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The effect of antimicrobial feed additives on the colonization of the alimentary tract of chickens by *Salmonella typhimurium*

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SUMMARY

Groups of 33 chickens were fed continuously on diets containing feed additives that are employed commercially for a variety of purposes, and were infected orally when 4 days old with a nalidixic acid-resistant mutant of *Salmonella typhimurium*. The amount of *S. typhimurium* organisms excreted in their faeces was estimated by culturing them at intervals and in a standard manner on brilliant green agar containing sodium nalidixate; when the chickens were killed their caecal contents were examined by the same technique.

Avoparcin and lincomycin, like nitrovin and tylosin (Smith & Tucker, 1975*b*), favoured colonization of the alimentary tract by the *S. typhimurium* organisms as shown by the fact that the chickens to which they were fed excreted these organisms in their faeces in higher concentration and for longer periods of time than did chickens fed on non-medicated diets. Amprolium, monensin, dimetridazole, arsenilic acid and nitro-hydroxyphenylarsonate had no obvious effect on the salmonella excretion pattern.

When only five chickens in each group were experimentally infected so that the effect of the feed additives on infections acquired by contact could be monitored, avoparcin, lincomycin, nitrovin and tylosin again favoured colonization of the alimentary tract with the *S. typhimurium* organisms and so did dimetridazole. Arsenilic acid, in contrast, hindered the development of infection. Amprolium, monensin and nitro-hydroxyphenylarsonate were without obvious effect.

Many of the chickens that were fed on diets that favoured *S. typhimurium* colonization, but not those fed on non-medicated diets, were still excreting *S. typhimurium* organisms in their faeces when they were killed at 56 days of age, the age at which broiler chickens kept under commercial conditions are usually slaughtered.

INTRODUCTION

In a recent study (Smith & Tucker, 1975*b*), the feeding of diets containing nitrovin, tylosin and, to a lesser extent, flavomycin, antibiotics commonly used for growth promotion, prolonged the carrier period and increased the amount of *Salmonella typhimurium* organisms excreted by experimentally-infected chickens. Because of this finding, it was decided to determine whether other commercially-

available antimicrobial feed additives possessed this undesirable property. These included amprolium and monensin, used for controlling coccidiosis, dimetridazole, used for controlling histomoniasis, avoparcin and lincomycin, used for growth promotion, and arsenicals, substances that are added to poultry feed for a variety of reasons. The results are reported in this paper.

In our previous study we infected all the chickens in each group fed on antibiotic-containing diets. In the present study, a similar procedure was adopted except that in some experiments only five chickens in each group were infected so that the effect of medicated diets on infections acquired by contact could be monitored; this additional procedure was adopted because it was thought to simulate natural conditions more closely.

MATERIALS AND METHODS

The kind of chickens, their management and the method of infecting them orally when 4 days old with a spontaneous mutant of *S. typhimurium* resistant to nalidixic acid (*nal*^{r}) have been described previously (Smith & Tucker, 1975*a*, *b*). Chickens infected at this age do not become unwell; in the first day of life they are highly susceptible to fatal infection with this strain. The method of assessing the concentration of the salmonella organisms in their faeces and caecal contents has also been described. It depends on specimens being inoculated in a standard manner on plates of brilliant green agar containing sodium nalidixate and novobiocin – very few faecal bacteria grow on this medium and the colonies of those that do can easily be differentiated visually from those of the infecting salmonella strain. Unless stated, chickens (33 per group) were fed on the medicated diets continuously from the time they were hatched.

RESULTS

The faecal excretion of Salmonella typhimurium *by groups of infected chickens fed on medicated diets*

Lincomycin

The effect on faecal excretion of *S. typhimurium* of feeding diets containing 100, 10 or 0 mg/kg of lincomycin to groups of 33 chickens that had been infected orally with the *nal*^{r} strain of *S. typhimurium* is summarized in Table 1. Except in the earlier examinations, *S. typhimurium* was found in much greater amounts in the specimens from the groups fed on diets containing 100 or 10 mg/kg of lincomycin than in those from the groups fed on the non-medicated control diet; the lincomycin-fed chickens, too, continued to excrete the organisms for a longer period than the others did. The results for pairs of groups receiving the same diet were, in general, closely similar.

At the conclusion of the experiment, 79 days after infection, the caecal contents of many of the lincomycin-fed chickens, especially those given the 100 mg/kg diet, contained large numbers of *S. typhimurium* whereas those fed on the control diet did not contain any.

When the above experiment was repeated, the period during which *S. typhi-*

Table 1. *Concentration of* Salmonella typhimurium *organisms in the faeces of groups of* 33 *chickens fed on diets containing lincomycin*

Time* (days)	% of chickens whose faeces had the following concentrations of *S. typhimurium* organisms when fed diets containing lincomycin at (mg/kg)																	
	100						10						0					
	Group 1			Group 2			Group 1			Group 2			Group 1			Group 2		
†	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T
9	24	82	97	48	91	100	45	82	100	52	91	100	30	91	100	24	94	100
16	12	73	88	33	82	100	27	73	100	18	79	88	30	70	85	12	64	85
23	33	79	91	36	91	100	18	45	70	24	52	88	0	15	70	6	9	79
30	21	82	91	12	67	85	15	85	88	33	85	88	3	36	58	3	33	42
37	0	48	61	3	42	42	0	36	39	6	52	58	0	6	6	0	9	33
44	18	45	76	6	39	52	12	45	58	9	48	64	0	12	15	0	3	6
51	6	36	64	3	39	55	6	30	36	3	39	61	0	3	3	0	3	3
58	24	36	55	12	45	52	3	24	27	0	36	55	0	0	9	0	3	3
65	24	42	58	3	27	39	3	12	24	3	15	39	0	0	0	0	0	3
74	18	52	58	6	39	42	3	15	24	0	18	33	0	0	0	0	0	0
79	6	24	30	6	27	27	0	0	9	3	9	27	0	0	0	0	0	0
79 (Caeca)‡	42	76	79	55	70	79	21	33	45	15	30	39	0	0	0	0	0	0

* After the chickens were infected when 4 days old; antibiotic-containing food was fed from day 1.

† > 50 = > 50 colonies of *S. typhimurium* grew on the culture plate; D = *S. typhimurium* isolated by direct culture; *T* = *S. typhimurium* isolated by selenite enrichment or by direct culture.

‡ Results of examining caecal contents when the chickens were killed at the end of the experiment (79 days after they were infected).

Table 2. *The isolation of* Salmonella typhimurium *from the faeces of groups of* 66 *experimentally infected chickens fed on diets containing different concentrations of avoparcin*

	% of chickens from which *S. typhimurium* was isolated when fed on diets containing avoparcin at (mg/kg)															
	100				20				10				0			
Time (days)	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T
9	15	64	94	97	11	41	91	100	3	30	86	94	3	21	77	88
16	21	55	92	100	14	38	89	98	14	45	79	94	0	6	45	73
23	24	67	91	97	30	50	89	100	8	39	79	89	0	0	12	24
30	11	50	88	95	5	32	85	85	5	24	73	74	0	0	2	21
37	8	44	83	94	6	26	74	92	9	26	79	95	0	0	2	6
44	2	35	89	97	5	23	59	76	2	17	76	92	0	0	0	9
51	6	27	83	97	2	15	56	82	2	12	48	80	0	0	0	3
51 (Caeca)	89	98	100	100	65	91	94	94	59	97	100	100	0	0	2	6

+ = The culture plate was covered with mainly discrete colonies. For other details and abbreviations see Table 1.

murium was excreted in the faeces of both the lincomycin-fed and the control groups was shorter, but the results were much the same. The percentage of faecal excreters in the two 100 mg/kg lincomycin groups, the two 10 mg/kg groups and the two control groups at 23 days after infection was 48 and 77, 77 and 77 and 13 and 13 respectively. The corresponding figures at 37 days after infection were 7 and 10, 42 and 31 and 3 and 3, and at 65 days after infection they were 0 and 3, 5 and 13 and 0 and 0.

Avoparcin

The effect of feeding diets containing different concentrations of avoparcin is summarized in Table 2; each diet was fed to two groups of chickens, the results for each pair of groups being amalgamated in the table because they resembled each other closely. *S. typhimurium* was excreted in greater amounts and for longer periods of time by the chickens fed on the avoparcin-containing diets, especially the 100 mg/kg one. These organisms were found in the faeces and caecal contents of only a few of the control chickens killed when they were 55 days old. In contrast, they were still present in the faeces of most of the avoparcin-fed chickens, often in high concentration; their numbers/g in 14 of these faecal specimens varied from 50000 to 40000000 (median 700000). The caecal contents of the avoparcin-fed chickens were even more heavily infected than the faeces, many of them yielding a confluent growth of *S. typhimurium* on the culture plates on which they had been inoculated. The plates used for examining the faeces and caecal contents of a random sample of the chickens fed on the 10 mg/kg diet and the control diet are shown in Plate 1.

The above experiment with the omission of the 20 mg/kg avoparcin diet was repeated twice. Similar results were obtained in the first repeat experiment, the percentage of chickens with *S. typhimurium* in their faeces at its termination being 94 and 97 in the 100 mg/kg groups, 37 and 21 in the 10 mg/kg groups and 6 and 6 in the control groups; the corresponding figures for the caecal contents were 100 and 100, 56 and 70 and 3 and 6 respectively. In the second repeat experiment, the results for the two groups fed on the 100 mg/kg diet resembled those obtained previously. During the first 24 days, the rate and amount of *S. typhimurium* faecal excretion was greater in the two 10 mg/kg groups than in the two control groups but after that time there was little difference between the results for all four groups, the percentage of faecal excreters at the 51st day in the 10 mg/kg groups being 9 and 9, and in the control groups 0 and 18.

The effect on *S. typhimurium* excretion of feeding the 100 mg/kg avoparcin diet for different periods of time is shown in Table 3. The pattern of *S. typhimurium* excretion by the chickens fed on this diet or on the control diet throughout the experiment resembled those observed in the previous experiments. The rate and amount of *S. typhimurium* excretion by the chickens fed on the avoparcin-containing diet from -3 to 11 days after infection ($-3 = 0$ days of age) decreased to that of the chickens fed on the control diet by the 37th day and remained low at the two subsequent examinations. Substantially similar results were obtained with a group of chickens fed on the avoparcin-containing diet from -3 to 4 days

Table 3. *The isolation of* Salmonella typhimurium *from the faeces of groups of* 33 *experimentally-infected chickens fed on diets containing* 100 *mg/kg of avoparcin for different periods of time*

Time (days)	% of chickens from which *S. typhimurium* was isolated when fed on diets containing avoparcin for the following days after infection																			
	−3* to 11				−3 to 44				−3 to 51				21 to 51				None			
	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T
9	12	58	100	100	12	58	100	100	18	70	94	94	6	48	91	97	0	24	85	100
16	12	55	94	100	30	64	97	100	12	45	88	100	0	15	70	91	0	3	33	73
23	3	6	33	73	27	64	85	97	21	70	100	100	0	3	36	40	0	3	21	39
30	0	0	15	36	42	67	88	100	6	33	85	91	15	45	91	94	0	0	12	39
37	0	0	3	15	9	39	88	97	6	48	79	91	6	67	88	97	0	0	3	30
44	0	0	0	3	0	30	85	97	3	39	94	100	0	21	88	100	0	0	3	6
51	0	0	0	9	12	21	85	100	15	33	82	94	12	39	88	100	0	0	3	12
51 (Caeca)	0	0	0	3	85	100	100	100	97	97	97	97	91	91	91	100	0	0	3	3

* −3 = From 0 days of age, 3 days before they were infected. For other details and abbreviations see Table 1.

Table 4. *Concentration of* Salmonella typhimurium *organisms in the faeces of groups of* 66 *chickens fed on diets containing arsenic preparations, dimetridazole, amprolium or monensin*

Time (days)	% of chickens whose faeces had the following concentrations of *S. typhimurium* when fed on diets containing																	
	Arsenilic acid at 250 mg/kg			Phenylarsenate* at 446 mg/kg			Dimetridazole at 150 mg/kg			Amprolium at 125 mg/kg			Monensin at 100 mg/kg			No additives		
	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T
9	29	70	79	11	61	71	45	79	82	9	65	76	17	62	71	29	65	70
16	12	38	58	30	58	70	35	65	73	5	56	83	2	21	64	11	52	65
23	9	30	41	2	17	35	5	14	21	17	42	53	8	18	23	8	21	29
30	11	27	33	2	15	29	5	9	17	6	20	23	6	12	14	5	9	20
37	5	18	30	2	14	18	3	14	20	3	12	14	0	0	2	0	2	8
44	0	6	20	0	6	17	0	12	18	2	6	8	0	2	3	0	2	3
51	0	5	9	0	3	8	0	3	3	2	2	3	0	0	2	2	2	2
56	0	0	2	0	2	9	0	0	3	2	2	5	0	2	2	0	0	2
56 (Caeca)	2	3	5	5	11	15	5	8	8	3	5	6	3	3	6	2	2	5

* Monosodium-3-nitro-4-hydroxyphenylarsenate. For other details and abbreviations see Table 1.

after infection (not shown in Table 3). Removing avoparcin from the diet one week before the conclusion of the experiment (−3 to 44 days in Table 3) had no effect on the pattern of *S. typhimurium* excretion; the faeces and caecal contents of the chickens in this group were as heavily infected at the final examination (51 days) as the chickens fed on this diet throughout. In the group that received the control diet for the first 21 days after infection and the avoparcin-containing diet thereafter, the pattern of *S. typhimurium* excretion during the 21 day period resembled that in the control group but afterwards the rate and amount of excretion increased greatly and came to resemble that in the group fed on the avoparcin-containing diet throughout.

Arsenic preparations, dimetridazole, amprolium and monensin

The effect on *S. typhimurium* excretion of feeding diets containing arsenilic acid, monosodium-3-nitro-4-hydroxyphenylarsonate, dimetridazole, amprolium and monensin at the concentrations commonly used commercially is summarized in Table 4; each diet and the control non-medicated diet was fed to two groups of chickens, the results for each pair of groups being amalgamated in the table because they resembled each other closely. None of the five additives had much influence on the pattern of *S. typhimurium* excretion and at 56 days after infection very few chickens in any of the groups were still excreting *S. typhimurium* organisms in their faeces.

In an additional experiment, four groups of 33 chickens were fed on the diet containing 250 mg/kg of arsenilic acid, four on a diet containing 250 mg/kg of sodium arsenilate and four on the control non-medicated diet. Two of each of the four groups given one or other of the arsenic-containing diets were given them from hatching time and the other two were given them from immediately after they were infected, at 4 days old. The pattern of *S. typhimurium* excretion observed in all twelve groups was similar, resembling those observed in control chickens in previous experiments. When they were killed 51 days after the start of the experiment, the percentage of chickens in the four arsenilic acid-fed groups with *S. typhimurium* in their faeces and caecal contents was 0, 3, 6 and 36 and 0, 6, 9 and 13 respectively. The corresponding figures for the sodium arsenilate-fed groups were 0, 0, 19 and 24 and 0, 3, 6 and 9 and for the control groups were 0, 3, 6 and 6 and 0, 0, 6 and 13 respectively.

The excretion of Salmonella typhimurium *by groups of chickens in contact with experimentally-infected chickens and fed on diets containing different additives*

The results of examining the faeces and caecal contents of groups of 28 chickens kept from hatching time in the same pen as five chickens experimentally-infected with *S. typhimurium* when four days old is summarized in Table 5; each group of chickens were fed continuously on diets containing different additives. *S. typhimurium* was usually isolated more often and in higher concentration from the faeces of the chickens fed on diets containing nitrovin, tylosin, lincomycin and dimetridazole than from the faeces of the chickens fed on the non-medicated control diet. Once infected, the chickens in these groups usually remained carriers for longer

Table 5. *The isolation of* Salmonella typhimurium *from the faeces of groups of* 28 *chickens in contact with five chickens infected with this organism and fed on diets containing different additives*

Time (days)	% of chickens from which *S. typhimurium* was isolated when fed on diets containing																							
	Nitrovin at (mg/kg)				Tylosin at (mg/kg)				Lincomycin at (mg/kg)				Dimetridazole at 150 mg/kg		Monensin at 100 mg/kg		Amprolium at 125 mg/kg		Arsenilic acid at 250 mg/kg		No antibiotics*			
	100		10		100		10		100		10										Group 1		Group 2	
	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
2	0	4	0	0	0	0	0	0	4	4	0	0	4	4	4	4	0	4	0	0	0	0	0	0
4	4	7	7	7	0	0	0	0	0	0	0	0	7	18	14	18	7	7	0	4	11	25	7	7
7	32	40	14	14	4	7	18	18	4	7	4	11	42	50	21	40	11	11	7	7	25	32	7	25
9	65	78	29	40	21	25	29	36	29	36	14	21	61	75	32	46	7	14	4	14	21	32	25	36
15	65	90	29	57	40	54	29	40	54	54	36	40	82	93	50	54	11	14	11	14	21	29	21	46
22	36	68	42	45	57	65	42	42	54	57	25	32	78	82	40	46	7	14	4	7	18	21	25	32
29	40	57	11	21	32	40	36	46	21	50	25	40	14	32	4	21	0	11	0	4	4	18	4	7
36	18	21	29	36	14	18	25	29	40	50	11	18	11	11	7	11	0	7	0	0	4	11	0	0
43	7	25	7	14	4	7	11	36	4	29	4	11	14	18	0	0	4	4	0	0	0	4	0	0
50	14	32	7	14	14	29	7	14	18	25	7	11	7	14	0	0	0	0	0	0	0	0	0	0
50 (Caeca)	67	70	52	70	26	41	44	59	56	67	22	26	36	40	11	11	0	4	14	18	0	4	0	0

The five chickens were infected when they were 4 days old and in contact with the non-infected chickens.

* Two groups were given non-medicated food. For other details and abbreviations see Table 1.

periods than the control chickens did – this is well illustrated by the results of the examinations of caecal contents. In contrast, *S. typhimurium* was isolated less frequently from the faeces of the chickens fed on diets containing amprolium or sodium arsenilate than from the control chickens; there was little difference between the results for the control chickens and those for the monensin-fed chickens.

The above experiment was repeated on several occasions, the effect of one or two additives being compared with that of the control diet on each occasion; the results of these experiments are described below.

Nitrovin and tylosin

Two groups of chickens in each of two experiments were given the control diet, two the 100 mg/kg tylosin diet, two the 10 mg/kg tylosin diet, two the 100 mg/kg nitrovin diet and four the 10 mg/kg nitrovin diet. The pattern of *S. typhimurium* excretion observed in the eight groups given the 100 or 10 mg/kg tylosin diets and in the four groups given the 100 mg/kg nitrovin diet resembled that shown in Table 5, the organisms being excreted at higher rates and in greater amounts by the chickens in these groups than by the chickens in the four control groups. The chickens in most of the groups fed on the 10 mg/kg nitrovin diet also became more heavily infected than the control groups but the results for some of them were little different from those for the control groups. When the two experiments were concluded, 37 days after their start, the percentages of faecal excreters in the four groups fed on the control diet were 4, 7, 12 and 18; in the eight groups fed on the 10 mg/kg nitrovin diet, 8, 12, 14, 23, 24, 32, 45 and 58; in the four groups fed on the 100 mg/kg nitrovin diet, 71, 82, 93 and 96; in the four groups fed on the 10 mg/kg tylosin diet, 56, 56, 59 and 83; and in the four groups fed on the 100 mg/kg tylosin diet, 52, 65, 78 and 78. The corresponding figures for the caecal examinations were 12, 15, 15 and 18 (control diet), 7, 28, 42, 46, 56, 59, 69 and 100 (10 mg/kg nitrovin diet), 89, 96, 100 and 100 (100 mg/kg nitrovin diet), 89, 96, 100 and 100 (10 mg/kg tylosin diet) and 93, 93, 96 and 100 (100 mg/kg tylosin diet).

Lincomycin

In one experiment two groups of chickens were fed on the 100 mg/kg lincomycin diet, two on the 10 mg/kg diet and two on the control diet. *S. typhimurium* spread more extensively than usual amongst the chickens in the control groups but the infections that developed in them were lighter than those that developed in the lincomycin-fed chickens. When the experiment was concluded at 31 days, the percentage of chickens from whose faeces *S. typhimurium* was isolated was 50 and 61 in the control groups, 78 and 88 in the 10 mg/kg lincomycin groups and 74 and 88 in the 100 mg/kg lincomycin groups. The corresponding figures for their caecal contents were 81 and 96, 96 and 96, and 93 and 96 respectively; the percentage of chickens whose caecal contents yielded more than 50 *S. typhimurium* colonies on the culture plates used for their isolation was only 22 and 35 in the control groups

Table 6. *The isolation of* Salmonella typhimurium *from the faeces of groups of 28 chickens in contact with five chickens infected with this organism and fed on diets containing avoparcin or arsenilic acid*

	% of chickens from which *S. typhimurium* was isolated when fed on diets containing																	
	Avoparcin at 10 mg/kg						Arsenilic acid at 250 mg/kg						No additives					
	Group 1			Group 2			Group 1			Group 2			Group 1			Group 2		
Time (days)	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	7	18	4	7	11	0	0	0	0	0	0	4	21	25	4	11	21
7	7	43	46	4	11	14	0	0	0	4	4	7	21	36	40	7	21	29
9	43	68	82	4	32	32	0	0	4	0	11	14	7	43	57	0	21	32
13	7	43	79	11	32	32	4	4	4	7	14	14	29	57	65	7	25	36
20	29	61	71	18	50	61	4	7	7	4	25	25	18	65	75	4	18	40
28	21	65	82	0	57	90	4	7	7	0	7	21	0	21	32	0	25	40
36	46	86	100	29	93	100	0	4	7	0	0	4	4	14	25	0	11	32
44	43	54	90	43	78	90	0	0	0	0	4	14	0	7	18	0	4	4
49	36	90	93	29	65	82	0	0	4	0	0	11	0	0	11	0	0	7
54	4	25	54	4	21	46	0	0	0	0	0	0	0	0	0	0	0	0
54 (Caeca)	50	90	90	90	100	100	4	4	18	0	0	0	0	0	0	0	0	0

For other details and abbreviations see Tables 1 and 5.

whereas in the groups fed on the 10 mg/kg lincomycin diet they were 85 and 93 and in the groups fed on the 100 mg/kg lincomycin diet they were 79 and 93.

Dimetridazole and amprolium

In one experiment four groups of chickens were fed on the diet containing dimetridazole, four on the diet containing amprolium and four on the control diet. As previously found (Table 5), *S. typhimurium* spread more rapidly and extensively in the dimetridazole groups than in the control groups, the infections that developed in the chickens in the dimetridazole groups being heavier and of longer duration than those that developed in the chickens in the control groups. In contrast, the pattern of *S. typhimurium* spread and excretion in the amprolium groups closely resembled that in the control groups. On the 30th day of the experiment, the percentage of chickens in the dimetridazole, amprolium and control groups that were excreting *S. typhimurium* in their faeces was 93, 93, 96 and 100, 4, 18, 18 and 29, and 4, 14, 14 and 29 respectively. On the 50th day, when the experiment was concluded, the corresponding figures were 11, 25, 29 and 75, 4, 4, 7 and 11, and 4, 4, 4 and 11 respectively. The percentage of chickens that were harbouring *S. typhimurium* in their caeca at this time was 41, 64, 67 and 100 (dimetridazole groups), 0, 4, 4 and 18 (amprolium groups) and 0, 0, 4 and 4 (control groups).

Arsenilic acid and avoparcin

The results of an experiment comparing the diet containing arsenilic acid, a diet containing 10 mg/kg of avoparcin (not included in the studies referred to in Table 5) and the control diet are summarized in Table 6; randomly-selected culture plates used for examining the caecal contents of some of the chickens at the end of the experiment are illustrated in Plate 2. The *S. typhimurium* infection spread throughout both avoparcin-fed groups, the infections that developed in the chickens in these groups usually being heavier and more persistent than those that developed in the chickens in the control groups. As previously found (Table 5), a smaller proportion of the chickens fed on the diet containing arsenilic acid became infected than was the case in the control groups and these infections were usually lighter and persisted for a shorter period of time. At the end of the experiment, *S. typhimurium* was not isolated from the faeces of any of the arsenilic acid-fed chickens or the control chickens but it was isolated from the faeces of about half of the avoparcin-fed chickens; furthermore, the caecal contents of most of the latter were heavily infected.

A further eight groups of chickens fed on the arsenilic acid-containing diet were compared with a similar number of groups fed on the control diet (Table 7). At all nine examinations performed before the 30th day, when the experiment was terminated, it was usual to find a lower proportion of faecal excreters of *S. typhimurium* in the arsenilic acid-fed groups than in the control groups; no spread of infection at all was observed in two of the arsenilic acid-fed groups. Only 22 % of the 216 chickens in the arsenilic acid-fed groups were found to be excreting *S. typhimurium* in their faeces on at least one of the nine examinations whereas 73 % of the 216 chickens in the control groups were. At the termination, *S. typhi-*

Table 7. *The isolation of* Salmonella typhimurium *from the faeces of groups of 28 chickens in contact with five chickens infected with this organism and fed on diets containing 250 mg/kg of arsenilic acid*

Dietary additive	Group no.	% of chickens that were excreting *S. typhimurium* in their faeces on the following days after exposure								
		2	4	7	9	11	16	18	22	29
Arsenilic acid	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	4	0	0	4	11	7	11	7
	4	0	0	0	7	14	14	11	4	0
	5	0	7	7	0	7	11	4	14	0
	6	0	0	7	14	18	32	25	32	14
	7	0	4	18	18	32	32	36	11	11
	8	4	4	18	25	29	25	36	29	4
		(1)*	(2)	(6)	(8)	(13)	(16)	(15)	(13)	(4)
None	9	0	0	4	11	7	25	32	39	21
	10	0	7	7	4	11	29	32	32	0
	11	4	7	11	11	11	32	14	43	18
	12	4	4	7	32	11	46	25	71	7
	13	0	7	36	32	39	32	36	25	11
	14	4	7	32	32	18	25	61	57	25
	15	4	36	64	89	79	71	54	25	4
	16	0	32	79	89	93	75	64	39	7
		(2)	(13)	(30)	(38)	(33)	(42)	(40)	(42)	(12)

* The figures in brackets refer to the % of chickens in all eight groups that were infected. For other details and abbreviations see Tables 1 and 5.

murium was isolated from the caecal contents of 16% of the chickens in the control groups but from only 6% of the chickens in the arsenilic acid-fed groups. Two groups of chickens fed on a diet containing 446 mg/kg of monosodium-nitro-hydroxyphenylarsenate were included in this experiment; their pattern of salmonella excretion resembled that of the control rather than that of the arsenilic acid-fed groups.

In additional experiments, the five chickens that were the source of infection for the other 28 chickens in the same group were infected when 3 instead of 4 days old. The inhibitory effect of arsenilic acid on the spread of infection was again noted – 10 days after the start of the experiment, 13% of the chickens in a group fed on the arsenilic acid-containing diet were excreting *S. typhimurium* in their faeces compared with 88% of a group fed on the control diet. The effect, however, was not noted in experiments in which the five chickens were infected at 2 days old. Feeding the arsenilic acid-containing diet, too, had no effect in controlling the high mortality that the strain of *S. typhimurium* used in these studies produces in chickens infected orally in their first day of life – 85% of a group of 34 chickens infected at this time and fed on the arsenilic acid-containing diet died compared with 97% in a group similarly infected but fed on the control diet.

DISCUSSION

The use of feed additives in animal husbandry is now widespread and the practice is likely to become more common and the kinds of additives employed more numerous. Particularly because of their anti-bacterial activity, some of these additives may possess harmful side effects and these should be identified. Favouring the colonization of the alimentary tract of chickens by salmonellas is an important one because chickens are commonly regarded as the main source of the salmonellas that cause food poisoning in man, a subject which, because of its emotive nature has implications extending beyond human health to impinge on the economic well-being of the poultry industry itself.

The results of the studies in which all the chickens in each group were infected experimentally with *Salmonella typhimurium* organisms indicated that avoparcin and lincomycin favoured the colonization of the alimentary tract by these organisms in that chickens fed on diets containing these antibiotics excreted them in greater amounts and for longer periods than did chickens fed on the non-medicated diet. In this respect they resemble nitrovin and tylosin (Smith & Tucker, 1975*b*). Avoparcin, particularly, resembled nitrovin in that the feeding of diets containing 100 mg/kg of antibiotics always favoured colonization by *S. typhimurium* whereas the reduction of the antibiotic concentration to 10 mg/kg sometimes failed to do so or did so to a lesser extent. A possible explanation for this is that the chickens used in all the experiments did not have the same kind of salmonella-antagonizing or salmonella-competing organisms in their caeca, the principal site of salmonella colonization, those in the chickens in the experiments in which the diets containing 10 mg/kg of nitrovin or avoparcin favoured salmonella colonization being more sensitive to these antibiotics than those in the chickens in the experiments in which these diets had little or no effect on colonization. Strong evidence that antagonizing or competing organisms were playing an important part in controlling salmonella colonization was provided by the experiment in which groups of chickens were fed on a diet containing 100 mg/kg of avoparcin for different periods (Table 3), the institution of this diet 21 days after infection being accompanied by a recrudescence in the rate and amount of faecal excretion of *S. typhimurium*. Because the group that were fed on the avoparcin-containing diet until the 49th day after infection were still excreting high concentrations of *S. typhimurium* organisms in their faeces one week later suggests that in practice a withdrawal period of one week before slaughter would do little to decrease the carcase contamination rate.

In the studies in which only five chickens in each group were experimentally infected, a procedure adopted because it probably more closely simulates natural outbreaks of salmonella infection, nitrovin, tylosin, lincomycin and avoparcin favoured a more rapid spread of infection to the in-contact chickens who then remained infected for longer periods and excreted higher concentrations of *S. typhimurium* organisms in their faeces than chickens fed on the non-medicated diets. This was not unexpected in view of the results obtained when all the chickens in each group were experimentally infected. Many of the chickens fed on the diets

containing these additives were still excreting *S. typhimurium* when they were 56 or so days old, the age at which broiler chickens are normally slaughtered for human consumption. These studies also revealed that feeding diets containing dimetridazole favoured colonization of the alimentary tract by *S. typhimurium* and feeding diets containing arsenilic acid hindered it – an effect that was not noticed in the studies in which all the chickens were experimentally-infected. To attribute these findings to the activity of the alimentary flora it is necessary to postulate that arsenilic acid and dimetridazole have no significant effect on the caecal flora but that they do bring about alterations in the flora of other regions of the alimentary tract sufficient to influence the small numbers of *S. typhimurium* that gain access to the tract of 'in-contact' chickens but not to influence the large numbers given to the experimentally-infected chickens. The alterations in the case of arsenilic acid, which is devoid of anti-salmonella activity *in vitro*, would be detrimental to *S. typhimurium* survival and in the case of dimetridazole would be beneficial to survival. Support for the view that arsenilic acid was acting in this manner was provided by the observation that it had no controlling effect on the spread of *S. typhimurium* in chickens in the first few days of life, a time when their normal alimentary flora is not completely established (Smith, 1965).

The experiments reported in this and our previous paper (Smith & Tucker, 1975*b*) have been concerned with the effect of feed additives on one strain of salmonella in one strain of chicken maintained under one method of management and it is conceivable that different results would be obtained under different conditions. Nevertheless they strongly suggest that, unless convincing evidence can be provided that those growth promoters that favoured the colonization of the alimentary tract with *S. typhimurium*, such as avoparcin, lincomycin, nitrovin and tylosin, have important advantages over others that did not, such as bacitracin and virginiamycin (Smith & Tucker, 1975*b*), their use in chickens should be discouraged. Those that should be sought in the future should ideally resemble arsenilic acid in hindering the development of salmonella infection. The position of dimetridazole is different from that of the growth promoting feed additives in that it is generally regarded as the most satisfactory agent currently available for controlling histomoniasis in turkeys, an economically important disease. This, obviously, should be given serious consideration before advocating a policy which would discourage its use for this particular purpose.

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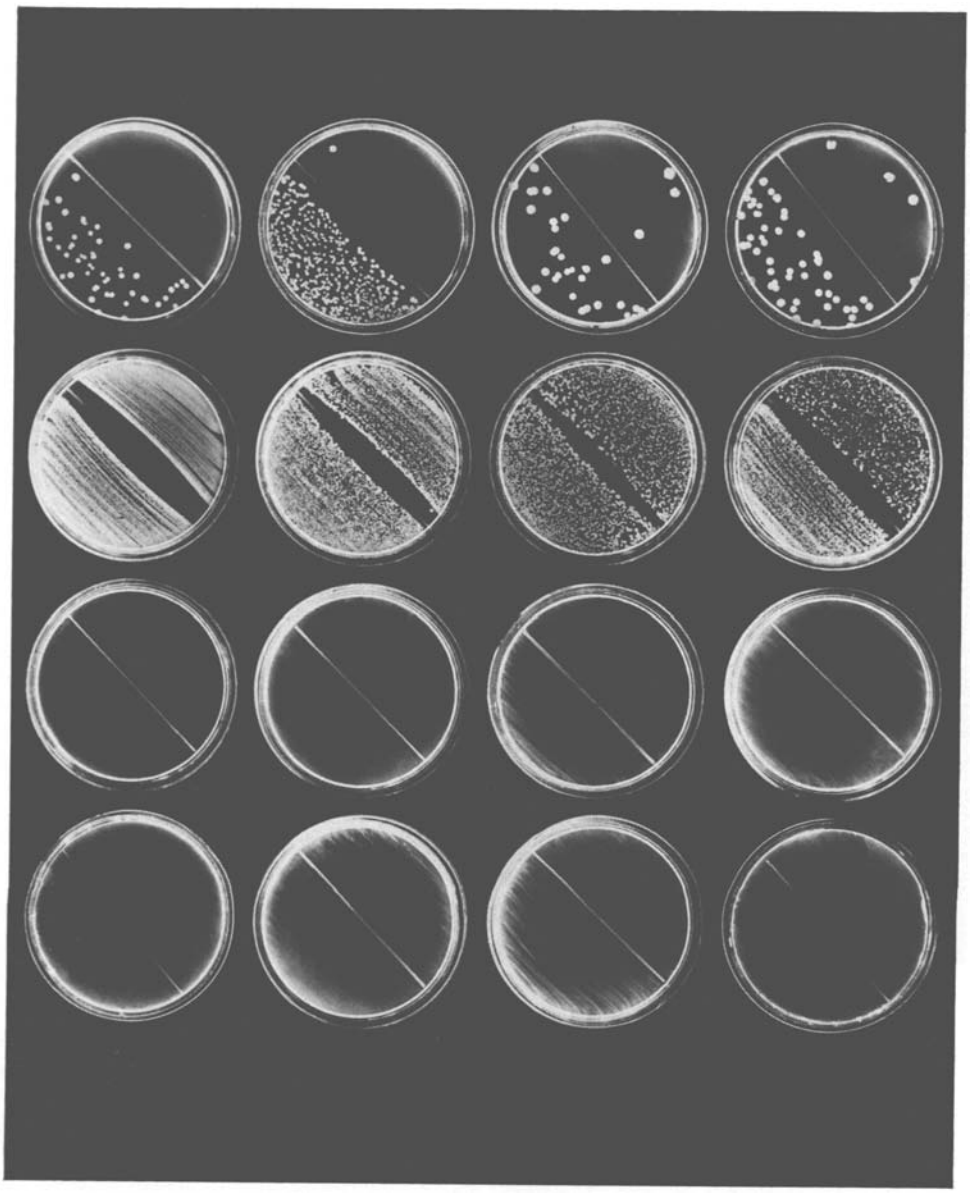

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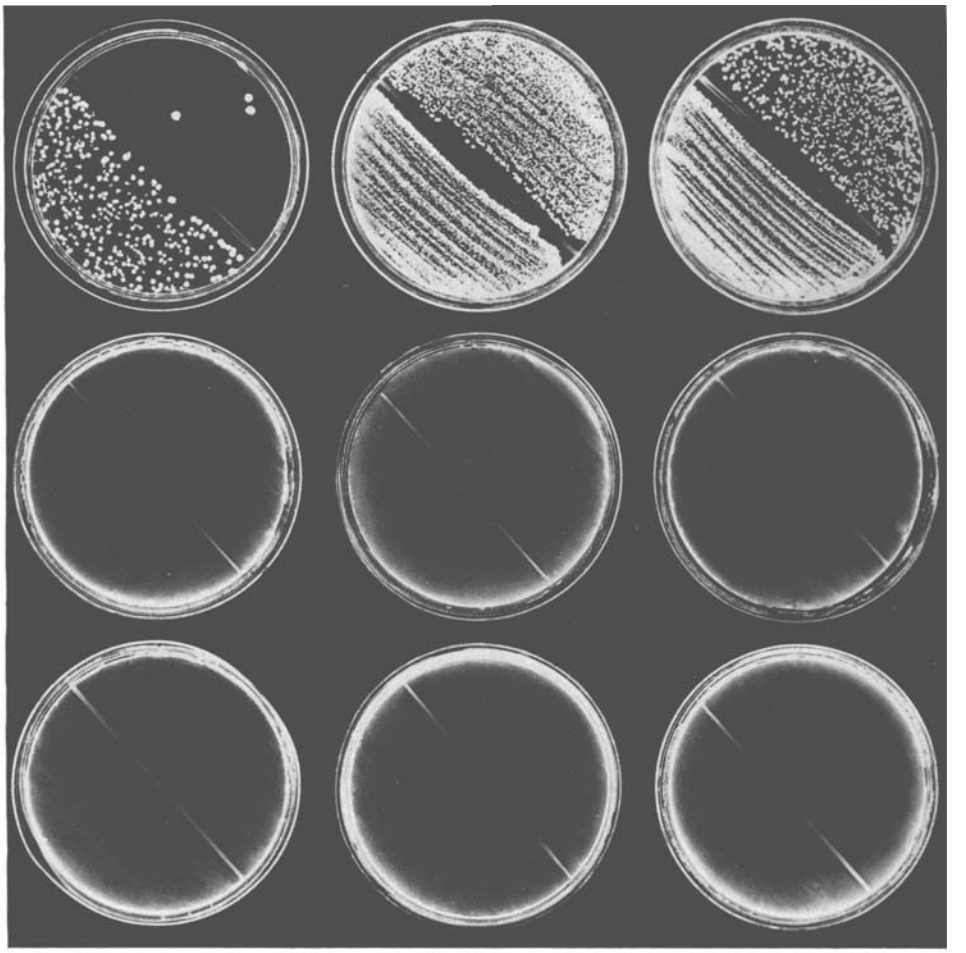

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EXPLANATION OF PLATES

PLATE 1

Culture plates used for examining swabs of faeces and caecal contents of chickens 55 days after they had been infected with *Salmonella typhimurium*.

The plates on the top (faeces) and second (caecal contents) rows had been inoculated with swabs, two per plate, from eight chickens that had been fed on the diet containing 10 mg/kg of avoparcin; those on the third (faeces) and bottom (caecal contents) rows were from eight chickens that had been fed on the non-medicated diet. All the bacterial growth on the plates is of *S. typhimurium*.

PLATE 2

Culture plates used for examining caecal swabs of chickens, 50 days after they had been in-contact with chickens experimentally-infected with *Salmonella typhimurium*.

The plates on the top row had been inoculated with swabs, two per plate, from six chickens fed on the 10 mg/kg avoparcin diet. Those on the second row were from chickens that had been fed on the 250 mg/kg arsenilic acid diet and those on the bottom row from chickens fed on the non-medicated diet. All the bacterial growth on the plates is of *S. typhimurium*.