REPORT
OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION (SCAN) ON THE EXTENSION OF USE OF DICLAZURIL (E-771) TO THE FEEDINGSTUFFS FOR TURKEYS
(Expressed, 26 September 1997; Text consolidated, 24 October 1997)

TERMS OF REFERENCE (July 1995)

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

1. Has the use as of Diclazuril (2,6-dicloro-alpha-(4-chlorophenyl)-4-(4,5 dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl) benzeneacetonitrile; E-771) under the conditions proposed for its use as an additive for turkeys (see background) significant effects on the prevention of coccidiosis in the turkeys?

2. Is this use safe for the turkeys ?

3. Does the proposed use result in residues on the meat?. If so, what is the qualitative and quantitative composition of these residues?

4. 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 [1], the use of Diclazuril (E-771) (E-771) is authorized at Community level in the Annex I, Section D (Coccidiostats and other medicinal substances), according to the conditions set up by Commission Directive 93/107/EEC[2], as follows (See Table I)

Table I. Annex I, Part D (Coccidiostats). Diclazuril (E-771)

Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuffs	Maximum content mg/kg of complete feedingstuffs	Other provisions
Chickens for fattening	-	1	1	Used prohibited at least five days before slaughter

1 Concerning additives in feedingstuffs (OJ No L270, 14.12.70 p.1.) as amended by Directives 84/58/EEC (O.J. No. L319 8/12/84 p 13) and 93/114/EC (OJ L334 31.12.93, p. 24)

2 O.J. No. L219, 4/12/93, p. 44.0

The Scientific Committee for Animal Nutrition has expressed its favourable opinion in its reports of 10 July 1991 on the use of Diclazuril in feedingstuffs for Chickens[3] .

It has been requested an extension of the use of Diclazuril (E-771) to turkeys under the following conditions of use (See Table II):

Table II. Annex I, Part D (Coccidiostats). Diclazuril

Species or category of animal	Maximum age	Minimum content mg/kg of complete feedingstuffs	Maximum content mg/kg of complete feedingstuffs	Other provisions
Turkeys	12 wks	1	1	Used prohibited at least five days before slaughter

A registration file has been submitted by the applicant firm

OPINION OF THE COMMITTEE

1. Laboratory, floorpen and field studies have been performed to demonstrate the efficacy of diclazuril as a coccidiostat in turkeys. Six experiments have been carried out in young turkeys to determine the efficacy of diclazuril using dose response titration against artificial infection with *Eimeria*. The *Eimeria* used were the most commonly found in turkeys: *E. meleagrinitis, E. adenoides, E. gallopavonis, and E. dispersa*. In five of these experiments conducted in Europe the animals were infected around three weeks of age (day 0). In the individual and battery studies carried out in America, an artificial infection was realized on 12 or 14-day of age.

   The efficacy of diclazuril was measured taking into account the lesion scores and dropping scores according to the systems described by Mc Dougald (1986), plus oocyst excretion. Zootechnical parameters such as daily weight gain and feed conversion were recorded over the week following an artificial infection. Dropping scores were measured at day 4, 5 and 6, the lesion scores at the end of the experiment (6-8 days).

   The common experimental design of these groups was performed using two types of turkeys, infected and uninfected. Dose response of diclazuril was studied on uninfected turkeys fed 0, 0.5, 1 and 1.5 mg/kg. Infection

3 Eight Series of reports, page 51. ISBN 92-826-7975-6

was provided by sporulated oocysts ranging between 500.000/bird for *E. meleagrinitis* and *E. dispersa*, to 50,000/bird for *E. adenoides*.

The conclusions of these studies were as follows: 1) *E.adenoides* and *E. gallopavonis* artificial infection produces more intensive symptomatic cocidiodiosis than the other *Eimeria* in the group of turkeys without diclazuril supplementation. 2) 0.5, 1 and 1.5 mg/kg have a statistically significant effect against a simple infection as well as against a challenge with a mixture of these species, 3) concentration of diclazuril higher than 1 mg/kg did not improve the parasitological or zootechnical parameters.

Five floorpen experiments were conducted in Belgium, Italy, Spain, Germany and U.K. Two additional experiments using similar conditions were performed in the U.S.A. Turkeys were infected on 21-day or 14-days of age with a mixed preparation of *E. adenoides, E. meleagrinitis and E. gallopavonis* in the feed. There was an untreated group as a negative control in all these studies. There were diclazuril dose response studies as well as some experiments conducted using other currently approved coccidiostats as positive controls. The turkeys fed with treated feed had better zootechnical performance up to eight weeks of age . Beyond eight weeks of age it can be concluded that the better coccidiosis control provided by diclazuril and the positive controls did not result in a significantly better zootechnical performance at slaughter time. This is probably due to the fact that turkeys were able to recover in the weeks following the challenge (3 weeks of age).Therefore it can be concluded there is a clear confirmation of the efficay of 1 mg/kg diclazuril when that dose is used in floorpen conditions with infected challenged turkeys at 21-day of age.

Three groups of field trials were conducted in Austria, Poland and Hungary. The efficacy of diclazuril was tested in turkeys fed using current management system applied in these countries. Diclazuril performance was compared with alternative coccidiostats such as lasalocid at 120 mg/kg and 100 mg/kg, and lerbek at 110 mg/kg. In the Austrian field studies diclazuril gave a better coccidiostatic protection than that provided by the competitor product. In the diclazuril groups a slight oocyst excretion was detected.

Summarizing the three group of studies, diclazuril at 1 mg/kg feed supplementation reduces lesion scores in turkeys infected at three weeks of age with pathogenic *Eimeria* species. Oocyst excretion is reduced to an almost non detectable level.

2. Owing to well known toxic side effects produced by some anticoccidial ionophores in avian species other than broilers, diclazuril was assayed in feed given to turkeys and Peking ducks for 7 consecutive days.

The levels of addition were 0, 10, and 100 mg/kg, i.e. 10 or 100 times the suggested level in turkeys. No clinical signs of discomfort were shown by the animals. They behaved similarly to control animals in respect of body weight gain, feed consumption and feed efficiency. At autopsy neither drug or dose-related lesions could be observed in these animals. Blood

collected from them and submitted to a complete set of hematological and hematochemical analyses revealed no important anomaly.

A safety test of diclazuril was performed on growing turkeys (males and females) under semi-field conditions. The animals were fed for 16 weeks with diets containing 1, 12.5 and 25 mg/kg diclazuril and were compared with untreated controls for body weight gain, feed consumption, feed conversion, clinical signs, mortality, haematology, serum biochemistry, and histopathology of liver, spleen, kidney, heart, bursa fabricii, brain, thymus, bone and bone-marrow, testes and ovary.

No drug-related finding was observed in turkeys up to the treatment level of 25 mg/kg.

These data reveal that growing turkeys will tolerate diets containing diclazuril at concentrations 25 times the recommended dosage for 16 weeks, or even diets supplemented at 100 mg/kg (100 times) for 7 consecutive days.

3. 14C-diclazuril labelled on a stable nitrile group was administered as a single dose (1mg/kg b.w.) to turkeys. A relatively slow excretion was observed in the droppings, i.e. 55% in 24 hours and 95% after 10 days, that correspond to 3.1 and 3.2 day half-lives for the male and female respectively. Over the 0-96- hour period most radioactivity (73.7 and 75.8%) was excreted as unchanged diclazuril. A major metabolite (DM5) corresponding to the hydrolytic cleavage of the 4,5-dihydro-3,5-dioxo-1,2,3-triazine ring represented 8.7 and 8.1% respectively. Seven other metabolites representing each less than 2.5% of the total radioactivity were identified. Without any indication of urine and bile excretion, the extent of diclazuril absorption cannot be assessed.. However, plasma radioactivity expressed as unchanged diclazuril showed a 1.78 mg/ml peak concentration at 6-hour post-administration, which indicates that a significant absorption occured. Lower concentrations were measured in the liver, kidneys, skin/fat and muscle in a decreasing order.

   The depletion of radioactivity from the tissues paralleled that from plasma and was mono-phasic with half-lives ranging from 33.7 to 45.6 hours. Multi-dose administration (1.1 mg/kg 14C-diclazuril for 14 days) showed that total diclazuril equivalent residues at 0-withdrawal were highest in the liver (0.41 and 0.61 mg/kg for the male and female respectively), followed in decreasing order by the kidneys (0.30 and 0.44 mg/kg), abdominal fat (0.19 and 0.31 mg/kg) and muscle (0.07 and 0.09 mg/kg). No data were provided to indicate that 14 days were sufficient to reach a steady state.

   The analysis of liver residues showed that 90.8 and 92.6% (male and female) of the total radioactivity was extractable, of which 71.1 and 82.7% corresponded to unchanged diclazuril, the remaining extracted compounds appearing as two minor (<4% each) and unresolved metabolites.

Turkeys fed all along their growing period (4 month) a feed supplemented with diclazuril at 1mg/kg b.w. and killed at 6h, 1, 3, 5, 7 and 9 days withdrawal, were sampled for analysis of diclazuril levels (HPLC method) in different tissues.

At 0-withdrawal (6h) residue levels were 0.57 mg/kg in liver, 0.3 mg/kg in kidneys, 0.16 mg/kg in skin/fat and <0.05 mg/kg in muscle. Depletion of diclazuril was mono-phasic and half-lives were approximately 3 days in liver and skin/fat, 2 days in kidneys.

Existing data on the metabolism, excretion and tissue kinetics of diclazuril in broilers (Reports of the SCAN, 8th series, 1992) indicate a very similar qualitative and quantitative fate as for turkeys. The same choice of marker residue (diclazuril) and target tissue (liver) is thus appropriate for both species.

4. Based on the satisfactory answers given to the former set of questions, on the great similarity of behaviour of diclazuril in the turkeys and broilers, and the fact that the conclusions drawn from the toxicological and environmental studies analyzed previously (Reports of the SCAN, 8th series, 1992) and those produced since (1), are not contradicted by new data, it can be concluded that the proposed conditions of use for this substance in turkeys are acceptable.

REFERENCES

- Registration file submitted by the firm in 1995

- Diclazuril (RO64433) Documentation assembled and submitted for evaluation by the 50th JECFA (1998) as reply to questions remaining after the 45th JECFA (1995).