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# Factors influencing salmonella shedding in broiler chickens

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## SUMMARY

Three variables were included in a study to determine their effect on the incidence of *Salmonella typhimurium* in broilers challenged at four days of age. Variables included the presence or absence of a feed additive, avoparcin; the use of new or used litter and the initiating dose of salmonella. Cloacal swabs were taken from approximately 600 chicks at weekly intervals for 45 days. At $10^4$, $10^6$ and $10^8$ c.f.u./chick there was a direct association of challenge dose and the incidence of positive chicks for the first several weeks. Chicks raised on used litter showed an appreciable reduction in susceptibility to salmonella when compared to control animals on fresh litter. As the birds approached slaughter age, the influence of litter hygiene and challenge dose diminished under the conditions of this study. Avoparcin in the diet at 10 p.p.m. had no enhancing effect on salmonella shedding at any time during the 45-day sampling period. The implications of competitive exclusion are discussed.

## INTRODUCTION

It is generally accepted that salmonella food poisoning in humans is often a consequence of improperly cooked or stored food, particularly meat. Although the proliferation and distribution of salmonella through the food chain is strongly influenced by hygiene in the slaughterhouse as well as the kitchen, it seems prudent to examine conditions under which livestock are raised and to determine whether certain management practices could have a significant influence on salmonella establishment.

In recent years, the scientific literature on salmonella in poultry has included an examination of the effect of feed additives. Those tested for salmonella enhancement have included broad-spectrum antibiotics (Rantala, 1974*a*; Jarolmen, Shirk & Langworth, 1976; Smith & Tucker, 1975*a*; Evangelisti *et al.* 1975); narrow-spectrum gram-positive growth promoters (Smith & Tucker, 1975*b*, 1978; Benazet & Cartier, 1980; Matthes, Leuchtenberger & Loliger, 1982; Gustafson, Beck & Kobland, 1982; Abou-Youssef & Di Cuollo, 1982); antiprotozoals (Smith & Tucker, 1975*b*, 1978) and organic acids (Matthes, Leuchtenberger & Loliger, 1981). The extensive variation in experimental protocols has yielded results leading to conflicting viewpoints on the effects of feed additives for salmonella enhancement in experimental models as well as the potential in large-scale broiler production. A related research area concerning salmonella colonization in broilers

has been the effect of indigenous bacterial flora. Several investigators have shown that the deliberate introduction of adult enteric microflora orally into newly hatched chicks may inhibit salmonella colonization (Nurmi & Rantala, 1973; Rantala, 1974; Lloyd, Cumming & Kent, 1977; Rigby & Pettit, 1980; Barnes & Impey, 1980; Dorn & Krabisch, 1981). This phenomenon has been termed competitive exclusion. If indigenous flora influences salmonella colonization, then the relatively clean conditions under which investigators normally carry out their tests may be inappropriate for conclusions on broilers in production. In some regions, especially the United States, the litter is often not changed between production cycles, and chickens in large broiler houses could be expected to develop competitive gut organisms much more rapidly than birds utilized in most experimental situations. Even producers practising extensive cleaning procedures at their production sites must expect that remnants of flora from previous chick batches, the presence of feral birds, rodents and insects could be significant sources of new microflora for young chicks. Such sources are not usually a factor under the more rigid experimental laboratory conditions. The work described here investigates the influence of avoparcin under dissimilar conditions of litter hygiene following oral challenge with 100-fold dilutions of *Salmonella typhimurium*. Avoparcin is a growth-promoting antibiotic used extensively in poultry and swine feeds in many European countries, and its antimicrobial activity is confined to gram-positive organisms (Redin & Dornbush, 1968).

## MATERIALS AND METHODS

Twelve individual small poultry houses were utilized as previously described (Gustafson, Beck & Kobland, 1982). All were cleaned and hosed with steam. Half of the houses were prepared with a layer of fresh pine shavings. The remaining six houses were supplied with used chicken litter from a recently completed nutrition study. This consisted of a combination of wooden shavings, feathers, and chicken excrement. Although no attempt was made to monitor the microbiological populations in samples of clean and used litter, they were tested for natural salmonella and found to be negative. Disposable gloves were used by the animal caretakers and technicians throughout the experiment, and separate work teams were used for the clean and dirty areas. Boots and disinfectant footbaths were used when entering and leaving each house.

### *Chicks*

The prinicipal study consisted of 600 day-old Hubbard × Hubbard chicks distributed randomly by sex with 25 males and 25 females in each house. The preliminary study consisted of 46 chicks per house in four houses with half of each sex per house. Diets for all chicks included a coccidiostat, monensin, 100 p.p.m. with half of the birds also receiving avoparcin at 10 p.p.m. Chicks were fed a commercial-type broiler starter diet from 0 to 4 weeks of age and a broiler finisher diet thereafter.

### *Salmonella challenge*

A nalidixic acid-resistant strain of *S. typhimurium* had been obtained from J. F. Tucker of the Houghton Poultry Research Station at Houghton, Hunting-

Table 1. *Salmonella shedding in chicks raised on used litter and challenged with $10^5$ c.f.u./chick*

| | Percentage positive* for *S. typhimurium* at stated days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4 | 11 | 18 | 24 | 31 | 38 | 45 |
| Avoparcin | 10 | 21 | 13 | 13 | 8 | 4 | 0 |
| Control | 12 | 20 | 13 | 14 | 14 | 10 | 4 |

* 92 chicks/group initially. By day 45, four chicks in the avoparcin group and nine chicks in the control group had died.

don, England. This strain had been designated TM F98. All chicks were infected by gavage at four days of age. An overnight liquid culture was adjusted turbidometrically and diluted to yield the indicated challenge levels.

### *Detection of challenge organism*

Primary recovery medium was brilliant green agar containing 20 μg/ml nalidixic acid and novobiocin sodium at 1 μg/ml novobiocin equivalent. Enrichment was carried out in trypticase soy broth containing nalidixic acid at 20 μg/ml, 5·0 ml/tube. Cloacal swabs were taken at indicated intervals from all chicks. Swabs were surface streaked on primary recovery medium and placed thereafter into a tube of enrichment broth. If after 24 h incubation of the agar plates and liquid medium at 37 °C no evidence of salmonella was seen on the primary plate, aliquots of the enrichment broth were placed on primary recovery medium, incubated for 24 h and examined.

## RESULTS

The results of a preliminary experiment on used litter are shown in Table 1. Soon after their arrival from the hatchery, 184 chicks were placed on used litter. The purpose was to determine whether avoparcin influenced salmonella shedding under hygienic conditions which were perceived to be somewhat closer to many production conditions than our previous tests. All chicks were challenged orally with $10^5$ c.f.u./chick at four days of age. The results show that the maximum incidence of infection was approximately 20 % in both groups. This was appreciably lower than the levels observed in our previous experiments with chicks challenged at 4 days of age with $10^5$ c.f.u. *S. typhimurium*. No influence of avoparcin was seen in the preliminary test reported in Table 1.

The second and principal experiment utilized 600 chicks and was designed to determine the effects of challenge dose, avoparcin and litter hygiene. The results may be seen in Table 2. A general inspection of the data confirms the results that were seen in the preliminary experiment. On used litter chicks challenged with $10^4$ c.f.u. reached a maximum infection rate of about 20 % as compared to levels up to 86 % in control chicks at $10^4$ on new litter. An unexpected result was observed on the last sampling day in several of the houses. This was particularly noticeable in houses 351, 356 and 358 where the percentage positive rose sharply on day 45 as compared to the previous sample on day 38. Cloacal swabs on day 45 were taken

Table 2. *Percentage of chicks positive for NA$^r$* S. typhimurium

| | Fresh litter | | | | | | Used litter | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Avoparcin | | | Control | | | Avoparcin | | |
| House | 350 | 351 | 352 | 353 | 354 | 355 | 356 | 357 | 358 | 359 | 360 | 361 |
| c.f.u./chick | $10^4$ | $10^6$ | $10^8$ | $10^4$ | $10^6$ | $10^8$ | $10^4$ | $10^6$ | $10^8$ | $10^4$ | $10^6$ | $10^8$ |
| Days after challenge | | | | | | | | | | | | |
| 3 | 30 | 88 | 98 | 28 | 66 | 98 | 6 | 24 | 94 | 8 | 30 | 94 |
| 10 | 86 | 92 | 96 | 54 | 84 | 100 | 22 | 54 | 88 | 20 | 90 | 84 |
| 17 | 76 | 80 | 66 | 42 | 52 | 92 | 20 | 48 | 45 | 16 | 49 | 71 |
| 24 | 58 | 50 | 54 | 54 | 48 | 64 | 22 | 28 | 37 | 8 | 35 | 63 |
| 31 | 28 | 46 | 64 | 36 | 10 | 16 | 6 | 12 | 2 | 2 | 24 | 19 |
| 38 | 16 | 10 | 10 | 2 | 6 | 14 | 16 | 8 | 18 | 2 | 14 | 21 |
| 45 | 29 | 34 | 2 | 4 | 4 | 14 | 69 | 2 | 41 | 0 | 19 | 15 |

before the birds were sacrificed, and no known change in procedure can account for the sudden increase in percentage positive. All three of the groups showing aberrant increases in salmonella were receving diets without avoparcin.

In order to determine graphically the effect of each of the variables on salmonella shedding, the data were segregated and the mean determined for each set of conditions. For example, the 200 chicks challenged with $10^4$ c.f.u. were averaged for each interval, irrespective of litter hygiene or antibiotic in the diet. The same

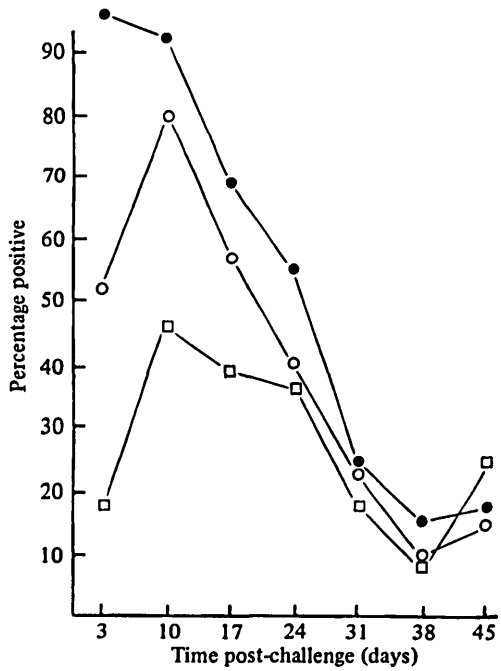


Fig. 1. Salmonella shedding in chicks challenged with 100-fold increments of *S. typhimurium*. Data were combined from 600 chicks receiving a control or avoparcin diet and raised on new or used litter ($10^4$ c.f.u., □; $10^6$ c.f.u., ○; $10^8$ c.f.u., ●).

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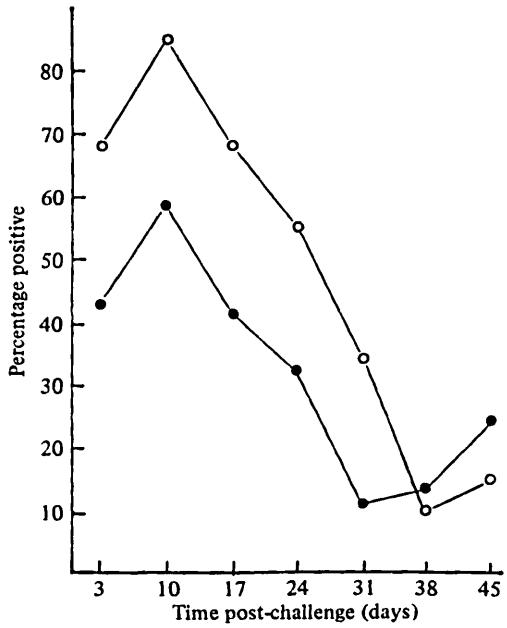


Fig. 2. A comparison of salmonella shedding in chicks raised on clean and used litter. Data were combined from chicks challenged with $10^4$, $10^6$ or $10^8$ c.f.u. and fed either control or avoparcin diets (used litter, ●; clean litter, ○).

was done for challenge with $10^6$ and $10^8$ c.f.u. and the results may be seen in Figure 1. As expected, the increasing 100-fold increments in challenge level resulted in consistently higher infection rates for the first few weeks. As the percentage positive decreased in succeeding weeks, the effect of initial challenge tended to become less noticeable. On day 31, 100-fold increases in challenge level resulted in 18, 23 and 25% positive. A week later on day 38, the figures were 9, 9·5 and 15·8%.

Figure 2 shows the effect of litter hygiene on salmonella establishment. It is apparent that chicks raised on used litter were far less susceptible to colonization by salmonella than those raised on new litter under very clean conditions. The effect of litter hygiene diminished in the last two sampling intervals with percentage positive on day 38 at 9·7% for new litter and 13·2% for used litter. The effect of litter hygiene on initial establishment may be seen in Figure 3. One may assume that the measurement of susceptibility to salmonella could be determined by observing percentage positive on days 3 and 10, before the infection rates had started to decline. If percentage positive is plotted for each challenge dose under the two conditions of litter hygiene, then a direct relationship exists between $\log_{10}$ challenge dose and percentage positive at the median infection level.

It may be seen that the dose required to infect 50% of the chicks was 100-fold higher on used litter than on clean litter. Thus the susceptibility of chicks to salmonella establishment was much greater under conditions which deprived them of rapid exposure to indigenous microflora. This is not surprising in view of the demonstrated relationship of salmonella colonization in young chicks with the

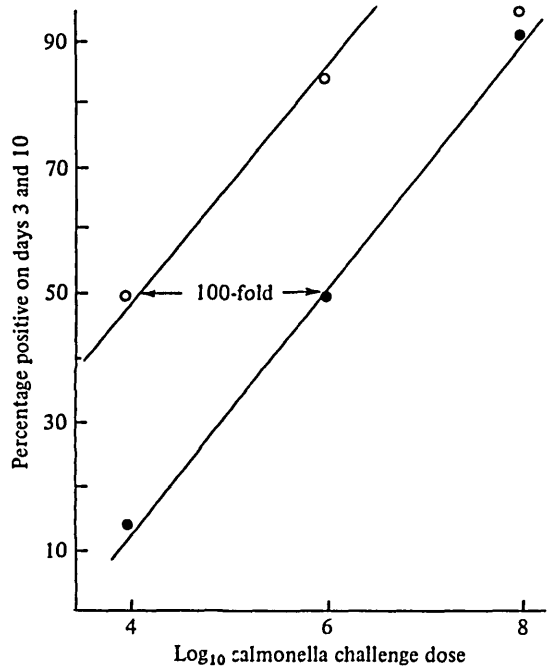


Fig. 3. Percentage chicks positive for *S. typhimurium* on days 3 and 10 at three challenge levels and raised on clean or used litter. Data were combined from chicks receiving control or avoparcin diets (clean litter, ○; used litter, ●).

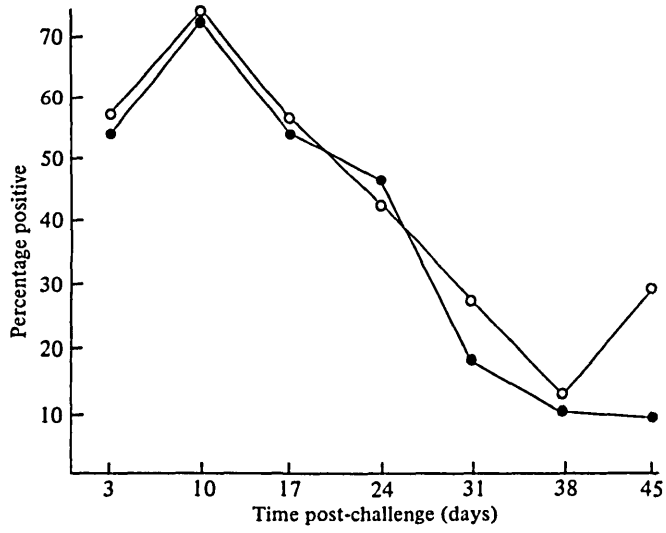


Fig. 4. The prevalence of *S. typhimurium* in chicks fed a control (○——○) or avoparcin (●——●) diet. Data were combined from 600 chicks challenged with $10^4$, $10^6$, or $10^8$ c.f.u. and raised on new or used litter.

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Table 3. *Summary of total samples positive for salmonella*

| | Positive/total | Percentage positive |
|---|---|---|
| $10^4$ c.f.u./chick | 329/1193 | 27·6 |
| $10^6$ c.f.u./chick | 522/1193 | 43·8 |
| $10^8$ c.f.u./chick | 699/1185 | 59·0 |
| Clean litter | 959/1795 | 53·4 |
| Used litter | 591/1784 | 33·1 |
| Control diet | 797/1787 | 44·6 |
| Avoparcin | 753/1784 | 42·2 |

status of competitive organisms in the gut. Figure 4 plots data segregated according to the presence or absence of avoparcin in the diet. The prevalence of salmonella at each interval has been determined by combining data from all three challenge levels and both litter variables. This resulted in close agreement between both groups except for the last sampling day. The abrupt increase in control chicks positive for salmonella may clearly be seen and it is not possible to explain this aberration.

In the principal study 4164 cloacal swabs were processed for salmonella with 40% positive. Half of these required liquid enrichment before salmonella was detected. Table 3 summarizes samples positive for each category for five sampling intervals. These figures include days 3, 10, 17, 31 and 38 and omit day 45 for the aforementioned reasons.

## DISCUSSION

The study of practices and conditions which influence the perpetuation of salmonella in poultry flocks may be pursued in a variety of ways. Searches for naturally occurring salmonella often yield either too much, too little or an incidence too variable to allow a conclusion. Nevertheless, this should be the preferred method if it is possible to process a sufficient number of samples. A very large number might be required, depending on the magnitude of effects which one hopes to detect. Even carefully designed challenge studies in which infection levels are controlled may show unexpectedly high variation among groups treated uniformly.

Some investigators have chosen to challenge chicks with a standard level of salmonella and sacrifice subgroups at intervals in order to assay specific organs, usually the caecum, for the challenge organism. Admittedly the caecum is usually a more sensitive indicator of the presence of salmonella, although studies in our laboratory have shown that when caecal and cloacal samples are taken at the same time in the same chicks, only a slight increase in positive birds is seen using the caecal method. Our view is that the economy of effort required and the advantages of repeatedly sampling a large number of the same birds favours use of the cloacal swab. However, this method might not be adequate when searching for naturally occurring salmonella when the prevalence is low.

In recent years attempts to define reasonable steps in the control of salmonella in poultry have included a consideration of feed antibiotics as a possible adverse

influence. In addition, a practical effort has been made to determine which organisms may provide a competitive influence which interferes with salmonella establishment (Barnes, Impey & Stevens, 1979; Impey, Mead & George, 1982). Unlike other meat animals, avian species may consume feed with growth promoters, coccidiostats, histomonostats and other additives within hours of hatching and while the intestine and caecum have a limited variety of organisms. The influence of an antimicrobial on such a developing bacterial population could be expected to be greater than on mammalian microflora, where first exposure to feeds and their antimicrobials usually comes after weaning. Recent studies have revealed that chicks arriving from the hatchery have high levels of presumptive coli-aerogenes bacteria, *Streptococcus faecales* and *S. faecium*, but on no occasion were lactobacilli found (Barnes, Impey & Cooper, 1980). *Lactobacillus* spp. had reached levels of 9 $\log_{10}$/g within three days, however. It is well established that poultry rapidly develop resistance to colonization by salmonella. If factors which influence this reduced susceptibility include the acquisition and increase in numbers of bacterial microflora, then an environment which is extraordinarily clean may present a paradoxical inverse relationship between good hygiene and salmonella establishment. Another factor which mitigates against the perpetuation of salmonella in chicks is the reduced survival of this organism in used litter (Snoeyenbos *et al.* 1967; Olesiuk, Snoeyenbos & Smyser, 1971; Turnbill & Snoeyenbos, 1973). Reinfection under such conditions is less likely over the long term. However, the data presented in Figure 3 were probably not influenced by the anti-salmonella effect of old litter, since percentage positive on days 3 and 10 should reflect initial challenge rather than reinfection.

One of the original purposes of this study was to determine whether an effect of avoparcin would be seen under hygienic conditions which allowed a more rapid establishment of competitive microflora. Some published studies have shown a prolongation of salmonella shedding in broilers receiving certain growth promoters, including avoparcin (Smith & Tucker, 1978, 1980; Matthes, Leuchtenberger & Loliger, 1982). Previous work carried out in our laboratory (Gustafson, Beck & Kobland, 1982) has failed to confirm more than a transitory effect of avoparcin on salmonella shedding. The present study showed no effect of avoparcin in broilers on either clean or used litter, and it was thus not possible to evaluate the mitigating influence of a more septic environment. It is difficult to assess the primary differences in experimental models which have led to conclusions which are not in agreement. The addition of monensin to all diets is a departure from the methods of other investigators measuring the effect of feed additives on salmonella shedding. Monensin is undoubtedly the coccidiostat currently used most extensively worldwide, and this ionophore has been reported to have no effect on salmonella shedding in models similar to those described in the present report (Smith & Tucker, 1978). Additional factors which have been reported to influence salmonella shedding have included diet and breed of chicken (Smith & Tucker, 1980), infection with *Eimeria tenella* (Baba, Fukata & Arakawa, 1982) and organic acids in the diet (Matthes, Leuchtenberger & Loliger, 1981). In surveys for natural salmonella during broiler production in Germany, we were unable to detect an influence of avoparcin on the incidence of caecal salmonella.

The natural incidence of *S. hadar* in a turkey production trial has also shown

no change in the presence of avoparcin or virginiamycin (Smith & Green, 1980). The present study further confirms that experimental inquiry into the effects of feed additives on salmonella shedding in chickens yields inconsistent results between laboratories using different protocols. This adds support to the belief that a policy based on public health considerations which significantly affects animal husbandry practices should, when possible, be supported by observations carefully made during commercial practice.

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