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Reports of the scientific committee on animal nutrition

(Seventh series – 1988)

Report

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FOREWORD

The Scientific Committee on Animal Nutrition was set up by Commission Decision 76/791/EEC of 24 September 1976 (1) in order to provide the Commission with informed opinions on scientific matters relating to the use of feed additives. The members of the Scientific Committee are independent and highly qualified scientists in the relevant fields of veterinary and human medicine, animal nutrition and environmental protection. The Secretariat of the Committee is provided by the Commission's Directorate-General for Agriculture.

The seventh series of reports of the Scientific Committee for Animal Nutrition (2) contains the opinions expressed by the Committee during the years 1987 and 1988 in answer to the questions of the Commission. They developed as the result of intensive discussions of the members of the Committee and reflect the present state of knowledge concerning the feed additives to be judged as well as the provisions of the relevant Community legislation, in particular the requirements of Council Directive 84/587/EEC of 29 November 1984 (3) amending Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (4) and of the Council Directive 87/153/EEC of 16 February 1987 fixing guidelines for the assessment of additives in animal nutrition (5).

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(1) O.J. No L 279 of 9.10.1976, p. 35

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(3) O.J. No L 319, 8.12.1984, p. 13
(4) O.J. No L 270, 14.12.1970, p. 1
(5) O.J. No L 64, 7. 3.1987, p. 19

C O N T E N T S

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(1) Set up by Commission Decision 76/791/EEC, 24/09/1976 (OJ No L 279, 09/10/1976, p. 35)
(2) Appointed by Commission Decision 86/C 173/02, 30/06/1986 (OJ No C 173, 11/07/1986, p. 2)
(3) Elected Chairman on 08/07/1986
(4) Chairman until expiry of his term of office on 08/07/1986
(5) Re-elected Vice-Chairman on 08/07/1986
(6) Elected Vice-Chairman on 30/09/1986
(7) Commission of the European Communities, Directorate-General for Agriculture

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF MADURAMICIN AMMONIUM IN FEEDINGSTUFFS FOR CHICKENS FOR FATTENING

Opinion expressed 27 April 1988

TERMS OF REFERENCE (October 1986)

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

1. Has the use as coccidiostat of the antibiotic maduramicin ammonium (ammonium salt of polyether monocarboxylic acid) at the dosage proposed for chickens for fattening (see background) significant effects on the prevention of coccidiosis in this animal species?

2. Is this use safe for chicken?

3. Can it result in the development of resistance in bacteria to prophylactic or therapeutic preparations?

4. What is the metabolic rate of maduramicin in the chicken? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative composition and persistence of these residues?

5. Do the toxicological studies allow to conclude that the proposed use does not present risks

   - for the consumer?
   - for the user?

6. What are the nature and the persistence of excreted products derived from maduramicin ammonium? Can these products be prejudicial to the environment?

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

BACKGROUND

Maduramicin ammonium was the subject of an application for admission in Section D (Coccidiostats and other medicinal substances) of the Annex to Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs(1) under the following conditions of use:

Species of animal : chickens for fattening
Use level : 5 mg/kg complete feedingstuff
Other provisions : use prohibited at least five days before slaughter.

OPINION OF THE COMMITTEE

1. Maduramicin ammonium is an ionophore consisting of 90% of the ammonium salt of a polyether monocarboxylic acid with an OCH_3 group at the C5 of the A ring (alpha-maduramicin) and 10% of the structurally similar compound with an -OH group instead of $-OCH_3$ at the C5 of the A ring (beta-maduramicin). The efficacy of maduramicin as a coccidiostat in chickens for fattening has been tested in battery chickens for fattening infected with four laboratory strains of Eimeria and in pen-raised chickens for fattening infected with six field strains of Eimeria. These trials established an effective but narrow dose range of 5-6 mg/kg complete feedingstuff as judged by improved weight gain, improved feed efficiency, reduced number of gut lesions and reduced mortality.

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

In battery trials in six countries 5 mg/kg complete feedingstuff was effective in preventing clinical coccidiosis and was comparable to treatment with other ionophores. A similar series of pen-raised chickens for fattening in seven countries confirmed the efficacy of that dosage regime when judged by similar parameters. In trials under commercial conditions in seven countries involving 3,25 million chickens for fattening 5 mg/kg complete feedingstuff was efficacious while higher levels caused a reduction in body weight gain.

These findings suggest that maduramicin is effective for the prevention of clinical coccidiosis in chickens for fattening at a dose of 5 mg/kg complete feedingstuff.

2. Administration of maduramicin to chickens for fattening at doses as high as 15 mg/kg feedingstuff resulted in significant depression of body weight gain. Feed efficiency was reduced at doses from 8-15 mg/kg feedingstuff. No other treatment-related effects were noted except a slight reduction in lymphoid tissue of the bursa and thymus at high doses.

Maduramicin had no adverse effects at 5 mg/kg feed on turkey poults, guinea fowl, laying turkeys, fattening rabbits, horses, grower and finishing pigs, lactating cows and finishing steers. Maduramicin has no deleterious effect on carcase quality and flavour in chickens for fattening.

3. Maduramicin ammonium is an ionophore antibiotic with moderate activity against many Gram-positive bacteria, but with no activity against Gram-negative organisms. Although no in vitro studies on the development of resistance and cross resistance were carried out, the in vivo studies showed that stable resistance caused by chromosomal mutation in sensitive Gram-positive organisms does not develop, even though a transient loss in sensitivity was observed. Maduramicin does not cause the selection of transmissible resistance factors in indigenous faecal coliforms nor in experimentally introduced salmonella. No effect was observed on colonisation or shedding of salmonella in chickens.

   The addition of maduramicin ammonium at the proposed dose of 5 mg/kg complete feedingstuff to the feed of chickens does not lead to the development of bacterial resistance to prophylactic or therapeutic preparations nor does it cause a persistence of Gram-negative bacteria in the gut of chickens.

4. The metabolism of maduramicin was studied using the compound labelled with 14-C in 7 well defined positions in the molecule. Rats metabolise alpha-maduramicin by O-demethylation at a site in the terminal G ring, the metabolite in the chicken being beta-maduramicin. The liver is the main site of metabolism in the rat and the other species. In the rat 96% of the radioactivity was extracted and consisted of 36% alpha-maduramicin and 64% metabolites.

   Maduramicin and/or its metabolites are rapidly eliminated, more than 70% of the ingested radioactivity being recovered in the excreta within the first 48 hours and more than 93% within the first 5 days of withdrawal. No measurable radioactivity was found in the carcase after 8 days withdrawal. Repeated administration leads to a steady-state plasma level after 72h. Detailed tissue level kinetics

have not been determined. However at zero withdrawal time residues are found mainly in fat and skin (1,29 mg/kg tissue), liver (0,49 mg/kg tissue) and kidneys (0,13 mg/kg de tissue), with very little appearing in muscle (0,05 mg/kg tissue). These tissue levels decreased rapidly to the limit of detection (0,025 mg/kg) in muscle (1 day), but more slowly in kidneys (3 days), skin (4 days), liver (5 days) and fat (7 days). The residue half-life was 20-27 hours. Tissue residues in chickens fed 5 mg/kg feed for 29 to 44 days, are measured by RIA (detection limit 0,025 mg/kg), closely correlated with the radiochemical estimates but were generally somewhat lower. Overall no significant residues were detectable by RIA (Radioimmunoassay) after 5 days withdrawal.

Of the tissue metabolites in chickens dosed with 5,5 mg/kg 14-C maduramicin in feed for 7 days, 93-99% were extractable. Most was alpha-maduramicin, the balance being beta-maduramicin. No other metabolites were detected.

The antibiotic activity of the residues in tissues was not determined but in the fat it would be essentially that of alpha-maduramicin, the major component. Roasting of the carcase had no effect on tissue residue levels.

5. Maduramicin has been tested thoroughly in acute toxicity, 28-day, 90-day and 12-month studies, in carcinogenicity, chronic toxicity, reproductive function and teratogenicity studies in mice, rats, rabbits and dogs. Mutagenicity was examined both in in vitro and in vivo tests.

The alpha and beta components had a high acute oral toxicity in the mouse and rat and a high dermal toxicity in the rabbit. The subchronic studies in the rat and dog showed adverse effects on growth, the heart being the target organ in the rat (lowest effective dose 0,35 mg/kg body weight), the heart, skeletal muscle and the eye being target organs in the dog (lowest effective dose 0,45 mg/kg body weight). The rat reproductive studies showed marginal effects on pup weight, litter size and pup survival in the F2b generation at 0,15 mg/kg body weight. Maduramicin is not teratogenic or foetotoxic but 3 mg/kg body weight caused 100% maternal mortality. Maduramicin is not carcinogenic. The NEL is based on the long-term rat study, giving an estimated ADI of 0,001 mg/kg body weight. Maduramicin is not genotoxic except for equivocal results in one test for chromosomal aberrations in mammalian cells.

Alpha-maduramicin has a preferential affinity for monovalent cations as shown in ion displacement studies (Chao-Min liu et al., 1983). Tests in model systems for cardiovascular and central nervous system effects, because of the known pharmacological activities of ionophores, produced no significant effects. These pharmacological studies produced no evidence of myocardial damage in the rat and dog except for ECG changes in the dog at very high dose levels (1 mg/kg b.w.). Maduramicin has been shown to be incompatible with the therapeutic antibiotic tiamulin.

Although maduramicin is irritating to the skin and corrosive to the eye the method of preparation of the premix prevents any formation of dust containing maduramicin. The allergenic potential has not been examined.

On the basis of these findings the Committee concludes, that at the doses proposed for use in chickens there is no risk for the consumer nor for the user. Intake from residues up to 60 ug/person per day is acceptable on the basis of the ADI.

6. The half-life of maduramicin in stored chicken excreta, as determined by antibiotic activity measurements, is about 55 days depending on the temperature of storage. The concentration in the soil following standard agricultural practice of fertiliser usage is of the order of 2-6 ug/kg soil. Further maduramicin is rapidly degraded in the soil to a large number of polar breakdown products only 7% remaining as maduramicin. Considering these low levels it would be unrealistic to require the identification of the degradation products in the soil.

The toxicity of maduramicin for the Daphnia and fish is similar, the NEL being approximately 1 mg/l. Neither methanogenesis nor soil nitrification are inhibited by maduramicin in chicken excreta when these are used as fertiliser nor does it have any significant phytotoxicity.

In the opinion of the Committee the excreted products derived from maduramicin are not prejudicial to the environment.

7. In the light of the information supplied the Committee is of the opinion that maduramicin ammonium is acceptable without risk for use in the feedingstuff for chickens for fattening at a level of 5 mg/kg complete feedingstuff subject to a withdrawal period of 5 days before slaughter.

References : Dossiers supplied by Cyanamid
Liu, Chao-Min, Hermann, T.E., Downey, A., Prosser, B. La T., Schildknecht, E., Palleroni, N.J., Westley, J.W. and Miller, P.A., J. Antibiotics (1983), 36 (4), 343-350

SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF FLAVOPHOSPHOLIPOL IN FEEDINGSTUFFS FOR RABBITS

Opinion expressed 27 April 1988

TERMS OF REFERENCE (April 1982)

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

1. Does the use of antibiotic flavophospholipol under the conditions proposed for feedingstuffs for rabbits (see background) result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. 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 Council Directive 88/228/EEC[2], the use of flavophospholipol is authorized at Community level under the conditions set out as follows in Annex I Section A of the Directive:

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(1) O.J. No L 270, 14.12.1970, p. 1
(2) O.J. No L 101, 08.04.1988, p. 30

Species of animal	Maximum Age	Minimum content	Maximum content
		ppm (mg/kg) of complete feedingstuff	
Turkeys	26 weeks	1	20
Other poultry, with the exception of ducks, geese laying hens and pigeons	16 weeks	1	20
Pigs	6 months	1	20
Piglets	3 months	10 (*)	25 (*)
Calves	6 months	6	16
	6 months	8 (*)	16 (*)
Animals bred for fur excluding rabbits		2	4
Laying hens		2	5
Cattle for fattening		2 (**)	10 (**)

(*) Milk replacers

(**) The following statement must be notified in the instructions for use : "For supplementary feedingstuffs the maximum dose in the daily ration must not exceed
- for 100 kg bodyweight : 40 mg,
- above 100 kg : add 1,5 mg for each additional 10 kg bodyweight"

It is proposed to complete the authorization of use of this additive by the following provisions:

Species of animal : rabbits

Minimum and maximum content of complete feedingstuffs : 2-4 ppm (mg/kg)

OPINION OF THE COMMITTEE

In its report of 22 January 1986 the Committee was of the opinion that the use of flavophospholipol under the conditions proposed (complete feedingstuffs for rabbits containing 2-4 mg/kg flavophospholipol) could be admitted provisionally. A reassessment of this use was envisaged when additional studies became available.

At that time the Committee noted that the data submitted to it did not permit the conclusion that the product is not absorbed from the digestive tract of the rabbit. It requested further studies on tissue residues in this species using analytical methods other than microbiological assays. Subsequently further information on the metabolic balance and residues in rabbits was supplied, on which the Committee commented in its opinion dated 30th September 1986. In its comments the Committee considered the additional information supplied to be unsatisfactory because it still did not permit a definite conclusion that the product was not absorbed from the digestive tract and therefore could not lead to tissue residues in the rabbit, particularly if caecotrophy existed. The analytical methodology used specifically permitted only the microbiological identification of flavophospholipol but not of any possible microbiologically inactive metabolites. The Committee accepted that the use of an HPLC method would not offer any better approach to the determination of residues. It therefore requested a study in rabbits of the metabolism and pharmacokinetics of flavophospholipol appropriately labelled with 14-C under conditions comparing the effect of the presence and absence of caecotrophy.

The Committee was eventually provided with additional documentation reporting the results of earlier preliminary experiments, carried out in 1975-1976. In these the labelling of flavophospholipol with 14-C was

attempted biosynthetically and metabolic balance studies in rats with this material were subsequently carried out. Despite the low specific activity of the administered material these studies demonstrated that some 29% of the administered radioactivity was recovered as 14-C CO_2 and some 50% appeared in the faeces and urine. These results conflicted with the finding of 100% excretion of flavophospholipol in the faeces in a metabolic balance study which used a microbiological assay procedure.

To clarify these discrepancies between apparent absorption and metabolism as shown in the experiment using labelled flavophospholipol, and apparent non-absorption as demonstrated in the experiments using microbiological activity measurements, the Committee was provided with additional information on the antibiotic activity of certain derivatives of flavophospholipol. It was shown that, when using a controlled chemical degradation of the molecule, the progressive removal of three of the glycosidic units did not change its specific antibiotic activity. Moreover, all the glycosidic units detached from 14-C-flavophospholipol were labelled. Even if such a chemical attack does not pretend to mimic the actual metabolism in the rabbit, the hypothesis may be reasonably put forward that such labelled fragments could be released in the digestive tract, especially in relation to the phenomenon of caecotrophy particular to this species, then absorbed and subsequently metabolized to 14-CO_2.

The Committee therefore accepted, in the special case of the rabbit, that these findings largely explain the observed discrepancies. Taking into account the relatively inoccous nature of flavophospholipol and very low residue levels, it is of the opinion that flavophospholipol could be used safely at a minimum and maxium content of 2-4 mg/kg in complete feedingstuffs for rabbits.

References : Dossiers supplied by Hoechst AG

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF AVILAMYCIN IN FEEDINGSTUFFS FOR PIGS

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 avilamycin (oligosaccharide) at the dosages proposed for feedingstuffs for pigs (see background) significant effects on the growth?

2. Is this use safe for the pig?

3. Can it result in the development of resistance in bacteria to prophylactic or therapeutic preparations, or exert an effect on the persistance of Gram-negative bacteria in the digestive tract of the pig?

4. What is the metabolic fate of avilamycin in the pig? Does the proposed use result in residues in animal tissues? If so, what is the qualitative and quantitative composition of these residues?

5. Do the toxicological studies allow to conclude that the proposed use does not present risks

   - for the consumer?
   - for the user?

6. What are the nature and the persistance of excreted products derived from avilamycin? Can these products be prejudicial to the environment?

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

BACKGROUND

Avilamycine was the subject of an application for admission in Annex II, Section A (Antibiotics), of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs [1] under the following conditions:

Species of animal : pigs
Dosages : - pigs up to 4 months : 40-80 mg/kg complete feedingstuff
- pigs of 4 - 6 months : 20-40 mg/kg compete feedingstuff

OPINION OF THE COMMITTEE

1. Avilamycine is an oligosaccharide which consists of 12 identified and a small number of unidentified factors, of which factor A represents about 60%, factor B about 7%, and factor D about 5%. The efficacy of avilamycin for promoting the growth of pigs has been tested in 21 trials conducted in Europe and in 11 trials performed in the USA. These trials involved a total of 2508 animals. During these trials several batches of product from two different manufacturers were used. The parameters considered were the daily increase in live weight and the indices of food consumption and daily ingestion of avilamycine.

   The dose-response relationship was studied in a series of experiments consisting of 9 European trials with starter pigs with a live weight of 9 - 40 kg, 9 trials with pigs weighing 24 - 80 kg and using a

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

combination of the products of the two manufacturers, and three European bridging trials with the product of one manufacturer. Eight of the 11 US trials used one product and three the other product on pigs weighing between 23 and 70 kg.

The composition of the feeds was very variable and often incompletely recorded. The protein content was frequently either above or below the norm and the energy values were never indicated. Evaluation of the carcases was frequently absent and the experimental protocol not always optimal. Most of the European feeding regimes included 100 - 125 mg/kg copper as well as avilamycine thus delivering two growth factors to the animals. This was done because of the current practice of adding copper to pig feed but it led to a considerable spread of the results. No attempts were made to demonstrate efficacy under different feeding conditions, although a 6% increase in body weight was demonstrated under optimal feeding conditions. The product was tested at the following concentrations: 0, 10, 20, 40, 60 and 80 mg avilamycine/kg final feed.

Statistical treatment of the results using curvilinear regression methodology indicated that the optimum doses for fattening starter or grower pigs lay between 22 and 64 mg/kg feed. Doses of 40 mg/kg feed significantly improved the daily weight gain of starter pigs up to four months of age by about 5.6%. In grower pigs a significant improvement in weight gain was already noticeable at doses of 10 - 20 mg/kg feed. Higher doses did not appear to show any further significant improvement in weight gain. The food conversion index was already significantly improved by 10 mg/kg feed, higher doses giving no better results.

These findings suggest that avilamycin is effective for fattening pigs at doses of 20 - 40 mg/kg feed for pigs up to the age of four months and 10 - 20 mg/kg feed for pigs aged 4-6 months.

2. Avilamycine administration to pigs at doses up to 3000 mg/kg feed caused no treatment-related adverse effects. It improved body weight gain significantly. Avilamycin is not toxic to cattle or sheep. Feeding of avilamycin has no effect on the quality of the pig meat as determined by taste panel tests.

3. Avilamycine is an oligosaccharide consisting of several factors with a limited antibacterial spectrum. It is only effective against Gram-positive bacteria. In-vitro tests on various bacterial species showed only a slight reduction in sensitivity to avilamycin, Clostridia remaining fully sensitive. No correlation exists between a possible resistance to avilamycin and resistance to other therapeutically used antibiotics. Although high doses of avilamycin briefly increased the number of E. coli in the faeces, this effect disappeared after a few weeks. Salmonella-infected pigs showed no increased or prolonged faecal excretion of Salmonella when treated with avilamycin.

   The addition of avilamycine at the proposed doses to the feed of pigs does not lead to the development of bacterial resistance to prophylactic or therapeutic preparations nor does it cause a persistence of Gram-negative bacteria in the gut of pigs.

4. The metabolic fate of avilamycin was studied using the compound uniformly labelled with 14-C on all constituent factors. Urinary

excretion was low (4.5%), most being excreted in the faeces (93.4%). There was only slight biliary excretion. Absorption was therefore minimal. Biotransformation yields two major metabolites of known structure. Excretion of avilamycin is very small, the major metabolites representing about 50% of the urinary and faecal excretion products.

Tissue residues are minute at zero withdrawal time, the maximum residues in the liver reaching 0.14 - 0.22 mg/kg tissue (limit of detection 0.012 - 0.017 mg/kg tissue). Residues in the kidneys were 0.10 mg/kg tissue, in the muscle 0.025 mg/kg tissue and there was slight accumulation in fat tissue. No residues with antibiotic activity were deteted in kidneys, muscle or fat by a radio-autographic method (limit of detection 50 ug/kg). No residues were detectable in muscle, liver, kidney and body fat after five days withdrawal. The residues are essentially inactive antimicrobially.

5. Avilamycin has been tested thoroughly in the mouse and rat in short-term, long-term, multigeneration-reproduction and teratology studies and in a relay toxicity study in rats without revealing any carcinogenic, mutagenic or reproductive effects. A 12-months study in dogs showed no significant toxic effects. However, doses of 3000 mg/kg feed of avilamycin activity, administered to rats in the multigeneration-reproduction study, caused borderline hepatic enlargement in some of the progeny in both sexes without any associated clinico-chemical or histological abnormalities. The substance is not genotoxic when examined in an adequate battery of mutagenicity tests. It has no allergenic potential but is slightly irritant to skin, eyes and the respiratory tract. The NEL is based on multigeneration-reproduction study. The ADI is estimated to be 0.15 mg/kg body weight avilamycin activity.

On the basis of these findings the Committee concludes, that at the doses proposed for use in pigs there is no risk for the consumer nor for the user.

6. Avilamycine has been studied extensively for persistence of excreted products in the environment. Only 5% of the excreted products are avilamycins, the remainder being hydrolysis products of which 50% are the two major metabolites. Avilamycin is not very stable in soil or water and is broken down within one day in sunlight. These factors suggest that it is unlikely to accumulate in the environment.

   It is poorly soluble in water and has a low n-octanol/water partition coefficient suggesting little or no risk of avilamycin passing from soil to water or to any life forms on land and in water. Its toxicity to Daphnia, fish and earthworms is small.

   The excreta of pigs given avilamycin do not affect methanogenesis or soil nitrification when used as manure nor do they have any deleterious effects on plant crops.

   In the opinion of the Committee the excreted products derived from avilamycine are not prejudicial to the environment.

7. The chemical composition of avilamycin is controlled by HPLC and its potency is standardised microbiologically. The range of factors used in the efficacy trials is about the same as that in the present

production. For official control microbiological tests are recommended.

8. In the light of the information supplied, the Committee is of the opinion that avilamycine may be used in the feedingstuff for pigs without risk at the following concentrations:

   - pigs up to four months : 20 to 40 mg/kg complete feedingstuff
   - pigs of 4 - 6 months : 10 to 20 mg/kg complete feedingstuff.

References : Dossier of Eli Lilly & Co

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)

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF ASTAXANTHIN IN FEEDINGSTUFFS FOR SALMON AND TROUT

Opinion expressed 8 March 1989

TERMS OF REFERENCE (June 1985)

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

1. Does the use of astaxanthin at the level of 100 mg/kg of complete feedingstuff result in effects on the fish other than the pigmentation of the muscle and skin ?

2. Does the desired pigmentation need the use of 100 mg astaxanthin/kg complete feedingstuff throughout the rearing period ?

3. Is the proposed use safe for the target species ?

4. Is the proposed use safe for the consumer ? Which is the qualitative and quantitative composition of astaxanthin residues in edible tissues and organs of the fish ?

BACKGROUND

It is necessary to establish whether the use of astaxanthin under the proposed conditions is in conformity with the requirements of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1) (2).

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(1) O.J. No. L 270 of 14.12.1970, p. 1
(2) O.J. No. L 319 of 8.12.1984, p. 13

OPINION OF THE COMMITTEE

1. Many species of crustacea and fish contain carotenoids naturally in their muscle and skin. However, only certain salmonid species have pigmented muscle. Astaxanthin is the major carotenoid of wild Atlantic salmon, while other salmonids may utilisé a wider range of carotenoids. Free astaxanthin and its isomers have been identified in the muscle of many sea and fresh water species of salmon. The carotenoids in the skin are usually esterifiéd. The source of all carotenoids in muscle and skin of salmonids is their food as the carotenoids cannot be synthesised by the fish. It is therefore necessary to add astaxanthin to salmon and trout feedingstuffs, when the fish are reared in fish farms to replace the natural sources. Astaxanthin occurs in the muscle of wild salmon and trout at levels up to 35 mg/kg. Because astaxanthin is the natural pigment in the muscle and skin of salmon and trout at levels higher than can be induced by feedingstuffs containing 200 mg/kg it cannot result in unforeseen biological effects.

2. The incorporation of 20-100 mg astaxanthin/kg complete feedingstuff over 15 days yields a level in the muscle of farmed salmon and trout of 3 and 8 mg/kg muscle respectively. 200 mg/kg feedingstuff produce about 10 mg/kg muscle and 400 mg/kg feedingstuff about 15 mg/kg muscle. To achieve a desirable colouring of the muscle, doses of 20 to 100 mg astaxanthin/kg complete feedingstuff need to be administered during the greater part of the growing period.

3. The data supplied show that astaxanthin is safe for salmonids. It may contribute to the development of fish and crustaceans and be involved in the physiology of the reproductive functions, like mating behaviour, stimulation of spermatozoa, protection of eggs against light etc. In a recent publication it has been reported that astaxanthin has a favourable effect on the immune reponse (3).

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(3) Bendich, A. and Shapiro, S.S., J. Nutrition, 116, 2254-2262, 1986
Al-Kalifa, A., Simpson, K.L., Comp. Biochem. Physiol.B. Comp. Biochem., 91 B, 563-568, 1988
Dossier from Hoffman-La Roche 1986

4. Astaxanthin has a low acute toxicity, the oral LD-50 being over 2000 mg/kg in the rat. A 14-week study in rats with doses of 300, 600 and 1200 mg/kg b.w. showed slight hepatotoxicity at the higher levels and some nephrotoxicity at the highest dose. The NEL was 300 mg/kg b.w. A 13-week study in dogs with doses of 40, 75 and 160 mg/kg b.w. showed no adverse effects.

A one generation reproduction study in rats with doses of 25, 100 and 400 mg/kg b.w./day showed no adverse effects on reproductive function. Litter parameters and pup development were normal except for a higher pup mortality during lactation at the highest dose level. The NEL was 100 mg/kg b.w. Embryotoxicity and teratogenicity studies in rats with doses of 250, 500 and 1000 mg/kg b.w. and in rabbits with 100, 200, and 400 mg/kg b.w. showed no adverse effects. There was no evidence of genotoxic potential in mutagenicity tests in Salmonella typhimurium and a micronucleus test in the mouse.

Because of the observation that moderately elevated doses of canthaxantin, a structurally closely related carotenoid, may cause retinal deposits of the carotenoid in the human retina, comparative studies on the metabolism and the pharmaco-kinetics of astaxanthin were carried out in the rat and man. Initial plasma levels of 14-C-labelled astaxanthin were much lower over the first 6 hours than those of equivalently dosed canthaxanthin but were subsequently similar. Canthaxanthin levels in the liver and spleen were much higher compared to astaxanthin, the highest levels of which were found in the small intestine. All other tissue levels remained comparable. Following a single oral administration only 10% was apparently absorbed, the remainder appearing in the faeces or gut contents, Of the absorbed astaxanthin some 66% appears in the urine, about 9% in the liver, about 7% in the gut and about 16% in the carcase. Astaxanthin is metabolised and excreted more quickly than canthaxanthin. About 10% of astaxanthin in feedingstuff is absorbed by fish, the proportion of isomers in the muscle matching that in the diet. No metabolism occurs in the muscle of fish but in the skin a small percentage is metabolised to zeaxanthin and other carotenoids.

Administration of 100 mg astaxanthin or canthaxanthin to a human volunteer resulted in almost 4 times higher peak plasma levels of canthaxanthin which would be equivalent to 7 kg fish containing 13 mg/kg astaxanthin. Astaxanthin was almost completely eliminated from the plasma after 48 hours while canthaxanthin levels had reduced to 50% only. After multiple administration the elimination half-time was about 17 hours for astaxanthin compared to 4.5 days for canthaxanthin. To reach similar steady-state plasma levels would require doses of astaxanthin 25 times higher than cantaxanthin.

The toxicity and pharmaco-kinetic data do not provide any evidence for a hazard to the consumer from the consumption of salmon or trout which had been fed up to 200 mg astaxanthin/kg complete feedingstuff. At this level of addition, the amount of astaxanthin appearing in the muscle of fish is about half that found in wild species. The question of residues or withdrawal periods therefore does not arise. The differences in the parmaco-kinetics of astaxanthin and canthaxanthin suggest that these compounds are metabolised differently by man. In addition man excretes astaxanthin much more rapidly so that retinal deposition of astaxanthin after consumption of pigmented fish muscle is unlikely. The use of astaxanthin up to 100 mg/kg complete feedingstuff is therefore acceptable in the opinion of the Committee.

Report of the Scientific Committee for Animal Nutrition on the use of Nitrovin in feedingstuffs

Opinion expressed 29/30 November 1988

TERMS OF REFERENCE (November 1988)

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the following question:

- Does the use of nitrovin as a growth promoter under the conditions given in the background give rise to dangers for human or animal health, particularly mutagenic, teratogenic or cancerogenic effects or present undesirable effects on the environment?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC (1) of 23 November 1970, concerning additives in feedingstuffs, Member States are authorized to use, by way of derogation up to 30 June 1989, nitrovin as an additive in feedingstuffs for use as a growth promoter in chickens for fattening, other poultry, calves and pigs under the following conditions:

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(1) OJ. no. L 270, 14.12.1970, p. 1.

Additive	Species or category of animal	Maximum age	Min. content mg/kg of complete feedingstuffs	Max. content mg/kg of complete feedingstuffs	Other provisions
1. Growth-promoters					
Nitrovin					For all feedingstuffs mixing or simultaneous use with antibiotics prohibited
	Chickens for fattening	-	10	15	
	Turkeys	26 weeks	10	15	
	Other poultry except ducks, geese, laying hens, pigeons	16 weeks	10	15	
	Calves	6 month	20	40	
			40	80	Milk replacers only
	Piglets	10 weeks	10	25	
			20	30	Milk replacers only
	Pigs for fattening	6 month	5	15	

Nitrovin had been authorized, in Annex I of the 4th Commission Directive 74/38/EEC (2) amending the annexes to Council Directive 70/524/EEC (1) as a growth promoter for chickens for fattening and in Annex II for pigs, calves and turkeys. In 1976 the Annex II uses for pigs were transferred to Annex I (13th Commission Directive 76/13/EEC (3)).

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(2) OJ no. L 30, 17.12.1974, p. 21
(3) OJ no. L 4, 09.01.1976, p. 21

Subsequently evidence arose of an apparant lack of stability transferred back to Annex II (46th Commission Directive 84/349/EEC (4)), whilst further work relating to stability was carried out. This work showed that the apparent lack of stability was due to the unreliability of the method of analysis then being used. A satisfactory method has now been developed resolving this problem (Dossiers Orphahell).

More recently doubts have been raised by certain Member States, partly based on a IARC publication of 1983, concerning the safety of the substance, in particular carcinogenicity, mutagenicity, teratogenicity and hazards for the environment. Data not previously known to the Commission have now become available.

OPINION OF THE COMMITTEE

The SCAN was provided with comprehensive documentation on nitrovin (see annex). In evaluating this evidence the working group noted that the data on metabolism in rats, pigs and chickens (Dossier Cyanamide) were adequate. Nitrovin is poorly absorbed (max. 1%), most being excreted in the faeces.

The available short-term studies in rat, dog and pig (Dossier Cyanamide) showed some evidence of hepatotoxicity and adverse effects on the kidneys and intestinal tract. From these a no-effect level of about 12,5 mg/kg body weight could be estimated.

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(4) OJ. no. L 183, 11.07.1984, p. 15

Chronic studies were available in three species:

The two chronic studies in rats (Dossier Cyanamide) were negative with regard to carcinogenicity. Both were inadequate regarding the number of animals, the parameters investigated and the duration of the studies.

The chronic study in hamsters (Dossier Cyanamide) showed no evidence of carcinogenic activity.

Of three chronic studies in mice, the study in C 57 black mice (Dossier Cyanamide) showed no evidence of carcinogenicity. The other two studies (Dossier Cyanamide) were performed in a lung-adenoma susceptible strain. Both showed an increased response to nitrovin administration which was dose-related concerning frequency, size and malignancy of tumors, particularly in females. However, the results of investigations in these susceptible strains are difficult to interpret. A whole series of mutagenicity tests, in bacterial systems and a test for sex-linked recessive lethals in Drosophila were positive, being evidence of a genotoxic potential of nitrovin.

The SCAN is of the opinion that information on the following aspects is required to enable a full toxicological evaluation to be made:

1. in vivo mutagenicity tests, particularly for their chromosomal effects

2. adequate multigeneration-reproduction and teratogenicity studies

3. adequate investigations of environmental impact.

For evaluating potential hazards to the user, additional information is needed on dust formation and whether an antidust formulation can be provided.

To evaluate the hazards to the consumer a better analytical method for tissue residues with a sensitivity of 10 ppb or less is needed. The SCAN draws attention to the fact that the apparent no-effect level in the rat and dog study would allow the establishment of an acceptable daily intake (ADI) of approximately 125 ug/kg bw. This is very close to the limit of sensitivity of the presently available method of analysis. In order to determine whether the ADI is likely to be exceeded, a much more sensitive method of analysis is required. Furthermore additional residue studies using such a more sensitive method are required.

On the basis of the information hitherto available the SCAN is unable to give a final opinion on the hazard for humans and target species, particularly the carcinogenic, mutagenic and teratogenic potential of nitrovin and on the possible hazards to the environment. Nevertheless the Committee wishes to draw attention to the advice given on the Nitrofuranes published in 1979.

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