Report of the Scientific Committee for Animal Nutrition on the use of lasalocid sodium in feedingstuffs for finishing cattle. (Opinion expressed : 27 July 1990).

Terms of reference (February 1984)

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

1. Has the use of lasalocid sodium under the conditions proposed for feedingstuffs for finishing cattle significant effects on animal growth?

2. Does this use result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

3. Could this use affect the development of resistance in bacteria?

4. Could the excreted products, derived from the additive, be prejudicial to the environment?

5. 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 of 23 November 1970 concerning additives in feedingstuffs[1], as last amended by the forty fourth Commission Directive of 29.11.1983[2], the use of lasalocid sodium is authorized at Community level under the conditions set out as follows in Annex 1, Section D, of the Directive.

Species of animal: chickens for fattening.
Minimum and maximum content in complete feedingstuffs: 75-125 mg/kg.
Other provisions: use prohibited for at least five days before slaughter.

The Scientific Committee for Animal Nutrition expressed a favourable opinion on this use in its report of 14 December 1982.

Opinion of the Committee

The file submitted to the Committee related to BOVATEC[3], a premix containing 15% lasalocid sodium.

1. Thirty-two trials with lasalocid sodium were conducted between 1978 and 1987 in several countries on some 2500 cattle of various breeds maintained under different husbandry conditions. They showed that lasalocid sodium, at concentrations between 10 mg and 35 mg/kg of complete feedingstuff, favourably influenced growth and improved the feed

[1] O.J. N° L 270, 14.12.1970, p. 1.

[2] O.J. N°L350, 13.12.1983, p.17.

[3] Registered trade name of Hoffmann-LaRoche

conversion ratio. The highest concentration employed did not achieve statistically significant greater effects than lower concentrations.

Evaluation of the efficacy in relation to the energy density of the animal diet showed the existence of an inverse relationship between feed intake and energy value of the ration. However, the addition of lasalocid sodium to low energy rations tended to increase body weight gain without concomitant effect on feed intake. This phenomenon has been observed also with other ionophore antibiotics. Overall it could be shown that lasalocid sodium, at a concentration of 35 mg/kg of complete feedingstuff, improved feed efficiency from 7% to 15% for low energy rations and from 3% to 5% for high energy rations. Grazing trials using lasalocid sodium at doses ranging from 100-300 mg/animal/day showed a greater improvement in daily body weight gain (the only parameter which can be measured in these trials) than feedlot trials (in these trials 3 parameters can be measured: feed consumption, feed conversion, and mean daily body weight gain).
Lasalocid sodium also reduced the incidence of meteorism (bloat) in cattle kept on dried leguminous feed.

Lasalocid sodium at concentrations of 50 mg/kg feedingstuff, altered the volatile fatty acid composition of the rumen contents by increasing significantly the molar proportion of propionic acid, though even concentrations as high as 176 mg/kg feedingstuff did not increase the total volatile fatty acid concentration in rumen.

Although the proposed levels of use ranging from 10-35 mg/kg of complete feedingstuff appear to be efficacious, there is a need to avoid the incorrect use in ruminating cattle of complementary feed containing lasaloid sodium. It would therefore be appropriate to fix a maximum daily dose for each animal according to its body weight because feed consumption during rumination does not increase proportionally with the body weight. The maximum dose of lasalocid sodium in the daily ration should not exceed 50 mg (constant value) plus 55 mg/100 kg body weight (variable value). The table below sets out the appropriate quantities.

Live weight (kg)	Mean daily feed consumption (kg)	lasalocid Na/head/day (50mg+55mg/100kg b.w.) (mg)	Equivalent dose in complete feed (mg/kg)
100	3.4	115	34
150	4.4	132.5	30
200	5.6	160	29
250	6.7	187.5	28
300	7.6	215	28
350	8.3	242.5	29
400	9.0	270	30
450	9.6	297.5	31
500	10.4	325	31
550	10.5	352.5	34
600	10.9	380	35

The use of lasalocid sodium in feedingstuffs for cattle did not affect carcase quality. Organoleptic tests carried out on beef meat showed no difference between samples from control animals and animals which had received 165 mg lasalocid sodium/kg feedingstuff. No adverse effects due to lasalocid sodium administration were noted in target animals such as chickens, laying hens, turkeys, heifers, cows and horses at doses up to 0.6 mg/kg body weight, equivalent to 35 mg/kg of complete feedingstuff. The tolerance of rabbits to repeated administration of lasalocid sodium has not been determined.

2. The oral acute toxicity of lasalocid sodium was about 100 mg/kg body weight in rats and about 150 mg/kg body weight in mice. The substance was not irritating to the skin and eyes of rabbits. A sensitization test in guinea-pigs was negative.

Mutagenicity studies in prokaryotic and eukaryotic organisms as well as *in vitro* assays for several genetic endpoints were all negative. Long-term studies in mice, rats and dogs showed no evidence of any carcinogenicity. The increased incidence of lymphosarcoma observed in the mouse study was within the range noted in historical controls. It was therefore not considered to be indicative of any carcinogenic potential. At high dose levels changes were noted in some haematological and clinico-chemical parameters and in some organ weights. The no-observable effect level (NOEL) in the rat was 0.5 mg/kg body weight. The highest dose of 6 mg/kg body weight in a three-generation-reproduction study in rats induced weight-loss-related effects on male and female fertility but no evidence of teratogenicity. The NOEL in this study was 1.8 mg/kg body weight. The ADI for man was established at 0.005 mg/kg body weight based on the NOEL in a long-term study in rats. Lasalocid sodium had no adverse effects in target and non-target animal species at doses up to 35 mg/kg feed.

The metabolic fate of lasalocid sodium was studied in steers using ^{14}C-labelled lasalocid. Single dose administration produced very low blood radioactivity levels, 89% of the administered radioactivity being recovered in the faeces and 0.18% in the urine after 24 hours. Some 80% of the faecal radioactivity was extractable, divided into 54% unchanged lasalocid and 26% other products, representing at least 5 metabolites. None of the metabolites was present at more than 4.5%. Lasalocid is partly absorbed and then excreted in the bile either as the parent compound or as related metabolites.

Repeated administration of ^{14}C-labelled lasalocid sodium results after three days in steady tissue levels, the main target organ being the liver. Of the labelled liver residues some 82% were extractable and consisted of 15% unchanged lasalocid, 7% identified metabolites (5 in number) and 60% unidentified fragments. The unidentified portion consisted of many products each representing less than 1% of the extractable hepatic radioactivity. Some 15% hepatic radioactivity was non-extractable. Both the extractable and non-extractable fractions were non-mutagenic when tested in the Salmonella reverse mutation test. The biotransformation products identified had no ionophoric properties. No information has been provided on the identity of the various metabolites.

Cattle treated under field conditions with ^{14}C-labelled lasalocid sodium for 2 weeks at a rate of 1.0 mg/kg body weight (approximately 40 mg/kg feedingstuff) had total labelled residues of 6.92 mg/kg tissue in the liver, 0.06 mg/kg tissue in the kidneys, 0.022 mg/kg tissue in the fat and 0.009 mg/kg tissue in muscle when slaughtered within 16 hours after the last dosing. After 9 days withdrawal period the total labelled residues were 0.713 mg/kg tissue in the liver, 0.019 mg/kg tissue in the kidneys and below the limit of detection in fat and muscle (limit of detection 0.0035 mg/kg tissue). The bioavailability of the liver residues was on average 24.4% as determined in the bile of rats fed with liver from steers treated with labelled lasalocid sodium.

Cattle treated for 4 weeks under field conditions with unlabelled lasalocid sodium at a rate of 0.6 mg/kg body weight/day (approximately 33 mg/kg feedingstuff) had liver residues of lasalocid sodium ranging from 0.025-0.539 mg/kg tissue at zero withdrawal period, less than 0.1 mg/kg tissue after a 2-day withdrawal period, 0.035 mg/kg after a 3-day withdrawal period and no detectable residues after a 4-day withdrawal period (limit of detection of HPLC method 0.025 mg/kg tissue). These figures do not include the 82% identified and unidentified metabolites of lasalocid sodium present in the liver but not determined by the HPLC method. For comparison with the total labelled residues deter-

mined by analysis of radioactivity these figures should be multiplied by a factor of at least 7.

Extrapolation from the data of the study using radiolabelled lasalocid sodium would yield a total liver residue of approximately 1.2 mg/kg tissue calculated as lasalocid sodium after a 5-day withdrawal period and about 0.71 mg/kg tissue after a 7-day withdrawal period. Extrapolation from the studies under field conditions yields total liver residues, also calculated as lasalocid sodium, of less than 0.18 mg/kg tissue after a 4-day withdrawal period and residues ranging from 0.18-3.7 mg/kg tissue at zero withdrawal period.

3. The Committee has already delivered its opinion on the possible effects of lasalocid sodium on the development of bacterial resistance when the use as additive to feedingstuffs for chickens was being evaluated. Like other polyether antibiotics it is generally effective against Gram-positive bacteria and ineffective against Gram-negative bacteria and also fungi. *In vivo* the substance inhibits specifically most of the lactic acid producing bacteria in the rumen. It has no effect on the development of Salmonella infections and does not prolong the shedding period of Salmonellae.

The ineffectiveness against Gram-negative bacteria, particularly *E.coli,* suggests that selection of enterobacteria carrying R plasmids is unlikely to occur. Enterococci and other Gram-positive bacteria show some degree of variation in resistance to lasalocid sodium but no persistent changes were noted. These effects were not accompanied by lowered sensitivity of the bacterial strains tested to antibacterial substances in common therapeutic use.

4. Lasalocid sodium, when added to feedingstuffs for cattle, is eliminated mainly in the faeces and in small amounts in the urine. Studies with ^{14}C-labelled material show that 50% of the excreted faecal matter is unchanged lasalocid sodium and the remainder at least 5 metabolites. The degradability of the product in cattle manure (approximate concentrations 10-12 mg/kg untreated manure, 40-48 mg/kg dry matter) has been studied in aqueous systems in relation to pH, light and temperature. Though very stable in dry faeces concentrations fall by 30% after one month in wet faeces kept at 37°C. Degradation varies with temperature after 1 week in slurry pits but always exceeds 50%.

Spreading of dung and liquid manure from treated cattle leads to soil concentrations of about 0.05 mg/kg soil of which about half leaches out. Static and dynamic trials with different types of soil showed considerable removal by leaching, as well as chemical and microbiological degradation over 2-3 weeks.

Lasalocid does not impair soil methanogenesis. The absence of effects on plant development, particularly soya, makes any deleterious effects on nitrifying soil bacteria unlikely.

As stated in the previous report of the Scientific Committee there was no evidence of any phytotoxic effects when tested on growing maize, barley, tomatoes and cucumbers nor does lasalocid sodium affect the germination of cereals and other plant seeds.

Lasalocid sodium is barely toxic to aquatic organisms such as Daphnia, goldfish, moonfish and fresh water algae. The LD_{50} ranges from 2.4 mg/l to 8.0 mg/l for periods of 48-49 hours.

5. CONCLUSIONS

Lasalocid sodium has been shown to be effective for promoting growth and improving feed conversion ratios in cattle during the finishing period at concentrations between 10 mg and 35 mg/kg of complete feedingstuff. There is, however, a need to fix a maximum dose according to the body weight of the animal as set out in the table in this report.

Residues of lasalocid sodium are found essentially in the liver but are low (about 0.25 mg/kg tissue) even at zero withdrawal periods. As the bioavailability is only about 25% these levels are well within the ADI and would not require a withdrawal period. However, as the identity of the residues is not well defined and as these residues, at the levels detected, have no biological activity, the Committee required additional mutagenicity tests with pure lasalocid sodium.

These additional tests establish adequately the absence of a genotoxic potential for the pure substance. Neither the extractable nor the non-extractable liver residues are mutagenic in a prokaryotic assay. Lasalocid sodium does not cause the development of significant bacterial cross-resistance to antibiotics in common therapeutic use.

The excreted products are not prejudicial to the environment and do not interfere with methanogenesis or with nitrification.

In the light of the above findings the Committee is of the opinion that the use of lasalocid sodium is acceptable in complementary feedingstuffs for finishing cattle at concentrations of 10-35 mg/kg but the maximum dose in the daily ration should not exceed the values given in the table in this report.

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