

PATENT SPECIFICATION

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(54) COMPOSITION AND METHOD FOR PROMOTION OF GROWTH OF CULTURED FISH

(71) We, ELI LILLY AND COMPANY, a Corporation organised and existing under the laws of the State of Indiana, United States of America, of 307 East McCarty Street, City of Indianapolis, State of Indiana, United States of America, 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:—

This invention relates to a method for promoting the growth of cultured fish and to a fish food containing a growth-promoting amount of at least one macrolide antibiotic.

More particularly, the invention provides a food composition adapted for feeding to fishes comprising at least one macrolide antibiotic selected from oleandomycin, triacetyl oleandomycin, erythromycin, leucomycin, carbomycin, picromycin, spiramycin, tylosin, the first-order derivatives thereof as herein-after defined, or acid addition salts thereof.

The invention further provides a method of promoting the growth of cultured fish which comprises administering thereto a growth-promoting amount of at least one macrolide antibiotic selected from oleandomycin, triacetyl, oleandomycin, erythromycin, leucomycin, carbomycin, picromycin, spiramycin, tyosin, the first-order derivatives thereof, and acid addition salts thereof.

In recent years, the cultivation of fresh water fish, such as trout, carp, eel and mountain trout, as well as sea water fish, such as Hamachi, globe fish, sea bream, shrimp (prawn), and the like, has assumed commercial importance. The type of fish food utilized depends on the species of fish cultivated, the differing nutritional requirements of the various species, as well as economic considerations determining the type of food employed in a given case. As a result, various combinations of materials, such as raw fish, fish meal, chrysalis, vegetable oil

[Price 25p]

meal, flour, powdered skim milk, yeast, bran, fish viscera, vitamins, and various kinds of oil and fats have been used as feed for the cultured fish.

It has now been unexpectedly found that the administration of macrolide antibiotics to fish, preferably by addition of such antibiotics to fish food, improves feed efficiency and promotes the growth of cultured fish.

An advantage of this invention is that it provides a method for promoting the growth of cultured fish by the addition of a sufficient amount of a macrolide antibiotic to the fish food. A further advantage of this invention is that it provides growth-promoting mixtures of fish food comprising a macrolide antibiotic and any kind of feed designed for administration to cultured fish.

When used herein, the term "first-order derivatives" means the derivatives of the macrolide antibiotics obtained by reaction of functional groups of the antibiotics with simple reagents well known in the art, for example, by acylation, by hydrogenation, as well as by reaction to give well known derivatives of carbonyl groups present in the macrolide antibiotic molecule, derivatives such as oximes, hydrazones, semicarbazones and bisulphite addition products. The procedures lead to such products as diacetyl-leucomycin, propionylleucomycin, tetrahydro-leucomycin and erythromycin propionate, all useful in the practice of the instant invention. Acid addition salts of the macrolide antibiotics likewise are prepared by methods well known to the art through the use of both organic and inorganic acid chosen to yield acid addition salts of the macrolide antibiotics possessing toxicities comparable to the usual toxicity possessed by the antibiotic free bases. Acids suitable for use in preparing acid addition salts are exemplified by hydrochloric, hydriodic, hydrobromic, sulfuric, nitric, tartaric, glucoheptonic, stearic, lactobionic, maleic, benzoic, adipic and

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laurylsulfonic acids.

Suitably, these macrolide antibiotics, derivatives or salts are used singly, or in the form of combinations of one or more of the pure antibiotics, or in the form of a concentrated extract of the fermentation mixture wherein the antibiotic was produced.

The effective dosage of macrolide antibiotic will vary with the species of fish, with the method of administering the antibiotic to the fish, with the type of food employed when the antibiotic is administered as part of the feed, and with the feed intake of the fish species. Suitably, a dosage of the macrolide antibiotic of from about 1 mg. to about 500 mg. per kilogram of body weight of fish, preferably from about 2 mg. to about 100 mg. per kilogram of body weight of fish, is employed to promote optimum weight gain. While a dosage of the macrolide antibiotic below the lower limit generally is less effective in promoting weight gain, a dosage level higher than that required for optimum weight gain produces no adverse effect on the fish.

The macrolide antibiotics useful in this invention may be readily incorporated into the fish food by premixing one or more of the antibiotics in commercial food during the usual preparation and processing thereof; or by adding measured amounts of one or more of the antibiotics to food, prepared immediately prior to each feeding. In either case, the incorporation is suitably accomplished by simple mixing or by soaking the food in a solution of the macrolide antibiotic; or by spraying the food with a liquid preparation of the macrolide antibiotic.

To minimize the possibility of loss in water due to solubility therein of the macrolide antibiotics, the antibiotics are preferably mixed or coated with non-toxic vehicles and adhesive substances to add bulk or provide a relatively water insoluble coating. Sub-

stances such as lactose, calcium carbonate, soybean phospholipids, cellulose, algininate, methylcellulose, carboxymethyl cellulose, polyacrylic acid, polyvinylpyrrolidone, and the like can be employed for this purpose. The macrolide antibiotics can also be suitably prepared for use in the form of powders, granules or pellets.

That the practice of the invention may be more clearly understood, the following examples are included. The scope of the invention, however, is not to be considered as being limited thereby.

EXAMPLE 1

The effect of tylosin on the growth of cultured Hamachi fish was determined as described below. (Hamachi is a specific designation for younger stage fish of the Yellow Tail [*Seriola quinqueradiata*] variety).

A total of 1000 fish were divided into two groups of 500 fish each and kept in sea water in fish-culturing nets. One group served as the control group and the other received tylosin incorporated into its food ration. The standard ration was comprised of dried fish food and ground frozen fish, such as sardine and mackerel. The ration was mixed prior to each feeding and thrown into the sea water inside the fenced-in nets. The total amount of the ration required per day was calculated to be about 30 percent of the total body weight of the cultured fish. The control group received the standard ration, while the treated group received the standard ration with tylosin mixed or incorporated therein at the rate of 226 mg. of tylosin base/kg. of body weight of fish per day. The tylosin used was in the form of gelatinized tylosin phosphate, 1000 mg. of which corresponds to 226 mg. of tylosin base. The experiment was conducted for a total period of 21 days. The results are shown in Table I.

TABLE I

	Tylosin treated group	Control group
Start of trial		
No. of fish	500	500
Average weight/fish	10.7 gm.	10.6 gm.
Total weight	5.35 kg.	5.3 kg.
No. of fish died during trial	33	28
No. of fish removed for autopsy	40	40
At the termination of trial		
No. of fish	427	432
Average weight/fish	37.6 gm.	35.1 gm.
Total weight	16.05 kg.	15.15 kg.
Total weight gain	10.7 kg.	9.85 kg.
Total weight of feed used	71.81 kg.	71.81 kg.
Feed Efficiency (kg. gained/kg. used × 100 percent)	14.9 percent	13.7 percent
Feed Required (kg. used/kg. gained)	6.71	7.29
Average daily gain/fish	1.28 gm.	1.17 gm.

EXAMPLE 2

In this example, the effect of tylosin on the growth of cultured fish was carried out using 300 fish of equal size, the total composed of 150 fish from each of the medicated group and the control group of Example 1. The study was conducted for a period of 11

days using a dosage level of tylosin of 22.6 mg/kg. of body weight of fish per day. The tylosin used was in the form of gelatinized tylosin phosphate, 100 mg. of which corresponds to 22.6 mg. of tylosin base. The results are shown in Table 2.

TABLE 2

	<i>Tylosin medicated group</i>	<i>Control group</i>
At start of trial		
No. of fish	150	150
Average weight/fish	55.3 gm.	54.7 gm.
Total weight	8.3 kg.	8.2 kg.
At the termination of trial		
No. of fish	150	150
Average weight/fish	92.3 gm.	85.0 gm.
Total weight	13.85 kg.	13.75 kg.
Total weight gain	5.55 kg.	4.55 kg.
Total weight of feed used	33.0 kg.	32.6 kg.
Feed Efficiency (kg. gained/kg. used × 100 percent)	16.8 percent	14.0 percent
Feed Requirement (kg. used/kg. gained)	5.95	7.15
Average daily gain/fish	3.36 gm.	2.75 gm.

EXAMPLE 3

The effect of macrolide antibiotics on growth promotion of carp (*Cyprinus carpio*) was studied.

The carp, having an average weight of 10 gm., were kept in running water tanks, the water temperature being kept at about 24°C. Each of the 9 tanks contained 25 carp which were fed a standardized ration composed of the following ingredients: white fish meal (70 percent), wheat flour (16 percent), active gluten (3 percent), cornstarch (5 percent), and a commercial premix containing multiple vitamins and nitrofurazone. The fish in each tank received a food mixture comprising the above-described ration and the specific test material to be fed to the fish in that tank, as indicated in the following list:

Tank	Testing material	Dosage (per 100 gm. of ration)
1	None (negative control)	
2	Tylocine premix AF-40	0.23 g. (202 mg.)
3	Tylocine premix AF-40	1.14 g. (100 mg.)

4	None (negative control)	
5	Tylocine premix AF-40	0.12 g. (10.6 mg.)
6	Tylocine premix AF-40	0.29 g. (25.5 mg.)
7	Tylosin	100 mg. potency
8	Acetyl-spiramycin	100 mg. potency
9	Erythromycin	100 mg. potency

Notes: (a) Bracketted value shows potency

(b) "Tylocine premix AF-40" is the Japanese equivalent of "Tylan premix AF-40" (manufactured in the U.S. by Eli Lilly and Company, Tylan being the U.S. trade-name for the antibiotic tylosin) and consists of the antibiotic tylosin and a soybean mill run (ground-up shells of soy bean).

Feeding was accomplished by dampening or moistening 3-4 gm. of the dried food just prior to each feeding and then feeding the carp with the thus-prepared ration. Feeding took place twice daily for a period of 25 days. The results are shown in Table 3.

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TABLE 3

Tank	1	2	3	4	5	6	7	8	9
At the start									
Total fish wt. gm.	268	270	264	268	263	263	268	257	253
5 Av. wt./fish	10.75	10.80	10.56	10.75	10.52	10.52	10.75	10.38	10.12
Intermediate									
Total fish wt. gm.	292	308	308	296	306	298	306	302	293
Av. wt./fish gm.	11.68	12.32	12.32	11.84	12.24	11.81	12.24	12.08	11.72
Wt. gain gm.	24	38	44	26	43	35	38	45	40
10 At termination									
Total fish wt. gm.	332	361	364	334	350	358	357	358	352
Av. wt./fish gm.	13.28	14.45	14.56	13.36	14.00	14.32	14.28	14.32	14.08
Total wt. gain gm.	64	91	100	66	87	95	89	101	99
Total feed consumed**	151	151	151	151	151	151	151	151	151
15 Feed efficiency (percent)	42	60	66	44	53	63	59	67	66

Note: **As dry feed.

EXAMPLE 4

The effect of macrolide antibiotics as growth promoters was conducted using carp (Cyprinus carpio) weighing about 20 gm. each. Eighty-four carp were divided into seven groups of 12 carp each, each group

being kept in a separate tank in the same type facilities as used in Example 3. The same basic ration described in Example 3 was used in Example 4 with the following testing materials for each specific group added to the basic ration:

Group	Testing Material	Dosage per 100 gm. of ration
30 1 and 2	None (negative control)	
3	Aureomycin	50 mg. (potency)
4	Tylocine premix AF-40	150 mg. (13.2 mg. potency)
5	Tylocine premix AF-40	600 mg. (52.8 mg. potency)
6	Oleandomycin	50 mg. (potency)
35 7	Leucomycin	100 mg. (potency)

Feeding was accomplished by dampening or moistening about 2 gm. of the dried feed just prior to each feeding. The fish were fed such a preparation twice daily.

period), the fish were fed the basic ration to which no antibiotics had been added. At the end of the nonmedicated period, the antibiotics were incorporated into the feed and the fish fed for an additional period of 11 days. The results are shown in Table 4.

During a period of 11 days (nonmedicated

TABLE 4

Group	1	2	3	4	5	6	7
50 Non-medication period							
Total fish wt.							
at start gm.	234	242	239	244	242	236	240
at termination gm.	253	260	259	262	260	256	259
Weight gain gm.	19	18	20	18	18	20	19
55 Medication period							
Total fish wt.							
at start gm.	253	260	259	262	260	256	259
at termination gm.	273	281	278	283	289	279	286
Weight gain	20	21	19	21	29	23	27
60 Total feed consumed	36	36	36	36	36	36	36
Ratio of weight gain	100	105	95	105	145	115	135

WHAT WE CLAIM IS:—

1. A food composition adapted for feeding to fish comprising at least one macrolide antibiotic selected from oleandomycin, triacetyl oleandomycin, erythromycin, leucomycin, carbomycin, picromycin, spiramycin,

tylosin, the first-order derivatives thereof as hereinbefore defined, or acid addition salts thereof.

2. A method of promoting the growth of cultured fish which comprises administering thereto a growth-promoting amount of at

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Intermediate									
Total fish wt. gm.	292	308	308	296	306	298	306	302	293
Av. wt./fish gm.	11.68	12.32	12.32	11.84	12.24	11.81	12.24	12.08	11.72
Wt. gain gm.	24	38	44	26	43	35	38	45	40
10 At termination									
Total fish wt. gm.	332	361	364	334	350	358	357	358	352
Av. wt./fish gm.	13.28	14.45	14.56	13.36	14.00	14.32	14.28	14.32	14.08
Total wt. gain gm.	64	91	100	66	87	95	89	101	99
Total feed consumed**	151	151	151	151	151	151	151	151	151
15 Feed efficiency (percent)	42	60	66	44	53	63	59	67	66

Note: **As dry feed.

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Feeding was accomplished by dampening or moistening about 2 gm. of the dried feed just prior to each feeding. The fish were fed such a preparation twice daily.

During a period of 11 days (nonmedicated

period), the fish were fed the basic ration to which no antibiotics had been added. At the end of the nonmedicated period, the antibiotics were incorporated into the feed and the fish fed for an additional period of 11 days. The results are shown in Table 4.

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Weight gain	20	21	19	21	29	23	27
60 Total feed consumed	36	36	36	36	36	36	36
Ratio of weight gain	100	105	95	105	145	115	135

WHAT WE CLAIM IS:—

1. A food composition adapted for feeding to fish comprising at least one macrolide antibiotic selected from oleandomycin, triacetyl oleandomycin, erythromycin, leucomycin, carbomycin, picromycin, spiramycin,

tylosin, the first-order derivatives thereof as hereinbefore defined, or acid addition salts thereof.

2. A method of promoting the growth of cultured fish which comprises administering thereto a growth-promoting amount of at



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least one macrolide antibiotic selected from
oleandomycin, triacetyl oleandomycin,
erthromycin, leucomycin, carbomycin, picro-
mycin, spiramycin, tylosin, the first-order
5 derivatives thereof, or acid addition salts
thereof.

3. The method of claim 2 wherein the
macrolide antibiotic is tylosin.

4. The method of claim 2 wherein the
10 macrolide antibiotic is oleandomycin.

5. The method of claim 2 wherein the
macrolide antibiotic is leucomycin.

6. A fish food containing growth-
promoting amount of at least one macrolide

antibiotic substantially as hereinbefore de- 15
scribed with particular reference to the
examples.

7. A method of promoting the growth of
cultured fish substantially as hereinbefore
described with particular reference to the 20
examples.

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