

SUSCEPTIBILITY OF CHICKEN INTESTINAL LACTOBACILLI TO ANTIMICROBIAL COMPOUNDS

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Abstract

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Fourteen strains of lactobacilli belonging to *Lactobacillus casei* subsp. *rhamnosus* and *Lactobacillus fermentum* were isolated from the small intestine, caeca, intestinal mucosa and colon of chickens. The susceptibility and resistance of isolates to 20 antimicrobial agents were studied in order to identify compounds, which can influence the establishment of lactobacilli in chicken intestinal tract. The list of agents included feed additives, drugs and pentachlorophenol.

All strains tested were inhibited at the concentration of 10 mg · l⁻¹ of ionophores (monensin, lasalocid, salinomycin and narasin), tylosin, virginiamycin and penicillin. Also high concentration (50 mg · l⁻¹) of nitrovin, bacitracin and pentachlorophenol inhibited the growth. The lactobacilli were resistant to aminoglycosides (kanamycin and streptomycin), quinoxaline derivatives (olaquinox and cyadox) and avilamycin at the concentration of 10 mg · l⁻¹. All strains tested were resistant to avoparcin, nourseothricin and aureomycin at the concentration of 50 mg · l⁻¹.

Intestinal bacteria, feed additives, antibiotics, pentachlorophenol

Lactobacilli constitute an important component of the intestinal flora in all farm animals. In chickens their numbers reach 10⁶–10⁷ per 1 g of the small intestinal contents and 10⁹ per 1 g of the caecal contents (Timms 1968; Barnes 1979). Many experiments have been performed to determine effects of lactobacilli cultures as growth stimulants in domestic avian species (Morishita et al. 1971; Tortuero 1973; Adler and Da Massa 1980; Damron et al. 1981; Miles et al. 1981ab; Szyliet and Charlet 1981; Watkins and Kratzer 1983, 1984). Stress was laid on the prevention of enteric infections and the improvement of growth rate and feed efficiency.

The establishment of the lactobacilli in the alimentary tract can be influenced by the presence of antimicrobial compounds in feed. Since very little is known about the susceptibility of avian lactobacilli to antimicrobials, the study reported herein was conducted to evaluate the effect of 16 antimicrobial agents on fourteen strains of chicken intestinal lactobacilli.

Materials and Methods

Lactobacilli strains

Fourteen lactobacilli strains were isolated from the small intestine, caeca, intestinal mucosa and colon of chickens (Table 1). Chickens used were sexed cockerels, 35 days old. One group of chickens was fed a commercial BR 2 diet based on ground wheat, barley, maize, soybean meal and a vitamin – mineral supplement. The mixture contained monensin in the amount of 100 mg · kg⁻¹. The second group was fed the same diet without monensin. Intestinal contents of cockerels were serially diluted and cultured on Rogosa agar (Oxoid). The strains displaying the general characteristics of lactobacilli were chosen for further study and identified by the criteria described in Bergey's Manual of systematic Bacteriology (Kandler and Weis 1984). One strain (No. 4) resembled *Lactobacillus casei*, subsp. *rhamnosus*. The remaining strains were identified

Table 1
Origin of lactobacilli strains tested

Strain No.	Isolated from:
5, 10	Small intestine (anterior)
14	Small intestine (posterior)
23	Caecum
32, 41, 4R, 6R, 8R, 18R	Colon
A23	Small intestinal mucosa (anterior)
141	Small intestinal mucosa (posterior)
A7	Caecal mucosa
127	Mucosa of the colon

as *Lactobacillus fermentum*. Four strains (4R, 6R, 8R, 18R) were isolated from chickens fed a diet without monensin. The other strains were isolated from chickens fed a monensin-supplemented diet. The MRS medium (De Mann et al. 1960) with glucose (20 g . l⁻¹) prepared anaerobically was used in this study. Cultures were routinely transferred every week or stored in agar stabs.

Susceptibility of isolates to antimicrobial feed additives

The following antimicrobial feed additives were examined: olaquinox, cyadox, nitrovin and aureomycin (Spofa, Prague, Czechoslovakia), virginiamycin (Smith Kline & French, Genval, Belgium), monensin, narasin and avilamycin (Eli Lilly & Co., Indianapolis, USA), lasalocid (Hoffman - LaRoche Inc., Nutley, USA), salinomycin (Hoechst, Frankfurt, Germany), bacitracin (Sigma Chemical Co., St. Louis, USA), avoparcin (Cyanamid of Great Britain, Gosport) and nourseothricin (Institute of Microbiology and Experimental Therapeutics, Jena, Germany). These compounds were dissolved in dimethylsulphoxide, ethanol, ethanol/dioxane or water and sterilized by filtration through sterilizing asbestos filtering films (John C. Carlson, London, UK). Solutions were added to sterile media to obtain concentrations 10 and 50 mg . l⁻¹. A control contained an equivalent amount of a solvent. Cultures were incubated at 37 °C for 40 h. The growth was assessed as visible turbidity.

Susceptibility of isolates to some antibiotics

The same procedure was used to examine the susceptibility of lactobacilli to six common antibiotics with a therapeutic use. Kanamycin was supplied by Serva (Heidelberg, Germany), the other antibiotics (erythromycin, tylosin, streptomycin, penicillin and ampicillin) were purchased from Spofa. Erythromycin, tylosin and kanamycin were dissolved in ethanol. Streptomycin, penicillin and ampicillin were dissolved in water.

Susceptibility of isolates to pentachlorophenol

Pentachlorophenol (Fluka Chemie AG, Bush, Switzerland) was dissolved in ethanol. The experimental procedure used was the same as described above.

Results and Discussion

The lactobacilli studied were resistant to quinoxaline derivatives (olaquinox, cyadox), avoparcin, nourseothricin, avilamycin (except two strains) and aureomycin (Table 2). They were also resistant to aminoglycosides (kanamycin and streptomycin) at the concentration of 10 mg . l⁻¹ (Table 3). All strains were inhibited by ionophores (monensin, lasalocid, salinomycin and narasin), virginiamycin and except one strain by macrolides (tylosin and erythromycin). Also high concentration (50 mg . l⁻¹) of nitrovin and bacitracin inhibited the growth. No monensin-resistant lactobacilli were found in monensin-fed chickens.

Pentachlorophenol, which is currently one of the most heavily used herbicide, insecticide and fungicide, inhibited the growth of all lactobacilli strains at the concentration of 50 mg . l⁻¹, but allowed slight growth at 10 mg . l⁻¹.

There are a few studies available in the literature regarding the susceptibility of poultry lactobacilli to antimicrobial compounds. Dutta and Devriese (1981)

Table 2
Susceptibility of chicken lactobacilli to antimicrobial feed additives

Additive	Mg. . 1 ⁻¹	Response ¹ of strain													
		5	10	14	23	32	41	4R	6R	8R	18R	A23	141	A7	127
Olaquinox	50	R	R	R	R	R	R	W	R	R	R	R	R	R	R
Cyadox	50	R	R	W	W	R	R	W	R	R	R	R	R	R	R
Nitrovin	10	R	R	R	R	R	R	S	R	R	R	R	R	R	R
	50	S	S	R	S	S	R	S	S	R	R	S	S	S	R
Bacitracin	10	R	W	R	R	R	R	R	R	R	R	R	R	R	R
	50	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Avilamycin	50	R	R	R	R	R	S	R	R	R	S	R	R	R	R

¹ All strains isolated were resistant to olaquinox, cyadox and avilamycin at the concentration of 10 mg . 1⁻¹. All isolates were resistant to avoparcin, nourseothricin and aureomycin at the concentration of 50 mg . 1⁻¹. All isolates were sensitive to monensin, lasalocid, salinomycin, narasin and virginiamycin at the concentration of 10 mg . 1⁻¹. S - Susceptibility; R - Resistance; W - weak growth.

Table 3
Susceptibility of chicken lactobacilli to some antibiotics

Antibiotic	mg. . 1 ⁻¹	Response ¹ of strain											
		5	10	14	23	32	41	4R	6R	A23	141	A7	127
Erythromycin	10	S	S	S	S	S	S	S	R	S	S	S	S
	50	S	S	S	S	S	S	S	R	S	S	S	S
Tylosin	10	S	S	S	S	S	S	S	S	S	S	S	S
	50	S	S	S	S	S	S	S	R	S	S	S	S
Kanamycin	10	R	R	R	R	R	R	R	R	R	R	R	W
	50	R	R	R	R	R	W	R	R	R	R	R	S
Streptomycin	50	R	R	R	R	R	R	R	R	S	R	S	R
Ampicillin	10	R	R	R	R	R	S	S	S	R	R	R	W
	50	R	R	R	R	R	S	S	S	R	R	R	S

¹ All strains were resistant to streptomycin at the concentration of 10 mg . 1⁻¹. All strains were sensitive to tylosin and penicillin at the concentration of 10 mg . 1⁻¹. S - Susceptibility; R - Resistance; W - weak growth.

studied the sensitivity and resistance of 49 strains from 29 birds to eleven growth promoting agents. Most of their strains were inhibited by virginiamycin, nitrovin, carbadox and bacitracin. Results of sensitivity tests made on Gram-positive intestinal bacteria revealed that poultry lactobacilli were as a rule less sensitive than clostridia and more susceptible than enteric streptococci (Dutta and Devriese 1984).

Our findings agree with results of similar tests made on rumen lactobacilli from calves (Marounek et al. 1988). Also rumen lactobacilli were sensitive to ionophores and macrolides and resistant to aminoglycosides and quinoxalines. The results reported here indicate on interaction between chicken intestinal lactobacilli and some antimicrobial agents (e. g. ionophores and macrolides) which are common growth promoters, drugs and biocides. On the other hand, some antimicrobial compounds (quinoxalines, avoparcin, nourseothricin, aureomycin) may support the lactobacilli colonization as they suppress other microbes.

Citlivost laktobacilů, izolovaných ze střevního traktu kuřat, vůči antimikrobiálním sloučeninám

Z tenkého střeva, slepých střev, střevní mukózy a kolonu jsme izolovali 14 kmenů laktobacilů, řadících se k druhům *Lactobacillus casei*, *Lactobacillus*

casei subsp. *rhamnosus* a *Lactobacillus fermentum*. U těchto izolátů jsme zjišťovali citlivost vůči 20 antimikrobiálním sloučeninám, ve snaze určit látky, které mohou ovlivnit osídlení střevního traktu laktobacily. Výběr antimikrobiálních látek zahrnoval krmná aditiva, některá léčiva a pentachlorfenol (běžný biocid). Růst testovaných kmenů inhibovaly ionofory (monensin, lasalocid, salinomycin, narasin), virginiamycin a penicilin. Růst rovněž inhibovaly vyšší koncentrace (50 mg . l⁻¹) nitrovinu, bacitracinu a pentachlorfenolu. Laktobacily byly rezistentní k aminoglykosidům (kanamycinu a streptomycinu), chinoxalinům (olachindoxu a cyadoxu) a avilamycinu při koncentraci 10 mg . l⁻¹. Všechny testované kmeny byly rezistentní k avoparcinu, nourseothricinu a aureomycinu při koncentraci 50 mg . l⁻¹.

Чувствительность лактобациллов, изолированных из пищеварительного тракта цыплят, к антимикробным средствам

В работе изучали чувствительность и устойчивость лактобациллов, изолированных из пищеварительного тракта, цыплят к 20 антимикробным средствам, в которые были включены кормовые добавки, антибиотики и петахлорофенол (гербицид).

Все штаммы лактобациллов были подавлены ионофорами (моненсин, ласалоцид, салиномицин, нарасин), макролидами (тилосин и эритромицин), виргиниамицином и пеницилином. Рост бактерий также подавляла высшая концентрация (50 мг.л⁻¹) нитровина, бацитрацина и пентахлорофенола. Лактобациллы были устойчивы к хиноксалиновым дериватам (олахиндокс, циадокс), авопарцину, ноурсеотрицину, стрептомицину, канамицину, авиламицину (исключая 2 штамма) и ауреомицину.

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