaction Functional diet x (Camp+Cals)

- ✓ **Inconsistent infection at 21d**
- ✓ **Clear effect at 35d; Lost of effect at 42d**



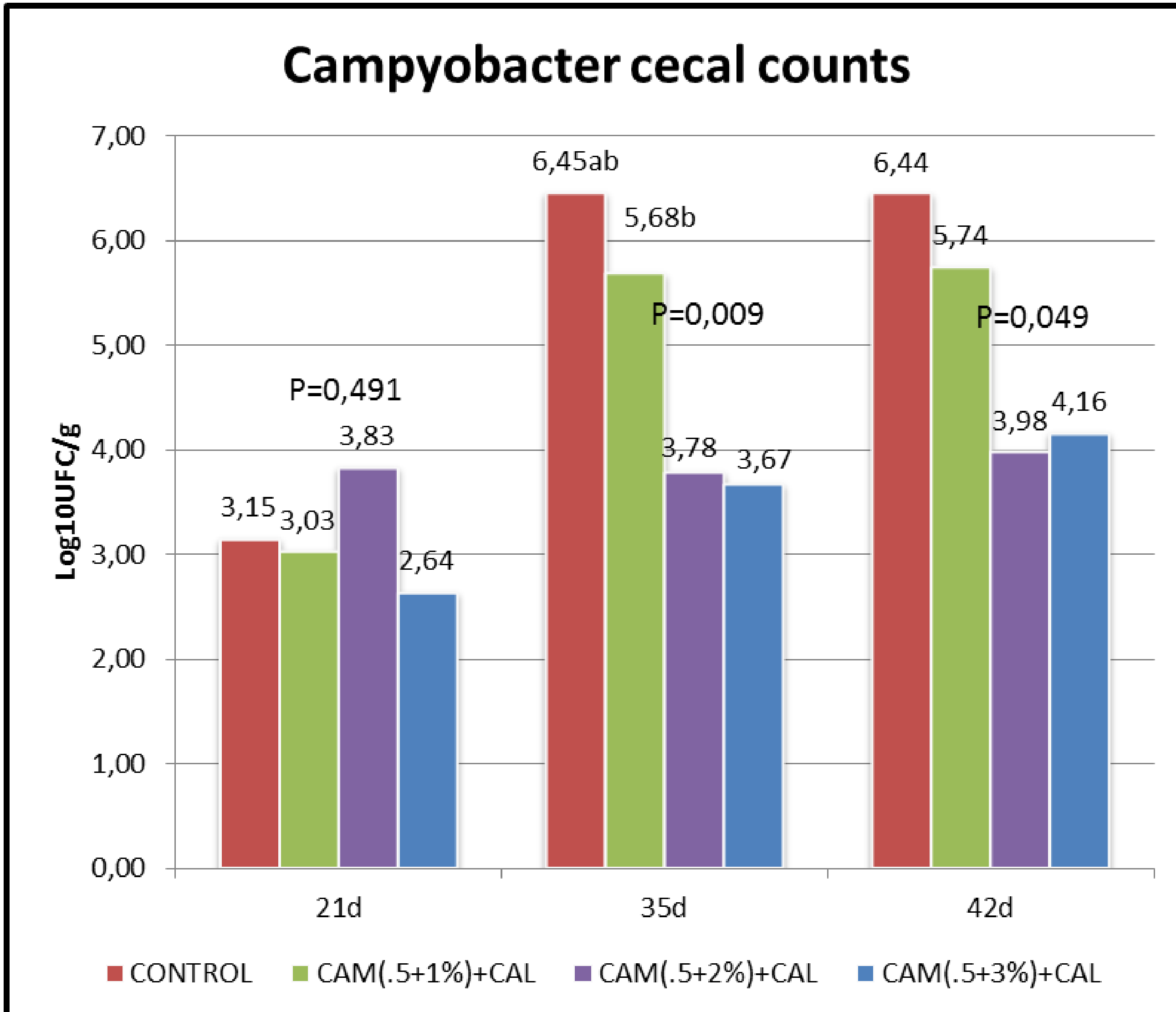
EXP.13. Interaction Camp x Cals in a control diet

- ✓ **Low infection level at 21d, but clear effect of Campylostat**
- ✓ **Clear effect at 35d; Synergism Campylostat x Calsporin**
- ✓ **Effect at 42d; Synergism Campylostat x Calsporin.**

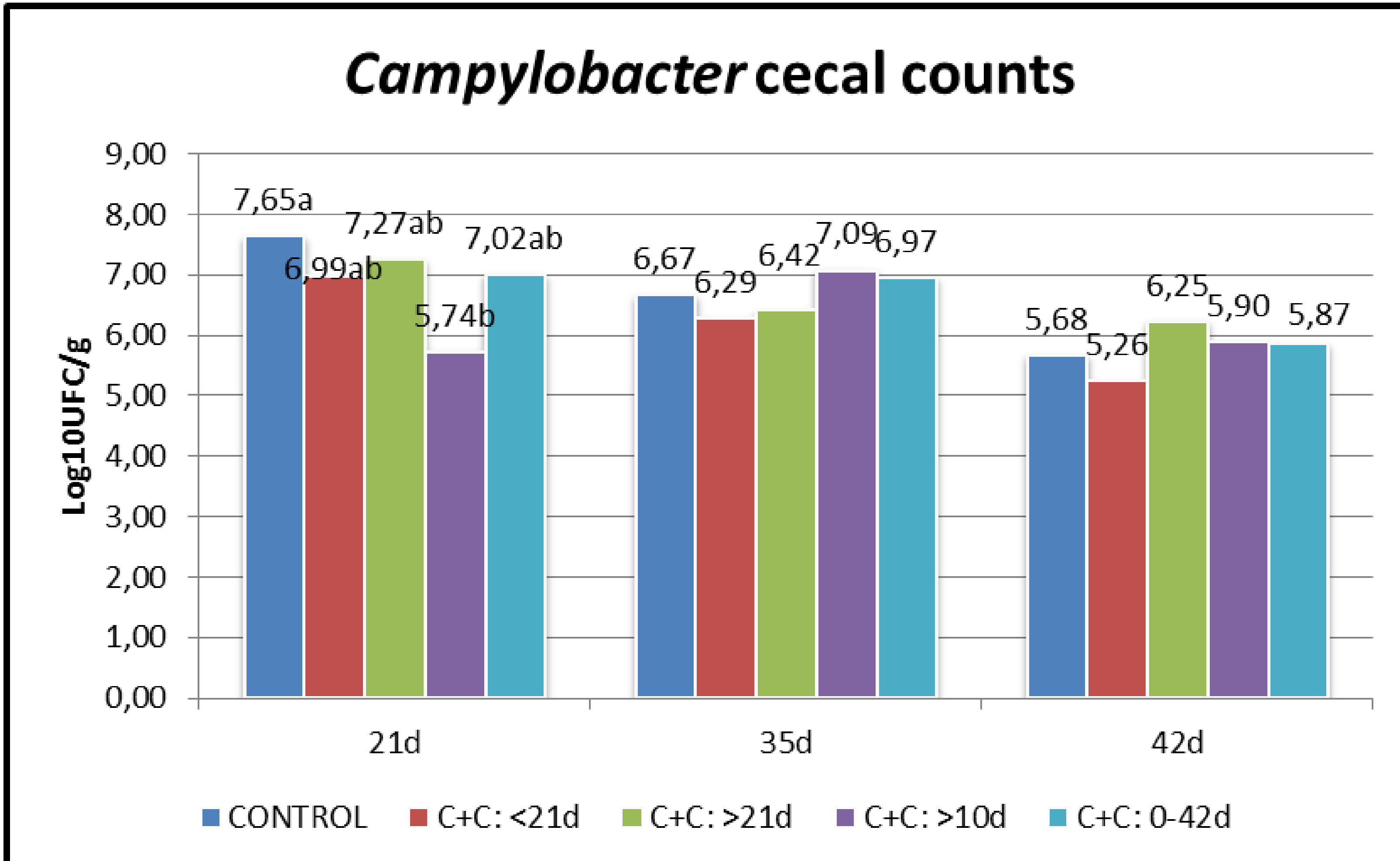


EXP. 14. Campylostat dose in a control diet

- ✓ Low infection level at 21d
- ✓ Clear effect at 35d; 1% decreased close to 1 log, but the biggest decreased was with 2%. No additional improvement with 3%
- ✓ Same effect at 42d



EXP.12. Time of administration of (Camp+Cals)



WP4: field trials

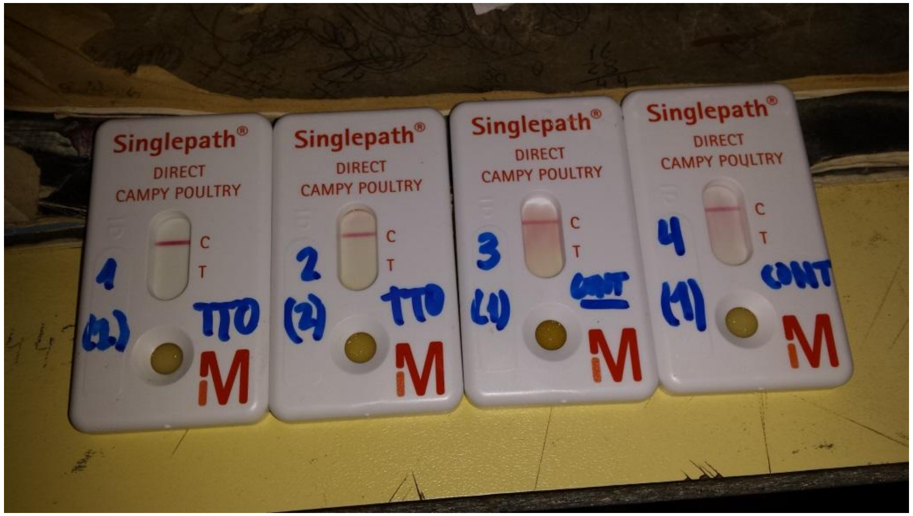
- Objective
  - Evaluate the efficacy of the combination of products in field conditions
    - Farm conditions (density, environmental, microbiota pressure)
    - In floor pens rather than cages
    - Barn vs barn, twin buildings
- Questions:
  - The efficacy is the same with field strains of C. jejuny (in challenge trials only two strains were used)?
  - Is it also efficiency for C. coli?
  - Is there any interaction?
  - Effect on performance?

WP4: field trials

- Trial at Redondo
  - Farm verified about its *C. jejuni* contamination
  - 4 farms expected, 2 farms finished, 2 running
  - In each farm twin barns, twins two-by-two
  - Typical Spanish diets.
  - 1-42/49d
  - Sampling
    - 5 chicks per barn at random (15 chicks per treatment and age)
    - 36 and 42d, prior to slaughter
  - Analysis
    - qPCR at IMASDE

WP4: field trials. Farm 1

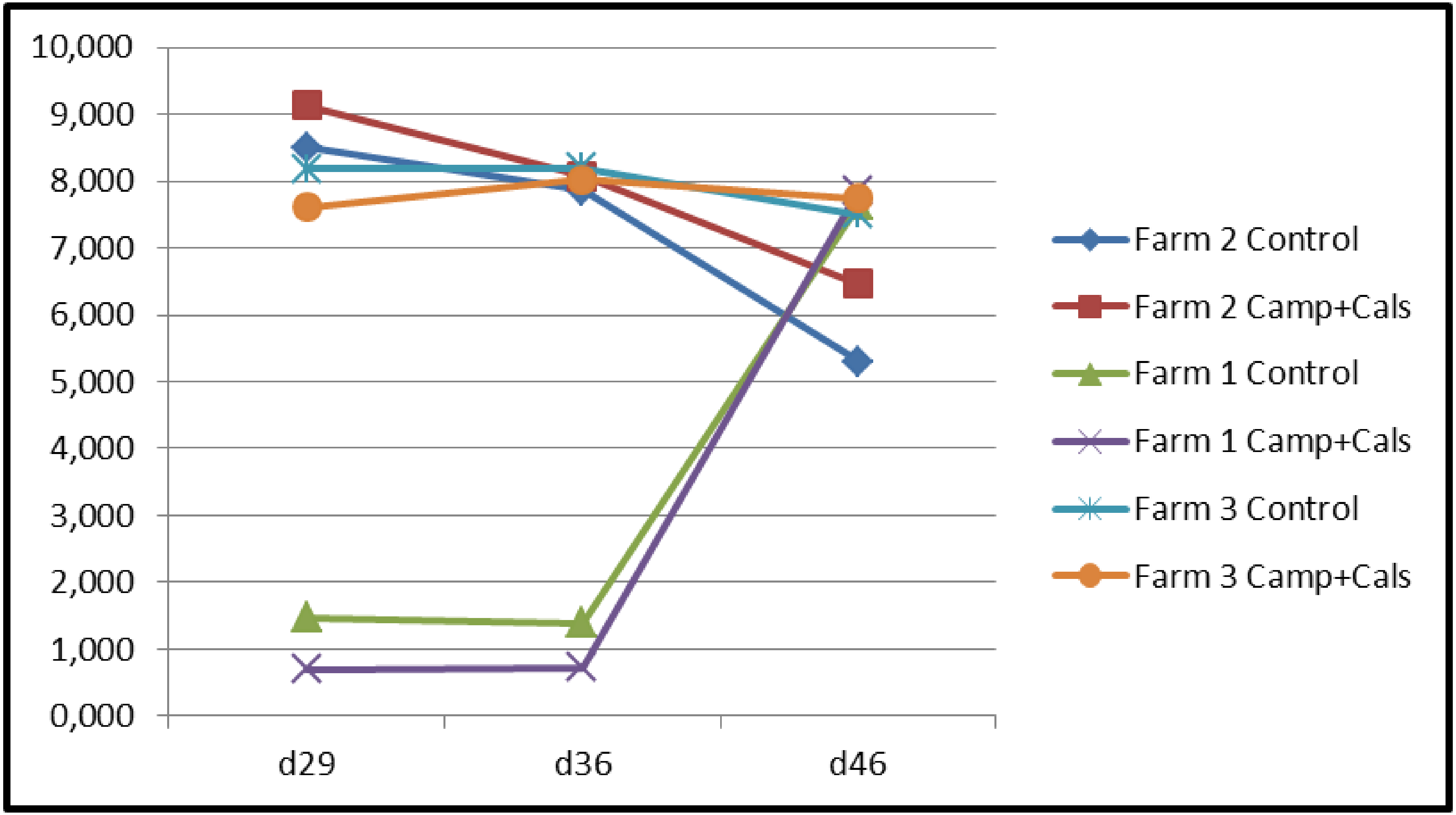
WP4: field trials. Farm 1



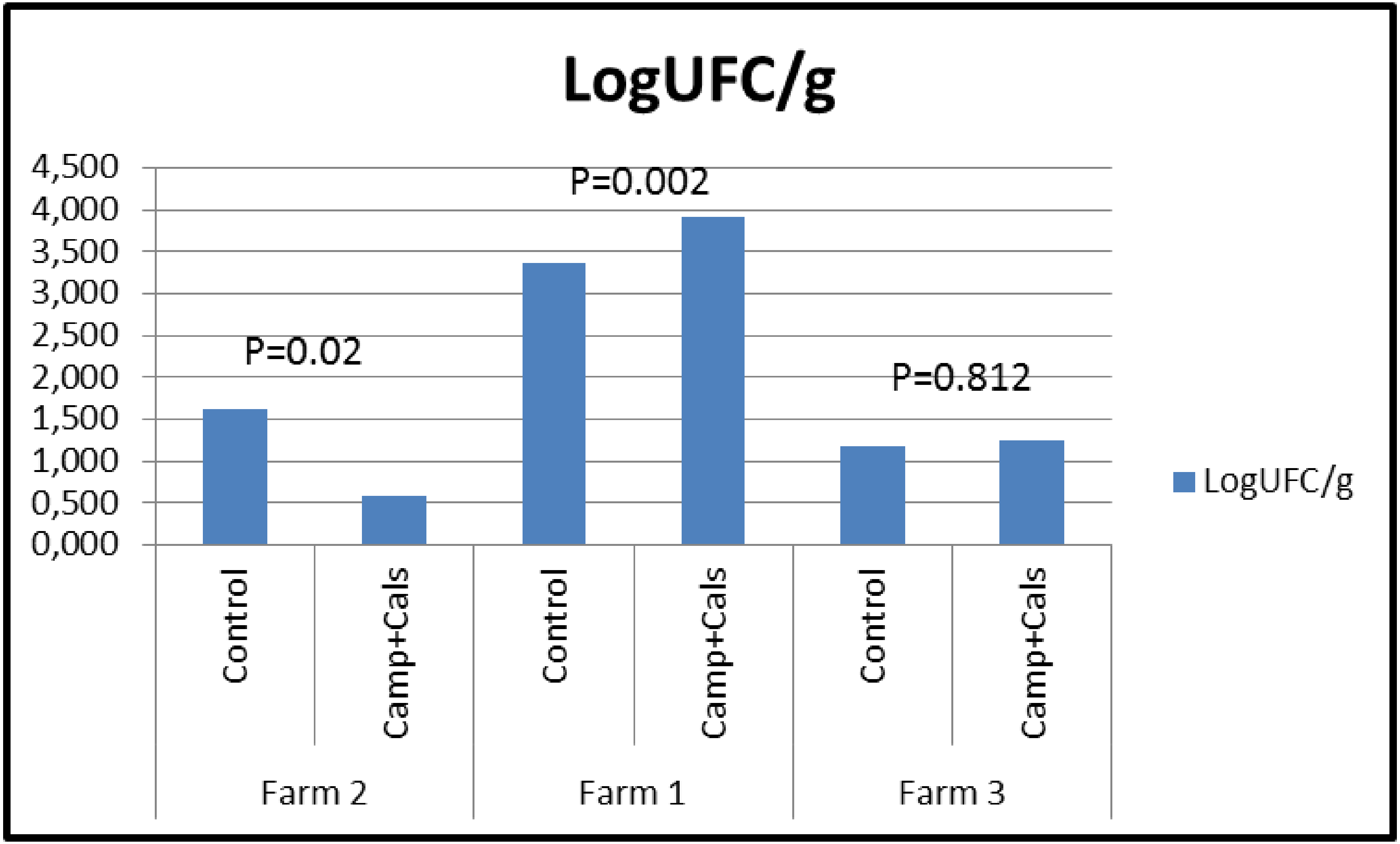
WP4: field trials. Farm 2

WP4: field trials. Farm 2

Field trials. Spain



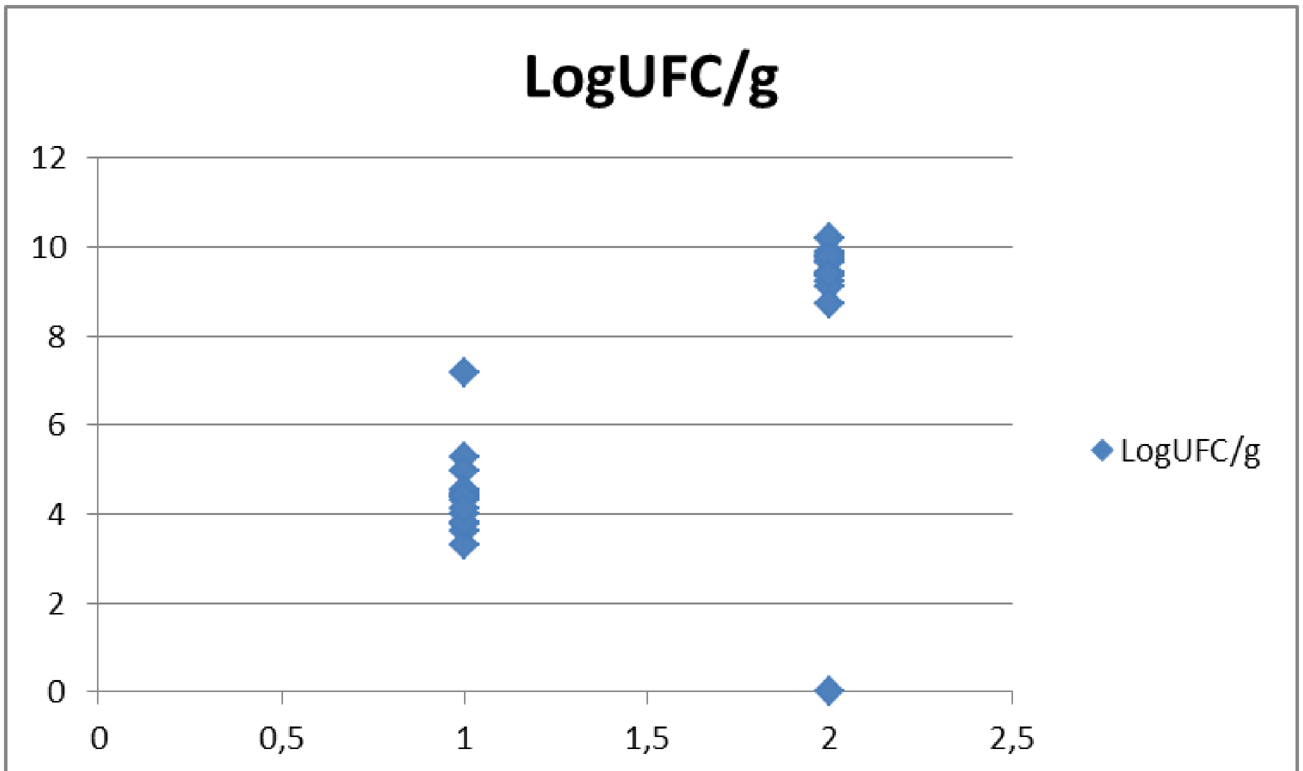
Field trials



Field trials. Hungary

Field trials. Hungary

	Log UFC/g
CONTROL	4,433
TREATED	8,912
SEM (n=15)	0,4854
P	<0,001



Objective of WP5

➔ Identify by reverse vaccinology strategy one or several antigens of *Campylobacter* that can be used as vaccinating antigens

-All the putative vaccine proteins will be ranked from the highest probability to be an antigenic protein to the lowest one

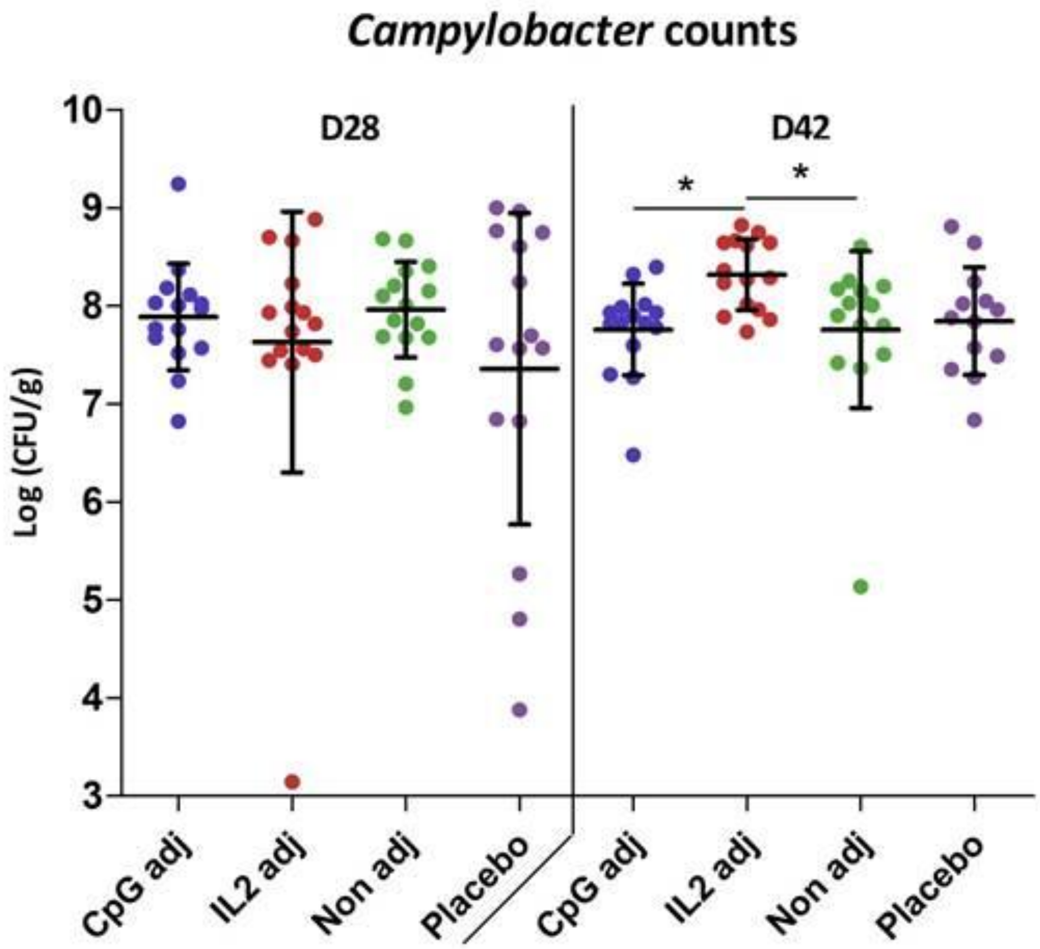
- Subselected proteins will be injected in broiler chickens and/or in ovo to evaluate their protective potentials against *Campylobacter*

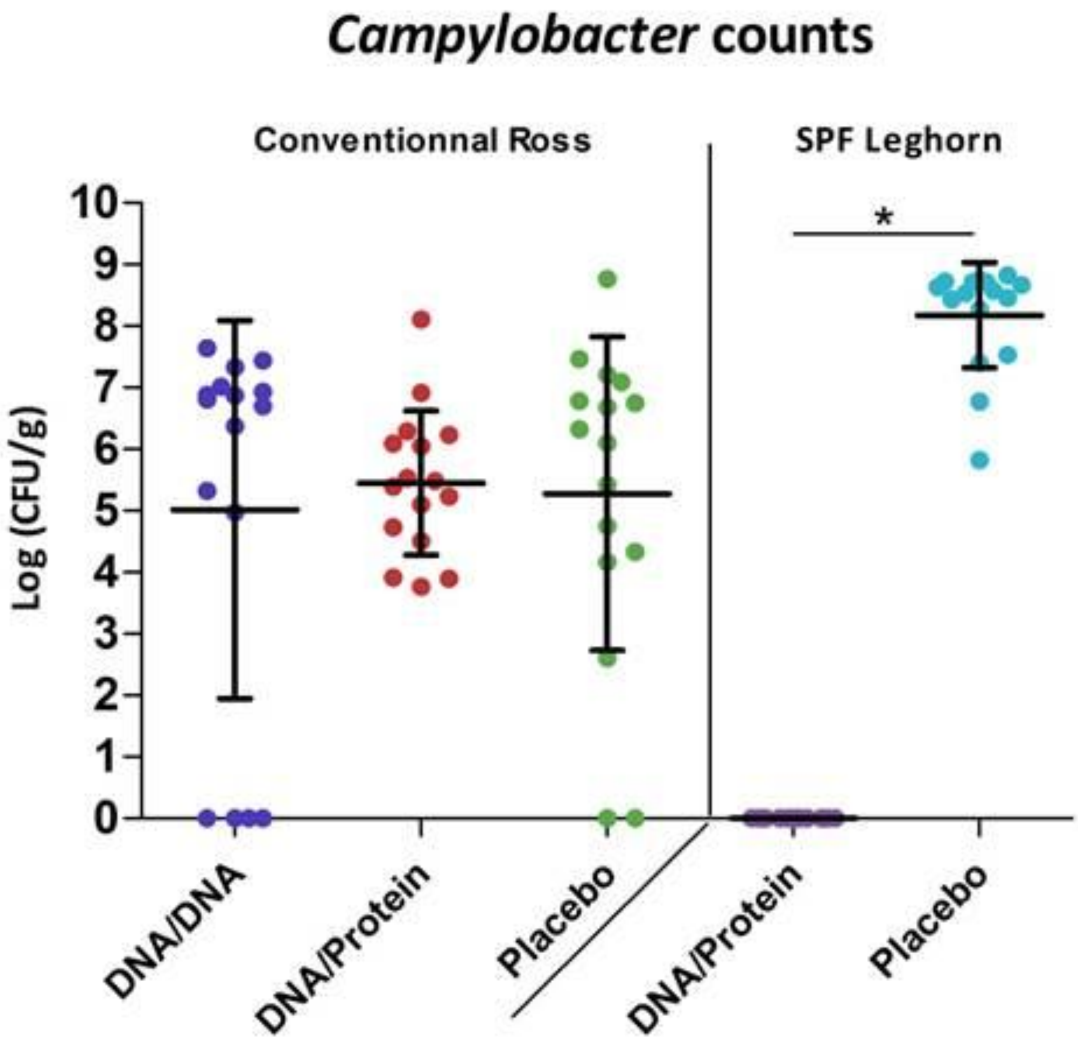
Identification of 12 potential vaccinating proteins

RANK	Protein Accession	Protein note	Localization
1	YP_001001371.1	flgE, flagellar hook protein FlgE	Extracellular
2	YP_001000562.1	flagellin family protein	Extracellular
3	YP_001000153.1	TonB-dependent receptor, putative, degenerate	OMP
4	YP_001000204.1	putative periplasmic protein	OMP
5	YP_001000248.1	fliD, flagellar capping protein	Extracellular
6	YP_001001115.1	flgK, flagellar hook-associated protein FlgK	Extracellular
7	YP_999769.1	flgE-1, flagellar hook protein	Extracellular
8	YP_001000945.1	N-acetylmuramoyl-L-alanine amidase	OMP
9	YP_001000437.1	putative OMP	OMP
10	YP_001001257.1	chuA, TonB dependent heme receptor	OMP
11	YP_999817.1	hypothetical protein	OMP
12	YP_999838.1	hypothetical protein	OMP
Flagellin B	YP_001000996.1	Flagellin B	Extracellular
Flagellin A	YP_001000997.1	Flagellin A	Extracellular

Fla A and B would be placed between FliD and FlgK

Is there a reduction of Campylobacter load at days 28 and 42?

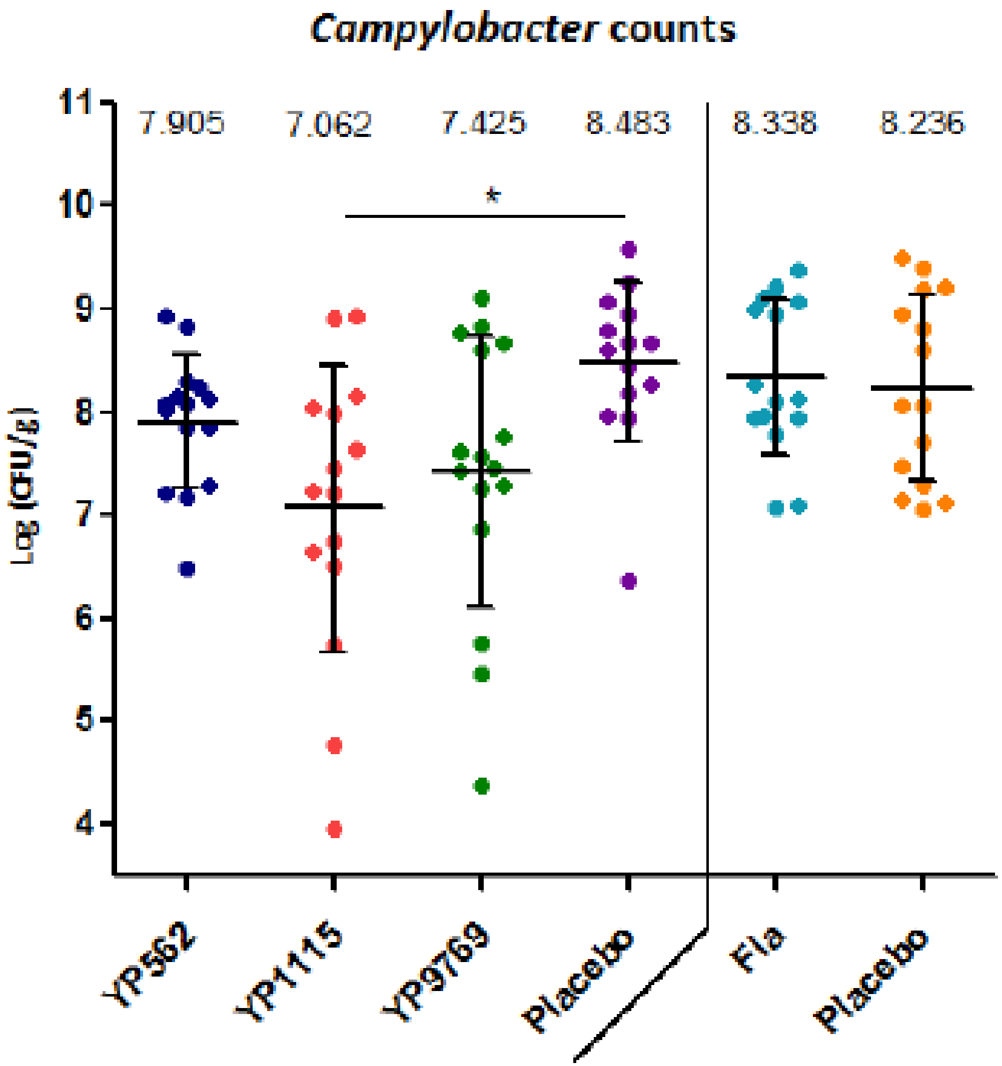




Campylobacter counts
Conventionnal Ross
SPF Leghorn
Log (CFU/g)
10
9
8
7
6
5
4
3
2
1
0
*
DNA/DNA
DNA/Protein
Placebo
DNA/Protein
Placebo

*p<0.01

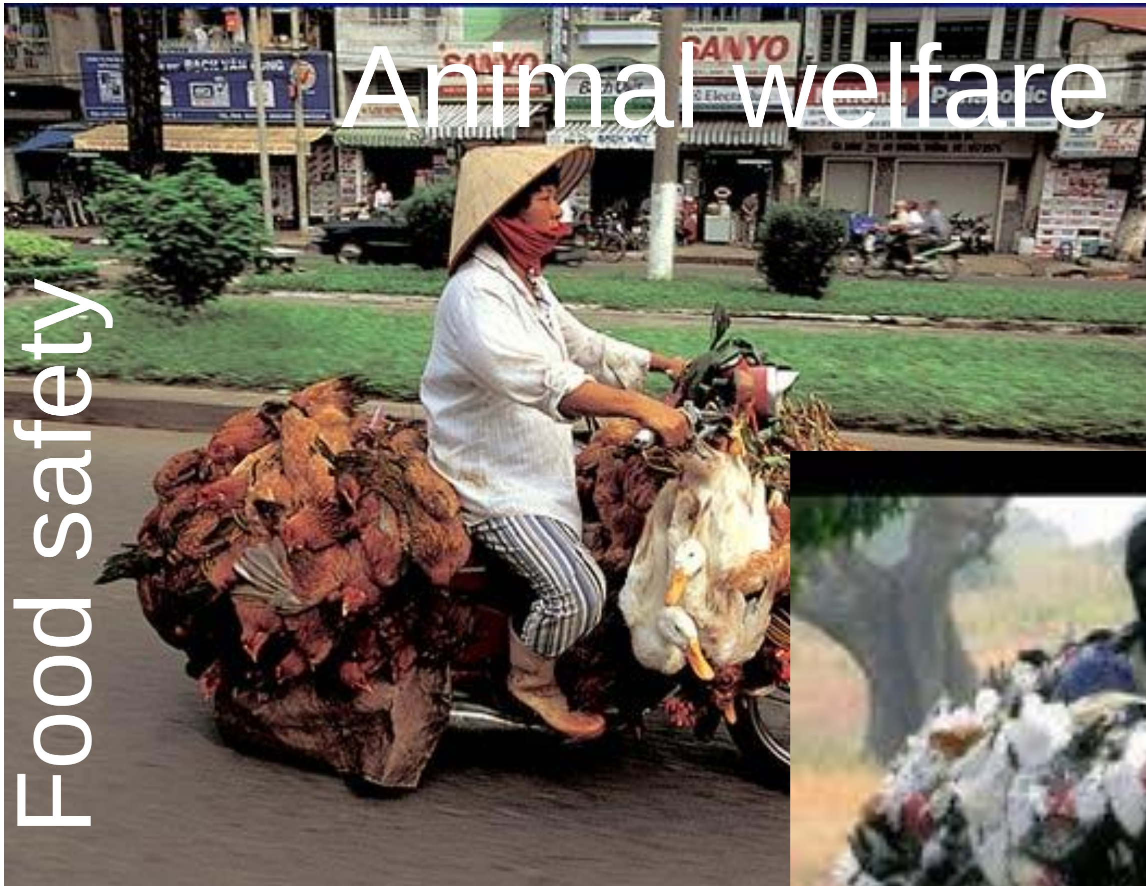
Vaccine development



p-value < 0,05

Conclusions

- **Diet modification (particle size, presentation, oat hulls, type of cereal, whole wheat) only affected the Campylobacter population in extremely high infection conditions**
- **A combination between MG MCFA+OA and B. subtilis repeatedly but heterogeneously decreased the Campylobacter infection, mainly through a decrease in the infected birds**
- **For the moment, this effect could not be demonstrated in field conditions**
- **New actual trials will define if the solution developed has a real capacity to control Campylobacter in practical conditions or not.**


Animal welfare
Food safety


Enviroment
Animal health