Control of Campylobacter infection in broiler flocks through two-steps strategy: nutrition and vaccination
-CAMPYBRO-
FP7-SME-2013-605835

Lajosmizse, 12/04/2016

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 605835"

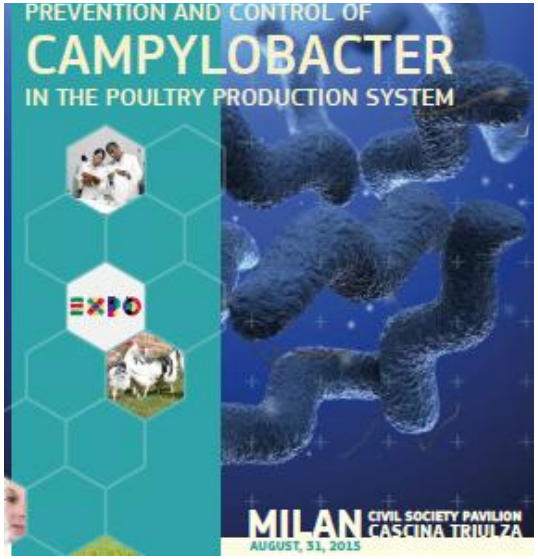
Campylobacter is on fashion

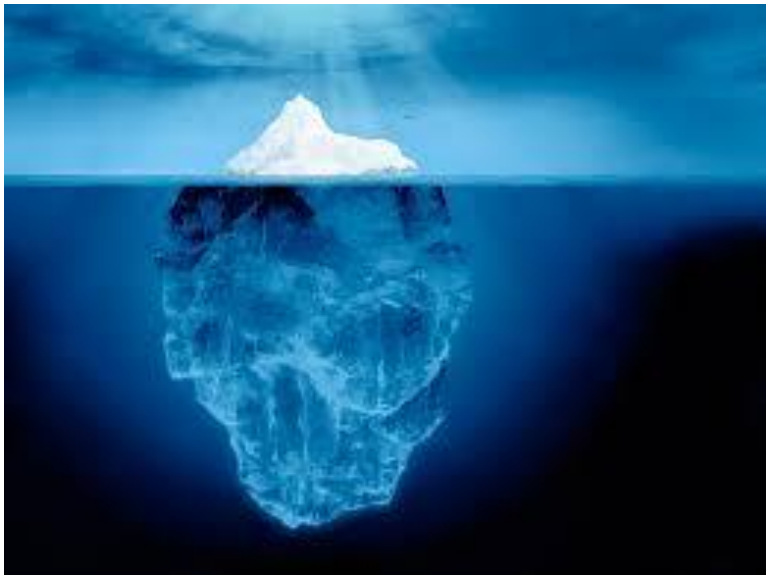
Campylobacterirosis

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.

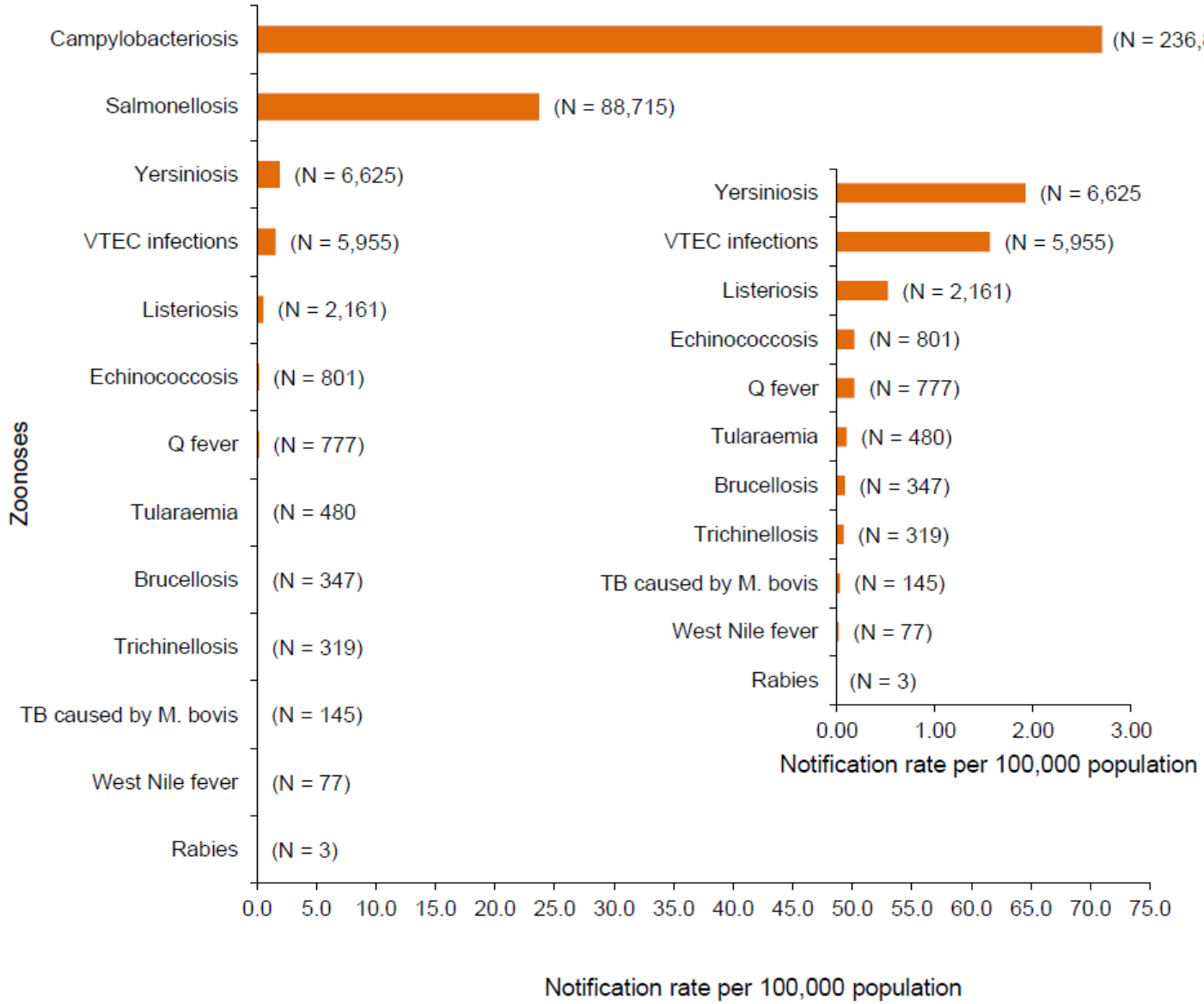
In the EU/EEA MS, the annual rate of exposure to *Campylobacter spp.* is estimated to be around 0.83 per person-year, translating in more than 420 million yearly infections. The vast majority of exposed cases do not develop the clinical disease and remain asymptomatic. Based on community studies, the 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





Campylobacterirosis EU, 2014



EFSA Journal 2015;13(12):4329, 191 pp. doi:10.2903/j.efsa.2015.4329

Campylobacterirosis EU, 2014

Country	2014					2013		2012		2011		2010	
	National coverage[a]	Data format[a]	Total cases	Confirmed cases & rates		Confirmed cases &rates		Confirmed cases & rates		Confirmed cases & rates		Confirmed cases &rates	
				Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
France[c]	N	C	5,958	5,958	45.2	5,198	39.6	5,079	38.9	5,538	42.6	4,324	33.5
Germany	Y	C	70,972	70,530	87.3	63,271	77.3	62,504	76.5	70,812	86.8	65,108	79.6
Greece[d]	–	–	–	–	–	–	–	–	–	–	–	–	–
Hungary	Y	C	8,490	8,444	85.5	7,247	73.5	6,367	64.4	6,121	62.4	7,180	72.9
Ireland	Y	C	2,595	2,593	56.3	2,288	49.8	2,391	52.2	2,433	53.2	1,660	36.5
Italy[b]	N	C	1,252	1,252	–	1,178	–	774	–	468	–	457	–
Latvia	Y	C	38	37	1.8	9	0.4	8	0.4	7	0.3	1	0.0
Lithuania	Y	C	1,184	1,184	40.2	1,139	38.3	917	30.5	1,124	36.8	1,095	34.9
Luxembourg	Y	C	873	873	158.8	675	125.7	581	110.7	704	137.5	600	119.5
Malta	Y	C	288	288	67.7	246	58.4	220	52.7	220	53.0	204	49.3
Netherlands[e]	N	C	4,159	4,159	47.5	3,702	42.4	4,248	48.8	4,408	50.9	4,322	50.1
Poland	Y	C	652	650	1.7	552	1.4	431	1.1	354	0.9	367	1.0
Portugal[d]	–	–	–	–	–	–	–	–	–	–	–	–	–
Romania	Y	C	256	256	1.3	218	1.1	92	0.5	149	0.7	175	0.9
Slovakia	Y	C	6,867	6,744	124.5	5,845	108.0	5,704	105.5	4,565	84.7	4,476	83.0
Slovenia	Y	C	1,184	1,184	57.4	1,027	49.9	983	47.8	998	48.7	1,022	49.9
Spain[f]	N	C	11,481	11,481	82.3	7,064	50.4	5,548	47.4	5,469	46.9	6,340	54.6
Sweden	Y	C	8,288	8,288	85.9	8,114	84.9	7,901	83.3	8,214	87.2	8,001	85.7
United Kingdom	Y	C	66,790	66,790	103.9	66,465	104.0	72,560	114.3	72,150	114.5	70,298	112.5
EU Total	–	–	237,642	236,851	71.0	214,784	64.8	214,316	65.9	223,998	69.0	215,395	67.0
Iceland	Y	C	142	142	43.6	101	31.4	60	18.8	123	38.6	55	17.3
Norway	Y	C	3,386	3,386	66.3	3,291	65.2	2,933	58.8	3,005	61.1	2,682	55.2
Switzerland[g]	Y	C	7,565	7,565	92.9	7,481	93.1	8,432	106.0	7,963	101.2	6,611	84.9

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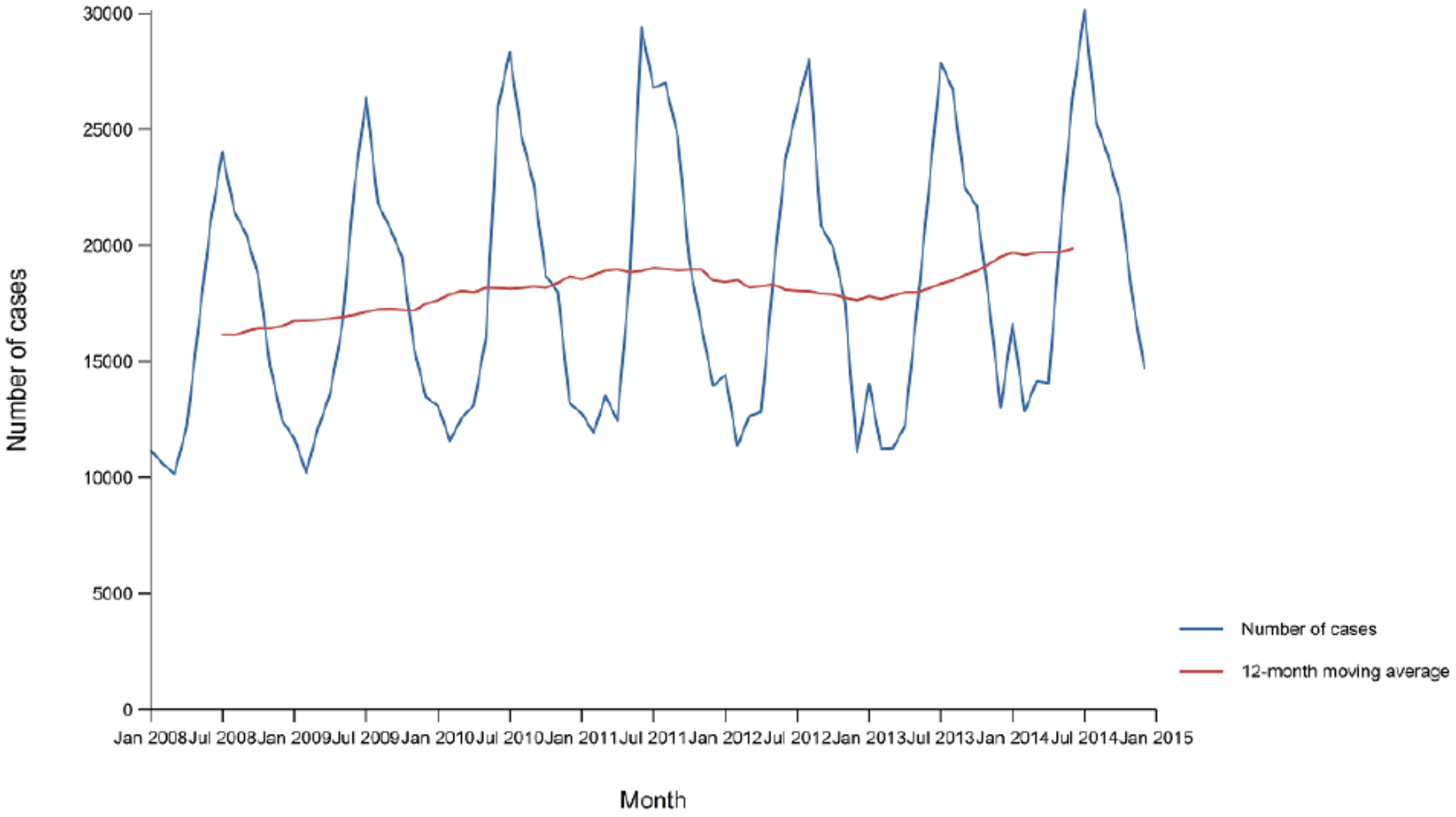
Campylobacterirosis EU, 2014



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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***

Regulation proposal

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

Year	Maximum Unsatisfactory, %	Criteria	c/n
2017	20	1,000 CFU/g	10/50
2018-2019	14	1,000 CFU/g	7/50
>2020	10	1,000 CFU/g	5/50

Corrective actions

…Improvements in slaughter hygiene, review of process controls, origin of animals and of the biosecurity measures in the farms of origin…

Key messages

- Campylobacteriosis: most important EU zoonosis
- Poultry meat: main source
- High level of contamination both in batches and carcasses
- Interventions at primary production is required
- No practical real tools are available to producers
- We still do not know about epidemiology
  - Literature full of inconsistent data
- Both consumers and EU authorities will ask producers to reduce Campylobacter in broilers
- CAMPYBRO will develop strategies to do so:
  - Short-medium term: through nutrition
  - Long term: through vaccination

Consortium


Pro Pollo
CIDEF
NEPLUVI
IMASDE
AGROALIMENTARIA
CAMPY BRO
FIA
Fédération
des Industries Avicoles
BTT
anses
French agency for food, environmental
and occupational health & safety
MIKROLAB és SPF Kft.
Redondo
CZV
CZ VETERINARIA

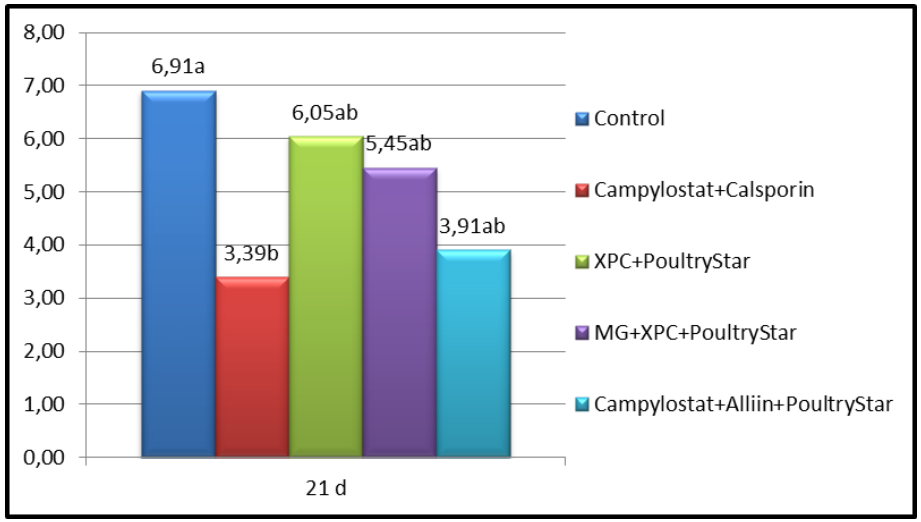
Work Packages

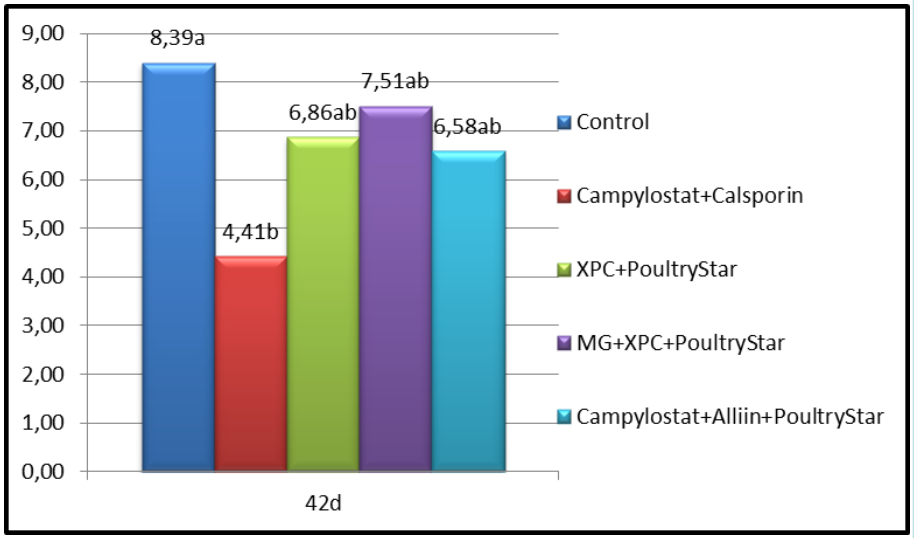
CAMPYBRO	WP	Year 1												Year 2												Year 3											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
WP 1. Efficacy of several compounds against Campylobacter in broilers orally infected looking for synergies	WP1																																				
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					

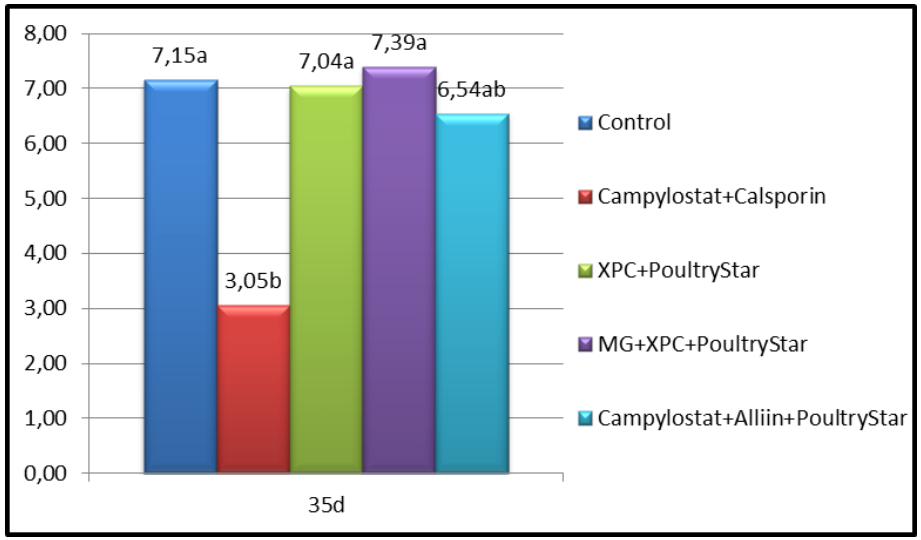
Experimental design

Treat.	Composition	Composition	Supplier	Dosage
T1	CONTROL			--
T2	T1 + Campylostat (2.5%) + Calsporin (100ppm)	Organic acids+Monoglycerides[a] Probiotic. (*B. subtilis*)	3F Technology, S.L. ORFFA	2.5%(wt/wt) 100ppm
T3	T1 + XPC (0.125%) + Poultrystar (0.1%)	Prebiotic (yeast product) Probiotic (Multi-species)	Diamond V Biomin	0.125% (wt/wt) 0.1% (wt/wt)
T4	T1 + Monoglycerides (0.8%) + XPC (0.125%) + Poultrystar (0.1%)	Monoglycerides[b] Prebiotic (yeast product) Probiotic (Multi-species)	Silo s.p.a. Diamond V Biomin	0.8% (wt/wt) 0.125% (wt/wt) 0.1% (wt/wt)
T5	T1 + Campylostat (2.5%) + Excential Alliin Plus (0.1%) + Poultrystar (0.1%)	Organic acids+Monoglycerides[a] Plant extract Probiotic (Multi-species)	3F Technology, S.L. ORFFA Biomin	2.5%(wt/wt) 0.1% (wt/wt) 0.1% (wt/wt)

Results of combination of additives

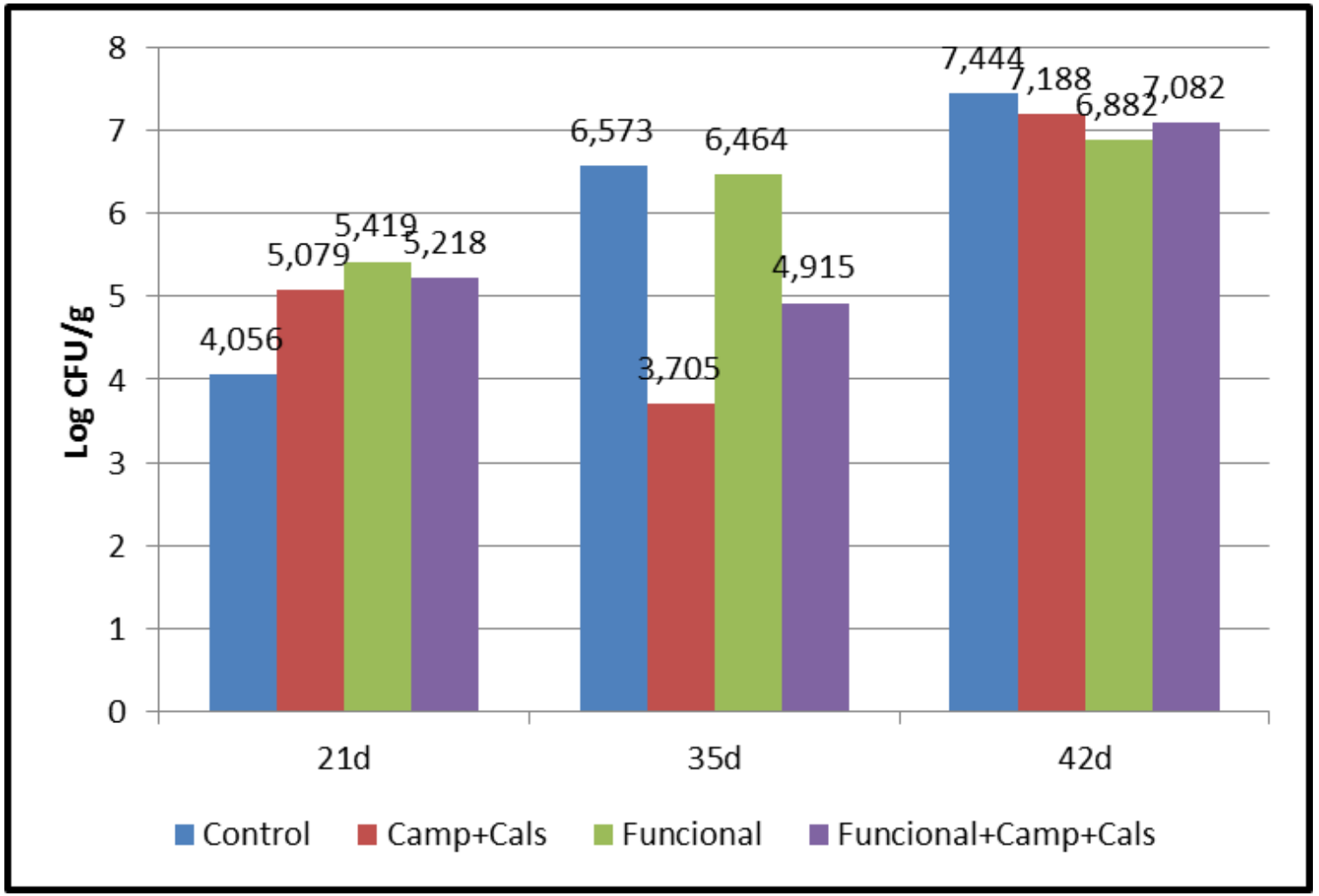






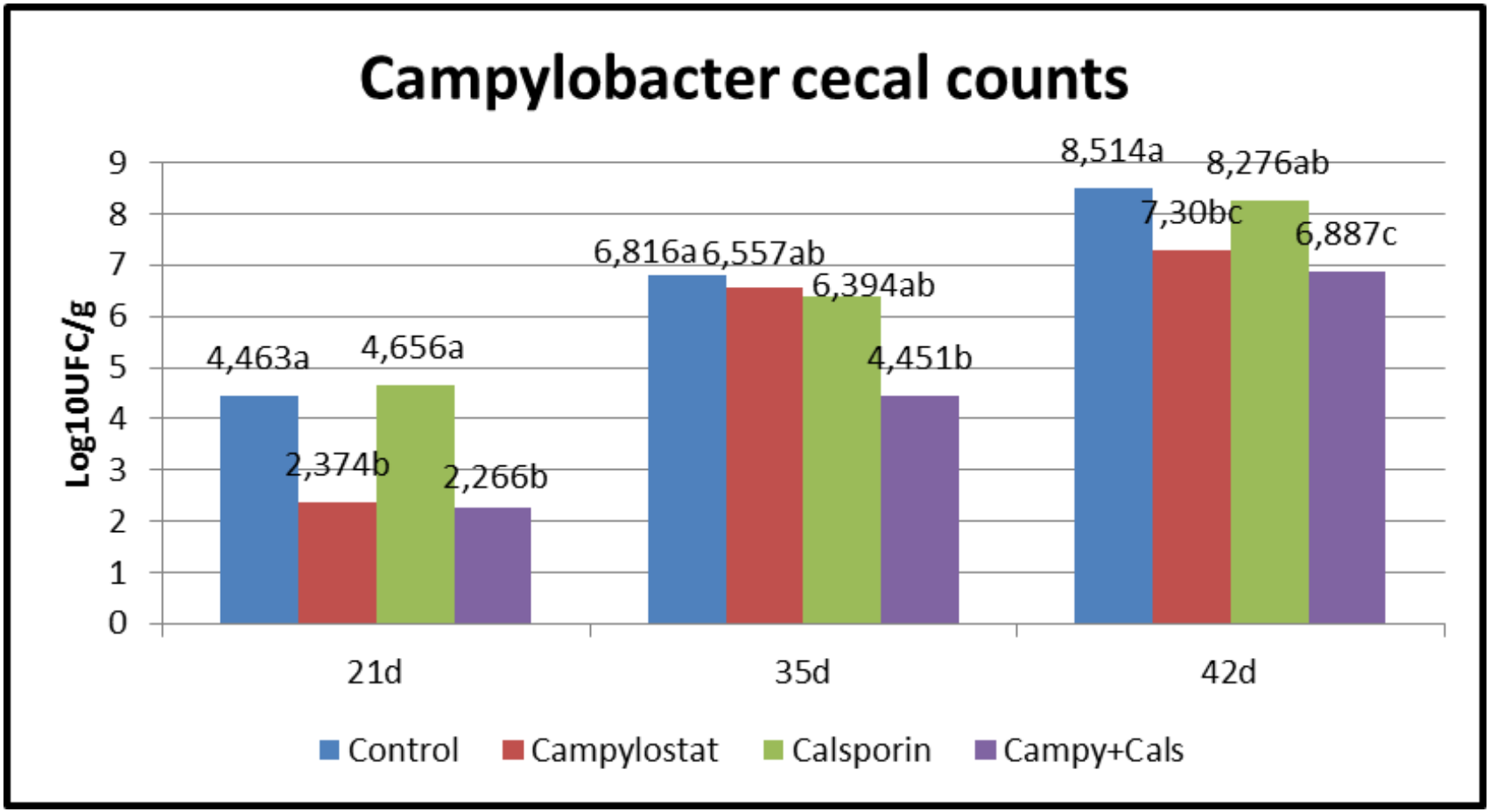
EXP.11. Interaction Functional diet x (Camp+Cals)

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



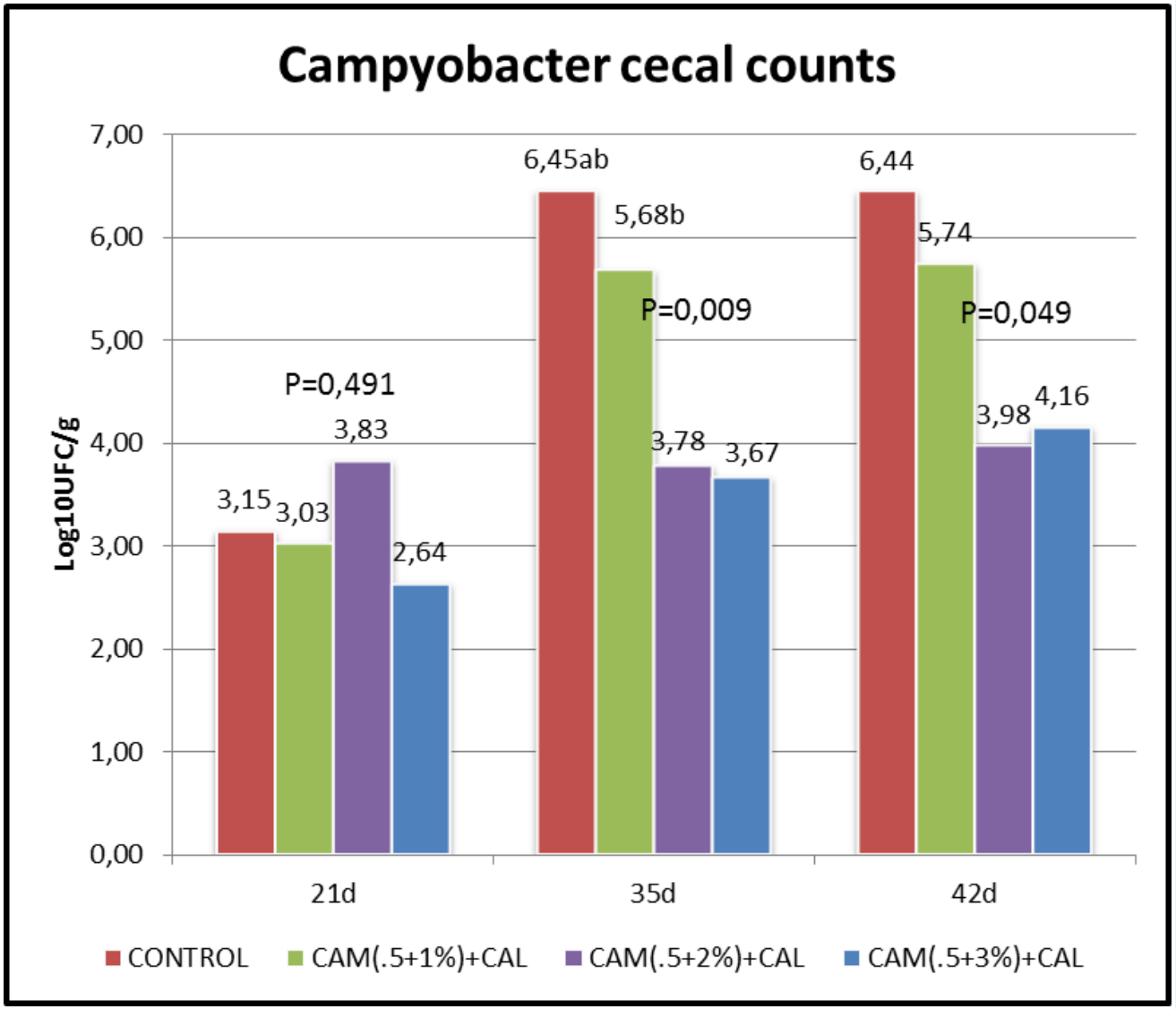
EXP.13. Interaction Camp x Cals in a control diet

- ✓ **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



WP4: field trials

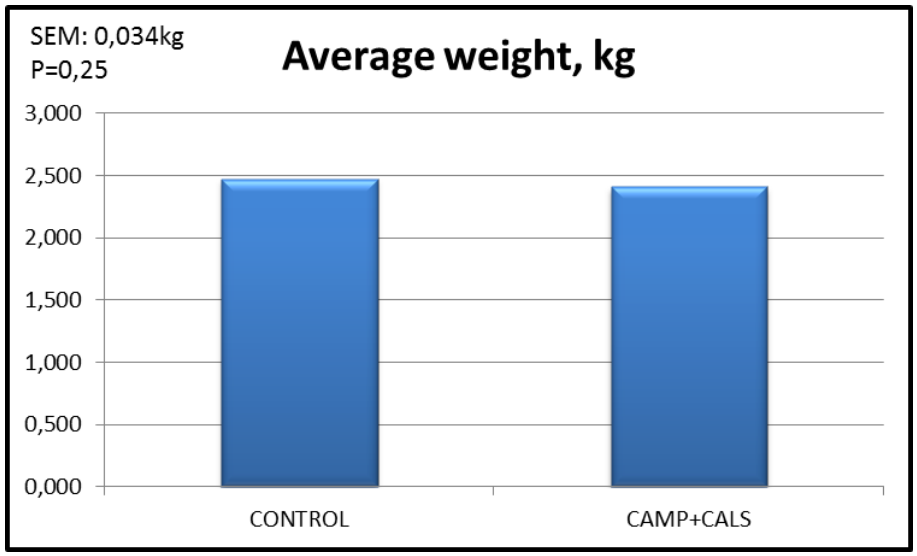
- Objective
  - Evaluate the efficacy of the combination of products in field conditions
    - Farm conditions (density, environmental, microbiota pressure)
    - In floor pens rather than cages
    - Barn vs barn, twin buildings
- Questions:
  - The efficacy is the same with field strains of C. jejuny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

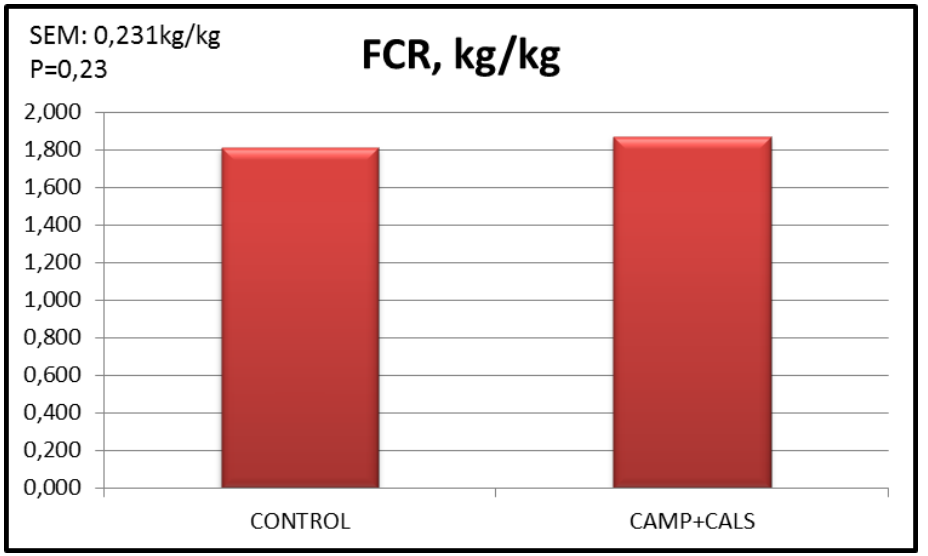
WP4: field trials

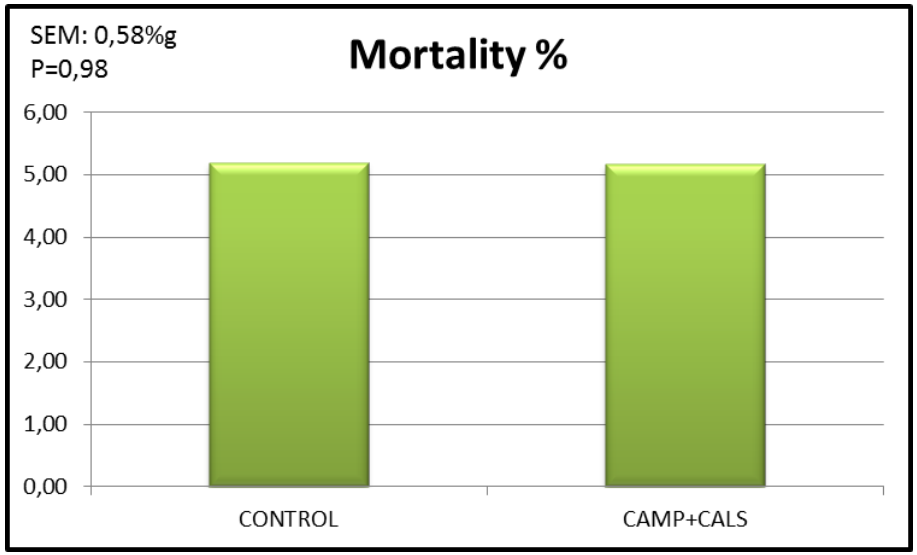
- Trial at Her-Csi
  - Farm verified about its *C. jejuni* contamination
  - 6 barns, twin two-by-two
  - Typical Hungarian diets (starter, grower I and II, finisher). Agrifirm.
  - Cobb chicks, 1-42d
  - Sampling
    - 5 chicks per barn at random (15 chicks per treatment and age)
    - 36 and 42d, prior to slaughter
  - Analysis
    - MIKROLAB
    - qPCR

WP4: field trials

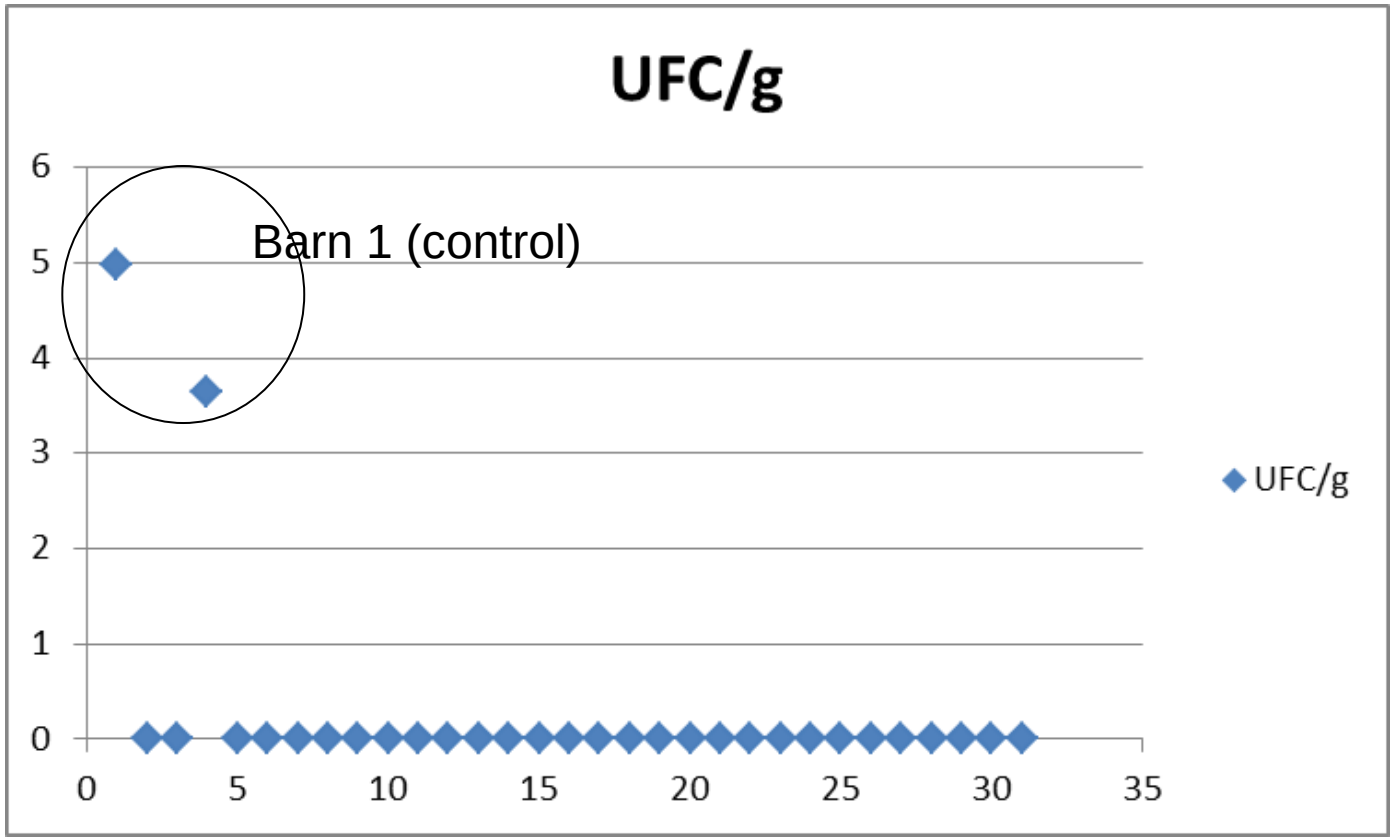
WP4: field trials



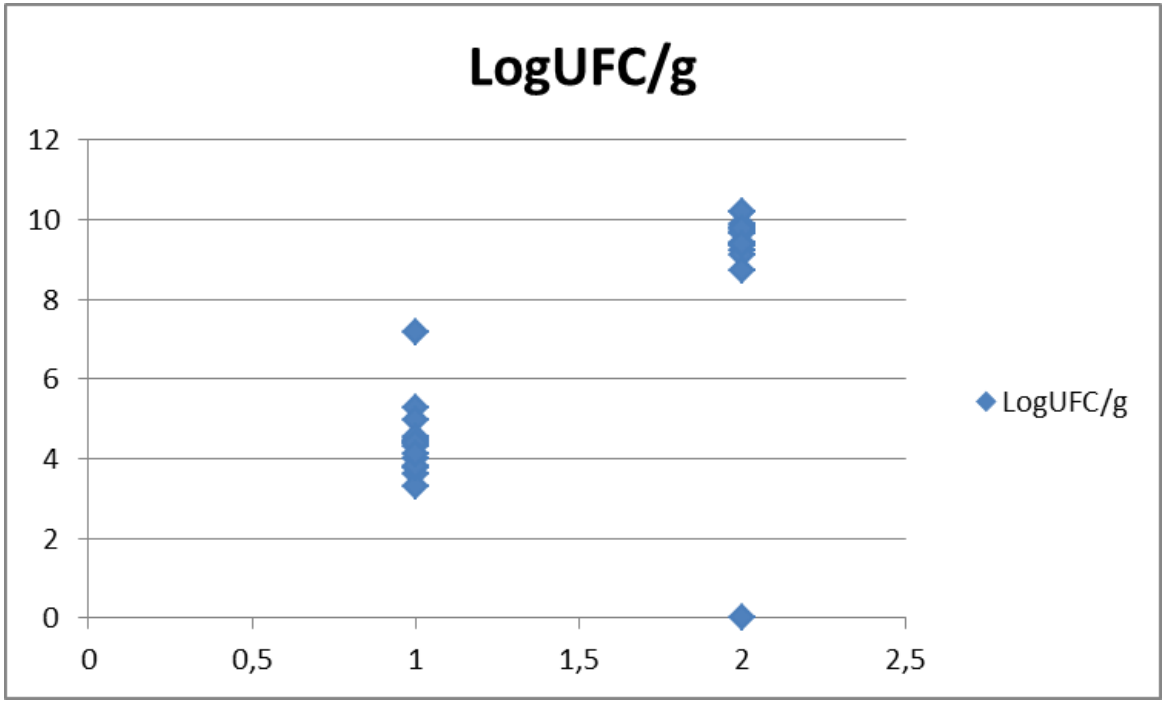




Field trial. 35d



Field trial. 42d



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

WP4: field trials

- Results interpretation
  - Clear effect of diet. No doubt.
  - No explanation
    - Protective effect of control diet?
      - Improbable
    - Mistake in manufacture of diets, distribution of diets in the farm, sampling?
      - Improbable. Checked
  - These results should be confirmed

WP6: Demonstration activity

- Open to Hungarian candidates
  - Trials in typical farms, as small as possible (8-10k)
  - If possible in twin buildings
  - The company should
    - Make the experimental feeds
    - Take the samples
      - 5 cecal samples/barn. Frozen.
        - Either in farm or slaughterhouse
- Interest
  - First approximation to the contamination
  - Free analysis of *Campylobacter*
- Cost of products: it will depend on the number of farms, partially covered by the project.

WP6: Demonstration activity

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Thanks for listen and interest to participate into the last part of the project!