SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF VIRGINIAMYCIN IN FEEDINGSTUFFS FOR FATTENING CATTLE

Opinion expressed 27 April 1988

TERMS OF REFERENCE (July 1986)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions:

1. Has the use of the antibiotic virginiamycin at the dosages proposed for feedingstuffs for fattening cattle (see BACKGROUND) significant effects on the growth?

2. Can this use result in the development of resistance in bacteria to prophylactic or therapeutic preparations or exert an effect on the persistence of gram-negative bacteria in the digestive tract of bovines?

3. What is the metabolic rate of virginiamycin in bovines? Does the proposed use result in animal tissues? If so, what is the qualitative and quantitative composition of these residues?

4. Do the toxicological studies of the product allow to conclude that the proposed use does not present risks:

   - for the consumer?
   - for the user?

5. What are the nature and the persistence of the excreted products derived from virginiamycin? Can these products be prejudicial to the environment?

6. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs(1), as last amended by the Commission Directive 88/228/EEC(2), the use of virginiamycin is authorized under the conditions set out as follows in the Annexes to the Directive:

Species of animal	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff
Turkeys (up to 26 weeks)	5	20
Other poultry, excluding ducks, geese, laying hens and pigeons (up to 16 weeks)	5	20
Piglets (up to 4 months)	5	50
Pigs (up to 6 months)	5	20
Calves (up to 16 weeks)	5	50
Calves (up to 6 months)	5	20
		80*
Laying hens**	10	20

An extension of the use of virginiamycin under the following conditions has been requested:

Species of animal : fattening cattle

Dosages : - in complete feedingstuffs : 15-50 mg/kg

- in the daily ration : 150 mg/100 kg live weight + 6 mg for each 10 kg live weight exceeding 100 kg.

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* milk replacers

** authorized by derogation up to 30 November 1988 (Annex II)

(1) O.J. No L 270, 14.12.1970, p. 1

(2) O.J. No L 101, 08.04.1988, p. 30

OPINION OF THE COMMITTEE

1. The efficacy of virginiamycin for fattening cattle has been tested in 22 trials involving 1852 animals. These trials were conducted in the USA and several European countries on cattle of various breeds and baseline weights, for different lengths of time and under different feeding regimes, energy intakes and climatic conditions.

   The dose/response relationship was studied over a range of doses (5, 10, 15, 25, 40 and 50 mg/kg complete feedingstuff) equivalent to doses of 0,2-1,2 mg/kg liveweight/day or 60-500 mg/animal/day. The results show that, at doses between 10 and 50 mg/kg feedingstuff, the addition of virginiamycin significantly improves the daily liveweight gain and the feed conversion ratio. The dose/response relationship is curvilinear. A largescale trial on 800 animals showed that the carcase weight at slaughter (hot carcase weight) increases with dose and that carcase quality is not affected.

   These findings show that the minimum and maximum levels proposed are appropriate.

   To prevent incorrect use of supplemented feed in ruminating cattle it is advisable to fix a maximum daily dose of virginiamycin for each animal in relation to body weight. As feed intake in ruminating cattle does not increase in proportion to body weight, it is necessary to adjust the quantity of virginiamycin in the ration according to the formula: 90 mg + 80 mg/100 kg b.w. as set out in the table below:

Animal weight (kg)	Average daily feed intake (kg)	Virginiamycin mg/head/day 90 mg + 80 mg/100 kg b.w.	Equivalent in mg of virginiamycin/kg complete feedingstuff
100	3,4	170	50
150	4,4	210	47,7
200	5,6	250	44,6
250	6,7	290	43,3
300	7,6	330	43,4
350	8,3	370	44,6
400	9,0	410	45,5
450	9,6	450	46,8
500	10,4	490	47,1
550	10,5	530	50
600	10,9	560	51,3

2. The antibacterial properties of virginiamycin and the question of the development of resistance by organisms within the spectrum of activity of this substance have been investigated repeatedly because this antibiotic has been used for several years already as a feed additive for other farm animals, e.g. pigs, calves, laying poultry. The fact that the antibiotic consists of two separate antimicrobially active substances probably explains why there has been no increase in the resistance levels of Gram-positive bacterial species against this antibiotic and no change in the sensitivity to antibiotics used under clinical conditions for the treatment and the prevention of infectious diseases.

An additional study was carried out on fattening cattle to ascertain the influence of virginiamycin on the Gram-negative intestinal bacterial flora (E. coli, Salmonellae). It showed that there was no significant increase in the bacterial counts of E. coli either in the jejunum and ileum or in the faeces of treated animals compared to

controls, when doses of 65 ppm and 80 ppm were used in the fattening period. It may be concluded therefore, that the inclusion of virginiamycin in feedingstuffs at the proposed levels does not favour the growth of salmonella and does not result in persistence and increased excretion of Gram-negative bacteria in the faeces of fattening cattle.

3. Metabolic studies in rats with 14-C-labelled virginiamycin showed rapid excretion of radioactivity, only 15% being absorbed from the gut. 80% of the blood radioactivity was found in the plasma, of which 75% was protein-bound. Radioactivity was also present in liver, lung and muscle. In adult male cattle 93.6% of radioactivity is excreted rapidly in the faeces over 120 hours and 1.3% in the urine over 72 hours. Radioactivity is excreted in the bile only for 72 hours which confirms the absence of significant enterohepatic cycling. Overall there is very little absorption of virginamycin from the gut in cattle.

In vitro studies have shown that only factor M is partially metabolised in the rumen into 3 major metabolites. Two are reduction products from bacterial action and inactive antibiotically. The third has about 50% of the antibiotic activity of factor M. Animals treated orally for 7 days with 1 mg/kg body weight of 14-C-labelled virginiamycin had no detectable residues in muscle and fat at zero withdrawal time (limit of detection: 50 ug/kg and 250 ug/kg respectively). Residues in the liver and kidneys decreased with a half-life of 5 days to 0,24 and 0,11 mg/kg tissue after 120 hours.

No tissue residues were detectable microbiologically (limit of detection 50 ug/kg).

40 % of the radioactive residues in the liver are extractable. They consist essentially of numerous metabolites, none exceeding 6.5% of the total radio-activity. Their nature has not been determined. The non-extractable liver residues are associated with the protein fraction. Rats and cattle metabolise virginiamycin similarly. The presence of 14-C-labelled cholesterol indicates that some of the virginiamycin is metabolised to acetate which latter is then used in the synthesis of cholesterol. The non-extractable fraction yields on hydrolysis 3 components of factor M. The contribution of specific amino acids from hydrolysis of factor S is very small.

4. The acute oral toxicity of virginiamycin is low in rats and mice, the LD_{50} being greater than 7000 mg/kg body weight. No adverse effects were noted in 90-day studies in rats and dogs given doses from 5 to 100 mg/kg body weight. Studies of similar length in pigs with doses up to 500 mg/kg body weight and in calves up to 80 mg/kg body weight showed no toxic effects. The NEL in a 6-months study in dogs given 25,200 or 750 mg/kg body weight was 25 mg/kg body weight. Higher doses caused lower erythrocyte counts, an increase in relative kidney weight and only at the highest dose bile duct proliferation.

An oral study extending over 2.5 years in rats with doses of 25, 50 and 250 mg/kg body weight per day established an NEL of 25 mg/kg body

weight. At higher levels there were changes in haematological parameters and testicular weights were increased. There was no evidence of carcinogenicity. A 2-year feeding study in mice with doses of 25, 75 and 1000 mg/kg body weight per day showed increased incidences of malignant lymphoma in males and endometrial stromal sarcomas in females. However these incidences were within the range of historical controls and were therefore not considered to be treatment related. The NEL in this study was 25 mg/kg body weight. Higher levels showed increased food intakes in males and increased kidney weights in females. This study also revealed no evidence of carcinogenicy. From these long-term studies an ADI of 0.25 mg/kg body weight/day may be established.

No adverse effects on reproduction were noted in 1-generation reproduction studies in rats, rabbits and pigs fed virginiamycin from mating to delivery with doses up to 500 mg/kg body weight in rats and 20 or 100 mg/kg feed in rabbits and pigs. The NEL of a 2-generation reproduction study in rats was 65 mg/kg body weight. Higher doses caused a reduction in pup weight of the F_{1b} generation during lactation. Teratogenicity studies in mice showed an NEL of 160 mg/kg body weight and in rats of 75 mg/kg body weight. Higher doses were toxic to the dams but caused no embryotoxicity or teratogenicity.

In vitro mutagenicity tests in various strains of Salmonella typhimurium were negative. However the mouse lymphoma test was

positive. An in vitro test for Unscheduled DNA Synthesis in rat primary hepatocytes was negative. An in vitro SCE test was negative as well.

Only 2 cases of dermal allergy to virginiamycin in at least 60 chronically exposed workers have been reported during 20 years of production. No animal tests to establish irritancy and sensitization potential have been carried out.

The toxicological data establish an ADI of 0.25 mg/kg body weight and suggest the absence of any health hazard to the consumer from any residues of virginiamycin.

5. Many studies have been carried out on the environmental impact of the use of virginiamycin. Most of the substance present in excreted products is the unaltered compound and about 20% is present as the three metabolites of factor M in approximately equal proportions. Virginiamycin is unstable in the environment and disappears quickly from excreta, soil and water. Its half-life is 24 hours. It is very slightly toxic to land and aquatic animals and plants. Trials conducted with cattle slurry containing 1.2 - 150 mg/kg virginiamycin have shown no deleterious effects on methanogenesis. Contamination of the environment would appear unlikely on the basis of these data.

6. For the reasons set out above the Committee is of the opinion that the use of virginiamycin in feedingstuffs for cattle at the dosages proposed is acceptable.

References : Dossier from Smith Kline Animal Health Products (1986)