

The invention relates to an enteral solid medicinal feed preparation for fish, which preparation contains at least one of the active substances malachite green, brilliant green, gentian violet, crystal violet, pararosaniline, fuchsin, rivanol, quinine, hexamethylenetetramine, transition metal compounds and/or a quaternary ammonium salt as an active substance in a concentration of 0.005 to 10 % by weight alone or in combination with other active substances, with the active substances being bound by the carrier or feed substances in such a way that no washing out by water takes place, and also to processes for the preparation of said medicinal feed preparation and the use thereof.

In the case of the care, breeding and maintenance of fresh water and marine fish in hobby aquaria and ponds and, to an even greater extent, in intensive maintenance installations of fish farming and aquaculture, parasitic diseases occur that endanger the attacked creatures and can result in considerable financial losses. The following organisms which are pathogenic to fish thereby occur especially frequently: dinoflagellates: *Oodinium pillularis* (fresh water), *Oodinium ocellatum* (sea-water); flagellates: *Costia necatrix*, *Hexamita symphysodonis*; ciliates: *Ichthyophthirius multifiliis* (fresh water), *Cryptocarion* (sea-water), *Chilodonella*, *Trichodina* (fresh water); monogeneans: skin and gill trematodes, for example *Gyrodactylus* or *Dactylogyrus*.

The treatment of ectoparasitoses, i.e. of diseases with parasites which live in or on the epidermis of fish, has until today been effected exclusively by means of the so-called bath therapy in which antiparasitic active substances are added to the maintenance water in appropriate biocidal concentrations.

Frequently, only the free-swimming-parasitic stages, the so-called swarms, are thereby damaged and not, however, the stages sitting fast in the skin or under the epidermis.

5 Consequently, the total treatment is based on killing off the swarms and thus the prevention of a new or re-infection and on the immune system of the fish, which system additionally or exclusively combats the parasitizing pathogenic stages.

10 According to the prevailing expert opinion, ectoparasitic diseases can only be treated from without and not by means of systemically administered curative agents, for example medicinal feeds.

On the other hand, endoparasitic diseases, for
15 example parasites of the gut, are treated successfully usually by systemically administered medicinal preparations, for example medicinal feeds (cf. N. Herwig, "Handbook of Drugs and Chemicals used in the Treatment of Fish Diseases", pp. 128 and 136, published
20 by Charles C. Thomas, Springfield, Illinois, U.S.A., 1979; E. Amlacher, "Taschenbuch der Fischkrankheiten", published by Gustav Fischer Verlag, Stuttgart, 1981; L. Zemer, Das Aquarium, 220, 515/1987).

The therapy of ectoparasitic diseases of fish
25 (ornamental fish and useful fish) is usually carried out, on the other hand, with addition to the maintenance water of biocidally or antiseptically acting dyestuffs, for example of the triphenylmethane and acridine group of derivatives. These dyestuffs
30 include, for example, N-[4-[[4-(diethylamino)-phenyl]-phenylmethylene]-2,5-cyclohexadiene-1-ylidene]-N-ethyl-ethanaminium sulphate (brilliant green); N-[4-[[4-(dimethylamino)-phenyl]-phenylmethylene]-2,5-cyclohexadiene-1-ylidene]-N-methyl-methanaminium chloride or
35 oxalate (malachite green); N-[4-[bis-[4-(dimethylamino)-phenyl]-methylene]-2,5-cyclohexadiene-

1-ylidene]-N-methyl-methanaminium chloride (gentian violet); 3,6-diamino-10-methylacridinium chloride mixed with 3,6-diaminoacridine (acriflavine); 9-aminoacridine; and 2-ethoxy-6,9-diaminoacridinium
5 lactate monohydrate (rivanol) and, optionally, the leuco forms thereof. In addition, (in bath therapy) antiparasitic active substances from other classes of chemical compounds are also used, for example: quinoline derivatives such as, for example, quinine;
10 quaternary ammonium salts; silver and copper salts; metal colloids; nitrofurans, for example nifurpirinol; nitrothiazoles, for example 2-amino-5-nitrothiazole.

The bath therapy with the described antiparasitic active substances, however, involves a series of
15 disadvantages: parasites in or under the epidermis of the fish are mostly not affected. Since usually all the maintenance water (aquarium, pond, intensive installation) must be treated with the antiparasitic active substance, in addition a very large amount of
20 active substance must be used. Furthermore, it is difficult to adjust the required concentration of active substance since, during the treatment, due to decomposition processes (chemical, biological and photochemical) and adsorption on surfaces, losses of
25 active substance occur that cannot be calculated. Often, the intensive colouration of the water caused by dyestuffs and the tendency of the bath solution to colour various materials are also disturbing factors. Since the whole of the maintenance system is affected,
30 the presence of the biocidally active substances leads to undesired and, under certain circumstances, even harmful side-effects, for example damage to other important water organisms, such as aquatic plants, planktonic organisms or damage of the biological flora
35 in filter systems. Thus the biological breakdown capacity is impaired. The therapeutically active

compounds mentioned also sometimes have a toxic effect upon the gills when used externally.

The object of the invention was therefore to provide possibilities for the therapy of ectoparasitic and ectobacterial diseases in fish that avoid the above-mentioned disadvantages.

The subject-matter of the invention is therefore a medicinal feed for fish and also the use of this medicinal feed for the systemic treatment of ectoparasitic and ectobacterial diseases.

The described disadvantages of the bath therapy can be completely avoided by the possibility according to the invention, which is surprising to the expert, of the enteral, systemic treatment of ectoparasitic and ectobacterial diseases of ornamental and useful fish with medicinal preparations, in particular medicinal feed.

The surprisingly found and unexpectedly high systemic effectiveness of active substances previously only applied externally against ectoparasites and ectobacteria, i.e. organisms which have attacked the fish on and/or under the external skin and mucous membrane layer, offers therapy possibilities which were not previously realizable. According to the invention, controlled oral administration of the medicinal feed to the fish can namely take place. Parasites or bacteria in or under the epidermis of the fish are thus completely attacked therapeutically. The extraction of the active substance from the medicinal feed taking place due to the maintenance water is very low and since, in addition, the absolute administered amount of active substance is very small, the maintenance system is only loaded with negligibly small amounts of active substance. The toxic stressing of water organisms is thereby reduced practically to zero, the breakdown activity of the filter is not impaired, a colouration

of the water is no longer recognizable and the gill-toxic action is eliminated. Incalculable losses of active substance during the treatment also no longer occur. A most desirable side-effect, however, is the simultaneous combatting of endoparasites which might be present.

As a result of the choice of appropriate active substances, with the novel medicinal feed the following ectoparasites which are listed by way of example, can be combatted in the case of ornamental and useful fish, namely, dinoflagellates, such as Oodinium; flagellates, such as Costia, (Hexamita); ciliates, such as Ichthyophthirius, Cryptocarion, Chilodonella, Trichodina; monogeneans, such as skin and gill trematodes, for example Gyrodactylus and Dactylogyrus.

Surprisingly, it has also proved possible to combat ectobacterioses, for example all skin and fin infections due to gram-negative and gram-positive bacteria, such as Aeromonas, Pseudomonas and Flexibacter.

As a side-effect, in the case of the systemic treatment of the ectoparasites and ectobacterioses, naturally at the same time all the internal diseases of ornamental and useful fish due to parasites and bacteria are also treated systemically if the active substances used have an appropriate spectrum of activity.

Surprisingly, for the systemic treatment of ectoparasitoses and ectobacterioses according to the invention, in the first instance it is possible to use all those active substances which were previously either used exclusively for bath therapy or, in exceptional cases, even already in the form of enteral medicinal preparations, but exclusively for the treatment of internal parasitoses, preferably of gut parasitoses.

The following were previously exclusively used for bath therapy:

triphenylmethane derivatives, for example malachite green (all salts, for example chloride, oxalate, sulphate), malachite green carbinol base, brilliant green (all salts), crystal violet (all salts), gentian violet (all salts), pararosaniline (all salts, base), fuchsin (rosaniline) (all salts) as well as all leuco forms of the cations of the dyes; in addition also thionine derivatives, with the exception of methylene blue B; furthermore, thionine derivatives with lower degree of N-methylation as well as all leuco forms of the thionine derivatives; and also nitrothiazole derivatives, for example 2-amino-5-nitrothiazole and transition metal derivatives, for example copper salts, silver salts or complexes, copper or silver metal colloids; all microbicidal quaternary ammonium salts.

In addition, some of the active substances have also been used in the already mentioned medicinal preparations intended for peroral intake, but not used for the treatment of ectoparasitic diseases, as this indication was not known. These substances are acridine derivatives, for example acriflavine, methylene blue B; nitrofurane derivatives, for example nifurpirinol, enheptine and its N-acetyl derivative acimitrazole as well as tryptaflavine and acriflavine.

As matrix or vehicle for the above-described anti-ectoparasitic and anti-ectobacterial active substances to be used systemically, it is possible to use the following forms of administration of carrier or feed types for ornamental and useful fish, namely film-like feed, flake feed, pelletized feed, extruded feed, expanded feed, granulated feed, ground feed, pulverized feed, tablet-form feed, feed pastes and feed suspensions, feed gels, aspic feed or other carrier substances, if applicable, enriched with attractants or

baits.

The introduction of the therapeutic active substances is effected in a technologically appropriate preliminary stage of the production process, for

5 example in the raw material powder mixture stage and the water-containing process stages thereof in such a manner that a distribution of the active substances in the finished feed is attained that is as fine and homogeneous as possible.

10 It has been shown that most of the described active substances, in particular when they are present in cationic form (for example malachite green, acridinium salts, quaternary ammonium salts and the like), are bound by the carrier or feed substances in a
15 greatly adsorptive manner. The result of this pronounced active substance immobilisation is that washing out by water no longer occurs and there is no loss of colour, for example onto the hands of the user. In this way, a high level of safety of use, even in the
20 case of active substances which are toxic or dangerous to humans, is achieved and therefore as well no safety measures are necessary during the production, packaging, storage and during the therapeutic use.

Similar advantages are also achieved if reduced
25 precursors of the cations of the dyes, the so-called leuco forms, or other derivatives, which are also very difficult to dissolve in water, are used.

The ranges of concentrations in the feed are compiled in the following Table I by way of example for
30 some active substances. All the details relate to mg. of active substance per kg of feed or to weights percent.

TABLE I

	Active substance	Concentration range		Preferred concentration range	
		mg/kg	%	mg/kg	%
5	malachite green chloride (and other salts)	100-	0.01-	200-	0.02-
		10000	1.0	4000	0.4
10	malachite green carbinol base	100-	0.01-	200-	0.02-
		10000	1.0	4000	0.4
	crystal violet (gentian violet)	100-	0.01-	200-	0.02-
		10000	1.0	4000	0.4
15	9-aminoacridine	100-	0.01-	500-	0.05-
		50000	5.0	20000	2.0
	ethacridine lactate (Rivanol)	200-	0.02-	1000-	0.1-
		50000	5.0	30000	3.0
	acriflavine (trypaflavine)	200-	0.02-	500-	0.05-
		50000	5.0	30000	3.0
	methylene blue	200-	0.02-	500-	0.05-
		20000	2.0	10000	1.0

With the medicinal feed variants according to the invention, it is possible to treat ectoparasitoses, ectobacterioses and, as a therapeutic side-effect, also systemic diseases due to endoparasites and endobacteria in all types of fish of the ornamental fish group (fresh water and sea-water) and of the useful fish group (fresh water and seawater) in all closed and non-closed maintenance systems that occur and are common.

Even fish in their natural environment can be treated therapeutically by taking in the feed.

The following demonstration of effectiveness and the following examples are given for the purpose of further explaining the invention, but without limiting the subject matter of the invention in any way:

Production of medicinal feed:

A medicinal feed according to the invention is produced in the manner familiar to the expert in that

either the desired active substance is admixed with the raw feed mixture before the final processing to give the necessary quantity of flakes, pellets, tablets, capsules, extrudates etc., or the finished feed product is sprayed with a solution of the active substance and is subsequently finally dried. A typical medicinal feed contains 0.005 to 10% of the active substance, preferably 0.05 to 3%.

For the effectiveness tests, a flake feed for ornamental fish was enriched with 0.2% malachite green chloride and administered to ectoparasitically diseased ornamental fish instead of the non-medicated flake feed used for normal feeding. The following results were thereby attained:

1. Treatment of Ichthyophthirius

30 giant loach (*Botia macracantha*) and 30 thread fish (*Trichogaster trichopteri*), which were severely infected with *Ichthyophthirius*, were fed once a day with the medicinal feed containing malachite green. In comparison with the untreated control, within 5 days a 100% success of treatment was achieved.

2. Treatment of Costia and Chilodonella

40 red neons (*Paracheirodon axelrodi*), which were doubly infected with *Costia* and *Chilodonella*, were fed once a day with the medicinal feed containing malachite green. In comparison with the untreated control, within 5 days a 100% success of treatment was achieved.

3. Other ectoparasitic diseases

Analogously to the Examples mentioned above, in 3.1 and 3.2, with the described medicinal feed containing malachite green the following ectoparasitoses could be completely healed: *Oodinium pillulare*, *Trichodina spec.* and *Gyrodactylus*.

Corresponding healing successes are also achieved with the other medicinal feeds listed in the description of the invention.

5 The complete harmlessness in this connection is particularly advantageous in the case of the treatment of ectoparasitoses with the described medicinal feeds: none of the treated fish showed any signs of indisposition or of damage during and after the treatment.

10 Furthermore, the effectiveness of triphenylmethane dyestuffs, for example brilliant green and malachite green, against gram-positive bacteria makes these active substances in the form of the medicinal feeds according to the invention suitable, in addition, as a
15 further possible application for the treatment of external and internal bacterioses with gram-positive pathogens, for example with *Corynebacterium*, *Nocardia* and *Mycobacterium*.

The known effectiveness of crystal violet and
20 gentian violet against internal worm diseases in humans and mammals can also be utilized for combatting external and internal helminthoses in fish. Helminthoses, which are brought about, for example, by monogeneans (for example *Gyrodactylus*, *Dactylogyrus*),
25 Cestoda, Trematoda (for example *Diplostomum*) or Nematoda (for example *Capillaria*), can be treated in this connection.

A further, fundamental possibility of a comprehensive therapy lies in the combined use of the
30 medicinal feed according to the invention and a healing agent bath, in which case a therapeutically effective amount of similar or other antiparasitic or antibacterial active substances have been dosed into the water. This combined therapy presents the
35 possibility as well of combatting the pathogenic stages which are non-parasitizing, freely swimming or firmly

fixed (to plants, stones or any surfaces). Advantages resulting therefrom are partial or complete disinfection of the system, a reduction in the re-infection rate of the fish by freely swimming

- 5 pathogenic stages, as well as treatment of parasitizing pathogens on the fish from within by the medicinal feed and from without by the healing bath.

In the final analysis, though, it must be observed that, naturally, in the case of the additional
10 administration of the active substance via the water, all the initially mentioned fundamental disadvantages of a bath therapy take effect.



Claims

1. Enteral solid medicinal feed preparation for fish, characterised in that said preparation contains at least one of the active substances malachite green,
5 brilliant green, gentian violet, crystal violet, pararosaniline, fuchsin, rivanol, quinine, hexamethylenetetramine, transition metal compounds and/or a quaternary ammonium salt as an active substance in a concentration of 0.005 to 10 % by weight
10 alone or in combination with other active substances, with the active substances being bound by the carrier or feed substances in such a way that no washing out by water occurs.
2. Preparation according to claim 1,
15 characterised in that the proportion of active substance amounts to 0.05 to 3 % by weight.
3. Preparation according to claims 1 or 2, characterised in that the active substance is malachite green or a suitable salt or derivative thereof.
- 20 4. Preparation according to claims 1 or 2, characterised in that the active substance is quinine or a suitable salt or derivative thereof.
5. Process for the preparation of a medicinal preparation according to claim 1 to 4, characterised in
25 that either (A) the active substance or the active substance combination is admixed with the mixture of adjuvant substances before final processing to form flocks, pellets, tablets, capsules, extrudates etc. in the required amount or (B) the finished product of the
30 preparation is sprayed with a solution of the active substance or the active substance combination and is subsequently finally dried.
6. Use of triphenylmethane derivatives or thionine derivatives, nitrothiazole derivatives,
35 nitrofurane derivatives, quinoline derivatives, acridine derivatives, compounds splitting off aldehyde,



transition metal derivatives, metal colloids,
hexamethylenetetramine and/or quaternary ammonium salts
or combinations thereof for the preparation of an
enteral medicinal preparation, a medicinal feed or feed
5 mixtures for the systemic prevention or treatment of
ectoparasitic and ectobacterial fish diseases.

7. Use of quinine in the form of its salts alone
or in combination with active substances according to
claim 6 for the preparation of an enteral medicinal
10 preparation, a medicinal feed or feed mixtures for the
systemic prevention or treatment of ectoparasitoses or
ectobacterioses in fish.

8. Use of quinine in the form of its salts alone
or in combination with active substances according to
15 claim 6 for the preparation of an enteral medicinal
preparation, a medicinal feed or feed mixtures for the
systemic prevention or treatment of endoparasitoses or
endobacterioses in fish.