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

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

TERMS OF REFERENCE (March 1996)

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

1. Has the use of Avilamycin (C57-62 H82-90 Cl1-2 O31-32, mixture of oligosaccharide of the orthomycin group produced by *Streptomyces viridochromogenes*) under the conditions proposed for its use as an additive for the feedingstuffs for Turkeys for fattening significant effects on meat production or animal performances?

2. Is this use safe to Turkeys for fattening?

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

4. What is the metabolic fate of Avilamycin in Turkeys for fattening? 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?

5. In the light of the answer to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

According to the provisions of Council Directive 70/524/EEC[1] the use of Avilamycin for pigs and piglets and chickens for fattening is authorized at Community level according to the conditions set up by Commission Directives 92/64/EEC[2] and 95/37/EEC[3] respectively (See Table 1).

1 O.J. No. L270, 14/12/70, p.1, as last amended by Directive 93/114/EC (O.J. No. L334, 31/12/93 p.24)

2 Amending Council Directive 70/524/EEC concerning additives in feedingstuffs (O.J. No. L221, 06/08/92 p. 51)

3 Amending Council Directive 70/524/EEC concerning additives in feedingstuffs (O.J. No. L 172, 18/07/95 p. 21)

Table 1:

Annex I, Part A: Antibiotics				
Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Piglets	4 months	24	40	-
Pigs	6 months	10	20	-
Chickens for fattening	-	2,5	10	-

The SCAN expressed his favourable opinion regarding the use of Avilamycin in feedingstuffs for pigs and chickens for fattening, in his reports of 27 April 1988[4] and 28 April 1995[5] Respectively.

In May 1994, a request was made for an extension of the use of Avilamycin to turkeys for fattening according to the conditions set up in Table 2.

Table 2:

Annex II, Part A: Antibiotics				
Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuff	Maximum content mg/kg of complete feedingstuff	Other provisions
Turkeys for fattening	-	5	10	-

New dossiers supporting this request have been provided by the applicant firm.

OPINION OF THE COMMITTEE

1. The efficacy of avilamycin was evaluated examining the growth performance in turkeys up to 16 weeks of age, kept under several European conditions, using different levels of avilamycin in complete feedingstuffs (0, 5, 10, 15 and 20 mg/kg).

4 Seventh Series of reports of the Scientific Committee for Animal Nutrition. Report EUR 12824 (Catalogue No CD-NA-12824-EN-C) p. 13

5 Tenth series of reports. In preparation

This evaluation was based on (1) a dose titration study using 3 224 turkeys in four European countries (France, Hungary, Italy and United Kingdom) and (2) a dose confirmation study carried out pooling data from the dose titration studies of turkeys fed with 5 and 10 mg/kg and another study conducted in the United Kingdom (2 367 birds).

All four dose-titration experiments were conducted using a common protocol and contained five treatments in a randomized split plot design.

The duration of each trial was identical (112 days) except in one experiment conducted in the United Kingdom which was ended at 108 days of life. The determination of growth performance was based on feed intake and weight of birds (bulk) at 28 day, 56 day, 84 day and last day of trial. Death and *post-mortem* data were recorded daily. Results from each individual experiment (dose titration study) showed a positive response to avilamycin in weight gain and feed to gain ratio at all doses in comparison with control treatment. The final liveweight of turkeys were statistically increased compared to the controls when data was pooled ($p<0.001$). The feed to gain ratio was also improved in the pooled data ($P<0.05$); however in that case only 10 and 20 mg/kg were statistically different from the control treatment. From the dose titration study it may be concluded that there was no evidence for a dose related response in any of the parameters. Avilamycin at the level of 5 mg/kg tends to increase the liveweight. At levels of 10 mg/kg or 20 mg/kg the best response were obtained in feed conversion. The dose confirmation study (five trials) (data of turkeys fed 0, 5, and 10 mg/kg of avilamycin in complete diets) confirmed the results observed in the dose-titration experiment.

To test the flavor and quality of the meat, two studies were conducted with turkeys fed control or experimental diet (10 mg/kg). There was no treatment effect in the first experiment. A panel test was unable to find any significant differences on meat flavour between the treated and untreated birds. On the basis of these studies it can be concluded that the 5 mg/kg (minimum content) and 10 mg/kg (maximum content) of avilamycin in feedingstuffs for turkeys for fattening sustain the claim regarding the efficacy of this additive as a performance enhancer for turkeys.

2. Five field studies were reported in full. At all inclusion levels studies (5, 10, 15 and 20 mg/kg in complete feedingstuffs), avilamycin seems to be safe for turkeys. The wide margin of safety for this substance has previously been demonstrated in laboratory animal studies as well as in pigs and chickens for fattening.

   A tolerance study of avilamycin in turkeys was also provided. To assess the safety of avilamycin in turkeys for fattening, it was administered continuously in complete feedingstuffs at levels of 0, 20 and 100 mg/kg for a period of 16 weeks. This new study was carried under GLP conditions.

   No symptoms of toxicity were observed during the trial and the mortality rate is considered to be within normal limits for the species. *Post-mortem* examination

did not reveal any lesions which could be related to treatment. Pale foci were found in the livers of 4 out 6 male birds on 20 mg/kg avilamicyn but were judged to be non-treatment related.

No other lesions were reported and no dose related symptoms were seen. These data demonstrate the safety of prolonged administration of avilamycin to turkeys at levels up to 10 times the maximum proposed levels.

3. No microbiological studies in turkeys following administration of avilamycin with the highest proposed level were presented. Isolates from each bacterial species were tested to determine the antibacterial spectrum of avilamycin.

   The microbiological studies were taken from the chicken dossier. The MIC-values were determined for 3 batches of avilamycin. Gram-positive bacteria were represented by streptococci, staphylococci and clostridia; the MIC-values were from 0.4-1.6 □g/ml. Lactobacillae showed a MIC-value about 3 □g/ml.

   The avilamycin factors A, B and D were tested separately, and showed A as the most active with a decline in activity from A to D. The Gram-negative bacteria were represented by *Pseudomona aeroginosa*, *Escherichia coli*, *Aerobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Shigella flexnerii*, *Proteus vulgaris*, *mirabilis*, *morganii* and *rettgeri*, *Salmonella typhimurium*, *choleraesuis*, *gallinarum* and *pullorum* ; they were cultured both aerobically and anaerobically, and for all tests the MIC-values were >100 □g/ml. Thus, avilamycin was active against most Gram-positive bacteria and very little active against Gram-negative bacteria. *In vitro* avilamycin readily selects for resistance in Staphylococci, Streptococci and in some Bacteroides, but not in Clostridia. Lactobacillae showed some sensitive and some resistant strains. Gram-negative bacteria were, with the exception of Bacteroides mentioned in one report, resistant to avilamycin.

   Avilamycin at the dose of 15 mg/kg did not select for cross-resistance, but 30 mg/kg selected for cross-resistance in four out of ten strains of Staphylococci. The modal MIC was calculated from samples taken (from pharyngeal Staphylococci of broiler chickens) before supplementing the feed. The bacteria were considered cross-resistant if the MIC was equal to or greater than 3 doubling dilutions above the modal MIC.

   A study was carried out in order to evaluate the effect on indigenous coliform bacteria on the selection of antimicrobial resistance on two groups of chickens one of which was fed 10 mg/kg avilamycin over 8 weeks. The differences between the two groups were not significant. The results of this study conclude that continuous feeding with avilamycin 10 mg/kg to chickens for 8 weeks did not have any significant effect on the incidence of resistant coliforms.

   To evaluate the duration of Salmonella-shedding, day old chickens were infected with about 10^9 organisms per animal of a nalidixic acid-fast culture of *Salmonella choleraesuis.* The birds were fed avilamycin from two batches and at the levels of 10 and 50 mg/kg. For each component 20 chickens served as non-medicated control. The results indicated that neither batch prolonged nor

shortened with statistic significance the time of Salmonella-shedding as compared to non-medicated control groups.

Avilamycin has no undesired effect on colonization with Salmonella in young chickens. Birds received feed supplemented with avilamycin 2.5 and 10 mg/kg either with or without monensin 100 mg/kg. In this experiment no difference was observed in the number of positive samples and number of Salmonellae in caecal contents

All of these results although obtained in chickens can be taken to apply to turkeys.

4. Studies concerning the safety of avilamycin in laboratory animals were taken from the documentation on pigs and chickens. Acute oral DL_{50} values in rat and mouse, two 28-day oral toxicity studies in rat and mouse, a 6-month oral toxicity study in Beagle dogs, two 2-year oral toxicity studies in mouse and rat, a multigeneration study in rat and a teratology study in rabbit were provided. Furthermore, two Amest tests (Salmonella and *Escherichia coli* strains), a DNA-repair unscheduled DNA synthesis in rat cells in vitro, a mouse lymphoma cell, and an *in vitro* sister chromatid exchange in Chinese hamster ovary cells were performed.

   Avilamycin did not reveal any carcinogenic, mutagenic or reproductive effects. While diarrhoea ocurred in the teratology study at all doses and some increase in liver weight was seen in rats at 3 000 mg/kg, these effects were discounted. Antibiotics with a Gram-positive spectrum like avilamycin frequently cause diarrhoea, even death in rabbits. The liver weight effect was seen only in low dose males and high dose females. It has no allergenic potential but was slightly irritant to skin, eyes and respiratory tract (SCAN Reports, 7th series, 1988), however the method of preparation of the premix formulation (oil is used) prevents any formation of dust. The lowest long-term no-observed-effect level was 3 000 mg/kg in male rats, equivalent to 100 mg/kg bw per day. A toxicological ADI of 60 mg/person was calculated by applying a safety factor of 100 to the NOEL.

   Following the administration of a concentration of 20 mg/Kg avilamycin in feedingstuffs (double concentration of the application) during sixteen wk no measurable residues were detected 6 hours, 2 and 4 days of withdrawal time in skin + fat, abdominal adipose tissue, muscle, liver and kidney sampled after slaughter. The detection limit (LOD) was 0.05 mg/kg (same as in chicken studies). Presuming avilamycin residues of 0.05 mg/kg at zero withdrawal in all edible tissues, standard food intake would lead to 0.025 mg of these residues, which corresponds to 0.04% of the ADI.

   Avilamycin does not persist in the body of turkeys. Its absorption is low. After ingestion avilamycin is eliminated as such by direct transit through the intestinal tract. This pattern of absorption, distribution, metabolism and excretion of avilamycin in turkeys is consistent with the previously reported for chickens.

In addition, environmental studies included in the chicken dossier show that avilamycin is rapidly degraded in soil, and accumulation is not expected. Whether leaching could occur was somewhat equivocal, but hydrolysis is rapid and no significant adverse effects were reported on various relevant environmental organisms.

The predicted environmental concentration (PEC) was low, certainly lower than the no-effect concentrations (NECs) in water for *daphnia* , rainbow trout and bluegills (23.8, 48 h.; 47.5, 96 h. and 35.4, 96 h. mg/l, respectively). No appreciable effect of avilamycin on methanogenesis and nitrification as well as no interaction with sewage microorganisms were observed. No avilamycin effect on seed germination (6 species) or plant growth (5 of 6 species) under simulated commercial conditions were observed. Tomato plants suffered a 20% inhibition of growth under these conditions (21 days after planting in manure soil, 20 t/ha). No data on eventual accumulation of avilamycin in crops were available. Therefore taking into account the rapid disappearance of avilamycin no adverse effect on the environment is to be expected from chicken excreta. The same conclusion can be presumed to be valid for turkeys.

5. Based on the above, the SCAN is of the opinion that the use of avilamycin in feedingstuffs for turkeys for fattening at the dosages proposed (5-10 mg/kg) is acceptable. In view of the ADI and the residue depletion studies, a withdrawal period is not required.

REFERENCES

- Registration file provided by Lilly Industries Ltd.
- Reports of the Sciencific Committee on Animal Nutrition (Seventh series-1988). Commission of the European Communities. Agriculture. Report EUR 12824 EN. The use of avilamycin in feedingstuffs for pigs, pp.13-20.
- Draft Reports of the Sciencific Committee on Animal Nutrition (Tenth series). File updated on 11 February 1997. The use of avilamycin in feedingstuffs for chicken for fattening, pp. 59-68.