Spectinomycin and Rat Large Bowel Microflora Associated Characteristics

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Three groups of adult male rats were fed chow supplemented with spectinomycin (SPC 0, 0.1 and 10 ppm, respectively). After 42 days there was no difference in the mean weight gain of the three groups. SPC did not change the levels of *Escherichia coli* in the rats' ceca. The distribution of resistance to SPC among *E. coli* from the three groups was not altered by SPC in the diet. SPC had no significant effect on pH, ammonia levels and stool moisture. It had no impact on the total short chain fatty acid production though the relative abundance of individual analytes was changed. Sterol and bile acid metabolism were unaffected. Comparison of cellular fatty acid (CFA) profiles showed small but measurable differences between the three groups. The no-drug controls and rats in the 0.1 ppm group had highly similar CFA profiles while the profile of the 10 ppm group was more distinct, suggesting a possible dose-dependent response to SPC. *Key words*: spectinomycin, cellular fatty acids, short chain fatty acids, bile acids, neutral sterols, rats microflora, resistance, bowel.

INTRODUCTION

Spectinomycin (SPC) is one of approximately 30 antimicrobial products approved for use in food animals in the US. It is an aminocyclitol, active against facultatively anaerobic, gram-positive and negative bacteria and mycoplasmas. It is used to treat and control enteritis of pigs caused by *Escherichia coli* and salmonella, other bacterial diseases of pigs, chronic respiratory diseases of poultry, enteritis and pneumonia in calves, and pneumonia in sheep. Its therapeutic usefulness against obligately anaerobic bacteria is negligible. Inappropriate agricultural practices may permit low levels of spectinomycin to enter the human food supply.

The impact of antibiotic residues on human flora remains an ill-defined hazard. Even so, the US Food and Drug Administration Center for Veterinary Medicine recommends an acceptable daily intake (ADI) for any microbiologically active residue in edible tissues from food-producing animals of 1.5 mg per person per day (1). So, in the absence of a specific recommendation by the FDA-CVM regarding SPC, 1.5 mg per day remains the ADI. For an adult of 60 kg, this corresponds to 25 μg/kg body weight (body wt.) per day. The Joint WHO-FAO Expert Committee on Food Additives (JECFA) fixed an ADI of 40 μg/kg body wt. per day (2). The JECFA's ADI was arrived at after consideration of several factors, including the minimum inhibitory concentrations of spectinomycin towards the most susceptible representatives of the normal flora (in this instance, the bifidobacteria), the average daily fecal bolus (150 g) and the mean adult body wt. (60 kg). The JECFA also reviewed the extent of any metabolism by the host and the host's gut flora, the amount consumed versus the amount that can be recovered from feces and the degree to which it is absorbed. Spectinomycin, given orally, is only poorly absorbed from the alimentary tract and almost all the consumed drug can be recovered from the feces, in the active, unmetabolized form (2).

Your cecum is full to capacity with more than 10^{11} bacteria in each gram representing over about 500 species. It is a stable ecosystem that is essential to your continued good health. When it is disturbed the consequences can be dire. An unintended side effect of the therapeutic levels of broad-spectrum agents is the potentially fatal antibiotic-associated diarrhea caused by *Clostridium difficile*. Even so, studies into the risks associated with antibiotics in our food supply are routinely limited to the more traditional toxicological testing of body fluids and tissues. Should they include attempt to quantify any impact on the gut microflora, testing is generally confined to only the broadest bacterial groupings, e.g. anaerobes and enterobacteria, though in general they do consider the frequency of sensitivity to the test compound (4, 5). Biochemical markers of microbial activity may be measures of more subtle changes than shifts in bacterial counts. Midtvedt has called these parameters microflora associated characteristics or MACs and has proposed their use in monitoring the stability of gut floras exposed to antimicrobial agents (6).

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It seems probable any effect of SPC will be proportional to the amount consumed and so low levels in foods might affect the flora at a metabolic (i.e. at the MAC) rather than at the microbial level. To investigate this we fed rats chow supplemented with 0, 0.1 and 10 ppm of spectinomycin. Since a 350–375 g rat eats about 20 g of chow per day, a rat in the 0.1 ppm group will eat about 2 μg per day or approximately 6 μg/kg body wt. per day. A rat in the 10 ppm group will eat about 600 μg/kg body wt. per day. Table I compares these levels with the FDA and JECFA ADIs.

MATERIALS AND METHODS

Animals and diet

Three groups of adult, male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), each weighing 350–375 g, were set up. They were housed in accordance with local, state and federal regulations and were in good health throughout the study. They were offered non-sterile chow (TekLad 4% mouse/rat diet; Harlan TekLad, Madison, WI) and chlorinated water *ad libitum*.

Spectinomycin

Basal chow was supplemented with spectinomycin dihydrochloride (Sigma, St Louis, MO) at one of the three levels; zero ($n = 12$), 0.1 ppm ($n = 10$) and 10 ppm ($n = 10$). The supplemented chow was powdered each morning daily in a Braun Multipractic food processor, set on high speed, for two periods of 5 min, with 1 min interval in between. The high dose diet (10 ppm) was made by mixing 10 mg active spectinomycin with 1 kg of diet. The low dose diet (0.1 ppm) was made by mixing 10 g of the higher dose diet with 90 g of unsupplemented diet. The control diet was unsupplemented chow powdered in the food processor. The food processor was thoroughly washed and dried between each preparation. After each day the left-over chow was discarded.

Bioassay for spectinomycin in chow

Assays were run to measure SPC in samples of chow prepared as described above. Standard aliquots of *E. coli* ATCC 10536, containing $10^{6.3}$ (7) cell per ml after a single freeze-thaw cycle, were stored at $< -70°C$ (4). As required, aliquots were thawed at room temperature. Inoculum (100 μl) was spread onto Wilkins Chalgren agar plates (20 ml; Difco, Detroit). Sterile discs of predetermined absorbency were loaded with an aqueous extract of chow. To prepare the aqueous extract, 10 g chow was mixed with 20 ml deionized water for 2 h at room temperature after the mixture had been thoroughly agitated on a vortex mixer for 5 min continuously. The discs were placed aseptically onto the agar plates and the plates were incubated overnight at 37°C. After incubation the zones of inhibition around the discs were measured. The concentration of SPC in the disc and hence in the chow was read from a standard curve. Three times during the feeding trial, a lot of each diet (i.e. chow with 10, 0.1 and 0 ppm) was tested in triplicate. Mean test concentrations are reported in Table I.

Collection and preparation of gut content

After 42 days on their respective dietary regimens the rats were killed, their large bowels were cut at the ileocecal junction and the rectum to permit removal of the whole large bowel from the abdomen. Ceca were isolated and their contents were individually extruded into glass containers, thoroughly mixed and kept for no more than 30 min postmortem at room temperature and under anaerobic gasses. During those 30 min the pH of each was measured, after which the samples were stored, under anaerobic gasses and at a maximum of $-20°C$. In preparation for the analysis, the frozen specimens were thawed at room temperature and split into aliquots in readiness for subsequent processing on the day of thawing.

Table I

Spectinomycin levels in the 0.1 and 10 ppm rat chow and acceptable daily intakes for humans

	Spectinomycin level [a]		Acceptable daily intake	
	0.1 ppm	10 ppm	FDA (1)	JECFA (2)
Intake (μg per day)	2 [a]	200 [a]	1500	2400 [b]
Intake (μg/kg body wt. Per day)	6	600	25 [b]	40
Added to chow (μg/g)	0.1	10		
Level in chow by bioassay (μg/g)	Below the minimum detectable level	7.4 ± 0.9		

[a] Assuming that an average adult rat eats 20 g chow per day.
[b] Assuming that an average adult weighs 60 kg.

Escherichia coli counts

Serial 10-fold dilutions of each sample were prepared (7). Aliquots (0.1 ml) of the dilutions were plated on MacConkey agar (Difco). Plates were incubated aerobically at 37°C for 18–24 h. Plates with 30–300 colonies were counted. Counts are expressed as count per gram dry wt feces.

Resistance to spectinomycin

E. coli was isolated on MacConkey agar (see above). Plates with 30–300 colonies were replica-plated onto MacConkey agar, with and without added SPC (16 μg/ml), using sterile velvet pads and a replicating block and collar (Replicatech Inc., Princeton, NJ). The last plate in each series to be inoculated was a no-drug control, to check for contamination and to prove that if colonies failed to grow on SPC, it was not because of a failure of the replica plating. Plates were incubated aerobically for 18–24 h at 37°C.

pH

The pH of the samples was measured directly with an Accumet 610A pH meter and a silver–silver chloride pH electrode (#912600; Orion, Inc., Boston, MA). The means, standard deviations (SD) and *t*-tests were calculated using the negative antilogarithm of measured pH values (i.e. using actual hydrogen ion concentrations). For presentation, data were converted back into pH units.

Cecal moisture content

The percentage of sample that was water was calculated by comparison of the weights before and after lyophilization.

Ammonia

Ammonia was assayed with a commercially available kit form of the Bethelot-indophenol test (Ammonia; Wako Chemicals USA, Inc., Richmond VA) according to the manufacturer's instructions.

Short chain fatty acids

The short chain (or volatile) fatty acid (SCFA) components of the samples were measured by gas chromatography using published methods (7).

Bile acids and neutral sterols

The bile acids and neutral sterols, both plant and animal, were assayed by gas chromatography (GC) and identified by GC-mass spectroscopy (8).

Cellular fatty acids (CFAs)

Cecal material (about 1 g) was added to sterile aqueous magnesium sulfate (10 ml; 0.7% w/v, pH 7.2) containing three or four glass beads (2–3 mm diameter). This and subsequent manipulations were done aerobically and at room temperature. The intestinal material was broken up and evenly dispersed by vortexing for 5 min. Particulate matter was removed by centrifuging (1000 × *g*, 10 min) and the supernatant was retained. Bacteria in the supernatant were sedimented by a second centrifugation (10000 × *g*, 20 min). The pellet was dispersed and sedimented twice more in sterile aqueous 0.7% (w/v) magnesium sulfate (10 ml). The washed bacterial pellets were stored at −20°C until needed.

The analytical gas chromatographic analysis of derivatized CFAs was described by Holdeman et al. (9), and in the literature provided by Microbial ID, Inc., (Newark, DE) to support the MIDI system. Libraries were generated and the mean CFA profile of each group was compared using pattern recognition and cluster analysis software packages (Microbial ID, Inc., Newark, DE). The results are expressed in Euclidean distances (ED).

Highly dissimilar profiles are separated by a high number of ED, similar profiles by a low number of ED and so for ease we have adopted a number of 'end points' used to identify bacteria with the MIDI system. Profiles of 2 ED or less are effectively identical and so represent a single strain. Profiles separated by 2–6 ED, seen with members of the same sub-species, are highly similar. While 6–10 ED separate members of the same species and moderately related profiles (differences of 10–25 ED encompass genera, but we saw no differences of that size.)

Statistical analysis

Data were analyzed by independent *t*-tests using Sigmaplot (version 5.0) and Excel (version 4.0). Proportional data, such as percentages, were transformed to arcsines prior to analysis (10).

RESULTS

Spectinomycin levels in diet

At 0.1 ppm, the level of spectinomycin in the chow was below the minimum we could detect by bioassay. We found a mean level of 7.4 ± 0.9 μg/g in chow supplemented with 10 ppm (10 μg/g chow, Table I).

E. coli counts and sensitivity to spectinomycin

Levels of *E. coli* in each group ranged from $10^{5.1}$ to $10^{6.0}$/g dry weight. They did not differ significantly (Table II). The frequency of sensitivity among isolates of *E. coli* to 16 μg/ml SPC was 71–78%. There were no significant differences between the groups.

Microflora associated characteristics

Table III shows the mean weights of each of the three groups, as well as the means of pH, moisture content and ammonia concentration of the cecal samples. None of these differed significantly ($P \leq 0.05$) between the three groups.

Table II

Effect of spectinomycin on E. coli in the rat cecum [a]

Spectinomycin in diet (nominal level)	None	0.1 ppm	10 ppm
Mean Log_{10} *E. coli*/g dry wt. ± SD	5.5 ± 1.4	6.0 ± 1.3	5.1 ± 0.9
Percentage with reduced sensitivity to spectinomycin (16 μg/ml) ± SD	32 ± 5	39 ± 9	37 ± 8

[a] No significant differences were seen.

Although total SCFA did not discriminate between the three dietary groups (Table IV), levels of some individual SCFA did so. Levels of acetate were significantly higher in the 10 ppm group (71.9 ± 3.1%) than in either the no-drug (68.8 ± 2.2%) or the 0.1 ppm group (69.3 ± 3.7%). The increase was offset by significantly decreased butyrate levels in the 10 ppm group (18.9 ± 2.4%) compared with the no drug controls (21.2 ± 2.0%) and the 0.1 ppm rats (20.5 ± 2.9%).

Although the relative distribution of total and individual bile acids varied between the groups, a series of large standard deviations around each mean made significant differences unlikely. For example, the mean total bile acids in the control group was 569.9 ± 442.3 μg/g feces. The same value for rats in the 10 ppm group was 768 ± 305.4 μg/g feces (Table V). The last parameter relevant to bile acids that we studied was the amount of the total comprised by secondary or bacterially produced metabolites. For the three groups of rats, secondary bile acids were about 65–75% of the totals (Table V). In contrast, the equivalent data for animal sterols and neutral phytosterols were just over 50% and just below 60%, respectively (Table VI). Mean levels of individual sterols showed the same general consistency seen with the bile acids and the same wide standard deviations around those means (Table VI).

The mean CFA profiles distinguished the 10 ppm groups from the no-drug controls and the 0.1 ppm fed animals (Table VIIFig. 1). The no-drug and 0.1 ppm groups were highly similar to each other having profiles separated by only 5.3 ED. The profile of the 10 ppm rats was distinguishable from the cluster formed by the no-drug and the 0.1 ppm profiles by more than 8 ED (Fig. 1). Of the individual CFA analytes, the most abundant were not the most discriminatory (Table VII). For example, 16:0 FAME was about 18% of the total CFA recovered in all three groups, whereas 18:2*cis* 9,12 FAME was significantly lower ($P \leq 0.05$) in the 10 ppm rats (9.8 ± 1.8%) than either the 0.1 ppm (15.0 ± 3.1%) and the no-drug rats (14.0 ± 4.0%).

DISCUSSION

There were no statistical differences in the mean weight of the test and control groups, implying that spectinomycin neither affected body weight, nor chow consumption and that any significant differences we saw were the consequence of spectinomycin in the diet. The luminal cecal pH (Table III) matched published data (11). pH is the balance between the bacterial production of SCFA neutralized by microbial ammonia and host secretions. SPC had no effect on the outcome. There were no significant differences in the level of cecal moisture between the test and control groups (Table III). It was 65–70%, though other workers have reported levels between 50 and 55% (3, 12). However, since cecal and fecal moisture are in part related to the amount and type of dietary fiber, differences in the fiber in chow between our study and the cited authors may explain the difference. McIntyre et al. (12), for instance, fed a chow containing only half the insoluble fiber of our chow.

Though quick and easy to measure, cecal ammonia levels did not change in response to spectinomycin (Table III). Though gained through a different method, our data are similar to those reported for freshly passed human stool (13), but are up to 10-fold lower than was detected 4 h post-mortem from human cecal and colonic matter (14). However, since ammonia is produced when feces are incubated (15), the 4 h period after death and before sampling may account for the discrepancy. The selective elimination of *Enterobacteriaceae* from the rat large bowel has no effect on ammonia production by the remaining, largely anaerobic bacteria (16). Thus, spectinomycin, which is more inhibitory effect to *Enterobacteriaceae* than it is to anaerobes, would by inference be expected to have little effect on the ammonia levels, even had SPC reduced the carriage rate of *E. coli*, which it did not (Table II).

Table III

Effect of spectinomycin on weight gain and some microflora associated characteristics in the rat cecum

Parameter	Spectinomycin level in diet		
	None	0.1 ppm	10 ppm
Starting weight ± SD (g)	374 ± 8	380 ± 9	379 ± 9
Final weight ± SD (g)	454 ± 16	441 ± 19	443 ± 14
Cecal pH (1)	6.71	6.72	6.77
SD range	6.52–6.84	6.61–6.83	6.60–6.95
% moisture ± SD	65.7 ± 6.0	66.2 ± 3.6	69.6 ± 8.6
Ammonia concentration ± SD (mM)	8.8 ± 3.2	8.5 ± 2.6	7.8 ± 2.1

Table IV

Effect of spectinomycin on short chain fatty acids in the rat cecum [a]

SCFA	Spectinomycin level in diet		
	None [b]	0.1 ppm	10 ppm
Acetic	68.8 ± 2.22 [c]	69.3 ± 3.7	71.9 ± 3.1*
Propionic	8.1 ± 1.0	7.9 ± 1.2	7.9 ± 1.4
Isobutyric	0.3 ± 0.2	0.4 ± 0.1*	0.3 ± 0.2
Butyric	21.2 ± 2.0	20.5 ± 2.9	18.9 ± 2.4*
Isovaleric	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Valeric	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.1**
Caproic	0.7 ± 0.5	0.8 ± 0.2	0.2 ± 0.4*,**
Total [d]	46.2 ± 9.6	39.8 ± 10.4	44.5 ± 7.5

[a] Mean cecal pH levels did not vary (Table III) so the undissociated SCFA levels were not calculated.
[b] Two control animals contained trace amounts of heptanoic acid (data not shown).
[c] Data expressed as percentage of total SCFA measured ± SD.
[d] Data expressed in mmol/kg dry wt. cecal material ± SD.
*Significantly different from the control group ($P \leq 0.05$); ** significantly different from 0.1 ppm group ($P \leq 0.05$).

In each group the concentration of total SCFAs was close to 40–45 mmol/kg dry wt. The levels of acetic acid were highest in the 10 ppm group. The level of butyrate was correspondingly low. Even so the calculated percent molar ratios of acetic, propionic and butyric acids were still very similar (about 70, 8 and 20%, respectively). The outstanding 2% were isobutyric, isovaleric, valeric, caproic and heptanoic acids; their combined distribution was highly inconsistent throughout the groups. Our data are similar but not identical to those reported by others (17–19). While the other workers generally found more propionic acid than we did, this was dependent on the type and amount of fiber in the diet. As SCFAs are produced by bacterial fermentation and because spectinomycin probably has little effect on the predominant, anaerobic bacteria, any difference between the control and 10 ppm groups may have resulted from changes to fermentation by the facultative anaerobes. However, as we have said, we saw no reduction in the rate of *E. coli* carriage from group to group (Table II).

Table V

Effect of spectinomycin on bile acids in the rat cecum

Bile acid	Spectinomycin level in diet		
	None [a]	0.1 ppm	10 ppm
Primary bile acids			
Cholic acid	13.6 ± 6.9	15.5 ± 6.8	14.7 ± 7.0
α-muricholic acid	25.0 ± 22.7	16.4 ± 9.5	42.2 ± 23.9
β-muricholic acid	42.9 ± 38.3	39.3 ± 11.0	65.9 ± 29.0
Secondary bile acids			
Lithocholic acid	28.6 ± 11.4	28.4 ± 6.9	29.9 ± 9.7
Deoxycholic acid	231.5 ± 210.4	226.4 ± 96.3	324.6 ± 144.4
Hyodeoxycholic acid	118.2 ± 99.1	165.8 ± 91.4	72.7 ± 75.1
Unsaturated ω-muricholic acid	26.5 ± 24.9	22.3 ± 13.0	54.6 ± 38.6
ω-muricholic acid	83.7 ± 93.9	51.6 ± 30.2	164.1 ± 102.0
Total bile acids	569.9 ± 442.3	565.8 ± 224.4	768.8 ± 305.4
Secondary as % of the total	72.4 ± 6.8	77.6 ± 19.5	63.6 ± 11.5

[a] Values expressed in μg/g feces ± SD: No statistically significant differences were seen.

Although the concentration of cecal bile acids rose from 5.7 mg/g dry wt. in the controls to 7.7 mg/g in the 10 ppm spectinomycin group, the differences were not significant. The degree to which the intestinal bacteria metabolized the primary bile acids to secondary forms was consistent (from 87 ± 3 to 83 ± 5%) across the groups. Our data match reports (20, 21) that, under similar conditions, the total bile acid levels were between 5 and 10 mg/g with slightly more than 80% occurring as secondary forms.

Neutral sterols have two origins, plant (hence 'phytosterols') and animal, of which cholesterol is best known. Just as with bile acids, bacteria can metabolize primary neutral sterols, the products of metabolism are known as secondary neutral sterols. Once again our data showed no significant differences between the levels of animal and phytosterols and the extent to which they were metabolized to their secondary forms. Our data were similar to those reported by others (21).

It is interesting to note that the varying extent to which these three groups of closely related molecules, bile acids, animal sterols and phytosterols are converted to their secondary components. The percentages were about 85, 60 and 50%, respectively. Although we have no explanation for the differences, in an earlier study (8) we found the secondary compounds to be 60, 70 and 80% of their respective totals in rats fed a standard chow diet very similar to the one we used in this trial.

The mean CFA profiles of the mixed microflora recovered from the large bowels of control rats and those receiving only 0.1 ppm spectinomycin were very similar. They differed by 5.3 ED. The mean profile of the 10 ppm differed from the controls' by slightly more than 8 ED. The data are summarized in Table I and Fig. 1. Although 16:0 FAME was the most common analyte, an unpaired t-test of the mean showed that its mean concentrations were not significantly different between the groups; 18:2 *cis* 9,12 FAME and 18:1 *cis* 9 FAME were both significantly lower in the rats fed 10 ppm spectinomycin. We have been unable to correlate these changes with any particular bacterium or group of bacteria.

Of the parameters we assayed, only one, bacterial counts, reflects directly what a test drug may or may not be doing to members of a bacterial flora. Accordingly counts may be the least sensitive feature that we studied.

Table VI

Effect of spectinomycin on neutral sterols levels in the rat cecum

Neutral sterol	Spectinomycin level in diet		
	None [a]	0.1 ppm	10 ppm
Primary phytosterols			
Sitosterol (24β-ethyl cholesterol)	575.4 ± 326.2	560.4 ± 175.8	539.5 ± 149.6
24β-methyl cholesterol	128.1 ± 74.2	118.5 ± 34.5	122.1 ± 43.6
Secondary phytosterols			
24β-methyl coprostanol	139.3 ± 68.4	139.3 ± 26.8	142.8 ± 36.5
Campestanol (24α-methyl coprostanol)	197.3 ± 101.7	205.7 ± 61.9	209.1 ± 61.5
Sitostanol (24β-ethyl coprostanol)	719.4 ± 282.5	760.7 ± 139.4	764.7 ± 172.7
Stigmastonal (24α-ethyl coprostanol)	231.0 ± 99.2	240.0 ± 45.1	237.9 ± 97.6
Total phyotsterols [a]	2132.3 ± 855.1	2183.2 ± 356.1	2163.8 ± 458.7
Secondary as% of the total	61 ± 10	62 ± 5	63 ± 5
Primary animal sterols			
Cholesterol	369 ± 243	316 ± 86	283 ± 61
Secondary animal sterols			
Coprostanol	282 ± 122	293 ± 98	274 ± 83
Total animal sterols	649 ± 272	609 ± 101	557 ± 110
Coprostanol as% of the total	46 ± 17	48 ± 12	49 ± 8

[a] Values expressed in μg/g feces ± SD. No statistically significant differences were seen.

Of the normal gut flora *E. coli* is the most abundant facultative anaerobe and so the most likely to be adversely affected by SPC in the diet. However, counts were consistent between and within the three groups. Levels in each group were 10^5–10^6/g throughout the trial (Table II). Furthermore, while bacterial counts may be the most direct measure of change to the flora, altered sensitivity to SPC is arguably the feature with the greatest relevance to public health, since any rise represents a reservoir that may eventually extend to human pathogens. We saw reduced sensitivity to 16 μg SPC/ml in about 35% of *E. coli* isolated from each of the groups. In comparison, half of all *E. coli* isolated from cows' feces were resistant to 32 μg/ml or more (22).

Of the several *in vivo* and *in vitro* models available to researchers, modeling the human colon's response to trace levels of antibiotics, conventional rats provide the most convenient *in vivo* method. Our study shows that it is possible to monitor large bowl flora ecology using conventional rats and by monitoring a range of MACs. However, in this study we did not address several potentially confounding issues. These include differences between human and rat flora, the relative dryness of both rat diet and feces that will impact the concentration of SPC in the bowel. In

Table VII

Effect of spectinomycin on microbial cellular fatty acids in the rat cecum

Fatty acid analyte	Spectinomycin level in diet		
	None	0.1 ppm	10 ppm
16:0 FAME [a]	18.5 ± 3.0 [b]	17.4 ± 1.2	18.2 ± 1.8
18:2 *cis* 9,12 FAME	14.0 ± 4.0	15.0 ± 3.1	9.8 ± 1.8*,**
18:1 *cis* 9 FAME	9.4 ± 1.8	10.5 ± 1.2	7.3 ± 1.2*,**
18:1 *trans* 11 FAME	6.1 ± 3.9	9.4 ± 1.8*	7.9 ± 2.5
UNK 18.199 ECL and/or 18:0 anteiso DMA	6.5 ± 2.4	7.0 ± 2.6	8.0 ± 4.3
20:0 FAME	8.4 ± 4.4	7.5 ± 2.6	2.8 ± 3.7*

[a] FAME, fatty acid methyl ester; DMA, dimethyl acetal; UNK, unknown compound; ECL, equivalent chain length in carbon atoms were the molecule linear and fully saturated.

[b] % of total ± SD. *Statistically significant ($P \leq 0.05$) compared with the control group and ** compared with the 0.1 ppm group.

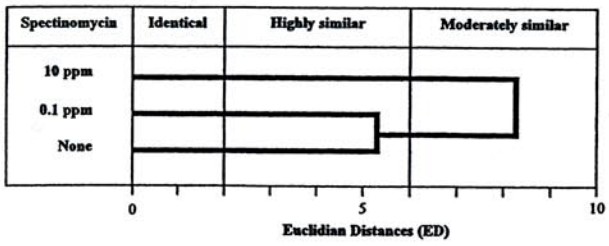

Fig. 1. Effect spectinomycin on microbial cellular fatty acid profiles in the rat cecum; dendrogram of relatedness.

addition, conventional rats are coprophagic and so will re-expose their fecal flora to any antibiotic that is excreted in feces. Short of feeding antibiotics to humans, these and other similar complications will always be present in this type of work.

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