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Medium chain fatty acid feed supplementation reduces the probability of *Campylobacter jejuni* colonization in broilers

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Abstract

Campylobacteriosis in humans is associated with handling and consumption of contaminated broiler meat. Reduction of the number of *Campylobacter*-colonized broiler flocks could potentially be realized by decreasing their susceptibility for colonization. The aim of this study was to determine the effect of feed supplementation with a mixture of medium chain fatty acids (C_8-C_{12}) on susceptibility of broilers for *Campylobacter* colonization, feed conversion and body weight gain. Two experiments were carried out with individually housed commercial broilers. The birds were fed with medium chain fatty acids supplemented feed (n=227), or received feed without supplement (n=87). The birds were inoculated with a dose of *Campylobacter jejuni* varying between $\log_{10}$ 1.19 - 5.47 CFU. During 14 days after inoculation, cecal or fecal samples were collected, in which the presence of *C. jejuni* was determined by bacterial culture. Beta-binomial dose response modeling of the colonization status at 14 days post-inoculation was performed to estimate the *C. jejuni* dose necessary to colonize 50% of inoculated broilers, which was estimated to be 200 times higher in broilers fed with supplemented feed ($\log_{10}$ 4.8 CFU) than in control broilers ($\log_{10}$ 2.5 CFU). Feed conversion was not affected by feed supplementation, while body weight gain was 49 g higher in broilers fed with supplemented feed. These findings indicate that susceptibility of broilers for *Campylobacter* colonization is decreased by supplementation with medium chain fatty acids, and that feed supplemented with this mixture may be a promising tool for the reduction of *Campylobacter* colonization in commercial broiler flocks.

Keywords: *Campylobacter*, broiler, feed supplementation, fatty acid, dose response

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Introduction

Meat products from broilers colonized with *Campylobacter jejuni* are considered an important source of human campylobacteriosis (Friedman et al., 2004), and reduction of human exposure is an important goal of public health programs (EFSA, 2005). One way to achieve this is to prevent intestinal colonization of broilers with *Campylobacter* spp., either by prevention of exposure or by reducing the susceptibility for colonization (Wagenaar et al., 2006). Because highly effective biosecurity measures to prevent exposure might be difficult to implement, reduction of susceptibility of broilers for colonization, here defined as the probability of colonization upon exposure, may be an alternative way to reduce the number of colonized flocks.

One way to reduce the susceptibility is to change the micro-environment in the gastro-intestinal tract in such a way that ingested *Campylobacter* bacteria are inactivated or unable to reach the lower intestines. It has been shown that short and medium chain fatty acids (SCFA and MCFA, respectively) have *in vitro* anti-*Campylobacter* activity that is additional to the *Campylobacter* inactivating effect of an acidified micro-environment (Chaveerach et al., 2002; Thormar et al., 2006). *In vivo*, feed supplemented with high level SCFA results in a reduced chance of colonization in broilers, but also in reduced body weight gain (BWG), which is economically undesirable (Heres et al., 2004). Lower fatty acid concentrations might be necessary to prevent BWG loss, preferably combined with improved effectivity at higher pH, to extend the anti-*Campylobacter* activity throughout a larger part of the gastro-intestinal tract.

Whereas SCFA activity ceases at pH $\geq$ 5.5 (Chaveerach et al., 2002), *in vitro* studies showed that 1-Monoglycerid of Capric acid, a MCFA, has anti-*Campylobacter* activity in feed mixed with a buffer at pH 5.5 and in feed mixed with tap water at pH 7.0 (Thormar et al., 2006). Consequently, MCFA might inactivate *Campylobacter* cells in the crop (pH 4.5) and the intestines, where the pH is approximately 5.8-6.0 (Chang and Chen, 2000; Farner, 1942). A low concentration mixture of MCFA could therefore be a promising tool to decrease the susceptibility without negative effects on weight gain or feed conversion.

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The aim of this study was to determine whether an acidified feed containing 1% MCFA was able to reduce the susceptibility of broilers for colonization with *Campylobacter. jejuni*. Additionally, the effects of supplemented feed on BWG and feed conversion rate (FCR) were determined.

Material and methods

Experimental Design

Two experiments were carried out subsequently. For each experiment hatching eggs, originating from one commercial broiler breeder flock (Ross 308) were purchased at 17 days of incubation. Chicks were hatched at the experimental facilities of the Faculty of Veterinary Medicine of Utrecht University. After hatching broilers were randomly assigned to control feed (CF) or supplemented feed (SF), which they received throughout the experiments. CF was an antibiotic and anti-coccidia drug free, mashed diet. SF was the same diet, with 1% soybean-oil substituted by 1% Lodestar™ C8-10 (Loders Croklaan, Wormerveer, the Netherlands), which is produced by fractional distillation of palm kernel oil free fatty acids, and typically consists of 56% C_{10}, 30% C_8, 10% C_{12}, <3% C_6, and <3% other lipids. The composition and calculated chemical analyses of both diets is shown in table 1.

In experiment 1 (exp. 1), a group of 150 day-old chicks was provided CF, based on random selection of the birds, and a group of 47 chicks was provided SF. In experiment 2 (exp. 2), chicks were placed in multiple groups: 192 day-old chicks were randomly divided into 32 groups of 6 chicks each, 22 groups receiving CF (132 chicks) and 10 groups SF (60 chicks). Birds were housed in a well-controlled facility, on litter floors. Groups were separated by walls. Water and feed was available *ad libitum*, and from 7 days of age a daily dark period of 6 hours was applied.

At 14 days of age, by random selection 114 of the initially 150 CF and 42 of 47 SF broilers (exp. 1), and 113 of 132 CF and 43 of 60 SF broilers (exp. 2) were weighed and housed individually in wired cages of 40x40 cm, with closed, littered floors, which were situated in four identical compartments. In exp. 1, broilers in both treatment groups were randomly divided over the cages in all compartments. In exp. 2, broilers per treatment group were evenly divided over the compartments, and

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within each compartment, broilers were randomly assigned to cages. The sides and back of each cage were covered with plastic sheets. The distance between the cages was 40 cm at least.

Inoculation

One dose of 10^9 CFU of *C. jejuni* C356, originating from a broiler flock (Jacobs-Reitsma et al., 1995) and stored in glycerol at –80°C, was orally administered to 3 five-day-old broilers, which were housed with 3 non-inoculated contact broilers. Three days post-inoculation (PI) *C. jejuni* was isolated from the ceca of one contact broiler. This chicken-passaged strain, here referred to as *C. jejuni* C356P (C356P), was stored in glycerol at –80°C and used in experiments 1 and 2.

Before inoculation, C356P was freshly cultured in Heart Infusion Broth (micro-aerobically, 37°C, overnight) and diluted in saline to obtain the intended inoculation doses. Based on power calculations, using results from a pilot study (data not shown), and presuming an additive effect on the inoculation dose required to colonized 50% of the birds (CD_{50}) of approx. $\log_{10}$ 1.5 CFU, SF broilers were inoculated with higher doses C356P than CF broilers (table 2). In each compartment, non-inoculated broilers were housed in 4 randomly selected cages (sentinel) to detect *Campylobacter* transmission. Broilers, except sentinels, were orally inoculated with 0.25 ml of the C356P inoculation suspensions at 14 days of age (exp. 1; n=140) or 18 days of age (exp. 2; n=139; one broiler died one day prior to inoculation). The inoculation doses were randomly divided over the compartments. To limit variation between individuals, all broilers were feed-deprived for 11 hours prior to inoculation, and provided feed directly afterwards. The concentration of *Campylobacter* in the administered inocula was determined by plating on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Biotrading Benelux B.V., Mijdrecht, the Netherlands).

Sampling and Testing

Six (exp. 1) or seven days (exp. 2) prior to inoculation, broilers were tested for the presence of *Campylobacter* by culture of a fecal dropping on mCCDA. All broilers tested negative. After inoculation, birds were sampled at 4, 8, and 11 days post inoculation (PI) by swabbing fresh cecal

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droppings if present, or otherwise a swab of a fresh fecal dropping. If neither could be obtained, a swab from cloacal content was taken. In addition, at 1, 2, 3, 7, and 9 days PI 1 sentinel per compartment and 1 to 5 inoculated broilers per inoculation dose were placed in cardboard boxes with wire floors for 4 hours, to collect cloacal swabs and fresh cecal droppings (or fecal droppings if not present). Different material was sampled with the intention to compare the sensitivity of each of these sampling methods. Because positivity was limited to one of two sample types only incidentally, sensitivity of different sample mediums was considered equal. At the end of the trial (14 days PI), cecal contents were sampled after euthanasia by cervical dislocation.

The person sampling the broilers was blinded to dose groups and could not distinguish sentinels from inoculated broilers. Long-sleeved plastic gloves were changed for each broiler to avoid cross-contamination. Samples were collected with sterile swabs and transported to the laboratory in modified Amies transport medium without charcoal (Biotrading, Mijdrecht, the Netherlands) within 6 hours. Samples were streaked on mCCDA plates and incubated micro-aerobically at 42°C, and examined for the presence of *Campylobacter*-suspect colonies after 24 and 48 hours. Microscopic examination of morphology and motility was used as confirmation. Broilers were considered *Campylobacter*-colonized and were excluded from further sampling when at least one sample tested positive. Ethical aspects of the experiments were judged and approved by the animal ethical committee of Utrecht University.

To assess the effect of MCFA feed supplementation on technical performance, BWG and feed intake during the period of individual housing (>14 days of age) were recorded in exp. 2.

Statistical Analyses

The effect of MCFA feed supplementation on susceptibility was assessed by fitting a Beta-binomial dose response model (Teunis and Havelaar, 2000). According to this model, inoculation with dose D results in a probability of colonization $\Pr_{inoc}(D)$:

$$\Pr_{inoc}\ D = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

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The underlying assumption of the model is that each bacterium can independently establish colonization, but that hosts may differ in their susceptibility (Teunis and Havelaar, 2000). The two parameters (instead of single probabilities for each dose) provide opportunities to assess the dose response relation over a wide range of exposure doses, and to compare treatments tested with different ranges of inoculation doses. Furthermore, the dose-response model could be used to calculate a CD_{50}: the colonization dose resulting in a 50% probability of infection.

Because sentinels were detected positive after day 4 PI in exp. 2 (table 2), colonization as a result of transmission could not be excluded for the inoculated birds either. Therefore, in the main analysis (using data up to day 14 PI) we corrected for transmission by estimating a transmission probability Pr_{tr}, different for each room and treatment group. Consequently $Pr_{+}(D)$, the probability of being colonized, was equal to

$$\Pr_{+}\ D = 1 - \ 1 - \Pr_{tr}\ \ 1 - \Pr_{inoc}\ D$$

Additionally, a more simple analysis was performed using the colonization status up to day 4 PI, without the correction for transmission. In the day-4 analysis, $Pr_{+}(D)$ was equal to $Pr_{inoc}(D)$.

For both analyses (day-4 and day-14), four different models were fitted to see if there were group and treatment effects: the first with separate dose response relations for each of the 4 treatment groups (CF_1 vs. CF_2 vs. SF_1 vs. SF_2), the second with combined CF groups (CF_{12}) vs. combined SF groups (SF_{12}), the third with combined exp. 1 groups vs. exp. 2 groups, and the fourth with all groups combined. All parameters were estimated by maximum likelihood. The corrected Akaike Information Criterium (AIC_c) (Hurvich and Tsai, 1989) was used to decide which model explained the data best and whether different dose response relations should be adopted for (combined) treatment groups.

Prior to inoculation, some groups of broilers got diarrhea at 1 week of age. To study the possible confounding effect of diarrhea on susceptibility, data of exp. 2 were analyzed as described above, in 4 groups, with diarrhea status instead of experiment. AIC_c was used to assess if the occurrence of this symptom was associated with an increased or decreased susceptibility to *Campylobacter* colonization.

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Linear regression analyses (SPSS 15.0.1.) were performed to assess if feed treatment affected BWG and FCR in exp. 2. Sixty-nine broilers, which were detected *Campylobacter*-colonized before or at 4 days PI, were weighed and sexed at 28 days of age, while the remaining 84 broilers (19 colonized and 65 non colonized) were weighed and sexed at 32 days of age. To correct for this, age and final colonization status were included as dependent variables, next to the variables sex and feed treatment, resulting in the equations

$$BWG_i = \beta_0 + \beta_1 Feed_{i1} + \beta_2 Sex_{i2} + \beta_3 Age_{i3} + \beta_4 Col_{i4} + \varepsilon_i,$$

and,

$$FCR_j = \beta_0 + \beta_1 Feed_{j1} + \beta_2 Sex_{j2} + \beta_3 Age_{j3} + \beta_4 Col_{j4} + \varepsilon_j.$$

Results

Colonization

In exp. 1, 35 of 46 colonized birds, and in exp. 2, 72 of 89 colonized birds were detected *Campylobacter*-positive in the first 4 days PI.(table 2). In exp. 1, the sentinels remained negative, but in exp. 2, 4 of 16 sentinels were detected *Campylobacter*-positive, at days 7, 8, 8 and 14 days PI. Four birds in exp. 1 and one bird in exp. 2 died after day 4 PI (table 2).

Dose Response: Effect on Susceptibility

The best fitting day-14 model (lowest AIC_c) included separate dose response relation for CF_{12} and SF_{12}, indicating equal relations in both experiments. Dose response relations of CF_{12} and SF_{12} show parallel sigmoid shapes (figure 1). CD_{50} for CF_{12} was $\log_{10}$ 2.5 CFU (95% CI: 2.2-2.8) and CD_{50} for SF_{12} was $\log_{10}$ 4.8 CFU (95% CI: 4.4-5.2) (figure 1). The AIC_c of alternative models were at least 3.95 higher.

The best day-4 model included separate dose response relations for all four treatment groups (CF_1, CF_2, SF_1, and SF_2). CD_{50} for CF_1 and CF_2 were $\log_{10}$ 2.8 CFU (95% CI: 2.6-3.3) and 2.1 CFU

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(95% CI: 1.8-2.3), respectively. CD_{50} for SF_1 and SF_2 were log_{10} 8.5 CFU (95% CI: 4.8 - ∞) and 4.7 CFU (95% CI: 4.2-5.2), respectively. The AIC_c of alternative models were at least 12.2 higher.

In exp. 2, diarrhea occurred around 1 week of age, with similar frequencies in both treatment groups (14/22 CF groups and 5/10 SF groups; Fisher exact test: P=0.70), suggesting that diarrhea was not feed related. In a separate analysis of data of exp. 2 inclusion of a variable describing whether a broiler originated from a group with diarrhea did not result in a better fit, with AIC_c being approx. 4 points higher in both the day-4 and day-14 models.

Effect on technical performance

The broiler that died during the exp. 2 (table 2) and a broiler that was lame during the last few days of the experiment were excluded from this analysis. BWG was estimated 49 ± 24 g higher in SF broilers compared to CF broilers (p=0.044) when correcting for the effect of sex (p<0.001), age (p<0.001), and final colonization status (p=0.398). FCR was estimated 0.061 ± 0.034 lower in broilers with were provided supplemented feed (p=0.075) when correcting for sex (p=0.179), age (p=0.183), and final colonization status (p=0.735).

Discussion

In two experiments the effect of MCFA feed supplementation on susceptibility and technical performance of broilers was studied. The effect on susceptibility was assessed by determining the relation between the *C. jejuni* inoculation dose and the subsequent occurrence of colonization, resulting in dose response curves of SF broilers shifted to the right compared to CF broilers, indicating that SF broilers required a higher inoculation dose to become colonized than CF broilers. The effect of MCFA feed supplementation could also have been assessed by comparing the percentage of colonized broilers exposed to equal inoculation doses (log_{10} 2.19 CFU in exp. 1, and log_{10} 2.47 and 4.37 CFU in exp. 2) at 14 days PI (table 2). Although this would also have illustrated the susceptibility reducing effect of the treatment (Fisher exact test: all p<0.05), we would not have been able to predict the

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probability of colonization for other inoculation doses, nor would we have been able to correct for transmission.

We used the Beta-binomial dose response model (Teunis and Havelaar, 2000) to estimate dose response curves and CD_{50}s. Besides the fact that this analysis resulted in less parameters to be estimated than separate analyses for different doses, and in the possibility to use different doses for different treatments, it also turned out useful to correct for transmission. We used the colonization status at 14 days PI to estimate the dose response relations, but when the *Campylobacter* colonization status at 4 days PI was used in the alternative (day-4) model, similar estimates were obtained. This similarity suggests that most broilers colonized after 4 days PI were colonized by transmission. Based on the results in this study, challenge experiments with *Campylobacter* might not necessarily have to last longer than 4 days to estimate the dose response relation properly.

Analysis of technical performance showed that BWG was increased in SF broilers, while FCR was not affected. The effect on BWG might have been caused by antimicrobial effects of the fatty acids, as feed supplementation with Capric Acid and Lauric acid, two MCFAs, has been shown to decrease the concentration of *Clostridium perfringens* in jejunum and ileum of *C. perfringens* challenged broilers (Jansman et al., 2006). Although feed supplementation with antimicrobial agents has the potential to improve feed efficiency (Dibner and Richards, 2005; Jansman et al., 2006), in this study no significant effect on FCR was observed.

Conclusion

The number of *C. jejuni* bacteria required to colonize 50% of inoculated broilers was estimated 200 times higher in broilers fed with supplemented feed than in control broilers. Although the working mechanism of supplemented feed remains to be elucidated, this effect on susceptibility is a promising finding for the implementation of MCFA feed supplementation as an intervention for reduction of susceptibility in broilers. As the *Campylobacter* exposure dose that broilers experience in the field is

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unknown, field trials are necessary to determine to what extent MCFA supplementation reduces *Campylobacter* colonization in the field.

References

Chang, M.H., Chen, T.C., 2000. Reduction of *Campylobacter jejuni* in a simulated chicken digestive tract by *Lactobacilli* cultures. J. Food Prot. 63, 1594-1597.

Chaveerach, P., Keuzenkamp, D.A., Urlings, H.A., Lipman, L.J., van Knapen, F., 2002. In vitro study on the effect of organic acids on *Campylobacter jejuni*/*coli* populations in mixtures of water and feed. Poult. Sci. 81, 621-628.

Dibner, J.J., Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84, 634-643.

EFSA 2005. Opinion of the Scientific Panel on biological hazards (BIOHAZ) related to *Campylobacter* in animals and foodstuffs. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620776955.htm

Farner, D.S., 1942. The hydrogen ion concentration in avian digestive tracts. Poult. Sci. 21, 445-450.

Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson, B., Tauxe, R.V., 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis. 38, S285-S296.

Heres, L., Engel, B., Urlings, H.A., Wagenaar, J.A., van Knapen, F., 2004. Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by *Campylobacter* and *Salmonella*. Vet. Microbiol. 99, 259-267.

Hurvich, C.M., Tsai, C.L., 1989. Regression and Time-Series Model Selection in Small Samples. Biometrika 76, 297-307.

Jacobs-Reitsma, W.F., van de Giessen, A.W., Bolder, N.M., Mulder, R.W., 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114, 413-421.

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ACCEPTED MANUSCRIPT

Jansman, A.J.M., Wagenaars, C.M.F., Schonewille, A., Snel, H., Van der Klis, J.D. 2006. Bestrijding van *Clostridium* en *Campylobacter* infecties in pluimvee via natuurlijke antimicrobiële voedingscomponenten (Animal Sciences Group - Wageningen UR), p. 50.

Teunis, P.F.M., Havelaar, A.H., 2000. The Beta Poisson dose-response model is not a single-hit model. Risk Anal. 20, 513-520.

Thormar, H., Hilmarsson, H., Bergsson, G., 2006. Stable Concentrated Emulsions of the 1-Monoglyceride of Capric Acid (Monocaprin) with Microbicidal Activities against the Food-Borne Bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. Appl. Environ. Microbiol. 72, 522-526.

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Table 1. Ingredient composition and calculated analysis of the diets

Item	Amount (%)	
Ingredients	Control Feed	Supplemented Feed
Wheat	36.53	36.53
Soybean meal	21.20	21.20
Corn	20.00	20.00
Peas	15.00	15.00
Soybean oil	3.95	2.95
Calcium carbonate	1.44	1.44
MCFA[b]	0.00	1.00
Monocalcium phosphate	0.57	0.57
Lysine 65%	0.35	0.35
Sodium chloride	0.26	0.26
Methionine	0.25	0.25
Premix	0.25	0.25
Sodium bicarbonate	0.10	0.10
Threonine	0.04	0.04
Choline chloride	0.03	0.03
Endoxylanase	0.02	0.02
Phytase	0.01	0.01
Calculated analysis		
CP	19.60	19.60
ME_n (kcal/kg)[*]	2790	2790
C8[†]	0.00	0.30
C10[b]	0.00	0.56
C12[b]	0.00	0.10

[*] Energetic value of MCFA and soybean oil was assumed to be equal
[†] 1% Lodestar™ C8-10

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Table 2. Colonization status of broilers (exp. 1 and exp. 2).

Rows represent different feed treatment groups (CF = control feed; SF = supplemented feed) and *C. jejuni* C356P inoculation doses ($\log_{10}$ CFU/broiler). Nominators in cells express the number of broilers detected *Campylobacter*-positive, and denominators express the number of broilers sampled throughout 4 or 14 days post-inoculation (PI).

Exp. 1				Exp. 2			
treatment	Dose	Time (days PI)		treatment	Dose	Time (days PI)	
group	C356P	4	14	group	C356P	4	14
CF	-	0/16	0/16	CF	-	0/16	4/16
CF	1.19	0/33	1/32[a]	CF	1.47	8/32	13/32
	2.19	9/33	12/32[b]		2.47	21/33	25/33
	3.19	20/32	21/31[a]		3.47	30/32	31/32
SF	2.19	1/21	2/21	SF	2.47	0/10	2/10
	4.49	5/21	9/20[a]		3.47	2/11	2/11
					4.47	2/10	3/9 [a]
					5.47	9/11	9/11

[a] A broiler died after being tested negative at 4 dpi.

[b] A broiler died after being tested positive at 4 dpi.

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Figure 1. Dose response curves for the final model of the day-14 analysis

The relation between inoculation dose en the probability of colonization occurring as a result of inoculation, $Pr_{inoc}(D)$, up to 14 days PI, for combined experiments (1 and 2). Best fitted curves, with 95% confidence bounds, represent relations for control feed (CF_{12}) and MCFA supplemented feed (SF_{12}) fed broilers. Dots represent raw data as observed in the experiments, not corrected for the occurrence of transmission.

<< Figure 1.tif >>

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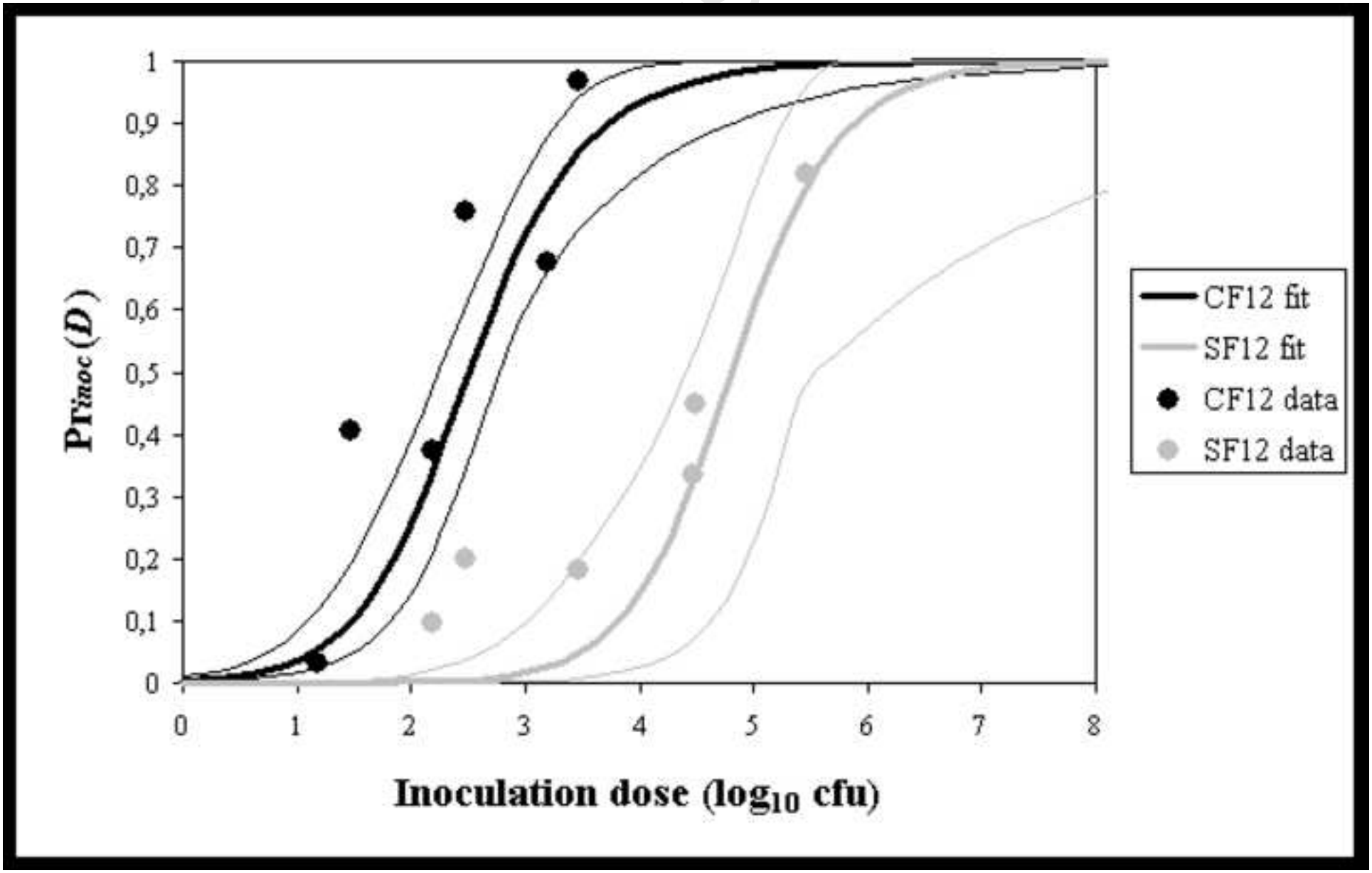
Figure


1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
$P_{Yinoc}(D)$
0
1
2
3
4
5
6
7
8
Inoculation dose (log_{10} cfu)
CF12 fit
SF12 fit
CF12 data
SF12 data