

## The vertical transmission of salmonellas and formic acid treatment of chicken feed

### A possible strategy for control

By T. J. HUMPHREY

*Public Health Laboratory, Church Lane, Heavitree, Exeter EX2 5AD, UK*

AND D. G. LANNING

*Lloyd Maunder Ltd, Willand, Nr Cullompton, Devon, UK*

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### SUMMARY

The treatment of feed given to laying hens with 0.5% formic acid reduced significantly the isolation rate of salmonellas and was associated with a reduction in the incidence of infection in newly hatched chicks. These improvements were not sustained until slaughter, however, as growing birds acquired salmonellas, probably from feed which was not acid treated. The data indicate that formic acid treatment of chicken food could have important benefits for the public health.

### INTRODUCTION

The link between the contamination of poultry carcasses with salmonellas and human salmonellosis has long been recognized. Live birds can become infected with the organism from a variety of sources (Smith, 1971), but contaminated feed is considered to be the principal source (Williams, 1981). The use of salmonella-free feed can bring about substantial reductions in the incidence of carcass contamination (Campbell *et al.* 1982). Its production, however, usually involves the use of heat (Blankenship *et al.* 1985) which can be relatively expensive, thus making the procedure unattractive to the poultry industry.

An alternative approach could be the use of organic acids. Formic acid has been used to decontaminate salmonella-positive animal feed (Watson & Kirby, 1985), and Hinton, Linton & Perry (1985) demonstrated that such treatment protected young chicks from infection with naturally-occurring salmonellas.

A typical modern poultry production and processing operation involves a number of separate but sequential stages. The egg-laying hens are kept on deep litter. The eggs are collected regularly, usually four times daily, from nest boxes, stored under cooled conditions for up to 6–10 days and transferred to large incubators where they are placed on setting trays lined with paper. After 18 days fertile eggs are moved to paper-lined hatching trays. Following hatching, the chicks are transported in large paper-lined trays to broiler houses where they are kept on deep litter and fed *ad libitum* until they reach slaughter weight after 6–7

weeks. They are then taken to a slaughter-house where they are killed at a rate which can reach 200 birds per min. The intensive nature of the egg laying, hatching, rearing and slaughter systems render the eggs, growing birds and carcasses prone to contamination with organisms such as salmonella.

The work of Hinton, Linton & Perry (1985) provided a valuable insight into the potential value of treating poultry feed with formic acid. Their findings, however, need to be substantiated on a commercial scale. We report here the effects of formic acid treatment of feed given to laying hens on the vertical transmission of salmonellas in a large poultry production unit.

#### MATERIALS AND METHODS

We investigated a poultry unit retrospectively and prospectively from January 1984 to May 1987 to assess the extent of salmonella infection. Samples of feed given to the adult hens and the growing birds, litter from the sheds accommodating the hens, hatchery waste (dead and dead-in-shell chicks), the papers on which chicks were taken to the broiler house (insert papers) and caecal contents collected at slaughter, were examined regularly for salmonellas.

From March 1986 feed given to laying hens was treated with a commercial brand of formic acid, thus providing an opportunity to assess the impact that acid treatment made on the vertical transmission of salmonellas.

##### *Formic acid treatment*

Sufficient formic acid to achieve a final concentration of  $0.5 \pm 0.1\%$  (w/w) was added to food in a high-speed mixer at the feed mill. Feed was delivered to farms in bulk and approximately 2 days elapsed between delivery and consumption.

##### *Microbiological examination of samples for salmonella*

Samples, 25 g, of all new batches of feed for adult hens, sampled at the mill, and many batches of broiler feed ingredients, including imported soya and fishmeal, were rehydrated in 225 ml buffered peptone water (BPW, Oxoid CM 509) and incubated at 37 °C for 18 h. Samples were then treated as recommended by Fricker *et al.* (1985). Litter samples, 100 g, were examined as above but using 1 l of BPW.

For macerated hatchery waste (dead and dead-in-shell chicks and hatching tray papers), 25 g was added to 225 ml selenite broth (Oxoid CM 395) and incubated overnight at 37 °C. A loopful was then streaked onto DCA (Oxoid CM 163) and XLD (Oxoid M 469) agars which were incubated at 37 °C for 24 h. Salmonella-like colonies were tested using standard laboratory techniques. Pieces of chick paper from six trays were added to 100 ml sterile tap water in a sterile plastic bag and shaken vigorously. The water was added to an equal volume of double strength selenite broth and cultured as above.

Infection in the broiler chicken was assessed by collecting 60 caecal samples at slaughter. These were mixed, macerated in a blender and 10 ml was inoculated into 90 ml selenite broth. Incubation and culture were carried out as described above.

### Analysis of data

The data obtained were to be divided into two groups, before and after acid treatment, and analysed statistically to determine the impact of such treatment on salmonella infection at various points on the production chain. Data were assigned to either group on the basis of date of collection, which varied according to sample type. April 1986 was taken as the critical date for breeder food, litter and hatchery waste, and all samples collected before then were placed in the untreated group. There is at least a 10-week gap between egg laying and slaughter of the resultant broiler chickens. For this reason, data on isolation rate of salmonellas from caeca were not considered to be influenced by acid treatment until July 1986.

Differences between the two treatment groups were analysed using the chi-squared test.

## RESULTS

Salmonellas were isolated from all sample types (Table 1). Nineteen different serotypes were identified, with *Salmonella typhimurium* (phage types 104C and 49), *S. stanley* and *S. sandiego* being the most common. Organisms isolated from feed given to the hens and growing birds were subsequently found in birds at slaughter (Table 1). The incidence of positive samples increased with each successive stage of the production process. Thus while only 2.0% of food samples contained salmonella, the corresponding figures for litter, newly hatched chicks (hatchery waste and insert papers) and broiler caeca were 3.4, 8.4 and 7.1 respectively.

Acid treatment reduced salmonella contamination of breeder feed (Table 2). The effect this had on the infection of day old chicks was profound and immediate (Table 2; Figure 1). Before March 1986 the rate of increase in the cumulative total of positive hatchery waste samples was 15.4% per month. Following acid treatment, the rate fell to 0.54% per month (Table 2;  $P < 0.00001$ ). Breeder litter was also positive for salmonellas less often (Table 2), suggesting that these organisms do not survive well in this environment since many samples were collected from houses which had been occupied for many months before acid treatment started.

The beneficial effects described above were largely negated, however, by salmonella contamination of broiler food ingredients (Tables 1 and 2). Imported fish and soya meal frequently contained this organism. This led to infections in the growing chickens and little difference was observed at slaughter between broilers examined before and after July 1986 (Table 2).

## DISCUSSION

Broiler chickens are produced using highly efficient systems of intensive agriculture and slaughtered in automated factories where up to 200 birds are killed per min. One consequence of this whole process is that finished carcasses are frequently contaminated with salmonellas and a recent survey of one factory's

Table 1. *Salmonella* serotypes isolated at various stages of poultry production

Sample	Salmonella serotype isolated*	
	Before acid treatment of breeder food	After acid treatment of breeder food
Breeder food	<i>S. altendorf</i> <i>S. senftenberg</i> <i>S. mbandaka</i> <i>S. agona</i> <i>S. sandiego</i>	<i>S. anatum</i> <i>S. mbandaka</i>
Breeder litter	<i>S. typhimurium 104C</i> <i>S. heidelberg</i> <i>S. albany</i> <i>S. anatum</i> <i>S. infantis</i> <i>S. agona</i>	<i>S. braenderup</i> <i>S. mbandaka</i> <i>S. heidelberg</i>
Insert papers† and hatchery waste	<i>S. typhimurium 104C</i> <i>S. sandiego</i> <i>S. senftenberg</i> <i>S. stanley</i> <i>S. saintpaul</i> <i>S. heidelberg</i>	<i>S. braenderup</i> <i>S. tennessee</i> <i>S. heidelberg</i>
Broiler food‡ ingredients	<i>S. typhimurium 49</i> <i>S. mbandaka</i> <i>S. agona</i> <i>S. ohio</i> <i>S. tennessee</i> <i>S. albany</i> <i>S. anatum</i> <i>S. havana</i>	<i>S. tennessee</i> <i>S. binza</i> <i>S. agona</i> <i>S. mbandaka</i> <i>S. livingstone</i> <i>S. anatum</i> <i>S. havana</i>
Broiler caeca	<i>S. typhimurium 49</i> <i>S. sandiego</i> <i>S. stanley</i> <i>S. saintpaul</i> <i>S. heidelberg</i> <i>S. mbandaka</i> <i>S. tennessee</i>	<i>S. typhimurium 49</i> <i>S. sandiego</i> <i>S. heidelberg</i> <i>S. senftenberg</i> <i>S. agona</i> <i>S. saintpaul</i> <i>S. stanley</i> <i>S. indiana</i> <i>S. kiambu</i>

\* Isolates are presented in chronological order of isolation.

† Insert papers were those that lined the trays in which chicks were transported to the broiler houses. Hatchery waste consisted primarily of dead and dead-in-shell chicks.

‡ Broiler food did not receive acid treatment.

production found that almost all carcasses sampled were salmonella-positive (Humphrey & Lanning, 1987).

There have been many attempts to eliminate salmonella contamination during slaughter (Patrick, Collins & Goodwin, 1973; Morrison & Fleet, 1985; Humphrey & Lanning, 1987), but the rapidity with which birds are killed and their close contact on the slaughter line has, as yet, presented too many problems for this approach to be wholly successful.

One way to ameliorate the problem would be to produce salmonella-free birds. The studies of Hinton, Linton & Perry (1985) demonstrated that young chicks can

Table 2. *The effect of formic acid treatment of food given to laying hens on salmonella contamination of feed, litter and chickens*

Sample	No. of salmonella-positive samples				$\chi^2$	Probability (P) of a significant difference
	Before acid treatment	(%)	After acid treatment	(%)		
Breeder food	11/270	(4.1)	7/642	(1.1)	8.8	< 0.01
Breeder litter	28/656	(4.3)	4/289	(1.4)	15.1	< 0.001
Hatchery waste*	180/1174	(15.3)	6/514	(1.2)	73.1	< 0.00001
Insert papers†	16/350	(4.6)	6/420	(1.4)	6.8	< 0.01
Broiler feed	30/366	(8.2)	36/480	(7.5)	0.13	n.s.
Broiler caeca	53/649	(8.2)	17/335	(5.1)	3.2	n.s.

\* Dead chicks, dead-in-shell chicks and hatching tray papers.

† Papers used to line trays in which chicks are transported to broiler house.

‡ Not acid-treated. Predominantly soya and fishmeal.

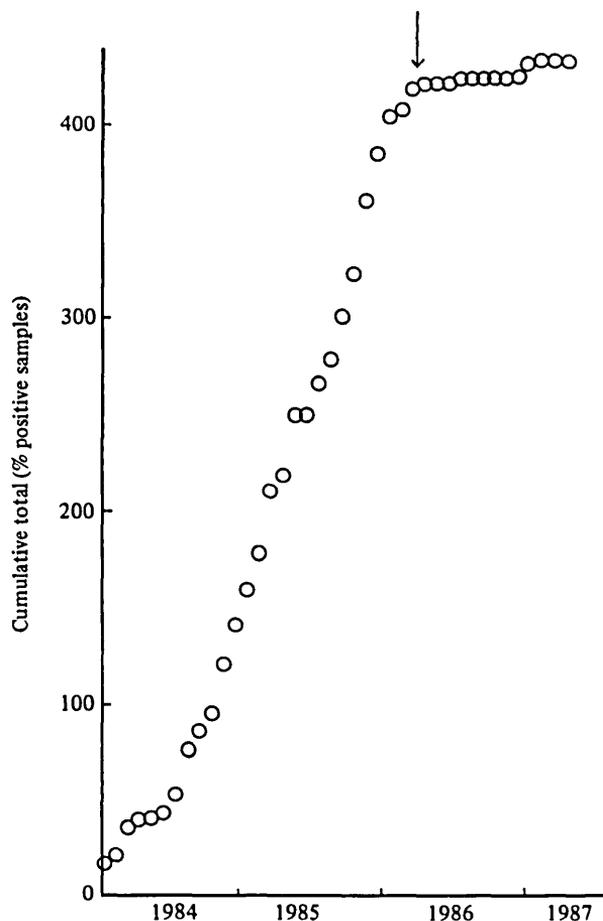


Fig. 1. The influence of formic acid treatment of feed given to egg-laying hens on the incidence of infection of newly hatched chicks with salmonellas. Infection in chicks was determined by microbiological examination of hatchery waste (principally dead and dead-in-shell chicks). The arrow denotes the point when chicks started to be hatched from eggs laid by hens being fed acid-treated food.

be protected from infection with salmonellas by formic acid treatment of contaminated food. Their work was on a small scale, while the data presented in this paper, however, demonstrate that such treatment could be potentially useful for controlling salmonella infections on commercial poultry farms.

The incidence of contamination in acid-treated food was significantly lower than that in untreated food. These apparent improvements were not due to an absence of salmonellas in the raw ingredients as many of these, including soya and fish meals, were also used in broiler food which was not acid-treated and was frequently salmonella-positive (Tables 1 and 2). Using the treatment regimen described here it was not possible to eradicate salmonella and the process could obviously be improved.

The effect, however, that such treatment had on the vertical transmission of salmonellas to the newly hatched chick was dramatic (Figure 1). During the 12-month period that acid-treated food was being fed to hens, only 1.2% of hatchery waste samples were salmonella-positive compared to 15.3% before treatment (Table 2). Such improvements were not sustained until slaughter, because broilers rapidly acquired the organism from their food which had not been disinfected. The most frequently positive food ingredient was imported soya meal which, as a vegetable protein, is not included in the current Protein Processing Order (Report No. 676, 1981).

Although many of the samples were found to contain salmonellas they were not usually serotypes associated with human disease (Palmer & Rowe, 1986), and it may be possible that the reporting of the incidence of infected carcasses without this qualification overestimates the role of poultry in human salmonellosis.

Further work is required to see whether broiler chickens can be kept free from infection by feeding them treated food. The differences in the isolation rates from hatchery waste and chick papers are of interest. They could indicate that salmonella was a significant cause of death in chick embryos. It is also possible that examination of lining paper is an insensitive way of monitoring for salmonellas.

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