

PATENT SPECIFICATION

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(54) ANIMAL FEEDS AND FEED ADDITIVES CONTAINING ORTHOSOMYCIN ANTIBIOTIC

(71) We, CIBA-GEIGY AG, a Swiss body corporate, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

5 The present invention relates to feeds and feed additives for productive animals. In the rearing of fattening animals and other productive livestock it is desirable for economic reasons that the animals show the greatest possible increase in weight in the shortest possible time and with the least possible consumption of feed. It has already been suggested in this connection that there be added to the feed active substances which improve the animals' utilisation of feed by favourably affecting the intestinal flora. Substances of this type which have been used hitherto are antibiotics, such as penicillin, oxytetracycline, virginiamycin, etc., as well as sulphonamides and other antimicrobics. 10

15 Since antibiotics are used on a large scale in human and veterinary medicine as therapeutics for the treatment of diseases, the following aspects should be taken into consideration with regard to the choice of such a substance for use as a feed additive for productive animals: the antibiotic should belong to those groups of substances which have no practical application in human medicine; the use of the antibiotic should not lead in the case of the treated animals to a selection of bacteria having cross-resistance to antibiotics used therapeutically; furthermore, the antibiotic should have on the one hand high affinity for fattening feedstuffs and for bacteria, and on the other hand only low affinity for animal organs, and should remain virtually unabsorbed; it should act principally against gram-positive germs, and should be able to fully develop this action even under anaerobic conditions. The aforementioned requirements are not satisfied or only partially satisfied by the antibiotics suggested hitherto for use as feed additives. 20 25

Feeds and feed additives are now suggested according to the invention which are characterised in that they contain avilamycin. The present invention provides a method of promoting the growth of economically valuable domestic and productive animals which comprises administering avilamycin thereto, as well as feeds and feed additives as defined below. 30

The feeds according to the invention contain, apart from the feedstuff, 1 to 500 ppm of avilamycin. The feeds according to the invention preferably contain 1 to 200 ppm, particularly 5 to 50 ppm of avilamycin. 35

The feed additives according to the invention contain animal feedstuff and/or edible carriers and other additives and 0.1 to 25 per cent by weight of avilamycin. The feed additives according to the invention preferably contain 1 to 5 per cent by weight of avilamycin. 40

Avilamycin is an antibiotic of the orthosomycin group. It can be produced by aerobic cultivation of *Streptomyces viridochromogenes* A 23 575 (NRRL 2860) (see, e.g., British Patent Specification No. 948,638. 45

The feeds and feed additives according to the invention are very suitable for feeding productive animals, especially pigs and poultry (e.g. fowl, turkey-hens, geese, ducks and quail), and also calves and ruminants (e.g. cattle, sheep and goats). The feeds according to the invention are exceptionally suitable for fattening pigs. 45

The feeds according to the invention can be produced by intimately mixing

avilamycin with the fodder concerned. For this purpose, avilamycin can be used either as pure substance or as crude product.

To produce the feeds according to the invention, it is advantageous to firstly prepare a premix containing, depending on the desired final concentration in the finished feed, 0.1 to 25% by weight, preferably 1 to 5% by weight, of avilamycin. These premixes constitute the feed additives according to the invention.

The feed additives according to the invention can be obtained by intimately mixing pure or crude avilamycin with a suitable carrier. Suitable carriers in a premix of this kind are organic and/or inorganic substances which have good mixing properties and good compatibility with the feed being used. Carriers which are suitable are, for example, maize flour, low-grade wheat flour, dried grain swill, soya-bean flour, calcium carbonate, calcium phosphate and bolus alba. In many cases, the crude avilamycin produced in the manner described above, for example by drying the whole fermentation broth, can be used directly as premix since this will contain the edible carrier. In certain cases, for example in the production of milk substitute for calves, it is expedient to formulate the premix as a wettable powder or soluble powder.

A feed (feedstuff) according to the invention can be prepared by diluting the premix with the other feed constituents until the desired final concentration of avilamycin in the finished feed is obtained. An advantageous procedure comprises firstly further diluting the avilamycin premix with a feed constituent to be contained in the final feed, and then adding the other necessary feed constituents. By this means a particularly homogeneous distribution of avilamycin in the finished feed can be obtained. This can also be obtained by firstly homogeneously mixing the avilamycin premix with the mineral substance/trace element/vitamin premix necessary for the finished feed, and to subsequently work up the resulting mixture with the main feed constituents to give the finished feed.

The content of avilamycin in a finished feed according to the invention can vary within the limits of 1 to 500 ppm, depending on whether the feed is used as a sole feed or only as a supplementary feed. A sole feed according to the invention usually contains avilamycin in amounts of 1 to 200 ppm, preferably 5 to 50 ppm. In the case of a supplementary feed, for example in the case of feeding fodder concentrate and hay and/or silage for fattening cattle, or fodder concentrate and silage for fattening pigs, and feeding protein concentrate and grain feed in the keeping of laying hens, the avilamycin concentration can be increased according to the proportion of supplementary feed. The content of avilamycin in the supplementary feed can be up to 500 ppm, depending on the ratio of supplementary feed to basic feed. Where avilamycin is used in the feeding of ruminants, it can moreover be advantageous to choose a formulation which renders possible an undisturbed passage through the rumen. This can be effected for example by coating or by the known rumen-by-pass methods.

By virtue of their content of avilamycin, which has a harmonising effect on the intestinal flora, the feeds according to the invention can be extensively used, which leads to an accelerated growth of the animals. The advantageous action of avilamycin is such that on the one hand it suppresses the clostridia and enterococci which are known to be toxin-formers, whilst simultaneously preserving the lactobacilli, but on the other hand it avoids any excessive development of the gram-negative coliform flora occurring at the expense of clostridia and enterococci. In this manner an equilibrium between lactobacilli and coli forms in the intestines, which is essential for the maintenance of a normal physiological intestinal flora, can be obtained. Furthermore, avilamycin has a high affinity for fattening fodder, cellulose and bacteria. On the other hand, the binding of the avilamycin to animal organs is slight, so that consequently an undesirable absorption of the avilamycin is largely avoided. Avilamycin produces under anaerobic conditions a greater effect than in the presence of oxygen, and acts exclusively against gram-positive germs. Gram-negative germs, especially enterobacteriaceae, are not inhibited by avilamycin. There occurs therefore no influencing of the resistance conditions in the enterobacteriaceae flora, which contains the R factor, of the intestines of productive animals. In addition, there is no therapeutic use for avilamycin in human medicine. There moreover exists no cross-resistance between avilamycin and antibiotics which are important in human medicine, such as dihydrostreptomycin, neomycin, benzyl penicillin, erythromycin, oxytetracycline, chloramphenicol and novobiocin.

The production and use of the feeds and feed additives according to the invention are further illustrated by the following Examples.

2	3	1,603,292	3
		Example 1.	
		<i>Production of avilamycin</i>	
5	5	a) By means of aerobic cultivation of <i>Streptomyces viridochromogenes</i> A 23575 (NRRL 2860) in a sterilised nutrient medium, which contains per litre of water 20 g of meat flour, 20 g of malt extract and 10 g of calcium carbonate, there is produced at pH 7 with an incubation time of 24 hours a culture solution containing avilamycin. After fermentation is finished, the aqueous solution containing the avilamycin is separated by filtration from the mycelium and other solid constituents, and the filtrate is extracted with chloroform. Avilamycin is precipitated from the concentrated chloroform extract by the addition of petroleum ether. The crude avilamycin obtained in this manner is in the form of a powder which is slightly yellowish-brown in colour and which contains about 50% by weight of avilamycin. Colourless crystals are obtained by crystallisation of the crude product from acetone/ether or acetone/water.	5
10	10		10
15	15	b) To produce a commercial crude avilamycin, the fermentation solution containing avilamycin can be processed as follows: The culture solution produced as described under a) is homogenised with the aid of a Dispax apparatus, and dried in a spray dryer, with two-component nozzles, in a parallel flow. The inlet temperature of the heated air is 180°C. The crude avilamycin, mixed with nutrient solution and cell constituents, is obtained in the form of a fine, light-coloured and virtually odourless powder, which can be used directly as a feed additive. The content of avilamycin in the spray-dried product is ascertained by extraction with chloroform or ethyl acetate, and subsequent microbiological or thin-layer-chromatographical determination.	15
20	20		20
25	25	Example 2.	25
	25	a) <i>Production of a feed additive</i>	25
		98 kg of low-grade wheat flour and 2 kg of crude avilamycin (produced according to Example 1) are intimately mixed in a mixer and subsequently ground. There is obtained in this manner a feed additive containing 1% by weight of avilamycin.	
30	30	b) <i>Production of a sole feed for fattening poultry</i>	30
	30	0.2 kg of the feed additive produced according to a) is mixed with 100 kg of a poultry feed consisting of	
35	35	maize 26.7% by weight	
		wheat 21.2% by weight	
		barley 18.2% by weight	
40	35	grass flour 1.2% by weight	35
		soyabean meal (44%) 16 % by weight	
		fish flour 7.2% by weight	
		meat flour 5.0% by weight	
		beef tallow 2.0% by weight	
45	40	dicalc. phosphate 0.7% by weight	40
		calcium carbonate 0.8% by weight	
		sodium chloride (iodinated) 0.4% by weight	
		methionine DL-98 0.1% by weight	
		vitamins+trace elements 0.5% by weight	
50	45	There is obtained in this manner a poultry sole feed containing about 20 ppm of avilamycin.	45
		Example 3.	
		a) <i>Production of a feed additive</i>	
55	50	49 kg of calcium carbonate and 1 kg of crude avilamycin (produced according to Example 1) are intimately mixed in a mixer, and subsequently ground. In this manner is obtained a feed additive according to the invention, which contains 1% by weight of avilamycin.	50
		b) <i>Production of a sole feed for fattening poultry</i>	
60	55	100 g of the feed additive produced according to a) is mixed with 100 kg of a poultry sole feed consisting of	55

	maize	30.3% by weight	
	wheat	27.0% by weight	
	soyabean meal	(44%) 26.0% by weight	
	fish flour	5.0% by weight	
5	beef tallow	5.0% by weight	5
	dicalc. phosphate	0.8% by weight	
	calcium carbonate	0.5% by weight	
	sodium chloride (iodinated)	0.3% by weight	
	methionine DL—98	0.1% by weight	
10	vitamins + trace elements	0.5% by weight	10

There is obtained in this manner a poultry sole feed containing about 10 ppm of avilamycin.

Example 4.

- 15 a) *Production of a feed additive*
10 kg of crude avilamycin (produced according to Example 1) and 90 kg of maize flour are mixed together and subsequently ground to obtain a feed additive containing 5% by weight of avilamycin. 15

- 20 b) *Production of a pig-fattening feed*
By mixing 0.25 kg, 0.5 kg, 1 kg and 2 kg of the feed additive obtained according to a) into (in each case) one ton of a basic feed comprising 20

	32.8% by weight of maize	
	23.0% by weight of wheat	
	15.0% by weight of barley	
	9.0% by weight of soyabean meal (44%)	
25	4.0% by weight of fish flour	25
	6.0% by weight of meat flour	
	1.8% by weight of beef tallow	
	1.0% by weight of oat chaff	
30	5.0% by weight of wheat bran	30
	0.5% by weight of dicalc. phosphate	
	0.9% by weight of calcium carbonate	
	0.5% by weight of sodium chloride (iodinated)	
	0.5% by weight of vitamins + trace elements,	

- 35 there are obtained pig feeds containing respectively 12.5 ppm, 25 ppm, 50 ppm and 100 ppm of avilamycin. 35

Example 5.

Poultry-fattening test

- 40 The effect of avilamycin on the growth and on the feed utilisation of day-old chicks (Hubbard) is tested in a test lasting two weeks. The basic feed used is an experimental stress diet containing a high proportion of white beans. A diet of this kind leads to a retarded growth and to an inferior feed utilisation compared with the growth and feed utilisation resulting from a normal diet. This depression can however be largely eliminated by the addition of substances having a growth-promoting action (see Fernandez et al., Poult. Sci. 52, 1973, 2299—2305). 40

- 45 Avilamycin is added to the feed in a concentration of 180 ppm. A negative control test using a feed not containing avilamycin is concomitantly carried out. For each treatment, 4 groups (2 ♂ and 2 ♀) each of 10 day-chicks are installed in heatable cages. Feed and drinking water are available ad libitum. The weight of the chicks is determined at the commencement of the test and after 2 weeks (end of test), the chicks being weighed in groups, and the feed utilisation is calculated on the basis of the feed consumed in this period. The results obtained are shown in the following Table. 50

Treatment — dose	GROWTH		FEED UTILISATION	
	absolute g	% diff with respect to neg. control	absolute kg	% diff. with respect to neg. control
neg. control —	137	—	1.95	—
avilamycin 180 ppm	182	+32.8	1.71	+12.3

Example 6.

Poultry-fattening test

The growth-promoting action of avilamycin is tested in a test lasting 4 weeks. The employed feed containing avilamycin is the diet with 20 ppm of avilamycin, produced according to Example 2. The feed is available ad libitum to the birds during the entire duration of the test.

For each of the two treatments (negative control/20 ppm of avilamycin) are used 8 groups (4 ♂ and 4 ♀) each consisting of 10 day-old chicks (Hubbard). The chicks are weighed in groups and installed in heated cages. Further weighings are made after 2 and 4 weeks (end of test). The consumption of feed is determined at the same time. The results of the test at the end of the test are shown in the following Table.

Treatment — dose	GROWTH		FEED UTILISATION	
	absolute g	% diff. with respect to neg. control	absolute kg	% diff. with respect to neg. control
neg. control —	634	—	1.782	—
avilamycin 20 ppm	705*	+11.2	1.713	+3.9

* = significantly different from neg. control $P \leq 0.05$.

Example 7.

Poultry-fattening test

The growth-promoting action of avilamycin is tested in a test lasting 6 weeks. The employed feed containing avilamycin is the diet with 5 ppm of avilamycin, produced according to Example 3. The feed is available ad libitum to the birds during the entire duration of the test. As the negative control feed is used the same basic feed but without any additive at all. During the test, the birds are kept in Ehret batteries with cage heating. To each of the two treatments are assigned at random 12 groups (6 ♂ and 6 ♀) each consisting of 12 chicks. The following climatic conditions are maintained during the test:

room temperature about 23°C
 cage temperature 1st week 32°C
 2nd+3rd week 30°C
 4th+5th week 26°C
 6th week 23°C
 humidity 35 to 50%.

For the evaluation of the test, the chicks are weighed before, during and at the end of the test, the consumption of feed being determined simultaneously. The test results are shown in the following Table.

Treatment — dose	GROWTH		FEED UTILISATION	
	absolute g	% diff. with respect to neg. control	absolute kg	% diff. with respect to neg. control
neg. control —	1402	—	1.904	—
avilamycin 5 ppm	1447	+3.2	1.849*	+2.9

* = significantly different from neg. control $P \leq 0.05$.

Example 8.

Pig-fattening test

The growth-promoting effect of avilamycin on the pig is determined in a 4-week test, in which the results are compared with those obtained with a negative control feed. The employed feed containing avilamycin is the diet having 25 ppm of avilamycin, produced according to Example 4. All the animals receive the same basic feed before the commencement of the test. The identical basic feed is used as a negative control feed during the test. Feeding occurs with rationed amounts (meal form, ground feeding, water being available ad libitum). Each treatment is carried out with 12 piglets in three groups each of 4 animals, which have at the start of the test an average weight of 9.5 kg. The test results are shown in the following Table.

Treatment	ppm	Initial weight in kg	Days 0 — 28	
			DI and % diff. with respect to neg. control	FU and % diff. with respect to neg. control
1 control	—	9.12	236 —	1.97 —
3 avilamycin	25	9.59	286 +21.2	1.74 +11.7

DI = daily increase in g

FU = feed utilisation (= kg of feed per kg of growth).

Example 9.

Pig-fattening test

The growth-promoting action of avilamycin is determined, in a test lasting 8 weeks, with the feed produced according to Example 4, containing 12.5 ppm, 25 ppm, 50 ppm and 100 ppm of avilamycin, respectively. The identical basic feed without any additive is used as a comparison (negative control). The test is carried out with 2 batches staggered with respect to time. In the case of the first batch to be installed, 20 male (castrated) piglets and 20 female piglets are divided up, taking into account sex and origin, into 10 groups each of 4 animals. One group of male piglets and one group of female piglets are randomly subjected to one of 5 different treatments. The animals are kept and fed as described in Example 8. This test procedure is repeated 3 weeks later with a second batch of piglets, so that altogether each treatment is tested on 2 groups of male animals and on 2 groups of female animals, each group consisting of 4 animals. The overall duration of the test amounts to 8 weeks. After 4 and 8 weeks, the average increase in weight and the utilisation of feed are determined. The results of the test are shown in the following Table.

TREATMENT	ppm	Initial weight kg Age in days	Days 1 - 28		Days 1 - 56	
			DI and % diff. with respect to neg. control	FU and % diff. with respect to neg. control	DI and % diff. with respect to neg. control	FU and % diff. with respect to neg. control
control	-	9.9/ 48	257 -	1.88 -	337 -	2.07 -
avilamycin (A-23575-N-OK 56)	12.5	10.5/ 48	263 +2.3	1.83 +2.7	348 +3.3	2.00 +3.4
" "	25	10.9/ 48	268 +4.3	1.80 +4.3	358 +6.2	1.95 +5.8
" "	50	10.3/ 48	281 +9.3	1.71 +9.0	371 +10.1	1.8 +9.2
" "	100	9.7/ 48	282 +9.7	1.70 +9.6	365 +8.3	1.90 +8.2

DI = daily increase in g.

FU = feed utilisation ($\frac{g}{kg}$ of feed per kg of growth)

The Examples in the foregoing show that avilamycin, when administered by way of the feed, has a clearly favourable effect on increase in weight and on feed utilisation both in the case of broilers and in the case of pigs.

Example 10.

5 *Wettable Powder*

A wettable powder which is suitable as an additive to be added to drinking liquids for domestic animals and productive animals, particularly to milk or milk substitute for calves, is obtained by mixing together

- | | | |
|----|--|----|
| | 25 parts by weight of crude avilamycin (50%), | 5 |
| 10 | 5 parts by weight of sodium dibutyl-naphthalenesulphonate (Tinovetin B), | 10 |
| | 5 parts by weight of sodium lignin sulphonate, | |
| | 60 parts by weight of bolus alba, and | |
| | 5 parts by weight of silicic acid K 320 (highly dispersed silicic acid). | |

15	Instead of using bolus alba, it is also possible to use kaolin or Champagne chalk as carrier.	15
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Example 11.

Soluble Powder

20	A soluble powder which is suitable as an additive for adding to drinking liquids for domestic animals and productive animals, particularly to milk or milk substitute for calves, is obtained by mixing together	20
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- | | | |
|--|--|--|
| | 40 parts by weight of crude avilamycin (50%), | |
| | 55 parts by weight of sodium chloride, | |
| | 5 parts by weight of silicic acid K 320 (highly dispersed silicic acid). | |

Instead of using sodium chloride it is also possible to use sugar as a carrier.

25 **WHAT WE CLAIM IS:—**

- | | | |
|----|---|----|
| | 1. A feed suitable for domestic animals and productive animals, which feed contains 1 to 500 ppm avilamycin. | 25 |
| | 2. A feed according to claim 1, which contains 1 to 200 ppm of avilamycin. | |
| | 3. A feed according to claim 1 or 2, which contains 5 to 50 ppm of avilamycin. | |
| 30 | 4. A feed additive suitable for domestic animals and productive animals which contains animal feedstuff and/or edible carriers or other additives and 0.1 to 25% by weight of avilamycin. | 30 |
| | 5. A feed additive according to claim 4 which contains 1 to 5% by weight of avilamycin. | |
| 35 | 6. A feed additive according to claim 4 or 5 in the form of crude avilamycin obtained by drying the whole fermentation broth after fermentation of an avilamycin-producing medium. | 35 |
| | 7. A feed additive according to claim 4 or 5 which contains crude avilamycin as defined in claim 6. | |
| 40 | 8. A feed or feed additive according to any one of claims 1 to 5 and 7 which contains one or more of ground or unground maize, wheat, oats or barley, dried grain swill, soya bean meal, fish or meat flour, dried grass, calcium phosphate, and calcium carbonate. | 40 |
| | 9. A feed additive according to any one of claims 1 to 5, 7 and 8 which contains one or more non-toxic wetting agents and/or solid inert edible carriers. | |
| 45 | 10. A feed or feed additive according to claim 1 or 4 substantially as hereinbefore described. | 45 |
| | 11. A method of promoting the growth of economically valuable domestic and productive animals which comprises administering avilamycin thereto. | |
| 50 | 12. A method according to claim 11 in which the avilamycin is administered in the form of a feed or feed additive as claimed in any one of claims 1 to 10. | 50 |
| | 13. A method according to claim 1 or 12 in which the animals are pigs, poultry, calves or ruminants. | |



The Examples in the foregoing show that avilamycin, when administered by way of the feed, has a clearly favourable effect on increase in weight and on feed utilisation both in the case of broilers and in the case of pigs.

Example 10.

5 *Wettable Powder*

A wettable powder which is suitable as an additive to be added to drinking liquids for domestic animals and productive animals, particularly to milk or milk substitute for calves, is obtained by mixing together

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 5 parts by weight of sodium dibutylphthalenesulphonate (Tinovetin B),
 5 parts by weight of sodium lignin sulphonate,
 60 parts by weight of bolus alba, and
 5 parts by weight of silicic acid K 320 (highly dispersed silicic acid). 10

15 Instead of using bolus alba, it is also possible to use kaolin or Champagne chalk as carrier. 15

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 55 parts by weight of sodium chloride,
 5 parts by weight of silicic acid K 320 (highly dispersed silicic acid).

Instead of using sodium chloride it is also possible to use sugar as a carrier.

25 WHAT WE CLAIM IS:— 25

1. A feed suitable for domestic animals and productive animals, which feed contains 1 to 500 ppm avilamycin. 25
2. A feed according to claim 1, which contains 1 to 200 ppm of avilamycin.
3. A feed according to claim 1 or 2, which contains 5 to 50 ppm of avilamycin.
- 30 4. A feed additive suitable for domestic animals and productive animals which contains animal feedstuff and/or edible carriers or other additives and 0.1 to 25% by weight of avilamycin. 30
5. A feed additive according to claim 4 which contains 1 to 5% by weight of avilamycin.
- 35 6. A feed additive according to claim 4 or 5 in the form of crude avilamycin obtained by drying the whole fermentation broth after fermentation of an avilamycin-producing medium. 35
7. A feed additive according to claim 4 or 5 which contains crude avilamycin as defined in claim 6.
- 40 8. A feed or feed additive according to any one of claims 1 to 5 and 7 which contains one or more of ground or unground maize, wheat, oats or barley, dried grain swill, soya bean meal, fish or meat flour, dried grass, calcium phosphate, and calcium carbonate. 40
9. A feed additive according to any one of claims 1 to 5, 7 and 8 which contains one or more non-toxic wetting agents and/or solid inert edible carriers.
- 45 10. A feed or feed additive according to claim 1 or 4 substantially as hereinbefore described. 45
11. A method of promoting the growth of economically valuable domestic and productive animals which comprises administering avilamycin thereto.
- 50 12. A method according to claim 11 in which the avilamycin is administered in the form of a feed or feed additive as claimed in any one of claims 1 to 10. 50
13. A method according to claim 1 or 12 in which the animals are pigs, poultry, calves or ruminants.



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