REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF MADURAMYCIN AMMONIUM IN FEEDINGSTUFFS FOR TURKEYS

(Opinion expressed, 25 September 1997, Text consolidated, 24 October 1997)

TERMS OF REFERENCE (January 1993)

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

1. Has the use of Maduramycin ammonium (C47H83O17N, ammonium salt of a polyether monocarboxylic acid produced by *Actinomadura yumaensis*), under the conditions proposed for its use as an additive for the feedingstuffs for turkeys significant effects on the prevention of coccidiosis in this animal species?

2. Is this use safe to the turkeys?

3. Can it be monitored in animal feedingstuffs?

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

5. What is the metabolic fate of Maduramycin ammonium in turkeys? Does this use result in the presence of residues in meat? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

6. Do the toxicology studies allow to conclude that the proposed use does not present risks

   - for the consumer?

   - for the user?

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

8. In the light of the answer 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 amended by Council Directive 84/587/EEC of 29 November 1884[2], the use of Maduramycin ammonium (E 770, ammonium salt of a polyether monocarboxylic acid produced by *Actinomadura yumaensis*) is authorized at Community level under the conditions set out in Annex I, Section D (Coccidiostats) of Commission Directive 91/248/EEC[3] at last amended by Commission Directive 92/64/EEC[4]:

D. Coccidiostats

Species or category of animals	Maximum age	Minimum mg/kg of complete feedingstuff	Maximum mg/kg of complete feedingstuff	Other provisions
Chickens for fattening	-	5	5	Indicate in the instructions for use: -Use prohibited at least five days before slaughter. Dangerous for Equidae. "This feedingstuffs contains an ionophore; simultaneous use with certain medicinal substances (e. g. tiamulin) can be contraindicated"

The Scientific Committee for Animal Nutrition (SCAN) expressed his favourable opinion regarding the use of Maduramycin ammonium in the feedingstuffs for chickens for fattening in its report 27 April 1988[5].

In September 1992 a request was made to a new usage for Turkeys, as follows:

1 OJ No L270, 14.12.70 p.1
2 OJ No L319, 8.12.84, p.13
3 OJ No L124, 18.5.91, p.1
4 OJ No L221, 6.8.92, p.51
5 Reports of the Scientific Committee for Animal Nutrition Seventh Series, 1988. Catalogue No CD-NA-12824-EN-C. Report EUR 12824. Luxemburg: Office for EC Publications. (p.1)

B. Coccidiostats

Species or category of animals	Maximum age	Minimum Content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Turkeys	16 weeks	5	5	Indicate in the instructions for use: Use prohibited at least five days before slaughter. Dangerous for Equidae "This feedingstuffs contains an ionophore; simultaneous use with certain medicinal substances (e. g. tiamulin) can be contra-indicated"

OPINION OF THE COMMITTEE (SCAN 25-26 September 1997)

1. Maduramycin is a polyether monocarboxyilic acid produced through aerobic fermentation by the microorganism *Actinomadura yumaensis.* The efficacy of Cygro l % premix under the proposed conditions of use (5 mg/kg of complete feed) as an anticoccidial for turkeys has been investigated in battery, floor pen and commercial conditions studies. Battery studies were performed with two kinds of experimental design : (1) on turkeys fed with medicated feed (4, 5, 6 and 7 mg/kg) from 2 days before to 7 days after infection with different *Eimeria* strains, i.e, *E. adenoides* (5 trials), *E. dispersa* (3 trials), *E. gallopavonis* (4 trials), *E. meleagrinitis* (4 trials) or with mixed isolates of the same *Eimeria* strains; (2) on turkeys fed with medicated feed (4, 5, 6 and 7 mg/kg) from 2 days before to 14 days after infection with mixed isolates of *Eimeria* strains. Anticoccidial efficacy was evaluated by measuring animals' mortality, weight gain, feed intake, feed conversion, dropping scores and oocyst counts and the data obtained have been analysed statistically for individual studies and for combined studies using two model analysis methods. No trial has been carried out on healthy animals, even in the short observation period (from -2 to +7 days), to verify possible effects of the different levels of medication. The best performance results under the point of view of zootechnical parameters have been observed in the group of non-medicated non-infected animals (e.g. within the group of *E. adenoides* challenge) while among the medicated and infected groups 7 mg/kg was the most useful level of medication *(E. adenoides, E. dispersa, E. meleagrinitis and mixed Eimeria* strains).

   Only during the *E. gallopavonis* challenge 5 mg/kg resulted the most sucessfull level of medication. The maduramycin ammonium medicated feedingstuffs succeded in improving the feed efficiency in animals with infection-dependent decreased performances.

A complete protection from mortalíty was reached only at the 7 mg/kg level in the *E. adenoides* challenge, at the 6 mg/kg level in the *E. meleagrinitis* challenge, at the 5 mg/kg level in the E. *gallopavonis* challenge and at the 4 mg/kg level in the *E. dispersa* and in the mixed *Eimeria* strains challenges. A discrepancy was observed between the severity of infection of mixed *Eimeria* strains for which the improvement of the feed efficiency requested a level of medication of 7 mg/kg and the possibility of protection from mortality which was possible with a level of medication as low as 4 mg/kg.

The results from the floor pen studies (4 trials with mixed *Eimeria* strains infections) confirmed the efficacy of the increasing levels of medication with maduramicin in improving feed efficiciency. The protection from mortality recorded in the long lasting experiments has to be considered good enough in the 4 mg/kg and in the 6 mg/kg level groups. The statistical analysis of the data obtained from three floor pen studies and from nineteen battery studies demonstrate that maduramycin at 5 mg/kg of complete feed is the optimum dose rate for the control of coccidiosis in turkeys up to 16 weeks of age.

The commercial condition studies were performed in USA, ltaly and U.K. The studies in USA were conducted on a total of some 31 000 turkeys fed with a 5 mg/kg maduramycin diet from 1 day to 10 weeks of age, or from 1 day to 7 weeks of age or from 10 to 52 days of age.

Neither clinical coccidiosis nor adverse effects were observed under this level of feedingstuffs medication with maduramycin. The study in ltaly involved 3 300 male turkeys reared up to 19 weeks of age. Half of them were reared on a 5 mg/kg maduramycin diet plus dimetridazole up to 12 weeks and the remaining ones received a diet containing metichlorpindol + methylbenzoquate up to 16 weeks. The two regimens of treatment provided similar results regarding coccidiosis control but the maduramycin fed turkeys showed slightly better zootechnical performances (mean body weight gain).

The studies in U.K. were conducted on a total of about 156 000 female and male turkeys fed with a 5 mg/kg maduramycin diet up to 8 weeks of age, or with a same diet and alternatively with a metichlopindol + methylbenzoquate + lasalocid diet for 8 weeks of age, or with a 5 mg/kg maduramicin diet and alternatively with a metichlopindol + methylbenzoquate + lasalocid diet for a 8 weeks.

These last two anticoccidial diets were moreover enriched by virginiamycin as a growth promoter and dimetridazole as an anti-blackhead drug. In all instances, even when compared with other traditional anticoccidial additives, maduramycin showed good effectiveness in controlling coccidiosis and in improving the zootechnical performances of the treated turkeys.

2. An experiment was conducted in the USA to determine the clinical safety of 5 mg maduramycin/kg of complete feedinstuffs in turkeys of different ages. Turkeys were assigned to group in pens of 15 males or 17 females. At days 1, 15, 29, 43 and 57, four pens of each male and female were transferred from the control diet to one containing maduramycin for 2 days.

   Throught the study there was no difference in performance parameters between the turkeys recently introduced to maduramycin and the control group. It was concluded that maduramycin at 5 mg/kg of complete feedinstuffs is safe when introduced to turkeys at any time during the growing period. Another study carried out in the USA involved 24 males and 24 females turkeys approximately of 20.5 weeks of age. They were fed diets containing maduramycin at 5.0(x), 10(2x) or 15(3x) mg/kg of complete feedingstuffs for 2 weeks. There were no apparent adverse effects for maduramycin at 5 mg/kg of complete feedingstuffs. The maduramycin at 10 mg/kg of complete feedingstuffs has a rather pronounced effect on the daily weight gain of the male turkeys, but was relatively unaffected in hens. There were no mortality neither signs of clinical adverse effects. At 15 mg/kg of complete feedstuffs (3 times the recommended dose) 5 turkeys died after an average of 8 days but daily weight gain of the survivors was relatively unaffected.

   No data has been provided on the compatibility of maduramycin with tiamulin in turkeys.

   On the basis of these experiments it can be concluded that the maduramycin at 5 mg/kg of complete feedinstuffs is well tolerated in turkeys.

3. No data were available on monitoring of maduramycin in feedingstuff for turkeys. In the SCAN summary report of the use of maduramycin ammonium in feedingstuffs for chickens for fattening (opinion expressed 27 april 1988) data on this subject were not mentioned.

4. No further data were available on microbiology for maduramicin in turkeys. Maduramicin has moderate activity against Gram-positive bacteria but no activity against Gram-negative organisms. The only available results on antimicrobial activity are quoted in SCAN's 7th series, pp. 4, and it only deals with chickens for fattening. No microbiological effects could be detected for maduramycin in chickens. The SCAN opinion expressed 27 April 1998 stated that maduramycin at 5 mg/kg of complete feedingstuffs does not lead to the development of bacterial resistance to prophylactic or therapeutic preparations not does it cause persistance of gram-negative bacteria in the gut of chickens. In conclusion, no microbiological effects could be detected for maduramicin ammonium in chickens.

5. The metabolism of maduramycin ammonium in turkeys was studied using the compound labelled with 14-C at seven positions in the molecule. No balance study was performed. However, biliary excretion represented 22,7% (10,4-44.6) of the administered dose, índicating considerable absorption. A tentative study to establish the shortest administration period to reach a

steady state showed that the plasma radioactivity level of only two animals (out of 15) was sliglitly over 0.025 □g/ml (limit of detectíon of the radioactivity), after 7 days repeated dosage.

A thorough examination of the nature of the excreted metabolites was carried out which showed unchanged maduramycin was the major compound (23,8%). The other identified metabolites corresponded to the double O-demethylation at C-44 and C-45 (G-ring) (17.9%), mono-O--demethylation at the same G-ring (4.2%), double O-demethylation at A-ring and G-ring (3.3%), O-demethylation at G-ring and hydroxylation at an undefined position (3.3%). A great number of very minor metabolites correspondíng to more polar compounds were not identified.

Following a 7-day repeated administration of ^{14}C-maduramycin ammonium (7 mg/kg) the tissue residue levels measured at zero withdrawal time were the following: fat 0,164 (0.097-0.303) □g/g, liver 0.137 (0.057-0.223), skin plus fat 0.061 (0.028-0.114) and muscle <0.025 (limit of detection). After 1-day withdrawal all the tissues except the liver were <0.025 □g/g, and after 3-days the radioactivity was not detected in any of the tissues. More than 93.5% of the residual radioactivity in the fat was extractable of which 92,5% was unchanged maduramycin.

The figure for the liver was >89.0% and 51.1 % respectively, and 14.2% (0.032 mg/kg) was tentatively identified as beta-maduramycin (or O-demethylated maduramycin at C-47). A more polar metabolite (11.2%) was not identified, as well as several very minor metabolites all <10% of the total radioactivity. However, as the analysis was performed on the zero withdrawal samples only, the marker-residue has not been determined.

Another study carried out in rearing conditions, i.e. unlabelled maduramycin for 100-107 days, confirmed the rapid elimination of the residual maduramycin (HPTLC and fluorescence detection at 0.025 mg/kg level); zero-day for the muscle, 1-day for the liver and 3-day for the skin and fat. Taking into account the fact that most of the residues in fat are unchanged maduramicin, this study establishes indirectly that maduramycin is the marker-residue and fat the target-tissue.

When compared to the chicken (present data and SCAN Reports, 7th series, 1988), the metabolic fate of maduramycin ammonium in the turkey appears to be very similar in terms of biliary excretion, and nature of the excreted metabolites and tissue residues. However, in the turkey, plasma and tissue residue levels are lower and the disappearance of the residues from the tissues, based on the same 0,025 mg/kg detection limit, is faster (3 days ínstead of 7 days).

6. Maduramycin ammonium has been evaluated by the SCAN in 1988 with respect to the use as a coccidiostat in feedingstuffs for chickens for fattening. Maduramycin has been tested throughly in acute toxicity, 28-days, 90-days and 12-month studies, in carcinogenicity, chronic toxicity, reproductive toxicity studies including teratogenicity studies on mice, rats, rabbits and dogs. Mutagenicity was examined both in *in vitro* and *in vivo* tests. Maduramycin is not genotoxic except for equivocal results in one test for chromosomal aberrations in mammalian cells (quoted from SCAN Reportd, 7th series, 1988). The no-observed-effect level (0,16 mg/kg bw)

was based on the long-term rat study, giving an estimated toxicological ADI of 1,6 □□□□□ bw (96 □g/person). A safety factor of 100 was applied.

The total residues in turkeys (following a 7-day repeated administration of ^{14}C-maduramycin ammonium, 7 mg/kg) calculated as total radioactivity in liver, skin + fat and muscle at zero withdrawal time were 137 □□□□□, 61 □□□□□ and 25 □□□□□ (limit of detection) respectively.

Using the reference standard daily consumption of liver, skin + fat and muscle, the total likely intake would be : 137 x 0.1 + 61 x 0.05 + 25 x 0.3 = 24.25 □g. The consumer intake of residues represents 25.2% of the ADI. Maduramycin is irritant to the skin and corrosive to the eye. The method of preparation of the premix prevents any formation of dust containing maduramycin (SCAN Reports, 7th Series, 1988). The allergenic potential has not been examined.

7. In the opinion of the SCAN (opinion expressed 27 April 1988 on maduramycin ammonium in feedingstuffs for chickens for fattening), the excreted products from maduramycin are not prejudicial to the environment. The half-life of maduramycin in stored chicken excreta is about 55 days depending on the storage temperature. The concentration in the soil following standard agricultural practice of fertiliser usage was of the order of 2-6 □□□□□ soil.

   Further maduramycin is rapidly degraded in the soil to a large number of polar breakdown products only 7% remaining as unaltered compound. The toxicity of maduramycin for *Daphnia magna* and fish is similar, the no-effect concentration (NEC) being approximately 1 mg/l. Neither methanogenesis nor soil nitrification are inhibited by maduramycin in chicken excreta when these are used as fertiliser nor does it have any significant phytotoxicity.

8. Based on the above the use maduramycin ammonium is acceptable for use in turkeys up to 16 weeks of age at a dose rate of 5 mg/kg of complete feedingstuffs. Taking into account the ADI and the residue depletion profiles, edible tissues are without risk for the consumer provided a pre-slaughter withdrawal period of 3 days is respected.

REFERENCES

- Previous registration-files by ROCHE

- Registration file provided by ROCHE

- Reports of the Sciencific Committee on Animal Nutrition (Seventh series-1988). Commission of the European Communities. Agriculture. Report EUR 12824 EN. The use of maduramycin-ammonium in feedingstuffs for fattening chicken, pp.1-8.