SUSCEPTIBILITY OF CHICKEN INTESTINAL LACTOBACILLI TO COCCIDIOSTATS

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Abstract

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Two strains of lactobacilli (26R and 51R) were tested for growth characteristics in MRS broth and in moistened chicken feed mixture BR–1–IT with and without coccidiostat supplements (monensin, maduramicin and robenidin). Minimal inhibition concentrations (MIC) for all three coccidiostats were measured in MRS broth.

The strains were most susceptible to monensin (MIC 2 $mg.l^{-1}$ for both strains) and most resistant to robenidin (MIC 90 $mg.l^{-1}$ for strain 26R and 110 $mg.l^{-1}$ for strain 51R). The onset of growth in moistened feed was delayed by monensin from 0.14 h to 7.07 h for strain 26R and from 0.87 h to 3.59 h for strain 51R. The other coccidiostat did not affect the growth of either strain to any significant extent. The results suggested that probiotics containing lactobacilli can only be combined with specific coccidiostats.

Feed additives, resistance, probiotics

Lactobacilli constitute an important component of the intestinal flora in all farm animals. In chickens their number reaches 10^9 per g of the ceacal contents (Barnes 1979). These bacteria are therefore the most frequently used as probiotics (Fuller 1990). However, it is well known that coccidiostats, which are commonly used in chicken rearing, can strongly inhibit lactic acid bacteria including lactobacilli (Dutta and Devriese 1981, 1984; Chow and Russel 1990).

The aim of this study was to determine interactions among three commonly used coccidiostats and two chicken intestinal lactobacilli.

Materials and Methods

Organisms

Two strains were isolated from the chicken caeca. Strain 26R was closely related to *L. salivarius* and strain 51R to *L. casei*. Both strains were rifampicin resistant and were used in chicken experiments studying lactobacilli survival in the gut.

Minimal inhibition concentration assessment

The following coccidiostats were examined: monensin, maduramicin and robenidin (Biofaktory Praha s. r. o.). These compounds were dissolved in ethanol and sterilized by filtration through filter membrane with 0.3 μm pores (Barvy a laky Praha s. r. o.). Solutions of coccidiostats (concentration 4 $mg.ml^{-1}$)were added to sterile MRS broth (De Mann et al. 1960) to obtain required concentrations. Control tubes contained an equivalent amount of ethanol. Cultures were incubated at 37 °C for 48 h. The growth was assessed as a visible turbidity.

Growth characteristics

Specific growth rate (μ) and lag time (L) were determined in MRS broth and in the chicken feed mixture BR–1–IT (international test) which was moistened by twice its volume of tap water. Chicken feed (maize meal, 59 %; soyabeen meal extracted, 25 %; fish meal, 10 %; vitamin supplement DB BR–1, 1 %; mineral supplement MKP2 SP, 3 %) was used alone or with the following supplements of coccidiostats: monensin (100 $mg.kg^{-1}$), maduramicin (5 $mg.kg^{-1}$) and robenidin (33 $mg.kg^{-1}$). These concentrations are usually used as coccodiostats in feed mixtures. The lactobacilli cultures in the MRS medium or in the moistened chicken feed mixture were cultivated in 500 ml vessels gassed with 0_2–free CO_2 at 42 °C. Culture vessels were clossed by rubber bungs with ports for sampling gas entry and exit. The overnight bacteria cultures served as inocula. At regular intervals the samples were removed and the total count of rifampicin-resistant lactobacilli was assessed. The rifampicin was used in order to distinguish our cultures from the wild lactobacilli strains. Colony-forming units (CFU) were counted on the acetate agar (Rogosa et al. 1951) with rifampicin additions (100 $mg.l^{-1}$). Rifampicin, dissolved in ethanol to a concentration of 8 $mg.ml^{-1}$, was added to the tempered (50 °C) acetate agar. The specific growth rate (μ) and lag time (L) were determined using a semi-logarithmic plot of CFU against time.

Results

Minimal inhibition concentration

The strains were most susceptible to monensin (Table 1). Strain 26R was also strongly inhibited by maduramicin, while strain 51R was more resistant. Robenidin was able to inhibit either strain only in higher concentrations.

Table 1

Minimal inhibition concentration (MIC) in $mg.l^{-1}$

Coccidiostats	Strain 26R	Strain 51R
Monensin	2	2
Maduramicin	2	13
Robenidin	90	110

Table 2

Growth characteristics of test strains in MRS broth and in moistened feed mixture with or without coccidiostats

Strain		MRS	Feed	Feed + monensin	Feed + maduramicin	Feed + robenidin
26R	μ^a	1.72	1.54	1.79	1.64	1.67
	L^b	0.17	0.14	7.07	0.72	0.99
51R	μ	1.83	1.83	1.80	1.99	2.23
	L	0.25	0.87	3.59	0.77	0.94

[a] Specific growth rate [h^{-1}]
[b] Lag time [h]

Growth characteristics

Results are shown in Table 2 and Figures 1 and 2. The strains examined were rapidly growing in MRS medium as well as in the moistened feed. The specific growth rate varied between $1.54.h^{-1}$ and $2.23.h^{-1}$. The growth chracteristics were similar in both strains and were not markedly affected by eiter maduramicin or robenidin. Monensin, on the contrary, had a strong inhibition effect and lengthened the lag time from 0.14 h to 7.07 h (strain 26R) and from 0.87 h to 3.59 h (strain 51R). Specific growth rate was unaffected by any coccidiostats.

Discussion

Gut-derived lactobacilli play the most important role in the probiotics use (Fuller 1990). Monensin and maduramicin are polyether antibiotics that inhibit the growth of Gram-positive bacteria (Russel and Strobel 1989) and these ionophores are routinely fed to chicken broilers as coccidiostats (Davison 1984). Our results revealed that the inhibition of chicken lactobacilli by these compounds was not so strong in moistened chicken feed as in MRS broth. It seems that MIC tests *in vitro* cannot reliably predict interactions among feed additives and probiotics administered. From this point of view there are probably three factors influencing probiotics administration: the resistance of certain probiotic bacteria to feed additives, the sort of additives used and their concentrations in the diet. Hence, our strains probably can be combined with robenidin (because of resistance) and with maduramicin (because of low concentration). It is unclear why monensin strongly inhibited both strains in MRS medium, but in the moistened feed merely delayed the onset of the growth.

Monensin, maduramicin and robenidin are commonly used coccidiostats in chicken rearing. Our results indicate that monensin probably should not be combined with probiotic

Figure 1. Growth of strain 26R in the feed with or without coccidiostats.

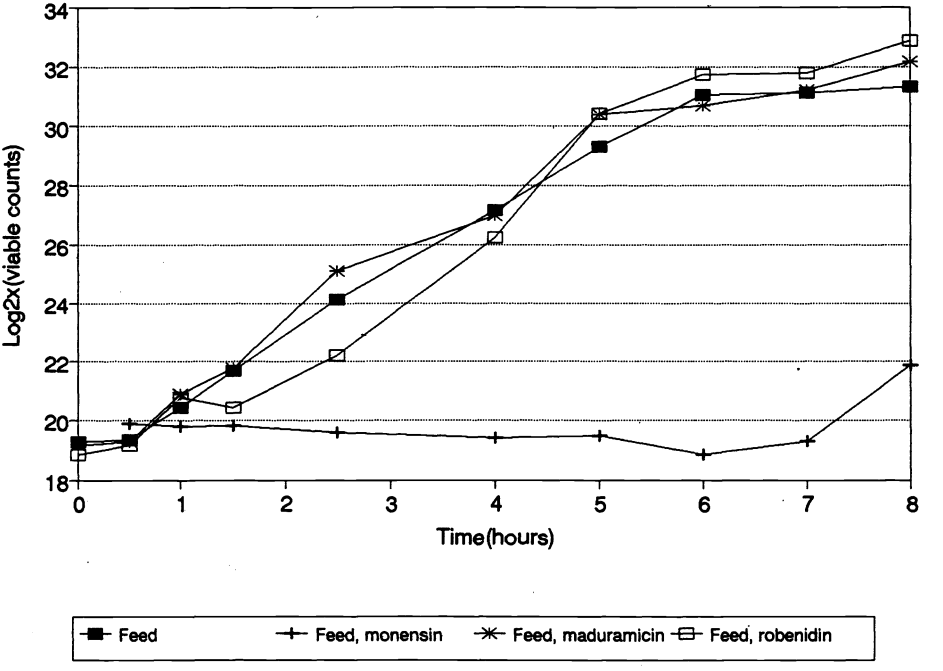
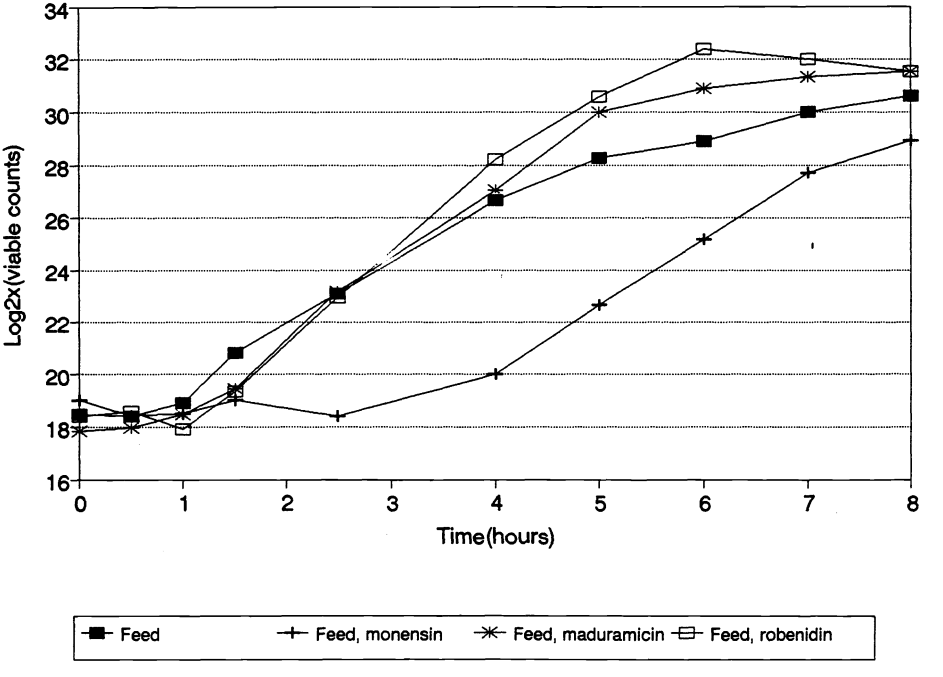


Figure 2. Growth of strain 51R in the feed with or without coccidiostats



treatment whereas maduramicin and robenidin are possible. The results suggest that probiotics containing lactobacilli could be combined only with certain specific coccidiostats and every combination should be evaluated individually.

Citlivost laktobacilů izolovaných z trávicího traktu kuřat ke kokcidiostatikům

U dvou kmenů laktobacilů (26R a 51R) byly testovány růstové charakteristiky v MRS bujónu a ve zvlhčené krmné směsi BR–1–IT s přídavkem kokcidiostatik (monensin, maduramicin a robenidin) a bez přídavku. Pro všechny tři kokcidiostatika byla stanovena minimální inhibiční koncentrace (MIK) v MRS bujónu.

Kmeny byly nejvíce citlivé k monensinu (MIK 2 $mg.l^{-1}$ pro oba kmeny) a nejvíce rezistentní vůči robenidinu (MIK 90 $mg.l^{-1}$ pro kmen 26R a 110 $mg.l^{-1}$ pro kmen 51R). Doba od inokulace do zahájení růstu ve zvlhčené krmné směsi byla prodloužena (z 0.14 h. na 7.07 h. u kmene 26R resp. z 0.87 h. na 3.59 h. u kmene 51R) přídavkem monensinu. Ostatní kokcidiostatika neovlivnila výrazně růst testovaných kmenů. Výsledky naznačují, že probiotika obsahující laktobacily by měla být kombinována pouze s určitými kokcidiostatiky.

Чувствительность из пищеварительного тракта цыплят изолированных лактобацилл к кокцидиостатикам.

У двух штаммов лактобацилл (ш. Но. 26R и ш. Но. 51R) определенны параметры роста в бульоне МРС и в увлажненной кормовой смеси BR-1-IT для цыплят с кокцидиостатическими дополнениями (монензин, мадурамицин и робенидин) и без дополнений. Минимальная тормозящая концентрация (МТК) всех трёх кокцидиостатиков выявлена в бульоне МРС.

Штаммы наиболее чувствительны к монензину (МТК 2 мг . $л^{-1}$ для обоих штаммов) и наиболее устойчивы к робенидину (МТК 90 мг . $л^{-1}$ для штамма 26R, 110 мг . $л^{-1}$ для штамма 51R). Начало роста в увлажненном корме отстало с 0,14 ч до 7,07 ч (штамм 26R), с 0,87 ч до 3,59 ч (штамм 51R) под влиянием монензина. Остальные кокцидиостатики не оказывали значительное влияние на рост обоих штаммов. В результатах указывается, что пробиотики содержащие лактобацилли можно комбинировать с определёнными кокцидиостатиками.

References

BARNES, E. M.: The intestinal microflora of poultry and game birds during life and after storage. J. Appl. Bacteriol. **46**, 1979: 407–419.

CHOW, J. M.–RUSSEL, J. B. (1990): Effect of ionophores and pH on growth of Streptococcus bovis in batch and continuous culture. Appl. Environ. Microbiol., **56**, 1990: 1588–1593.

DAVISON, K. L.: Monensin absorbtion and metabolism in Calves and Chicken. J. Agric. Food Chem., **32**, 1984: 1273–1277.

DE MANN, J. C.–ROGOSA, M.–SHARPE, M. E.: A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., **23**, 1960: 130–135.

DUTTA, G. N.–DEVRIESE, L. A.: Sensitivity and resistance to growth promoting agents in animal lactobacilli. J. Appl. Bacteriol., **51**, 1981: 283–288.

DUTTA, G. N.–DEVRIESE, L. A.: Observation on the in vitro sensitivity and resistance of gram-positive intestinal bacteria of farm animal to growth promoting antimicrobial agents J. Appl. Bacteriol., **56**, 1984: 117–123.

FULLER, R.: Probiotics in agriculture. Agbiotech news and information. Vol. 2. No. 2, 1990: 217–220.

ROGOSA, M.–MITCHELL, J. A.–WISEMAN, R. F.: A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol., **62**, 1951: 132–133.

RUSSEL, J. B.–STROBEL, H. J.: Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol., **55**, 1989: 1–6.