Report of the Scientific Committee for Animal Nutrition on Question 61 by the Commission on the use of Avilamycin in feedingstuffs for chicken for fattening
(Opinion expressed on 7 July 1995)

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 Avilamycin ($C_{57-62}H_{69}O_{11}Na$, Sodium salt of a polyether monocarboxylic acid produced by *Streptomyces albus*) under the conditions proposed in the feedingstuff for chickens for fattening significant effects on animal growth?

2. Is this use safe to chickens for fattening?

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 avilamycin in chickens for fattening? Does this use result in the presence of residues in animal tissues? 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 avilamycin? 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 1984[2,] the use of Avilamycin is authorised at national level under the conditions set out by Commission Directive 91/248/EEC[3]:
Annex II

1 O.J. No. L270, 14.12.70 p.1
2 O.J. No. L319, 08.12.84, p.13
3 O.J. No. L124, 18.05.91, p.1

A. Antibiotics

Species or category of animals	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 Scientific Committee for Animal Nutrition (SCAN) expressed his favourable opinion regarding the use of avilamycin in feedingstuffs for pigs in his report of 27 April 1988[4]. Before submitting a proposal for an entry to Annex I of the use of this substance for chickens for fattening, the Commission requests a favourable opinion of the SCAN.

OPINION OF THE COMMITTEE

1. Avilamycin dose-response relationship was studied in the chicken in two series of trials conducted in Europe in 1982 and 1986. The first consisted of 12 floor pens (0, 2.5, 5, 10, 15 and 20 mg/kg feed 28.560 birds) and the second of 6 trials (0, 2.5, 5 and 10 mg/kg feed 12.160 birds). Day-old chickens were reared to slaughter weight during 42 or 49 days, and fed commercial diets and libitum. The energy content of the diets expressed as metabolizable energy was in the range 3030-3106 Kcal/kg feed. The contents of protein, fibre and aminoacids were similar, but oil varied between 3.03 to 9.0 %.

   Statistical analysis of the data using curvilinear regression methodology in each series of trials indicated a dose related improvement of live weight gain and feed conversion rate between 2.5 to 10 mg/kg, but no further significant improvement for the 15 and 20 mg/kg dosages. At 10 mg/kg (versus control) the final live weight was increased by 3.6% in the first series and 5.8% in the second series of trials. The feed conversion rate was improved by 2.7 and 3.4% respectively. It is noteworthy that mortality was not affected by Avilamycin supplementation.

   The effectiveness of the claimed dosage (10 ppm) has been confirmed under practical husbandry conditions and against negative controls in four European countries. Moreover the additive had no adverse effect on carcass quality. These findings suggest that Avilamycin is effective for fattening chickens at doses of 2.5 to 10 mg/kg feed.

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

2. Avilamycin has been subjected to dose-titration studies (maximum 20 ppm), user studies under commercial conditions in several countries, and to overdose studies (0, 30, 300, 3000 ppm for 62 days, n=48/group). No adverse, treatment related effects on growth, clinical signs, gross pathology, histo- pathology, haematology or clinical chemistry were reported.

3. An HPLC method is proposed for the determination of total avilamycin, namely avilamycins A, A', B, C, D1+D2 and E in the bulk product and premixes. An agar diffusion method using *Micrococcus luteus* as the test microorganism is proposed for the determination of avilamycin in premixes and feeds. The recovery from spiked (2.5 to 20 ppm) samples is satisfactory as is reproducibility (Sd = 8.2% for ten replicates at 10 ppm level).

4. The induction of resistance to antimicrobials was studied in coliforms isolates collected weekly from 14 day caged chicks (n=24) for eight weeks during which 10 ppm Avilamycin activity was included in the diet. The resistance pattern remained essentially stable. Salmonella shedding was neither enhanced nor diminished by Avilamycin at 10 or 50 ppm in groups of 20 artificially infected broilers over three weeks of feeding. Colonisation of the gut by *S. kedougou* from contaminated feed was not enhanced by 2.5 or 10 ppm Avilamycin with or without the additional presence of Monensin in young broilers (n=10/group). In respect of the aspects studied, Avilamycin is without adverse effect on growing chicks.

   The effect on common Gram+ bacteria (*Streptococcus*, *Staphylococcus*, *Clostridia* and *Lactobacillae*) has been examined. The average MTC values for *Streptococci*, *Staphylococci* and *Clostridia* were between 0,4 and 1,6 ìg/ml., while the examined lactobaccillae had MIC values about 3 ìg/ml. Avilamycin had a higher activity under anaërobic as under aërobic conditions.

   Mono- and multiple resistant *Staph.aureus* were tried for development of cross-resistance to Avilamycin. The MIC-values were the same as seen for the other Staphylococci, and there was no cross-resistance to the 7 clinically used antibiotics to which the Staphylococci had shown resistance.

5. A balance study of C^{14}-Avilamycin has been established in the chicken using a uniformly labelled molecule whose composition into the different constitutive Avilamycin factors was similar to that of the commercial compound. Most the administered single dose had been recovered from the droppings after 4 days, 50-78% being recovered during the first 24-hour collection period. The absorption and distribution of Avilamycin was evaluated by determination in the blood of 240 treated chickens (22 ppm for 25 days) both of parent Avilamycin and its potential metabolites, i.e. those already found in the rat and pig containing rings A+B+C and sharing the dichloroeverninic moiety of the molecule. No Avilamycin was detected using a bioautographic method with a sensitivity limit of 0.04 mg/kg, neither were metabolites found using a gas chromatographic method with a sensitivity limit of 0.1 mg/kg.

The conclusions drawn from these data stating that very limited or no absorption of Avilamycin occurs do not take into consideration the limitations of the methodology used, i.e. the sensitivity of detection of unlabelled Avilamycin metabolites and the lack of investigation of biliary excretion. It must be recalled that the biliary excretion figure for the pig was about 7%.

However, a microbiological study revealed no intact Avilamycin in chick (20 ppm for 56 days, n=6) tissues at a zero withdrawal (detection limit 0.05 mg/kg). Therefore the kinetics and residue data imply an Avilamycin dietary exposure to man at zero withdrawal which is analytically indistinguishable from nil.

6. The toxicology package is the same as that produced and considered as satisfactory when the additive was examined for use in the pig (SCAN Reports, 8th Series, 1992). The lowest long-term NOEL is 3000 ppm in male rats, i.e. 100 mg/kg body weight, therefore ADI=66 mg/day. Presuming Avilamycin residues of 0.05 ppm at zero withdrawal in all edible tissues, standard consumption weights total 0.025 mg/day. This equals 0.04% of ADI at the most.

   The safety studies reveal no evidence for the likelihood of adverse effects in the consumer. The crude Avilamycin possesses reversible mild, dermal, ocular and respiratory tract irritancy. However, the rabbit eye study used 69 mg of the dried fermentation product (= 7 gm of premix) while the rat four hour inhalation study was to 0.115 mg Avilamycin activity/l (= 0.77 mg/l crude product or = 7.7 mg premix). This latter was achievable only with preliminary micromilling of crude Avilamycin. Hence as dusting is difficult to maintain and oil is used in the premix formulation, worker exposure is unlikely to be a problem under normal conditions of handling and use.

7. Accumulation in soil of Avilamycin and derived material was excluded in a study using manure from chickens fed 30 ppm radio labelled Avilamycin. No data are available concerning the nature of Avilamycin excreted products in chicken droppings. The only reference concerns the pig where less than 5% of Avilamycin A and B are excreted intact in the faeces, metabolite A resulting from the rupture of the ester bond between rings C and D being by far the major metabolite. The drug is rapidly degraded by photolysis (t 1/2 1.2 h), hydrolysis (T 1/2 230 h at pH 7), is a leacher, and Kow is around 2 (detection limit 0.2 ppm). It is without adverse effect on aerobic and anaerobic sewage digestion processes.

   The oral LD 50 of Avilamycin activity in bobwhite quail (n =60) is >372.5 mg/kg. In 5-day feeding studies in bobwhite quail (n =60) and mallard (n =60) this increased to >745 mg/kg Avilamycin. The average assays in water for NECs for Avilamycin in rainbow trout, bluegills and daphnia were (96 h), 35.4 (96 h) and 23.8 (48 h) mg Avilamycin/l, the highest tested concentrations. In a 14-day earthworm study (n =14) the NEL was 100 mg Avilamycin/kg soil. Avilamycin was without effect on seed germination (6 species) although tomato plants suffered a 20% inhibition of growth under the same simulated commercial conditions (21 days after planting in manured soil, 20 t/ha).

8. Although certain insufficiencies have been noted such as the lack of data on the nature of the metabolites in the excreta and the limited value of the methodology used to characterise tissue metabolites, it was considered that the question of the environmental behaviour and residue status were substantiated enough to conclude to the safety of avilamycin for the human consumer and the environment.

   Therefore on the basis of the above specific data on chickens and previous general toxicological assessment by the SCAN of data covering another target species (the pig), laboratory animals and environmental end-points, the Committee considers the use of Avilamycin for fattening chickens at 2.5-10 mg/kg complete feed acceptable.