

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE
OF ZINC BACITRACIN IN FEEDINGSTUFFS FOR POULTRY, PIGS AND RABBITS

Opinion expressed 30 January 1985

TERMS OF REFERENCE (September 1980)

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

1. Does the addition of zinc bacitracin to complete feedingstuffs in dose levels higher than 20 mg/kg produce a significant increase of nutritional effects, free of prophylactic or therapeutic effects, in the case of poultry (excluding turkeys, ducks, geese, laying hens and pigeons) over the age of four weeks, swine over the age of four months, and rabbits ?
If so, what is the dose/response ratio ?
2. Is the administration of complete feedingstuffs with a maximum zinc bacitracin content of 100 mg/kg justified from a nutritional point of view
 - in the feeding of poultry (excluding turkeys, ducks, geese, laying hens and pigeons) up to the age of 16 weeks,
 - in the feeding of swine up to the age of six months,
 - in the feeding of rabbits ?
3. Does the use of zinc bacitracin in feedingstuffs for poultry, pigs and rabbits, under the conditions of use being proposed (cf. Background), result in the presence of residues in animal products ?
If so, what is the nature and the amount of these residues ? Could they be harmful to consumers ?
4. Could these conditions of use lead to the development of resistance in bacteria ?

5. Could they be harmful to the environment ?

If so, what is the nature of the risks ?

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

BACKGROUND

In accordance with Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the 34th Commission Directive of 4 September 1980 (2), the use of zinc bacitracin is authorized throughout the Community, subject to the conditions of use set out as follows in Part A of Annex I to the Directive :

Species of animal	Maximum age	Minimum content ppm (mg/kg) of complete feedingstuff	Maximum content
Turkeys	4 weeks, from 4 to 26 weeks	5	50
Poultry, excluding turkeys, ducks, geese, laying hens and pigeons	4 weeks, from 4 to 16 weeks	5	50
Laying hens	-	15	100
Calves, lambs and kids	16 weeks, from 16 weeks to 6 months, 6 months	5	50
Piglets	4 months, 6 months	5	80 (*)
Swine	from 4 to 6 months	5	20
Animals bred for fur	-	5	20

(*) Milk replacers

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

(2) OJ No L 251, 24.09.1980, p. 17

The proposed amendments to these conditions of use are given in the table below :

Species of animal	Maximum age	Minimum content	Maximum content
		ppm (mg/kg) of complete feedingstuff	
Turkeys : no change			
Poultry, excluding turkeys, ducks, geese, laying hens and pigeons	16 weeks	5	100
Laying hens : no change			
Calves, lambs, kids : no change			
Swine, piglets	6 months	5	100
Rabbits	-	50	100

In the opinion delivered on 21 February 1978 (3), the Scientific Committee for Animal Nutrition reserved its position regarding any increase in the dose levels authorized for zinc bacitracin, given the lack of supporting data. As further relevant material is now available, the Commission considers that the matter should be re-examined.

OPINION OF THE COMMITTEE

The dossiers made available to the Committee were those on zinc bacitracin produced by A/S Apotekernes Laboratorium for Specialpraeparater.

(3) Reports of the Scientific Committee for Animal Nutrition, First Series (1979). Office for Official Publications of the European Communities, Luxembourg. Catalogue No CB-28-79-277-FR-C.

Considering that the data available up to 1982 did not enable a satisfactory assessment to be made of the effects of an increase in the dose levels of this antibiotic, the Committee requested that additional studies be carried out on the efficacy of the product, the metabolism and residues, mutagenicity, bacterial resistance and the effects on the environment. The results of these studies were submitted during 1984. The opinion delivered hereinafter is none the less restricted to broilers and pigs, the only target species to have been the subject of appropriate studies under the newly proposed conditions of use.

1. and 2. The nutritional effects of zinc bacitracin were established on the basis of 839 tests involving 178 000 broilers and 207 tests involving 6 400 pigs. The parameters considered were the increase in liveweight gain and the improvement in the feed conversion ratio among the groups given feed containing zinc bacitracin as compared with the control groups. The zinc bacitracin content of the additive-containing feeds varied between 3 and 275 mg/kg for the fowl and between 3 and 250 mg/kg for the pigs.

Processing the results by multiple regression analysis enabled a statistically significant dose/response ratio to be worked out for each parameter and allowed the optimum concentrations of the additive to be assessed for broilers and pigs (Morris 1983). On account of the high number of variables associated with the trials (breed, ration composition, breeding conditions, etc.), the parameters included in the multiple regression equation enable the variance to be explained in part only (21 to 32%).

For broilers, the optimum zinc bacitracin concentration is 110 mg/kg of feed for the increase in liveweight gain and 130 mg/kg of feed for the improvement in the feed conversion ratio. For pigs, the optimum concentration is 106 mg/kg of feed for the increase in liveweight gain and 125 mg/kg of feed for the improvement in the feed conversion ratio.

For both species, the favourable effects of the additive up to optimum concentration show a gradual reduction with the increase in concentration.

Although the yield is not proportional to the dosage used, the increase in the concentration of zinc bacitracin in feedingstuffs for broilers and pigs up to 100 mg/kg feedingstuff is justified by statistically significant nutritional effects. Since these studies were performed on healthy animals, the likelihood of prophylactic or specific therapeutic effects can be ruled out.

3. Bacitracin is a polypeptide antibiotic produced by Bacillus licheniformis; its main components are the dodecapeptides, bacitracins A, B₁, B₂ and F₁. The acute toxicity of bacitracin has been determined in the mouse, rat, rabbit and dog by various routes of administration. The subacute toxicity has also been tested in the mouse, rat, rabbit, dog and monkey by various routes, including a subchronic oral feeding test in rats extending over 90 to 365 days. No toxic effects were noted. Furthermore, bacitracin did not irritate skin or eyes.

Zinc bacitracin was tested for mutagenicity in the Salmonella reverse mutation test, in a mouse lymphoma gene mutation test, an in vitro test for chromosomal aberrations in human peripheral lymphocytes and in rat bone marrow cells. No mutagenic activity was detected. DNA metabolism in spleen cells in vitro showed also no adverse effects. Thus bacitracin lacks mutagenic activity and there is therefore no reason to suspect genotoxic carcinogenic activity.

Bacitracin is appreciably resistant to hydrolysis by mammalian proteases and peptidases due to its cycloheptapeptide substructure, its thiazoline ring in the N-terminal L-isoleucine-L-cysteine moiety and to the presence of four D-amino acids (D-glutamine, AA 4, D-ornithine, AA 7, D-phenylalanine, AA 9, D-aspartic acid, AA 11). Only some 4% of ingested bacitracin is broken down to di- and tripeptides and free amino acids.

Zinc bacitracin metabolism has been studied in rats, chickens and piglets using single oral doses of the antibiotic labelled with ¹⁴C-L-isoleucine in AA positions 1, 5 and 8 in the molecule. The doses administered were 80 mg/kg live weight for the rat, 20 mg/kg live weight for the chicken and 7 mg/kg live weight for the piglet; with the piglets dosing with the labelled product was preceded by the administration of a ratio containing 117 mg zinc bacitracin/kg feed for 18 days. Of the administered dosages, 102% was recovered in rat faeces and 96% in piglet faeces; with the chicken 95% of the administered dose was recovered in the combined excreta (faeces and urine). Analysis by TLC/autoradiography of the rat and piglet faeces and the chicken excreta showed the presence of oxidised derivatives (i.e. bacitracins F) of the bacitracins A, B₁ and B₂, of desamidobacitracins and di- and tripeptides in addition to the original bacitracin supplied in the feed. Rat and piglet urine contained respectively 3.2 and 3.6% of the administered dose while the comparable values for bile were 0.5% and 0.03% respectively. No bacitracins or their near derivatives were detected in the bile or urine, nor was there any ¹⁴C-labelled free isoleucine.

Traces of radioactivity were found in some samples of liver and kidney from the chick (limit of determination 0.027 mg/kg fresh weight) and in liver, kidney, lung and heart of the piglet (limit of determination 0.0038 mg/kg fresh weight). The negligible quantities of these tissue residues, which persisted for some 5-7 days in some animals after withdrawal of the antibiotic from the feed, were not identified. However, extracts of the liver failed to show any microbiological activity. Tissues from chicken and piglets kept on feeds containing 100 mg or more zinc bacitracin for periods up to one month or more contained no bacitracin detectable by microbiological assay (limit of determination 0.054-0.26 mg/kg). Therefore, no harm to the consumer arises.

4. The administration of zinc bacitracin in various concentrations up to 100 mg/kg feedingstuff to pigs and broilers only slightly altered the number of bacteria in the intestinal flora as far as the main species

were concerned, i.e. lactobacilli, enterococci, E. coli and staphylococci (Gedek, 1983; Walton, 1983).

The slight increase in the MIC values observed mainly in the enterococci vis-à-vis bacitracin used as a feed additive was not accompanied by reduced sensitivity to other antibiotics used therapeutically (inter alia β -lactam antibiotics, tetracyclines and chloramphenicol). Furthermore, after administration of zinc bacitracin to pigs and chickens for several weeks, a lowering of the resistance of enterococci and E. coli in the intestinal flora of these animals to several drugs (antibiotics, sulphonamides, nitrofurans, etc.) used in human and veterinary medicine was noted. The concentration of 100 mg/kg of feed led to a greater reduction than did 20 mg/kg (Walton 1978, 1983; Walton and Laerdal 1980; Siebert, 1982).

5. After administration of zinc bacitracin, the products excreted by pig and chicken were made up of bacitracin A, B and F, desamidobacitracins, breakdown products and non-extractable components bound to faeces constituents. Their antibiotic activity fell sharply in excreta and soils. The half-life of zinc bacitracin mixed with the excreta of chicken is two to seven days depending on the temperature. This rapid loss of antibacterial activity due to oxidative deamination means that any effect on the nitrifying bacteria in the soil may be ruled out. Nitrogen-fixing bacteria (Rhizobium trifolii) are not sensitive to zinc bacitracin, even in high concentrations. Under experimental conditions, bacitracin inhibits the formation of methane in sludges even in a 3 mg/kg concentration (Hilpert et al., 1983). On account of its rapid inactivation in excreta however, it does not seem that in practice, zinc bacitracin could affect the production of biogas.

Bacitracin does not present any risk to fish, shellfish or algae. No adverse effect was observed on trout (Salmo gairdneri) fed for 110 days on feed containing 57.5 and 104 mg zinc bacitracin/kg respectively. The CL_{50} after 96 hours for this species is 74 mg/l.

For Daphnia magna, the minimum concentration without any effect is 3.2 to 5.6 mg/l, and the CL₅₀ after 48 hours is 34 mg/l. The growth of green algae (Chlorella ellipsoides) is not affected by zinc bacitracin at a concentration of 10 mg/l.

No inhibiting effect on the growth of plants was observed in plant species grown in soils mixed with manure from poultry fed with feed containing from 20 to 100 mg zinc bacitracin/kg or poultry manure mixed with 150 mg zinc bacitracin/kg.

6. On the basis of the foregoing information, the Committee is of the opinion that the use of zinc bacitracin levels of 5-100 mg/kg complete feedingstuffs for broilers up to the age of 16 weeks and pigs up to the age of six months is justified for the animal production and does not present any risk for human or animal health or for the environment.

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