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PREVENTION OF SALMONELLA INFECTION BY CONTACT USING INTESTINAL FLORA OF ADULT BIRDS AND/OR A MIXTURE OF ORGANIC ACIDS

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ABSTRACT

This study was carried out to assess the ability of competitive exclusion and a mixture of organic acids to prevent *Salmonella* infection by contact between newly hatched chicks. A bird infected with *Salmonella* was placed in a box containing non-infected birds, previously treated with a broth culture of faeces of adult birds (CE) and/or a mixture of organic acids. The number of *Salmonella* organisms in the caeca of the contact birds was estimated at 4 and 8 days post-challenge. The birds were infected with *Salmonella* Typhimurium, *Salmonella* Enteritidis (both repeated 5 times), *Salmonella* Agona and *Salmonella* Infantis (3 repetitions). The same approach was used to test the mixture of organic acids alone. In this case the birds received feed containing 0.8% of a mixture of formic acid (70%) and propionic acid (30%). Also, a third trial was carried out with birds inoculated with the broth culture of faeces and fed with feed containing the mixture of organic acids. Appropriate controls were included. Whereas the birds from the control groups and the groups treated with the mixture of organic acids were heavily infected with *Salmonella*, those pre-treated with CE or CE plus the mixture of organic acids had no viable cells per gram of caecal contents.

Key words: *Salmonella*, competitive exclusion, organic acids, by-contact infection, poultry

INTRODUCTION

The perception of *Salmonella* infection as a problem to the poultry industry has changed as the industry itself has changed. In many countries Pullorum disease and fowl typhoid are largely under control although this is not the case in many countries that have only recently intensified their industries and in those in which the high ambient temperature indicates problems with environmental hygiene (10). Avian salmonellosis, not caused by *S.* Pullorum and *S.* Gallinarum, is named paratyphoid infection, and may be caused by many serotypes of *Salmonella*. This disease has an important economic impact, as it may affect the animal performance and provoke morbidity and mortality in younger birds. In addition, paratyphoid salmonellosis may result in food-borne disease in humans, due to the ability to colonise the alimentary tract of the chicken, resulting in carcass contamination. From the eighties there was a dramatic increase of outbreaks of food-borne salmonellosis caused by *S.* Enteritidis (28, 35, 36). Poultry products were considered the main means of transmission. According to Lister (19), the bacterium was transmissible vertically. Following infection of young birds, the bacterium may be disseminated throughout the environment of the poultry house making its control very difficult. Davies and Wray (8) demonstrated the presence of *Salmonella* in a poultry house after depopulation and cleaning and disinfecting. The authors demonstrated that rodents present in the house had in all probability re-contaminated the house.

Poultry are much more susceptible to *Salmonella* infection in the beginning of life. By preventing infection among young birds, including those that are infected by vertical transmission,

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the frequency of infection throughout the life of the bird may be reduced (6). Control of *Salmonella* infection in birds may be approached in several ways. Antibiotic therapy is very simple and is used extensively. However, it may not eliminate *Salmonella*, may actually increase excretion and also may select for resistance (9, 13, 31). Nurmi and Rantala (25) initiated a new approach of controlling *Salmonella* in birds by administrating to young birds a broth culture of faeces obtained from adult birds. This method, known as competitive exclusion (CE), is adopted in many countries (22, 23) using either an anaerobically (12, 21, 29, 33) or aerobically (27) incubated culture. In view of the worsening situation in recent years regarding the *Salmonella* problem in both humans and poultry production this study was carried out to assess the administration of aerobic broth culture of faeces from adult birds to prevent *Salmonella* in young birds placed together with infected (seeder) birds.

MATERIALS AND METHODS

Bacterial strains

The work was carried out with *Salmonella* Typhimurium*, S.* Infantis*, S.* Agona and *S.* Enteritidi*s* strains resistant to nalidixic acid and spectinomycin (Nal^rSpec^r). Broth cultures were prepared in Brain Heart Infusion (BHI) broth incubated at 37°C for 24hs in a shaking incubator (100 strokes/ min.).

Faecal culture (CE)

Faeces from adult birds, reared in the FCAV-UNESP – Jaboticabal, São Paulo State, were inoculated into BHI broth (1:10) that was incubated statically in air at 37°C for 24hs. The culture was tested for *Salmonella*. The birds were inoculated into the crop with 0.1 ml of a culture diluted a thousand fold.

Acid treatment of the feed

A mixture of formic acid (70%) plus propionic acid (30%) was added into the feed at the level of 0.8%.

Birds

Newly hatched chicks from a commercial broiler hatchery were used, six birds per box. Feed with no added antibiotics or other additives and drinking water were both available ad libitum, and also was provided a heat source. Swabs from cloacae and from transport boxes of the birds were taken to assess they were free of *Salmonella*.

Experimental procedure

The experiment consisted of three groups with three to five repetitions. In one group the birds received 0.1ml of faecal culture broth. In the second group the birds were provided with feed containing the organic acid mixture and in the third group the birds were treated with 0.1ml of faecal broth culture together with in-feed organic acids. On the following day, an infected bird was placed inside each box containing six birds. This approach was carried out using as a challenge, *Salmonella* Typhimurium*, S.* Agona*, S.* Infantis*,* or *S.* Enteritidis. In a separate box, birds were inoculated with 0.1ml of *Salmonella* from an overnight broth culture diluted to contain approximately 10^5 colonies cfu/ml. The experimental design is present in Table 1.

Data of viable counts were transformed logarithmically ($\log_{10}$) and subjected to analysis of variance. Media were compared statistically by t test ($p < 0.05$).

Bacteriological examination

Four and eight days post-treatment three birds from each box were killed for an estimation of the viable numbers of

Table 1. Experimental Design

Groups	Repetitions	Treatments	Chalenge Strain
A_1	5	Feed With Organic Acids 0.8%.	*Salmonella* Typhimurium $Nal^r/Spec^r$
B_1	5	Faecal Culture.	
C_1	3	Feed with Organic Acids and Faecal Culture	
D_1	5	None	
A_2	3	Feed With Organic Acids 0.8%.	*Salmonella* Agona $Nal^r/Spec^r$
B_2	3	Faecal Culture.	
D_2	3	None	
A_3	3	Feed With Organic Acids 0.8%.	*Salmonella* Infantis Nal^r/Spec
B_3	3	Faecal Culture.	
D_3	3	None	
A_4	5	Feed With Organic Acids 0.8%.	*Salmonella* Enteritidis $Nal^r/Spec^r$
B_4	5	Faecal Culture.	
C_4	3	Feed With Organic Acids And Faecal Culture	
D_4	5	None	

Number of birds for repetition = 7.

Salmonella in the caecal contents. This was done by an adaptation of the method of Barrow *et al*. (3) using Brilliant Green agar containing sodium nalidixate (100 ug/ml) and spectinomycin (100 ug/ml).

RESULTS

Table 2 presents the results of the evaluation of the faecal culture either alone or associated with treatment with a mixture of organic acids in newly born birds challenged with *Salmonella* Typhimurium, *S.* Agona, *S.* Infantis, *S.* Enteritidis.

The contents of Table 2 show that the competitive exclusion treatment alone or associated with in-feed organic acids was able to completely prevent intestinal colonization by *Salmonella*. The use of organic acids did not interfere with the effectiveness of the CE but alone was not able to prevent the caecal colonization by all *Salmonella* serotypes.

DISCUSSION

There is a great deal of current concern about avian salmonellosis with regard to avian disease and particularly to food-poisoning salmonellae. The control of paratyphoid salmonellae in birds is a difficult task. Newly hatched chicks are highly susceptible to infection with *Salmonella* organisms. Infection at this time may follow hatchery infection. Infected young birds excrete *Salmonella* in the faeces in greater number and for longer than do chickens infected when they are older (4, 24, 32). This increase in resistance is explained by the gradual acquisition by the chickens of the microorganisms that constitute its normal intestinal microflora (1, 7, 26, 30). Attempts to increase the resistance of the newly hatched chick to a level observed in the older chicken have centred on the administration of cultures or suspensions of caecal contents or faeces obtained from healthy adult chickens.

The competitive exclusion system first proposed by Nurmi and Rantala (25) has been used and studied extensively over many years (22). Despite the fact that previous authors (11, 21) have suggested the necessity of anaerobic incubation this methodology does not fit in with the finding that CE mixtures may be administered to birds either by spray or in drinking water (22) or that broth cultures incubated aerobically may also be successfully (27). Static broth cultures would anyway develop very low oxygen tensions after facultative anaerobic bacteria reach stationary phase and there seems no reason why such cultures should be effective. The observation of an effect against *S.* Infantis, has been noted previously (25, 34). The present and previous findings are important because the outbreaks of food-poisoning salmonellae originating from hatchery derived birds acquire their infection by vertical transmission (19) and *Salmonella* organisms may spread very easily among birds in the first few days after hatch (10).

Table 2. Number (log_{10}) of viable cells of *Salmonella* Nal^r $Spec^r$ strains present in the caecal contents of the birds challenged 24 hours after the treatment with faecal culture and/or feed containing a mixture of organic acids.

Salmonella serotypes	Group	Log_{10} viable number of *Salmonella* organism Nal^r $Spec^r$ per gram of caecal contents.	
		4 day post-treatment	8 day post-treatment
Typhimurium	A_1	8.34* (6.41 - 9.36)	8.27* (5.0 - 9.63)
	B_1	N* (N - 4.46)	N* (N - N)
	C_1	N** (N - N)	N** (N - N)
	D_1	6.27* (N - 8.90)	5.05* (N - 8.96)
Agona	A_2	8.90** (8.04 - 9.50)	7.60** (N - 8.86)
	B_2	N** (N - N)	N** (N - N)
	D_2	8.24** (7.56 - 8.74)	7.87** (7.04 - 8.49)
Infantis	A_3	7.92** (6.80 - 8.61)	8.03** (7.56 - 8.46)
	B_3	N** (N - N)	N** (N - N)
	D_3	8.13** (7.53 - 8.70)	6.59 ** (5.68 - 7.08)
Enteritidis	A_4	3.26* (N - 9.53)	5.94* (N - 9.08)
	B_4	N* (N - N)	N* (N - N)
	C_4	N** (N - N)	N** (N - N)
	D_4	5.21* (N - 9.25)	3.08* (N - 7.46)

N = <2, groups A = treatment with a mixture of organic acids, groups B = treatment with faecal culture, groups C = treatment with faecal culture plus a mixture organic acids, groups D = control (no treatment)

* = The median count per gram is shown to fifteen birds in parenthesis,

** = The median count per gram is shown to nine birds in parenthesis.

The addition of organic acids into the feeds is used to varying degrees to control *Salmonella* in the feed (2, 17, 37). In the present study the incorporation of organic acid into the feed did not prevent the infection of birds placed in contact with an experimentally infected bird. Humphrey and Lanning (17) had partial success working with adult birds, but they were unable to prevent the vertical transmission. In contrast, Berchieri Jr. and Barrow (5) observed a good reduction in the mortality by *Salmonella* Gallinarum again, it was thought, by affecting the rate of infection between infected and uninfected birds. These data show that the results may depend upon the *Salmonella* serotype used as a challenge organism. The approach including organic acid mixture into the feed was carried out to assess its interference on the CE action. It was concluded that the organic acid mixture did not interfere with the CE action. The choice of the acids, the concentration and the method of administration in this research were based on previously published work (5, 14, 16, 17, 18, 20).

The presented results using the combination of organic acids in the ration and faecal culture are in agreement with Hinton *et al*. (15) who showed that this combination was effective.

In conclusion an aerobically cultured CE preparation is able to prevent intestinal colonization by *Salmonella* Typhimurium*, Salmonella* Infantis*, Salmonella* Agona*, Salmonella* Enteritidis in birds infected by contact, and organic acids incorporated in the feed do not interfere with the CE method.

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RESUMO

Controle da transmissão de *Salmonella* por contato entre aves de exploração comercial pelo uso de flora intestinal de aves adultas e/ou uma mistura de ácidos orgânicos

O presente trabalho avaliou a prevenção da disseminação de quatro sorotipos de *Salmonella,* de interesse em avicultura e saúde pública (*Salmonella* Typhimurium*, Salmonella* Agona*, Salmonella* Infantis e *Salmonella* Enteritidis), entre aves recém-nascidas, com o intuito de diminuir a disseminação de salmonelas em rebanhos avícolas por aves que contraíram a infecção pela via vertical. Analisou-se experimentalmente a administração de microbiota intestinal de aves adultas em aves recém-nascidas, a incorporação de uma mistura de ácidos orgânicos na ração e a associação desses dois tratamentos, em grupos onde colocou-se uma ave infectada, para provocar a transmissão por contato. A microbiota intestinal de aves adultas mostrou-se eficiente no controle da colonização cecal nos quatro ensaios realizados, com os diferentes sorotipos. A mistura de ácidos orgânicos na ração não foi eficaz em prevenir a colonização cecal e a associação dos tratamentos demonstrou que não há interferência entre eles, permanecendo a ação da microbiota de aves adultas.

Palavras-chave: *Salmonella*, exclusão competitiva, ácidos orgânicos, infecção por contato, aves comerciais

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