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Effect of *Lactobacillus salivarius* administration on microflora in the crop and caeca of broiler chickens

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ABSTRACT

A rifampicin-resistant *Lactobacillus salivarius* was isolated from chicken caeca and administered orally to newly hatched broiler chickens. The resistance to rifampicin enabled to differentiate the administered organism from indigenous strains. After inoculation, rifampicin-resistant lactobacilli dominated among lactobacilli in the crop and caeca of inoculated chickens. In two different experiments the inoculation by *L. salivarius* lowered count of enterococci in the crop in the whole experimental period (6 and 3 days in the first and the second trial, respectively). Counts of coliform bacteria in the crop were lowered to a smaller extent, in the first day in both trials only. Effects of *L. salivarius* administration on caecal counts other than lactobacilli were generally small. Four methods of *L. salivarius* administration were compared: live cells per os, lyophilized per os, live cells via drinking water and lyophilized cells added to the feed mixture. Rifampicin-resistant lactobacilli became established in the crop and caeca using any method of inoculation tested. Provision of lactobacilli decreased the pH of the chymus, especially in the first day of experiment.

KEY WORDS: chicken, probiotic, *Lactobacillus*

INTRODUCTION

The supplementation of feeds with pure or mixed cultures of live microorganisms (probiotics) can have benefitial effects on animal growth and health. The effects of probiotics were attributed to the production of antibacterial substance antagonistic to harmful bacteria, destruction of antinutritional factors, synthesis of vitamins, and provision of nutrients and digestive enzymes (Fuller, 1986).

A wide variety of probiotics for poultry have been introduced in the market. Most of them are various *Lactobacillus cultures*, which are thought to colonize the crop and the small intestine. Beneficial effects of lactobacilli administration on performance of chickens or laying hens were reported by Krueger et al. (1977), Adler and DaMassa (1980) and Arends (1981). Little is known, however, about the bacteriological basis of these effects. The aim of this work was thus to administer lactobacilli per os to newly hatched chickens and enumerate lactobacilli and several specific groups of bacteria in the crop and caeca. We also compared four different methods of lactobacilli administration.

MATERIAL AND METHODS

Probiotic organism isolation

Rifampicin-resistant lactobacilli were isolated from caeca of broiler chickens (Ross), 8 weeks of age, fed a commercial feed mixture BR 2, which contained ground maize, soyabean meal, fish meal and vitamin-mineral supplement, no antibiotics and coccidiostats. Plates of Rogosa agar with rifampicin (100 μg/ml) were used for the isolation. The strain 51R was chosen for further experimentation out of sixty isolates on the basis of its rapid growth in the diet and the stability of its resistance to rifampicin. The resistance to rifampicin, which is atypical among lactobacilli, was tested according to Pedersen and Tannock (1989). The growth of lactobacilli in the BR1 feed mixture (containing ground maize 60%, soyabean meal 25%, fish meal 10% and a vitamin-mineral supplement 2%, no antibiotics and coccidiostats were present) *in vitro* was checked by the method of Fuller (1973), modified by the authors. The diet was mixed with distilled water in the 1:2 ratio. The moistened feed was inoculated with a *Lactobacillus* strain to obtain 10^4 cfu/g and incubated anaerobically under an atmosphere of CO_2 at 42°C. At regular intervals the samples were removed and counts of rifampicin-resistant lactobacilli were assesed. The isolate was identified on basis of its fermentation characteristics, using the API 50 CH test (API Products, La Balme les Grottes, France). Tests of the bile tolerance were performed in the MRS medium (0.1-2.0 %). Adherence of bacterial cells to the crop epithelial cells was examined microscopically according to Fuller (1973).

Experiment 1

One hundred and twenty 1-day-old broiler chickens (Ross) were divided into two groups, 60 birds each, and housed separately in different buildings to avoid any transfer of microorganisms. Chicken had free access to the BR1 feed mixture.

The strain 51R was grown in the MRS medium for 24 h at 37°C. Culture (100 ml) was centrifuged at 14.000 rpm for 3 min and resuspended in isotonic saline solution (25 ml). Each 1-day-old chicken of the experimental group was given per os 0.2 ml of 51R strain suspension (8×10^8 cfu). Two chickens from each group were killed by cervical dislocation at 6, 9, 24, 50, 72 and 144 h after inoculation of the experimental group. The contents of the crop and caeca were collected aseptically, serially diluted in sterile MRS medium, and plated on Wilkins-Chalgren agar, Endo agar, kanamycin, esculin azide agar, Rogosa agar with rifampicin (100 μg/ml) to enumerate total anaerobes, coliforms, lactobacilli, enterococci and rifampicin-resistant lactobacilli, respectively. Bacteriological media were purchased from Oxoid. Plates were incubated aerobically at 37°C for 24 h (coliforms, enterococci) or anaerobically under CO_2/H_2 atmosphere at the same temperature for 48 h (other bacterial groups). The pH of the crop and caecal contents was measured immediately after the slaughter. Ten chickens were killed for this purpose from each group at 24, 48 and 72 h after inoculation of the experimental group.

Experiment 2

Two hundred and thirty 1-day-old broiler chickens (Ross) were used to compare different methods of lactobacilli administration. Chickens were randomly assigned to five groups and treated as follows: Group I, per os inoculation with suspension of live cells of the strain 51R, equal to 10^8 cfu per chicken (70 chickens); Group II, per os suspension of lyophilized cells, equal to 10^8 cfu per chicken (30 chickens); Group III, culture of the strain 51R supplied in the drinking water, diluted to 10^6 cfu/ml (30 chickens); Group IV, lyophilized cells added to the feed mixture in amount of 10^6 cfu/g (30 chickens) and Group C, control without treatment (70 chickens). Groups were thoroughly isolated to avoid any transfer of microorganisms.

Three chickens from each group were killed at 0, 3, 6, 12, 18, 24, and 72 h after the beginning of the experiment. The pH of the crop content was measured at 9, 12, 30, and 54 h and the caecal pH at 9, 30, and 54 h after the beginning of the experiment, in Groups I and C. Enumeration of microorganisms was performed as described above.

RESULTS

The strain 51R was identified as *Lactobacillus salivarius*, using results of the API test and criteria of Kandler and Weiss (1986). Its resistance to rifampicin was stable and did not disappear after nine passages in an antibiotic-free medium.

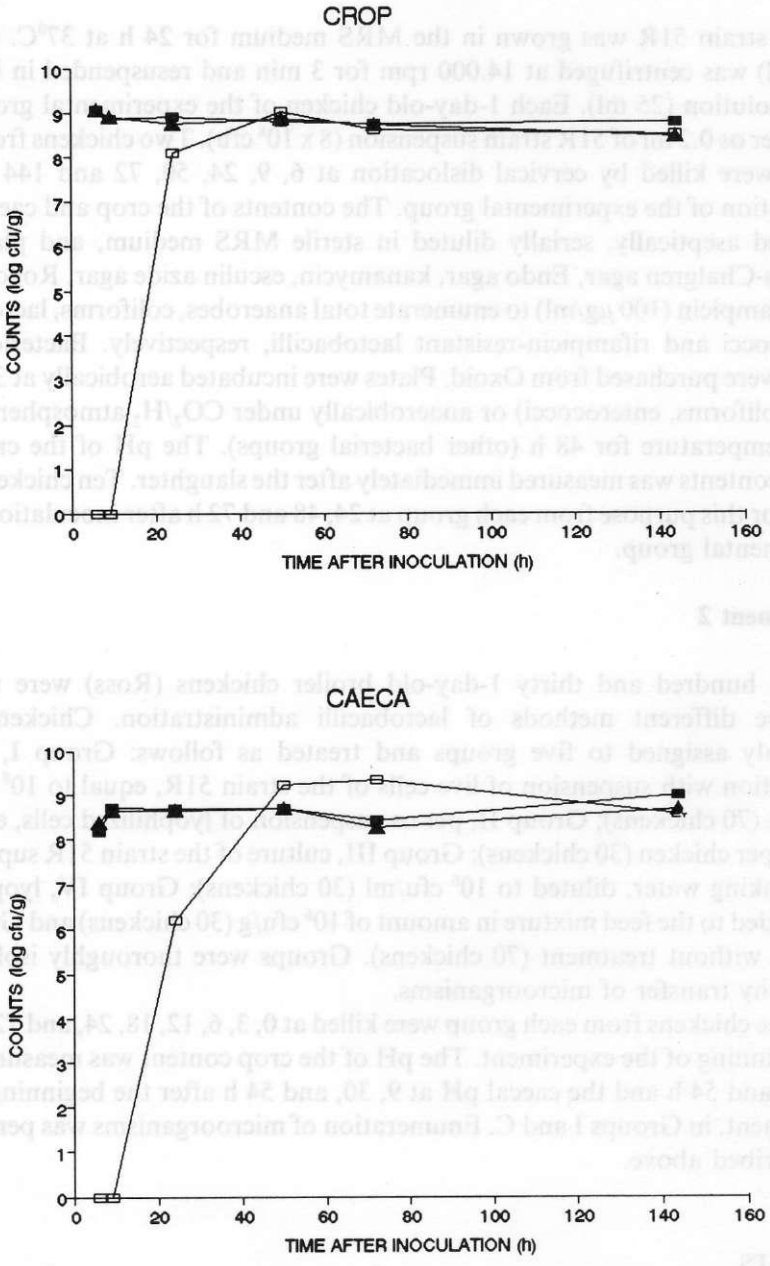

Figure 1. Counts of lactobacilli (LB) in the crop and the caeca of control (C) and inoculated (I) chickens. (LBR) lactobacilli resistant to rifampicin

—□— LB (C) —■— (LB) (I) —▲— LBR (I)

Bile at the concentration of 0.5% inhibited the growth of strain 51R in the MRS medium. Partial inhibition observed at bile concentration of 0.3%. The isolate did not adhere significantly to crop epithelial cells. Only 1-2 bacterial cells adhered per one epithelial cell were observed. The growth *in vitro* in the BR1 feed mixture rapid ($\mu = 1.54\ h^{-1}$) with a short lag period (0.57 h).

Table 1 presents counts of anaerobic bacteria, enterococci, and coliforms in the crop and caeca of chickens. Total viable counts of anaerobes in the crop were similar in both groups. Counts of enterococci were higher in the crop of control chickens. Counts of coliform bacteria were higher in the control group during the initial period of the life. The difference, however, disappeared at 50 h after inoculation of the experimental group. The crop of control chickens contained no lactobacilli in samples taken at 6 and 9 h after inoculation (Figure 1). The number of lactobacilli was the same in control and experimental chickens at 50 h after inoculation of the latter group. Whereas lactobacilli of the crop of experimental chickens were resistant to rifampicin, no rifampicin-resistant lactobacilli were found in the crop of control chickens.

Total viable counts of caecal anaerobes and coliforms were similar in both groups and generally higher than those in the crop (Table 1). Counts of enterococci were higher in the caeca of control chickens in samples taken at 6 and 9 h. Lactobacilli were absent in these samples in control chickens (Figure 1). Again, the differences disappeared at 50 h after inoculation of the experimental group. The rifampicin-resistant strain dominated among caecal lactobacilli of

TABLE 1

Counts of microorganisms in the crop and the caeca of control (C) and inoculated (I) chickens (Log_{10} cfu/g of chymus, mixed samples from two chickens)

Time after inoculation, h	Anaerobes		Enterococci		Coliforms	
	C	I	C	I	C	I
Crop contents[2]						
6	8.78	9.16	6.78	5.10	8.00	7.70
9	8.53	8.97	8.22	6.63	8.19	7.75
24	8.76	9.02	8.04	6.61	9.15	8.00
50	9.06	8.94	7.09	6.34	6.00	6.30
72	8.60	8.79	6.86	6.56	6.60	6.58
144	8.36	8.85	6.76	5.63	6.04	5.61
Caecal contents[2]						
6	9.39	9.56	6.78	4.45	8.00	8.00
9	9.79	9.66	10.00	6.27	8.51	8.43
24	10.17	10.04	8.92	9.08	11.03	10.57
50	10.03	9.94	8.51	8.42	9.71	9.92
72	9.79	9.36	8.72	8.96	8.88	8.68
144	8.93	9.10	7.88	7.08	8.67	8.77

TABLE 2

Counts of microorganisms in the crop of control (C) and inoculated (I-IV) chickens – comparison of four different ways of *L. salivarius* 51R administration (Log_{10} cfu/g of chymus, mixed samples from three chickens, Group I, per os live cells; Group II, per os lyophilized cells; Group III, live cells in drinking water; Group IV, lyophilized cells via feed mixture)

Treatment group	Time after inoculation, h							
	0	3	6	12	18	24	36	72
Lactobacilli								
C	0	3.04	4.24	5.18	3.6	9.02	9.29	8.44
I	0	8.77	8.92	8.69	8.47	8.68	8.92	8.47
II	0	8.24	8.51	7.71	8.43	8.75	8.76	8.54
III	0	6.55	7.87	8.49	8.69	8.80	8.92	8.73
IV	0	5.94	8.16	7.97	8.62	8.77	8.87	8.83
Rifampicin-resistant lactobacilli								
C	0	0	0	0	0	0	0	0
I	0	8.77	8.95	8.52	8.60	8.55	8.87	8.46
II	0	8.37	8.34	8.12	8.30	8.76	8.76	8.43
III	0	6.74	7.80	8.59	8.61	8.80	8.79	8.53
IV	0	5.98	8.05	8.33	8.64	8.72	8.74	7.95
Enterococci								
C	0	6.91	5.35	8.81	7.58	8.56	8.84	6.61
I	0	0	3.66	4.30	3.86	4.89	4.42	0
II	0	5.48	4.71	5.77	5.53	5.21	4.00	2.26
III	0	5.63	6.42	7.39	6.96	6.78	4.16	2.80
IV	0	4.87	7.46	7.43	6.78	7.35	5.88	3.32
Coliforms								
C	3.00	7.90	8.46	8.42	7.99	8.07	6.96	6.81
I	3.00	4.91	6.26	4.91	4.00	6.04	8.08	5.32
II	3.00	6.85	6.85	4.15	4.00	4.00	5.48	6.02
III	3.00	6.94	8.88	8.07	7.31	6.48	4.80	5.71
IV	3.00	5.30	7.50	7.41	5.60	6.65	5.44	4.89

inoculated chickens, whereas no rifampicin-resistant lactobacilli in the caeca of control chickens were found.

Table 2 present counts of lactobacilli, enterococci and coliform bacteria in the crop of chickens inoculated by *L. salivarius* 51R per os via drinking water or via feed mixture. Total viable counts of anaerobes were similar in all groups (data not shown) and approx, the same as shown in Table 1. Rifampicin-resistant lactobacilli prevailed in lactobacilli of inoculated chickens, whereas no lactobacilli of this type were present in the control group. Counts of enterococci were lower in all inoculated groups in comparison with the control. In groups III and IV this effect was not observed at 6 h after the beginning of the experiment. Also counts of coliform bacteria were lower in the crop of inoculated chickens in

TABLE 3

Count of microorganisms in the caeca of control (C) and inoculated (I-IV) chickens – comparison of four different ways of *L. salivarius* 51R administration

Treatment group	Time after inoculation, h							
	0	3	6	12	18	24	36	72
Lactobacilli								
C	0	0	2.60	3.86	3.60	8.75	9.34	8.85
I	0	8.46	8.84	9.62	9.72	9.62	9.03	8.31
II	0	7.89	7.19	7.96	8.95	9.85	8.94	8.35
III	0	5.23	4.74	7.08	7.94	8.94	8.85	9.08
IV	0	3.01	4.33	7.58	8.53	9.61	9.07	9.20
Rifampicin-resistant lactobacilli								
C	0	0	0	0	0	0	0	0
I	0	8.42	8.79	9.46	9.82	9.59	8.92	8.23
II	0	7.85	7.13	8.40	8.83	9.81	8.93	7.70
III	0	5.17	4.84	7.04	7.84	8.93	8.42	8.77
IV	0	2.41	4.35	7.59	8.54	9.53	8.70	8.78
Enterococci								
C	6.59	10.40	8.59	9.68	9.72	9.83	9.47	8.36
I	6.59	7.74	9.15	9.30	8.70	9.57	9.11	8.56
II	6.59	8.27	8.18	9.84	9.30	9.04	9.19	9.17
III	6.59	9.13	9.09	8.54	9.47	9.49	8.63	8.95
IV	6.59	7.93	9.27	8.98	9.45	9.64	9.28	9.49
Coliforms								
C	8.84	10.40	9.11	9.84	10.12	10.49	10.37	9.61
I	8.84	6.44	8.90	9.65	10.06	10.40	10.37	9.22
II	8.84	6.92	9.59	9.72	10.25	9.86	10.21	9.61
III	8.84	8.13	9.02	10.31	10.21	10.10	10.13	10.28
IV	8.84	6.19	8.18	9.88	10.34	10.03	10.17	9.13

See Table 2 for explanation

comparison with control, except of two samples. Table 3 summarizes data on counts of specific bacterial groups in the caeca of the same chickens. Again, there were no substantial differences in total viable counts of anaerobes among different groups (data not shown). Numbers of anaerobes were similar to those shown in Table 1. Lactobacilli in caecal contents of inoculated chickens were mostly resistant to rifampicin. Contrary to this, no rifampicin-resistant lactobacilli were detected in the caeca of control chickens. Counts of caecal enterococci and coliforms were lower in inoculated chickens, 3 h after inoculation only.

In the first experiment, inoculation produced a significant drop in the pH value of the crop content and caecal chymus in samples taken 24 and 48 h after *L. salivarius* 51R administration (Table 4). In the second experiment

TABLE 4

Values of pH in the crop and caeca of control (C) and inoculated (I) chickens

Time after inoculation, h	Crop contents[1]		Caecal contents[2]	
	C	I	C	I
Experiment 1				
24	5.38±0.37	4.83±0.38**	5.50±0.06	5.12±0.06**
48	4.70±0.23	4.97±0.25	6.15±0.35	5.50±0.33**
72	5.02±0.51	5.26±0.46	5.75±0.63	5.78±0.31
Experiment 2				
9	5.57±0.25	4.64±0.39**	5.56±0.21	5.64±0.51
12	5.42±0.24	4.48±0.06**	ND	ND
30	4.90±0.49	4.83±0.16	6.05±0.29	5.46±0.28**
54	4.77±0.43	4.76±0.14	5.74±0.58	5.17±0.22**

* significantly different from the control at $P \leq 0.01$, combined samples from ten (Experiment 1) and eight (Experiment 2) chickens

ND – not detected

L. salivarius administration significantly lowered the pH of the crop content at 9 and 12 h. Other differences were not significant.

DISCUSSION

Lactobacilli represent the major group of microorganisms in the digestive tract of chickens (Smith, 1965). A number of reports exists suggesting desirable effects of probiotic lactobacilli on the health and performance of poultry. Unfortunately, results of trials done by probiotics manufacturers have been published in commercial literature with little critical appraisal. Furthermore, in the majority of these trials only growth stimulation was measured, omitting any microbiological monitoring. Few reports on probiotic lactobacilli colonization and consequent microbiological changes in the digestive tract of poultry have been published in the scientific literature. Tortuero (1973) reported results of experiments in which *L. acidophilus* increased weight gains of chickens and counts of lactobacilli in the gut. The colonization of the chicken digestive tract by lactobacilli was described by Fuller (1973). The dosing with an intestinal strain of *Lactobacillus* suppressed the count of *E. coli* in the crop. The administration of *Sporolactobacillus sp.* improved weight gains of broiler chickens and reduced counts of staphylococci and coliform bacteria in large intestinal contents (Han et al., 1984). Results of Fuller (1973) and Han et al. (1984) suggest that the lactobacilli in the developing digestive tract of chickens exert a controlling effect on coliform bacteria. The increase in numbers of lactobacilli accompanied by a concurent reduction in numbers of coliform bacteria was observed also in

young calves fed milk containing *L. acidophilus* as a dietary adjunct (Gilliland et al., 1980).

The resistance of our isolate 51R to rifampicin represents a readily selectable phenotypic marker, which enables to differentiate administered lactobacilli from indigenous strains. Our results confirm the colonization of the digestive tract of newly hatched chickens with this probiotic organism, in spite of the fact that its adhesion to epithelial cells was negligible. Unfortunately, it was not possible to analyse our data statistically, as the number of samples was limited from technical reasons. However, the administration of *L. salivarius* lowered counts of enterococci and coliform bacteria in the crop and to some extent also counts of enterococci in caeca in two different trials. In both trials the establishment of lactobacilli was accompanied by the decrease of the pH of the crop contents. Oral provision of live bacteria seems to be the best methods of lactobacilli administration. The supplementation of feed by lyophilized "cells or addition of culture of *L. salivarius* to drinking water also gave good results.

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STRESZCZENIE

Wpływ podawania *Lactobacillus salivarius* na mikroflorę treści wola i jelit ślepych u kurcząt brojlerów

Wyizolowany z treści jelit ślepych kurcząt, odporny na rifampicynę, *Lactobacillus salivarius* podawano doustnie świeżo wyklutym kurczętom brojlerom. Odporność na rifampicynę umożliwiła odróżnienie podawanego mikroorganizmu od szczepów zasiedlających. Po inokulacji, lactobacilli odporne na rifampicynę dominowały wśród lactobacilli treści wola i jelit ślepych inokulowanych kurcząt. W dwóch doświadczeniach inokulacja *L. salivarius* spowodowała obniżenie liczby enterokoków w treści wola w ciągu całego okresu doświadczalnego (6 i 3 dni w pierwszym i drugim doświadczeniu, odpowiednio). Liczba bakterii coli w wolu zmniejszyła się w nieznacznym stopniu w pierwszym dniu obydwóch doświadczeń. Wpływ podawania *L. salivarius* na liczebność szczepów innych niż lactobacilli w jelitach ślepych był mały.

Porównano 4 metody podawania *L. salivarius*: żywe komórki *per os*, liofilizowane komórki *per os*, żywe komórki z wodą podawaną do picia oraz liofilizowane komórki dodane do paszy, i oznaczono w treści wola i jelit Lactobacilli odporne na rifampicynę. Wprowadzenie lactobacilli obniżało pH treści, specjalnie w pierwszym dniu doświadczenia.