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Transmission of *Salmonella* between broiler chickens fed with fermented liquid feed

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SUMMARY

In the light of food safety and the control of *Salmonella* at chicken farms, fermented liquid feed (FLF) was studied. This moistened feed reduced the susceptibility of chickens for *Salmonella*. To assess the effect of the fermented feed on the transmission of *Salmonella* between chickens, a transmission experiment was performed. *Salmonella* shedding was followed within groups of two susceptible chickens together with two previously inoculated chickens. The between-chicken transmission was quantified by calculating a reproduction ratio (R_0) and a transmission rate parameter (β). R_0 and β in the FLF-treated groups were reduced, but a typical infectious chicken fed with FLF, could on average still infect more than one new infectious case. FLF can therefore reduce the transmission of *Salmonella* in chicken flocks, but it will not prevent the occurrence of major outbreaks.

INTRODUCTION

Human salmonellosis is associated with *Salmonella* in poultry. The distribution of different serotypes is unequal in humans and poultry, and the different serotypes in poultry are probably not equally pathogenic for humans [1]. Nevertheless, considering the relationship between humans and poultry, all phases in the poultry production chain, including the farm, should seriously control *Salmonella*. Two major epidemiological processes determine the *Salmonella* status of a flock at a certain moment. The first process is the introduction of the pathogen into the flock. This introduction can occur either vertically or horizontally. At vertical introduction, the infection enters the flock via the hatchery. One or more eggs, originating from infected breeders, are contaminated and cause some chickens to be infected at hatch. The other way of introduction is horizontally with rodents, birds, or from the environment via footwear, etc. In an abstract way, introduction into a flock can be seen as transmission between flocks, i.e. the *Salmonella* infection originates from another flock in another pen or from a previous flock in the same pen.

The second process that determines the prevalence in a flock is the extent of transmission from infected chickens to susceptible flock-mates. The most important route of transmission of *Salmonella* to another animal is the faecal–oral route, where faeces or faecally contaminated substances are pecked from the environment. The susceptibility of an individual broiler and the amount of infectious agent at exposure are important for a successful infection. Thus, the transmission in a flock depends on the susceptibility of the chickens and the infection pressure. The infection pressure results from the structure of contact between infected and susceptible chickens, and also from the amount of *Salmonella* shed and the subsequent survival or multiplication of *Salmonella* in the environment (e.g. litter). The contact structure is determined by the number of shedding chickens per

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number of susceptible chickens, and by animal behaviour like pecking and mixing.

Fermented liquid feed (FLF) is studied for its role in the control of *Salmonella* at farm level. Broiler chickens fed with FLF were less susceptible for an infection with *Salmonella* [2]. Fermented feed has a low pH, a high concentration of lactic and acetic acid, and a high number of lactobacilli. These characteristics of fermented feed may be explanatory for the beneficial characteristics of this feed. In pig husbandry it was shown that feeding fermented products was correlated with a lower prevalence of *Salmonella* [3, 4], which illustrates the possible protective effect of FLF. Concurrent with the reduced probability for *Salmonella* to colonize in chickens that are fed FLF compared to chickens fed dry feed (DF) [2], transmission between chickens may be reduced, because FLF-fed flockmates will not be as easily infected with the faeces or contaminated litter particles they consume.

A transmission experiment was conducted to quantify the effect of fermented feed on the transmission of *S. enteritidis* (SE) between chickens. The reproduction ratio (R_0; the average number of secondary cases caused by one typical infectious case) and the transmission rate parameter (β; see Table 2) were estimated for a quantitative evaluation of the differences in transmission between DF-fed and FLF-fed chickens. The relevance of observed differences in susceptibility and transmission was substantiated by making inferences on the size of an outbreak and on the course of infection within a flock.

MATERIALS AND METHODS

To study the transmission of *Salmonella* between chickens two contact chickens were placed in a pen with two inoculated chickens. Transmission of the infection was followed in both type of chickens by measuring the faecal shedding of *Salmonella*. Eighteen repetitions per treatment group were used. To prevent the contact with artificially high amounts of *Salmonella*, which may be shed after an experimental infection, the inoculation dose was chosen at a level that was expected to cause a moderate infection. Approximately 80 % of the chickens should become infected. The chickens from the DF group were inoculated with 10^3 c.f.u. SE. The inoculated FLF chickens were inoculated with 10^7 c.f.u. SE (FLF7). These inoculation doses were deduced from our previous experiments [2]. In addition to the moderate inoculation level there was a waiting period of 3 days before contact chickens were placed with the inoculated chickens. This measure should avoid contact with artificially high shedding levels during the first days after inoculation. Another 18 pairs of FLF-fed chickens were inoculated with 10^3 SE (FLF3) to estimate the difference in transmission between FLF and DF groups inoculated with the same dose. Beforehand it was known that a limited proportion of the FLF-fed chickens inoculated with 10^3 SE would shed SE. Cloacal swabs were taken daily after inoculation to determine *Salmonella* shedding. The inoculated chickens were swabbed until at least three consecutive swabs were *Salmonella* positive or until the end of the experiment at day 13 or day 14 post inoculation (p.i.). Cloacal swabs of the 128 contact birds were also taken until at least three consecutive swabs were positive for SE or until the end of the experiment.

Animal experiment

The experimental layout is shown in Table 1. One-day-old broiler chickens (Ross type) were obtained from a parent flock with a *Salmonella*-free history. Down and paper pads from the hatching cabin and paper pads from the transport boxes were examined for the presence of *Salmonella*. All samples were *Salmonella* negative. Fresh faecal samples were gathered from the litter before the chickens were experimentally infected and before the susceptible animals were placed in the pen with an infected animal. These samples were examined for the presence of *Salmonella*. It was concluded that the chickens were reared free of *Salmonella*, because no *Salmonella* was detected in these samples.

At day 1, 256 broilers were randomly divided into two groups on litter. One group ($n = 144$) was fed FLF the other group ($n = 112$) a DF. At day 8, 72 FLF-fed chickens and 36 DF-fed chickens were inoculated orally. The inoculated chickens were housed in pairs in pens. Two susceptible chickens of the same feed group were placed in each pen with the two previously inoculated chickens at day 3 p.i.

In each compartment five pens housed sentinels to assess whether unwanted transmission between pens occurred. These 40 chickens were not inoculated and were fed with DF. Apart from omitting inoculation, all other treatments were the same for these negative controls.

Chickens were inoculated by giving them a 0·25 ml inoculum with a curved and blunted needle at the

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Table 1. *Experimental layout*

Day of experiment	Actions
Day 1	144 FLF-fed broilers and 112 DF-fed broilers in two rearing compartments
Day 8	Inoculation of 36 pairs of FLF-fed chickens (10^3 and 10^7 c.f.u.) Inoculation of 18 pairs of DF-fed chickens (10^3 c.f.u.) 10 pairs of DF-fed chickens as sentinels (not inoculated) All these pairs housed in pens in two compartments 72 FLF and 56 DF chickens remain in the rearing compartment
Days 8–11	Cloacal swabs of inoculated and sentinel chickens
Day 11 = day 3 p.i.	Pairs of susceptible chickens are placed in pens with the inoculated or sentinel chickens
Days after contact	Daily cloacal swab of all chickens
	Days 16, 18 = days 8, 10 p.i. – culling of chickens in some pens (all four chickens *Salmonella* positive or all chickens *Salmonella* negative). Caeca are sampled for quantitative and qualitative *Salmonella* detection
Days 21, 22 = days 13, 14 p.i.	Culling of chickens in all remaining pens, one compartment per day. Caeca are sampled for quantitative and qualitative *Salmonella* detection

pharyngeal side of the tongue. Care was taken that the chicken swallowed the entire inoculum. A *Salmonella enterica* serovar *enteritidis* PT4(SE), originally isolated from poultry meat, was used for inoculation. This was a naladixic acid-resistant strain to facilitate the detection with selective culture media. Before and after inoculation the concentration in the inoculation suspension was estimated by plating after serial dilution. The number of *Salmonella* in the inoculation dose was $2{\cdot}3 \times 10^3$ c.f.u./ml (DF and FLF3) and $4{\cdot}1 \times 10^7$ c.f.u./ml (FLF7).

During the experiment chickens were culled from pens where the four chickens had all shed *Salmonella*. This was done to prevent transmission between pens. Therefore, chickens were killed at day 8 p.i. (3 DF pens and 1 FLF7 pen) and at day 10 p.i. (7 DF and 4 FLF7 pens). In addition chickens were culled from three pens at day 8 p.i. and five other pens at day 10 p.i., both with FLF3 chickens. These pens housed four chickens with no detectable *Salmonella* shedding. Inoculation had apparently not been successful. Caecal culture confirmed that they were not colonized by *Salmonella*. In the analyses of the results it was assumed that the chickens that were culled during the experiment would have kept their positive or negative status until the end of the experiment.

At day 13 p.i. chickens were culled from the 23 remaining pens from the first compartment and at day 14 p.i. from the last 18 pens from the other compartment.

The caeca of all chickens were isolated aseptically after necropsy. One gram of caecal content from chickens that were shedding *Salmonella* was weighted in 9 ml buffered peptone water (BPW; Oxoid CM509). Serial dilutions were made and were plated for counting of *Salmonella*. The caecal content from animals that only had *Salmonella*-negative cloacal swabs was only cultured to detect the presence of *Salmonella*.

Feed and water

A sterilized (gamma radiation, 0·9 Mrad) compound broiler feed was used as control feed and to prepare the fermented feed (water content in the FLF was 1:1·4). The preparation of FLF was done as in previous experiments (Heres et al., unpublished results) and was as follows. Three 480 g starter batches of liquid feed, fermented at 30 °C for 24 h with *Lactobacillus plantarum* were mixed with 14·4 kg feed batches. After mixing the feed was fermented at 30 °C for 48 h. The final pH of the feed was 3·9, the number of lactobacilli was between 10^9–10^{10} c.f.u./g liquid feed. Before feeding the FLF was stored at 4 °C. Feed was administered in troughs with a wired cover. Chickens were fed *ad libitum*. During the first week the feed was refreshed twice a day. Thereafter, feed was refreshed daily.

Drinking water was acidified with fumaric acid, acetic acid, and propionic acid (7·5, 14·3 and 21·2 mmol/l respectively). Water was administered in 1 l round drinking vessels. The reason for addition of these acids was to prevent bacterial growth (including *Salmonella*) in the drinking vessel. Otherwise the vessel could cause a continuous re-infection of the chickens. For some farmers the addition of acids to water is a standard practice. It is worth emphasizing that 2·75 mg acid/ml was added, in comparison to the

high levels of lactic and acetic acid in the liquid feed, respectively 24·4 and 4·8 mg/g (Heres et al., unpublished results). It is difficult to imagine that this 10% of extra acids via the drinking water made the FLF effective.

Housing

The 1-day-old chickens were reared in separate compartments per feed treatment. The chickens were in 2·5 m² ground cages on litter.

Two other compartments with 32 pens in each were used to house the inoculated chickens. Inoculated chickens and contact animals were housed within these compartments in pens with a solid floor with 1 cm of wood shavings. The pen size was $0{\cdot}5 \times 0{\cdot}5$ m. The pens were placed against the wall, with two further rows of pens, back to back, placed in the middle of the compartment. There was a path of at least 1 m between the pens in the middle and the pens against the wall. There was at least a 20-cm space between neighbouring pens. The roof, sides and back of a pen were at the outside covered with plastic sheets. No contact between animals of different pens was possible. The front side of the pens was wired and open. Chickens, feed and water equipment were handled with gloved and 70% alcohol-disinfected hands to prevent the spreading of microorganisms between pens. In each compartment comprising 32 pens there were 9 FLF7, 9 FLF3, 9 DF and 5 sentinel pens. Feed group and inoculation dose were assigned randomly to a pen before inoculation was started.

Bacteriology

Litter samples, that were taken to determine the *Salmonella* status of the 1-day-old chickens and of the chickens before inoculation or placing in the pens, were enriched in BPW at 37 °C for 24 h. These enriched cultures were plated on Modified Semi-Solid Rappaport–Vassiliadis broth (MSRV, Oxoid CM910) and incubated for 24 h at 42 °C. Suspected cultures were plated on BGA (modified) (Oxoid, CM329) for 24 h at 37 °C. Cloacal swabs of inoculated and contact chickens were enriched in BPW (24 h at 37 °C) and were subsequently plated on BGA with 100 ppm naladixic acid (BGA^+, 24 h at 37 °C). Positive diagnosis was dependent on the presence of one or more typical colonies. Confirmation of the cultured colonies was done by serum agglutination.

Table 2. *Transmission model*

Event		Rate
A susceptible animal becomes infected	$(S, I) \rightarrow (S-1, I+1)$	$\beta (S I)/n$
An infected animal recovers	$(S, I) \rightarrow (S, I-1)$	αI

S, susceptible; I, infected; n, total number of animals present; β, transmission rate parameter; α, recovery rate parameter.

Serial dilutions from caecal content in BPW were plated on the BGA^+ and counted after overnight incubation at 37 °C.

Transmission rate and reproduction rate estimates

To quantify a reduction of transmission, the transmission rate parameter (β)and the reproduction ratio (R_0) were calculated. The R_0 is the average number of infected animals that follows from a typical infected individual during its infectious period. If $R_0 < 1$ only minor outbreaks will occur. If $R_0 > 1$, major outbreaks may occur, i.e. the infection can spread. With an increase of R_0 the probability of major outbreaks increases. R_0 was estimated by calculating the Maximum Likelihood estimator of R_0, based on the final size distribution, as previously described by De Jong and Kimman [5] and Kroese and De Jong [6].

β is the infection rate parameter shown in the equation in Table 2, where the number of infections per time period (C) depends on β and the number of susceptible and infected animals per total number of animals present. The β estimation was based on a method described previously [7, 8]. This method is as follows. Because chickens were swabbed every day after inoculation or contact it was possible to describe for every period between swabbing (day), the mean number of infectious animals (I), the number of susceptible animals at the beginning of that period (S_0), the number of new infectious cases (C), and the total number of animals present ($n = 4$). The number of cases can be explained by the SIR model from the number of infectious animals per total number of animals and the transmission rate (β). The number of cases has a binomial distribution.

$$C \cong \mathrm{Bin}(S,\ 1 - e^{-\beta \frac{I}{N} \Delta t}).$$

The β can be estimated with Generalized Linear Model (GLM) with a complementary log log

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Table 3. *Frequency of courses of SE infection in FLF-fed and DF-fed chickens in transmission experiments in small groups*

Freq.	Group	n	S_0	I_0	S_t	R_t	I_t	
1	FLF3	4	3	1	3	1	0	One positive inoculated chicken caused no contact infection
1	FLF3	4	2	2	1	3	0	Two positive inoculated chickens caused one contact infection
2	FLF3	4	3	1	1	3	0	One positive inoculated chicken caused two contact infections
14	FLF3	4	4	0	4	0	0	The two inoculated chickens were not infected, nothing happened
16	FLF7	4	2	2	0	4	0	Two positive inoculated chicken caused two contact infections
2	FLF7	4	2	2	1	3	0	Two positive inoculated chicken caused one contact infection
14	DF	4	2	2	0	4	0	Two positive inoculated chicken causes two contact infections
4	DF	4	4	0	4	0	0	The two inoculated chickens were not infected, nothing happened

n, total animals per pen; S_0, I_0, number of chickens at start that are susceptibles, respectively that shed *Salmonella* after inoculation; S_t, R_t, I_t, number of chickens at the end of experiment that are still susceptible, that were shedding *Salmonella* during the experimental period, or that are still infectious respectively; Freq., frequency of this observation. FLF3, FLF7, fermented liquid feed-fed groups inoculated with respectively, 10^3 and 10^7 c.f.u. SE. DF, dry-feed groups, inoculation with 10^3 c.f.u. SE.

link function:

$$G(E(C)) = \log\left(\log\left(1 - \frac{E(C)}{S}\right)\right).$$

$E(C)$ is the expected value of the number of cases in a certain period based on the binomial distribution. Therefore

$$E(C) = S(1 - e^{-\beta\frac{I}{N}}).$$

If this is combined with the complementary log log function it follows that

$$G(E(C)) = \log\beta + \log\frac{I}{N}.$$

The log I/N is known for every period and is used to offset, allowing log β to be estimated.

Within-flock transmission

In addition, calculations were performed to extrapolate the results observed in this experimental setting to the flock level. In a minor outbreak a limited number of chickens become infected. The probability of minor outbreaks was approximated by calculating $1/R_0$. The number of contact-infected animals per day was calculated using the expressions in Table 2. The number of susceptible animals that become infectious during the following period (1 day in this study) is $S \times \beta \times (I/N)$, and the number of animals that recover, i.e. no longer being colonized with SE, is $\alpha \times I$. The β in this calculation is the point estimates of β from the GLM method. The proportion of infected chickens at introduction in the flock (I/N) was assumed to be 0·1 %.

Statistics

For calculations of transmission rate by GLM, Genstat 5.0 was used [9]. For inferences on effect at within-flock level Microsoft Excel was used.

RESULTS

The numbers of detected infections are shown in Table 3 and Figure 1. This show the status of the animals (susceptible or infectious) at the start of the transmission experiment, day 3 p.i., and the status of the animals in a pen at the end of the experiment. In some pens, 4 DF pens and 14 FLF3 pens, nothing happened because the inoculated animals did not shed *Salmonella*. In other cases there were one or two infected chickens at the start of the experiment and at the end all, three or just one chicken was infected.

In Table 4 the results are shown in more detail. The numbers of shedding animals and the number of pens with shedding chickens detected by cloacal swabbing are summarized. Here again it is shown that in 4 out of 18 pens with DF-fed chickens and in 14 out of 18 pens with FLF-fed chickens, the birds did not shed *Salmonella* after inoculation with 10^3 c.f.u. SE. The eight FLF3 groups that underwent necropsy at days 8 and 10 were all *Salmonella* negative. The proportion of shedding chickens from the inoculated FLF7 group steadily increased to 100 %.

The contact chickens fed with DF started to shed *Salmonella* shortly after the first contact. The contact-shedding interval is longer for the FLF7 group. Eight

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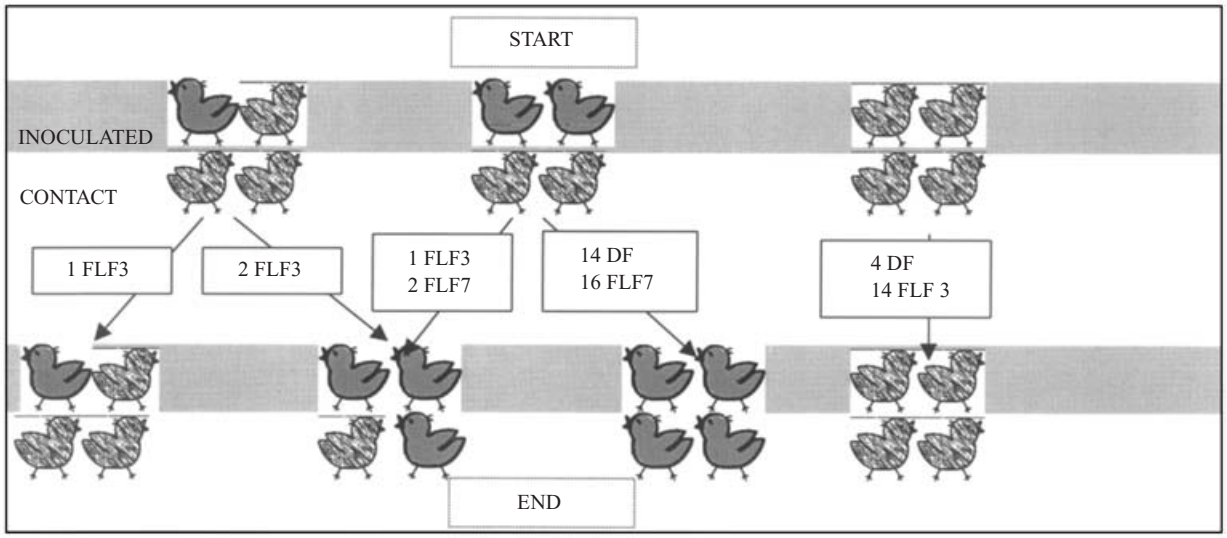
Table 4. *Cumulative number of SE-positive FLF-fed and DF-fed broiler chickens several days after inoculation or contact with inoculated chickens*

	FLF3*		FLF7		DF	
Days p.i.	Inoculated ($n=36$)	Contact ($n=36$)	Inoculated ($n=36$)	Contact ($n=36$)	Inoculated ($n=36$)	Contact ($n=36$)
1	0		8 (6)		8 (8)	
2	0		12 (10)		14 (11)	
3	0		15 (10)		18 (12)	
4	0	0	19 (14)	1 (1)	23 (14)	9 (6)
5	0	1 (1)	21 (14)	9 (5)	26 (14)	14 (9)
6	0	0	22 (15)	14 (10)	26 (14)	19 (11)
7	1 (1)†	0	24 (16)	20 (12)	27 (14)	24 (15)
8	2 (2)	1 (1)	25 (16)	25 (15)	27 (14)	27 (14)
9	2 (2)	0	28 (17)	25 (15)	27 (14)	28 (14)
10	3 (2)	1 (1)	29 (17)	25 (16)	27 (14)	28 (14)
11	3 (2)	1 (1)	29 (18)	26 (16)	28 (14)	28 (14)
12	4 (3)	1 (1)	31 (18)	28 (16)	28 (14)	28 (14)
13	4 (3)	1 (1)	32 (18)	30 (17)	28 (14)	28 (14)
Positive caeca (n)	4	6	36	34	28	28
Positive caeca (%)	11	17	100	94	78	78
(pens with pos. caeca)	(4)	(4)	(18)	(17)	(14)	(14)

* FLF3, FLF7, fermented liquid feed-fed groups inoculated with respectively, 10^3 and 10^7 c.f.u. SE. DF, dry-feed groups, inoculated with 10^3 c.f.u. SE.

† Figures within parentheses are the number of pens with one out of two or two out of two positive chickens.



= shedder; = non-shedder; FLF3, FLF7, fermented liquid feed-fed chickens inoculated with respectively, 3 and 7 log c.f.u. SE; DF, dry feed chickens, inoculated with 3 log c.f.u. SE.

Fig. 1. Course of infection in *Salmonella enteritidis* transmission experiment illustrated by shedding status at start of the contact and at the end of experiment in FLF-fed and DF-fed broiler chickens.

DF-fed chickens were negative at the end of the experiment. These were chickens from four pens. All FLF7 pens had positive chickens at the end of the experiment. Only two FLF7 contact chickens were *Salmonella* negative. Five FLF3 and eight FLF7 chickens with a SE-negative cloacal swab at day 13, had a *Salmonella*-positive caecal content. There was a very limited number of positive samples in sentinel

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Table 5. *Mean colonization level in caeca of* Salmonella *shedding inoculated and contact infected chickens in DF-fed and FLF-fed broiler chickens* (*Values are mean* ± *S.D.*)

	All	Colonized after inoculation	Colonized after contact
DF	$6{\cdot}5 \pm 1{\cdot}5^{a}$	$6{\cdot}5 \pm 1{\cdot}5$	$6{\cdot}4 \pm 1{\cdot}7$
FLF7	$4{\cdot}4 \pm 2{\cdot}0^{b}$	$5{\cdot}0 \pm 1{\cdot}8^{c}$	$3{\cdot}8 \pm 2{\cdot}0^{d}$

Significance level ANOVA analyses: ^{a,b} $P=0$; ^{c,d} $P=0{\cdot}04$.
FLF7, fermented liquid feed-fed groups inoculated with 10^7 c.f.u. SE. DF, dry-feed groups, inoculated with 10^3 c.f.u. SE.

chickens. Three chickens from two pens with sentinels had a positive cloacal swab at day 1 (respectively days 9 and 12). Two chickens had a positive caecum at the end in only one of the pens. It was concluded that the transmission between pens could be ignored for further analyses.

The average *Salmonella* counts in the caeca of colonized chickens are shown in Table 5. Analyses of variance showed that the feed group effect is highly significant. The differences between inoculated and contact-infected chickens tend to be significant, but this significance disappeared when the numbers of days at which the chickens were positive is accounted for in the analyses of variance.

Transmission

The results as shown in Table 3, and illustrated in Figure 1, were used for the calculation of R_0 with the final size approximation. The results of the calculations are shown in Table 6. Only the FLF3 group has a significantly lower R_0 value than the DF group ($P=0{\cdot}02$), based on observations in 4 FLF3 groups and 14 DF groups. The R_0 value of the FLF7 group (6·8) tended to be lower ($P=0{\cdot}062$) than the DF group.

With the complementary log log link function the transmission rate parameters (βs) were calculated for the DF group and the FLF7 group. Results are shown in Table 7. The FLF3 group was omitted from this analysis, due to insufficient records being available for this group. The β estimates were 1·15 (95% CI 0·76–1·75) for the DF groups and 0·58 (95% CI 0·22–1·53) for the FLF7 groups. These estimates are significantly different ($P=0{\cdot}02$).

Table 6. *Maximum likelihood estimators of transmission rate* (R_0) *for DF-fed and FLF-fed broiler chickens*

Treatment	R_0	95% CI	$P(R_0 \leqslant 1)$	$P(R_0 \geqslant 1)$
Dry feed	∞^{a}	$4{\cdot}8$–∞	$>10^{-5}$	1
FLF3	$1{\cdot}3^{b}$	0·4–13·1	0·23	0·88
FLF7	$6{\cdot}8^{a,b}$	3·3–51·4	$>10^{-5}$	1

^{a,b} Reproduction ratios with different superscripts are significantly different: R_0 FLF$10^3 \geqslant R_0$ DF, P value = 0·022; R_0 FLF $10^7 \geqslant R_0$ DF, P value = 0·54; R_0 FLF $10^7 \geqslant R_0$ FLF 10^3, P value = 0·062. R_0 dry feed and R_0 FLF7 are both significantly >1 (H_0: $R_0 \leqslant 1$ rejected).
The hypothesis that R_0 FLF3 is $\leqslant 1$ was not rejected.
FLF3 and FLF7, fermented liquid feed-fed groups inoculated with respectively, 10^3 and 10^7 c.f.u. SE. DF, dry-feed groups, inoculated with 10^3 c.f.u. SE.

Table 7. *Estimated transmission rate parameter* (β) *for DF-fed and FLF-fed broiler chickens*

Group	β	95% CI
DF	1·15	0·76–1·75
FLF3	n.e.	
FLF7	0·58	0·22–1·53

FLF3, FLF7, fermented liquid feed-fed groups inoculated with respectively, 10^3 and 10^7 c.f.u. SE. DF, dry feed groups, inoculated with 10^3 c.f.u. SE. n.e., not estimated due to shortage of data.

Modelling

The probability of a minor outbreak was approximated by the reciprocal of R_0. For the DF this is zero. In DF-fed flocks outbreaks will always be major. The probability of a minor outbreak is 76% (1/1·3) in FLF-fed flocks where the index case is infected with 10^3 c.f.u. If the index case is infected with 10^7 c.f.u. SE this probability is 15% (1/6·8). These are all probabilities of minor outbreaks originating from an infection of one single chicken in a flock. For each individual chicken that is infected at introduction there is this probability of a minor outbreak and the opposite probability of a major outbreak. The estimated βs were used as input data in the calculations for the within-flock transmission. These deterministic calculations illustrate what occurs during an outbreak in a flock of 25000 broiler chickens with an initial 0·1% infected chickens. The course of infection is shown in Figure 2. (The recovery rate is assumed to be zero, for both feed groups). The slowed transmission

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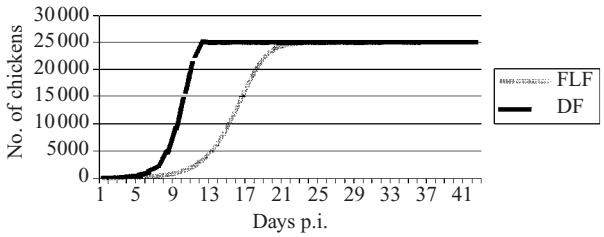

Fig. 2. Modelled number of infected chickens in a FLF-fed and DF-fed flock of 25 000 broiler chickens.

between FLF-fed chickens causes a postponed infection peak. In both DF- and FLF-fed flocks, all animals will eventually be infected.

DISCUSSION

Transmission within a flock and transmission between flocks are the predominant factors that determine the number of infected chickens for a certain pathogen, e.g. *Salmonella*, at a certain moment. A quantitative estimate of the reduction of transmission is therefore helpful in evaluating the effectiveness of new intervention strategies. The transmission between chickens was studied in an animal experiment to evaluate the effect of FLF. Inoculated chickens (also called seeders) were housed with susceptible chickens. Similar experiments have been previously performed to study the effect of other intervention strategies, for example the use of vaccines or probiotics [10, 11]. Epidemiological quantification of transmission in animal experiments was performed in experiments with viral diseases (e.g. Pseudo Rabies Virus [12]), and recently for a bacterial disease in pigs [7].

Fermented feed reduces the susceptibility for colonization with *Salmonella* [2]. This protective effect of FLF was confirmed by the present experiments. An inoculation dose of 10^3 c.f.u. SE resulted in infection in 14 out of 18 pens in the DF-fed chickens and in only 4 infected pens out of 18 in the FLF-fed chickens. FLF also reduced the level of colonization, and the FLF-fed chickens shed SE more intermittently than the DF chickens. In DF chickens a SE-negative cloacal swab followed a SE-positive swab on four occasions. In FLF-fed chickens this was observed 30 times (results not shown). A lower level of SE in the caeca of FLF-fed chickens is the probable cause of this intermittent shedding. The culturing of a cloacal swab is not 100% sensitive at these lower shedding levels. A difference in the caecal colonization level between DF and FLF groups was not observed in the previous experiments. Nevertheless, in the previous experiments an enrichment step for culturing *Salmonella* from cloacal swabs of FLF-fed chickens, and intermittent shedding in FLF-fed chickens was also more frequently observed than in chickens fed with DF [2].

Two methods of transmission calculations were applied to quantify the effect of FLF on transmission. The advantage of the GLM method is that no assumption is made about the final stage of a chicken at the end of an experiment, whereas in the final-size method it is assumed that chickens continuing to shed *Salmonella* are no longer infectious. Both the smaller R_0 in the FLF3 group and the significant reduction of β in the FLF7 group show the reduced transmission between FLF-fed chickens in comparison to DF-fed chickens. The R_0 with FLF is, however, not smaller than 1. In that case only small outbreaks would occur, because every infectious animal infects on average less than one other chicken. Therefore, the infection comes to a dead end. The estimated R_0 values show that in the FLF-treated groups a spread of infection still occurs. However, in a larger proportion of cases ($1/R_0$) the infection might as a matter of chance come to a dead end in FLF-fed groups, i.e. it results in a minor outbreak. A reduced transmission in FLF3 and FLF7 groups was expected because FLF chickens are less susceptible for *Salmonella* after a single oral inoculation. Besides this reduced susceptibility, the reduced colonization level in the caeca should contribute a reduced transmission. With lower colonization levels in the FLF-fed chickens the number of faecally shed *Salmonella* will also be lower.

However, the transmission reduction was expected to be higher than observed in the present experiments. This expectation was based on the previously observed increased individual resistance. The smaller reduction of transmission is even more surprising as the caecal colonization level was 10^5 c.f.u./g in the FLF-inoculated chickens. Such a moderate level of infection could only colonize a small proportion of FLF-fed chickens in the previous experiments [2]. The within-animal transmission is apparently facilitated by repeated ingestion of infectious material against a single infection in inoculation experiments. Additionally, airborne transmission might have played a role. *Salmonella* can float through the air connected to dust particles. *Salmonella* may enter the body via the respiratory mucosa and be transported via white blood cells to the caecum, as in pigs [13]. With this alternative route of infection *Salmonella* enters the

caecum without passing the crop and gizzard. FLF reduces *Salmonella* especially in the crop and gizzard [14]. The airborne route of infection is not affected by FLF.

The inference in assessing the effect of FLF on the within-flock prevalence of *Salmonella* shows that the transmission rate observed in the FLF7 group is high enough to infect all broilers in a flock. The final number of infected chickens is ultimately not different between feed treatment groups. Only if introduction takes place shortly before slaughter is the proportion of contaminated broilers lower in FLF chickens. In practice, however, *Salmonella* introduction mostly occurs in the early weeks and not just before slaughter.

To make inferences about the course of infection, assumptions and simplifications were necessary. One simplification is that transmission and recovery rate do not change if chickens grow older, this, however, does not take into account that older chickens are less susceptible for an infection [15]. That infectious chickens are randomly mixed and mixing in a flock, is an assumption that was made. This assumption is supported by the literature [16, 17]. Because we did not observe recovery after infection with SE in this and other experiments lasting 14–35 days, no recovery was assumed. In some experimental infections with other *Salmonella* types in young broilers a recovery in approximately 30 days was observed [18, 19]. In other inoculation experiments it is seen that inoculated chickens housed in groups shed for longer than do individually housed chickens, probably due to continuous re-infection [20].

Because of the assumptions and simplifications, conclusions must be carefully drawn. The modelled outcome of a quick increase in *Salmonella*-positive chickens, transmission to all susceptible chickens and no decrease after the infection peak is in accordance with experimental transmission experiments with SE but not with other *Salmonella* serotypes [21]. This 100% level of contamination at slaughter age is however higher than the 10% level that is estimated from Dutch field data [22]. Regrettably, there is a lack of data from systematic sampling about the course of infection within flocks. This prevents the validation of the modelled outcome. Nevertheless, the calculations illustrate that lower numbers of infected chickens at time of slaughter are not achieved by reduced transmission in flocks fed with FLF alone. However, the prevention of introduction, i.e. transmission between flocks, might be another important feature of FLF. The reduced susceptibility of broiler chickens indicates that FLF can prevent introduction. In commercial circumstances the number of c.f.u.s introduced or residential salmonellas in rodents, insects, and dust will in most cases be low. Therefore they are infrequently detected. It was shown that FLF-fed chickens are less susceptible for these low levels of *Salmonella*. Moreover, FLF might make R_0 between flocks smaller than 1 if it is combined with hygienic barriers, like separation of flocks and bio-sanitary measures, or other intervention strategies, like competitive exclusion.

The presented epidemiological infection model suggests that there is a probability of only small outbreaks when *Salmonella* is introduced in a FLF-fed flock, major outbreaks can nevertheless occur. The experimental results and epidemiological model calculations indicate a significant and biologically relevant reduction of transmission in addition to the reduced probability of introduction into the flock. Experiments under field conditions are necessary to validate these modelled outcomes for between- and within-flock transmission.

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