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(54) Title: ORAL VETERINARY DRUG DELIVERY SYSTEM AND/OR FEED ADDITIVE DELIVERY SYSTEM, PROCESS FOR THE PREPARATION AND USE THEREOF

(57) Abstract: An oral veterinary drug delivery system and/or feed additive delivery system is disclosed that contains at least one biologically active substance at least to a substantial extent preferably uniformly sorbed into the bulk of a porous carrier, preferably feed; said drug or feed additive delivery system is obtainable by mixing the feed with solution of the biologically active substance for sorption, followed by the evaporation of the solvent. The medicated feed is readily ingested by animals. Processes for the preparation of the oral veterinary drug or feed additive system and uses thereof as pharmaceutical or feed additive are also disclosed. The preparation is carried out in a closed system that is occupationally and environmentally safe. Products of the invention can be used for farmed animals and pets, preferably for farmed fish in freshwater and seawater.

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**Oral veterinary drug delivery system and/or feed additive
delivery system, process for the preparation and use thereof**

The present invention concerns an oral veterinary drug delivery system
5 and/or feed additive delivery system that contains at least one biologically
active substance preferably uniformly sorbed into a porous carrier, a method
for the preparation of the delivery system, and the application of the product
to farmed and pet animals, especially to fish.

10 **Background Art**

The oral administration of biologically active substances to animals in
feed is a diverse field concerning the type of the delivery system, i.e. the
properties of the active substance and the properties of the feed. Another
variable is the distribution of the active substance in the feed, whether it is
15 located throughout the feed or only on its surface. The medicated feed can be
prepared by adding the active substance during feed preparation to the feed or
combining the active substance with the prepared feed product. Medicated
feed can be prepared on farms, in feed mills or by drug and premix
manufacturers.

20 Methods for mixing feed ingredients and medication are reported.
Chinese patent 1999-115312 describes the production of furazolidone- and
terramycin-containing fish feed; the drugs are mixed with trace elements,
maggot protein, other ingredients of plant origin, and pelleted.

Chinese patent 1999-111364 describes the preparation of an
25 antiparasitic premix and the usage with fish feed. Abamectin was dissolved
in ethanol, mixed with rice husk and dried with hot air. The carrier-drug
product was then mixed with the feed ingredients that were fish meal, soy
sauce lees, calcium hydrogen phosphate, vitamins and minerals.

When drugs and feed additives (medication hereafter) are added
30 directly to the feed mix ingredients during feed preparation, prior to
the formation of the pellets or granules, the medication is distributed
uniformly within the produced pellets, granules etc. Adsorbing the
active substance on one of the feed ingredients as carrier and using
it for mixing with other ingredients improves the stability of the delivery

system. Concerning the equipment that is involved in the preparation of the feed, all equipment in the production line is contaminated with the medication. The cleaning of elaborate equipment, e.g. extruders, spray dryers, is time consuming and expensive. Moreover, the feed has to be medicated or
5 the medication has to be added to the feed without knowing the exact demand, due to unknown factors e.g. the outbreak of diseases, the animal species that are affected, their number, the need for preventive or therapeutic treatment, body weight, growth stage, hatching rate, litter size, survival rate etc.

Examples for adding the medication to feed pellets, granules etc. are
10 widely described.

The recommended preparation of medicated fish feed on farms is surface coating with additional sealing. The procedure is carried out in cement mixers or equivalent equipment: the unmedicated fish feed, the calculated amount of drug, and edible fish oil or vegetable oil are mixed
15 together for several minutes to ensure uniform coating. An example for the preparation of Aquaflor (florfenicol)-containing feed is given on the Syndel International Inc. Aqua Life homepage (www.syndel.com). To adhere Tribissen (trimethoprim and sulfadiazine) to food pellets, oil is first added to the pellets in the mixer and then the powder is sprinkled into the mixer. In
20 another example Romet-30 (combination of sulphadimethoxine and ormetoprim) is first suspended in edible vegetable oil and the slurry is used to coat the pelleted fish feed.

Similar technique is given in the Japanese patent 2002-58435 for the preparation of medicated fish feed. Docosahexaenoic acid and/or its ethyl
25 ester are first mixed with fish oil or vegetable oil and then added to the basic feed.

The mixing of feed and drug results only a loose binding of the drug; the sealing with oil does not assure the penetration of the drug into the feed. Thus, leaching cannot be avoided. The drug that is concentrated on the
30 surface of the feed spoils the taste of the feed and thus reduces the likeliness of medication ingestion by sick animals with reduced appetite.

Experimental scale fish food medication is usually performed by spraying the active ingredient solution onto the food. WO 02/30215 A2 published patent application that concerns the growing of marine fish in

freshwater describes a method for preparing food that contains 7 w/w% sodium chloride and tryptophan. A solution of the calculated amount of sodium chloride and tryptophan was prepared, poured into a hand held sprayer and was then applied to standard freshwater salmonid diet that was rotated in a cement mixer. After absorption of the sodium chloride-tryptophan solution the diet was dried on window screens in a heated rack system. After drying, the pellets were returned to the cement mixer to receive a top dressing that was composed of 50% krill hydrolyzate and 50% Menhaden oil. Generally a spraying process cannot be used safely; the open system is an occupational and environmental hazard. The spraying does not allow the drug solution to penetrate into the feed; the drug is absorbed only into the surface layer. An extra step is required for sealing that involves the use of additional equipment and contamination of the equipment.

A clean technology is reported for the preparation of medicated animal foodstuffs in US 6,387,393 that involves the coating of the foodstuff with a cohesive gel containing the drug. The cohesive gel is prepared by mixing the drug with a gelling agent (e.g. cellulose polymer) and other ingredients in a mixer and keeping the dry mixture in sachets. Upon usage the mixture is added to sufficient water to form a suspension for coating the pellets. Pellets are weighed into a suitable mixer (e.g. ribbon blender) and the required amount of drug suspension is added. The mixture is blended for 5 minutes and dispensed into paper sacks. The mixing vessel becomes coated with the gel only to a minimal extent. Medicated food for cattle, pigs and fowl can be prepared by this technology.

The technology allows for the flexible preparation of medicated foodstuffs and reduces cleaning efforts. The product is not suitable for fish medication; the drug is distributed only on the surface, and the gel coating does not resist leakage.

The juvenile hormone analogue compound 3-(3',7'-dimethyloctyloxy)pyridine was dissolved in capelin oil for coating the ordinary fish feed pellet for Atlantic salmon in WO 01/07047 A2 published patent application on the control of crustacean infestation of aquatic animals. This method has the advantage that no additional sealing step is required. The application is limited to oil-soluble substances.

Efforts have been made for embedding-type or encapsulation-type incorporation of the active substance into the feed.

A layered pharmaceutical dosage form for the medication of fish is concerned in WO 89/12442. A drug layer is surrounded with an outer layer
5 that is substantially impermeable to water and the drug, and it contains substances to enhance taste and smell of the product to stimulate the ingestion. The drug layer contains the drug and an aqueous gel-forming carrier material. The dosage form is prepared by co-extruding the outer layer with the medicated inner. Dosage forms of oxytetracycline, flumequine and
10 oxolinic acid for Salmoniformes are claimed. The drug is well enclosed in the system and thus has a low leakage tendency, and is palatable. The drug has to be prepared in advance before knowing the demand. There is also the difficulty of cleaning the extruders in order to avoid cross-contamination.

German patent DE1974 1114 concerns granules, pellets or tablets for
15 veterinary use with a drug-containing core, an inner coating which is acid resistant but soluble at neutral and basic pH values, and an outer coating which is acid labile and insoluble but swellable in neutral and basic media. These compositions are useful for oral administration of antibiotics, vaccines, etc. to fish with the feed; it is placed in the water since the active agent is not
20 immediately released in the water or in the stomach, but is released in the intestine.

The preparation of these drugs is tedious; first the core has to be prepared by kneading the drug or vaccine with starch, lactose, polyvinylpyrrolidone and water, formation of granules, coating the granules
25 with the acid resistant inner layer and drying, followed by coating with the acid-labile layer.

It is apparent from the above review that medicated food has hitherto been prepared principally by two methods: by applying the active substance onto the surface of the carrier after said carrier has been formulated, or by
30 mixing the carrier ingredients with the active substance, thereby introducing said active substance into the mass of the carrier prior to formulation. Both methods have disadvantages as detailed above. In the prior art no process for the preparation of medicated foodstuff has been known wherein the active substance is introduced into the bulk of the carrier after the carrier has been

formulated. In all the prior cases wherein the active substance is applied to a formulated carrier, the resulting products have the active material on their surface.

The terms used herein have meanings as follows.

5 Drug: substance used to treat and prevent diseases, for contraception, for anesthesia, to increase the resistance of the animal, or to influence its gender.

10 Feed additive: substance added to feed to enhance the growth efficiency of the animal, to control parasites, reduce bacterial infections, stimulate certain enzymes, increase appetite, control bloat and feed spoilage.

Formulated animal feed: feed composed from natural and/or synthetic ingredients as opposed to non-formulated feed and live feed (fish, meat, plants, seeds etc.).

Medicated feed: feed containing drugs and/or feed additives.

15 Medication, biologically active substance / material: drugs and feed additives.

Sorption: adsorption and absorption.

Objects of the Invention

20 In traditional livestock production as well as in aquaculture the type and amount of required drugs and feed additives cannot be always planned ahead. The same is true for pet animals. There are many factors that affect the usage of medication, e.g. the outbreak of a disease, its type and time; the number of animals that have to be treated; the need for preventive treatment;

25 the hatching rate, litter size, survival rate; the growth stage and weight of the animal that determines the dose, etc. The administration of biologically active substances increases the cost of farming; weak, unhealthy, sick animals are reluctant to ingest drugs orally. Unconsumed and non-absorbed drugs and feed additives pollute the environment. The aim of this invention is to prepare

30 in a flexible manner an oral drug delivery system or feed additive delivery system that contains the biologically active substance in a bioavailable form preferably uniformly sorbed into the bulk of a palatable carrier, preferably animal feed. According to the invention the medicated feed is prepared from a formulated porous feed that the animal is likely to consume and the

medication. On animal farms and aquacultures the feed is usually on hand; the medication can be stored or easily obtained from the manufacturer. On the other hand, drug manufacturers, premix manufacturers and feed mills can produce the medicated feed as orders arrive in a flexible way. The amount of medication that will be included per unit feed can be varied according to the recommended dosage. The medication is preferably uniformly distributed in the feed, not as a coating; thus the palatability of the feed is not altered. The sorption of the medication within the feed assures that no leaching or only insignificant leaching occurs. The method for the preparation allows for laboratory scale, pilot scale and production scale. Closed systems are used at any scale, thus occupational and environmental hazards are excluded.

Brief Description of the Invention

The present invention relates to an easily ingestible and bioavailable oral veterinary drug delivery system and feed additive delivery system which contains the medication preferably uniformly sorbed into the bulk of a porous carrier, preferably feed, and is simply prepared by mixing the solution of the medication with the feed to allow for sorption, followed by the evaporation of the solvent. The process is flexible and can be carried out in laboratory, pilot and industrial scale when need for medication occurs. The technology involves closed systems, e.g. rotary evaporators, drum dryers, thus occupational and environmental hazards are excluded. The product can be used for the medication of farmed animals and pets, preferably in aquaculture. The medication is sorbed within the pores; it is adhered to the feed and thus it is not likely to leak easily into the environment. In those cases when the carrier contains lipophilic substances, the low wettability delays the leaching of the drug as well.

Detailed Description of the Invention

In the first aspect the present invention relates to an oral veterinary drug delivery system and/or feed additive delivery system comprising at least one biologically active substance sorbed at least to a substantial extent into the bulk of a carrier and which is obtainable by the steps:

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- a) making available the solution of the biologically active substance and the carrier in a vessel;
- b) mixing the content of the vessel for a time period sufficient for the sorption of at least a substantial part of the biologically active substance into the carrier, preferably for 10 minutes to 2 hours, more preferably 20 to 30 minutes to promote sorption of the solution of the biologically active substance by the carrier; and
- c) evaporating the solvent while maintaining mixing until dryness.

As used herein the terms "at least to a substantial extent/ at least a substantial part of the biologically active substance" means at least 40 w/w%, preferably at least 60 w/w%, more preferably 80 w/w%, even more preferably 90 w/w% and most preferably up to 100 w/w% of the active substance.

Here it should be pointed out that previously uniformly distributed medication in the feed could only be achieved when medication was added during feed production. The novel product according to the present invention is a medicated feed that, as a result of the above described preparation process, contains the active substance uniformly sorbed into the bulk of the feed matrix, unlike products that were prepared by applying the biologically active substance onto the surface of the formulated carrier. Further, the skilled person will readily be able to make a distinction between products prepared by premixing the active substance with the carrier prior to formulation and the product according to the present invention e.g. on the basis of the differences between their active substance distribution profile.

In a preferred embodiment according to the present invention the biologically active substance is uniformly sorbed into the carrier.

In an embodiment according to the present invention the solvent is selected from the group consisting of acetone, methanol, ethanol, propanol, isopropanol, ethyl acetate, halogenated hydrocarbons, water and any solvent of the active substance having a boiling point of less or equal to 120 °C, and their mixtures.

In a further embodiment according to the present invention the solvent is evaporated in vacuum and/or in an air or nitrogen stream.

In a further embodiment according to the present invention, mixing is carried out in a vessel selected from the group consisting of rotary

evaporator, mixing vessel, agitated vessel, drum dryer, vacuum drum dryer and fluid bed.

In a further preferred embodiment according to the present invention process steps a) to c) are performed in a closed system.

5 In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the carrier is a porous material.

10 In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the carrier is a formulated animal feed selected from the group of feed pellets, granules, extrudates, tablets, flakes and other particulate feed in dry, semi-moist or moist state.

15 In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the carrier is a palatable substance for animals.

20 In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the biologically active substance is selected from the group consisting of parasiticides, anthelmintics, antibacterial agents, antistress agents, animal growth promoters, anti-inflammatory agents, antibiotics, hormones, hormone analogues, oral vaccines, vitamins and any other drugs, and any pharmaceutically acceptable combinations thereof.

25 In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the biologically active substance is selected from the group consisting of 17-alpha-methyltestosterone, 17-alpha-ethyltestosterone, 17-alpha-methyl-dihydrotestosterone, 17-beta-estradiol, flumequine, oxolonic acid, sarafloxacin, oxytetracycline, sulfadimethoxine, sulfamonomethoxine, ormetoprim, sulfamerazine, ampicillin, penicillin, enrofloxacin, buserelin, 30 chloramphenicol, furazolidone, gentamycin, ivermectin, hexaflumuron, levamisole, metronidazole, nalidixic acid, nitroxynil, nitrofurazone, piromidic acid, streptomycin, sulfadiazine, trimethoprim, teflubenzuron, diflubenzuron, tetracycline, tobicillin, albendazole, bicozamycin, bicomycin, dimetridazole, doxycycline, flubendazole, josamycin, kanamycin, leucomycin, lincomycin,

novobiocin, praziquantel, rifampicin, spiramycin, tiamulin, emamectin, erythromycin, florfenicol, fumagillin, simazine, ofloxacin, norfloxacin, enoxacin, ciprofloxacin, tosufloxacin, benofloxacin, danofloxacin, enrofloxacin, orbifloxacin and any of their pharmaceutically active salts, 5 esters and other derivatives and pharmaceutically acceptable combinations thereof.

In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the biologically active substance is a hazardous material for the environment 10 and/or the operating personnel.

In a further embodiment in the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention the biologically active substance is radiolabeled.

In a further embodiment the oral veterinary drug delivery system and/or 15 feed additive delivery system according to the present invention has good storage stability.

In a further embodiment according to the present invention no or low leaching of the active substance can be experienced from the oral veterinary drug delivery system and/or feed additive delivery system according to the 20 present invention.

In a further embodiment the oral veterinary drug delivery system and/or feed additive delivery system according to the present invention is bioavailable.

The second aspect of the present invention is a process for the 25 preparation of the oral veterinary drug delivery system and/or feed additive delivery system as disclosed above comprising the steps of

- a) making available the solution of the biologically active substance and the carrier in a vessel;
- b) mixing the content of the vessel for a time period sufficient for the 30 sorption of at least a substantial part of the biologically active substance into the carrier, preferably for 10 minutes to 2 hours, more preferably 20 to 30 minutes, to promote sorption of the solution of the biologically active substance by the carrier; and
- c) evaporating the solvent while maintaining mixing until dryness.

In an embodiment according to the process of the present invention the solvent is selected from the group consisting of acetone, methanol, ethanol, propanol, isopropanol, ethyl acetate, halogenated hydrocarbons, water and any solvent of the active substance having a boiling point of less or equal to
5 120 °C, and their mixtures.

In a further embodiment according to the process of the present invention the solvent is evaporated in vacuum and/or in an air or nitrogen stream.

In a further embodiment according to the process of the present invention mixing is carried out in a vessel selected from the group consisting
10 of rotary evaporator, mixing vessel, agitated vessel, drum dryer, vacuum drum dryer and fluid bed.

In a further embodiment according to the process of the present invention steps a) to c) are performed in a closed system.

15 The third aspect of the present invention is the oral veterinary drug delivery system and/or feed additive delivery system as disclosed above for use as a pharmaceutical or feed additive.

In a further embodiment according to the present invention the oral veterinary drug delivery system and/or feed additive delivery system as
20 disclosed above is for use in aquaculture, especially farming of fish, farming of poultry, swine, cattle, and for pet animals.

The following examples are offered by way of illustration and not by way of limitation.

25 **Analytical methods**

The HPLC system included two Model 125 programmable pumps, a Model 406 digital-analog interface, Model 155 UV detector set for 254 nm (Beckman Instruments, Irvine, CA), a Pharmacia model FRAC-100 fraction collector, and an IBM model 55X PC computer with System Gold Version
30 8.10 Gold software.

Methyltestosterone was determined by reverse phase HPLC using a 3 μ m C18 column (Supelco LC18-DB 15cm x 4.6 mm) and a gradient elution program. Mobile phase A was a mixture of 40 volumes methanol, 30 volumes

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acetonitrile, and 50 volumes water. Mobile phase B included 40 volumes methanol, 30 volumes acetonitrile, and 10 volumes water. The program was 0-5 min. 100% A; 5-12 min. linear gradient to 40% A, 60% B; 12-15 min. linear gradient to 100% A; 15-20 min. 100% A. Flow rate was set to 1 mL/min. Under these conditions the methyltestosterone peak appeared at 11 min and was separated from matrix components and possible metabolites.

Flumequine was analyzed on the same HPLC system using isocratic elution. The mobile phase was composed of 60 volumes buffer (20 mM potassium phosphate, pH 3.2), 20 volumes methanol and 20 volumes tetrahydrofuran. The C18-column was Beckman Ultrasphere IP, 25cm x 4.6 mm. The flumequine peak appeared at 9.5 mm.

For liquid scintillation counting, 1 minute fractions of the HPLC effluent were collected into 6 mL vials.

Calibration curves for the methyltestosterone determinations were prepared from standard solutions of 17 α -methyltestosterone and of possible metabolites 4-androsten-17 α -methyl-11 α ,11 β -diol-3-one and δ -4,6-androstadien-17 α -methyl-17 β -ol-3-one in mobile phase A.

For flumequine determination calibration curves from standard solutions of flumequine and 7-hydroxy-flumequine were used.

Radioactive analysis was carried out in a Beckman Liquid Scintillation system Model LS 6000IC; the scintillation cocktail was ScintiVerse E (Fisher Scientific, Fair Lawn, NJ).

Plasma samples were analyzed using an isotope dilution method. A cold spike that contained known amounts of the analyte (17 α -methyltestosterone or flumequine and their possible metabolites) was added to the radioactive samples, and the specific activity was calculated from the HPLC quantification and the corresponding peak radioactivities. The amount of analyte per gram sample (W_x) was calculated using the equation $W_x = W_f / (((S_o / S_x) - 1) W_s)$, where W_f was the amount of added unlabeled analyte, W_s was the weight of the plasma sample, S_o the specific activity of the analyte in the sample before the addition of the unlabeled analyte (S_o value was assumed to be equal to the specific activity of the medicated feed), S_x was the specific activity of the analyte after dilution with the cold spike. S_x was

$A_{\text{dpm}} / A_{\text{analyte}}$, where A_{analyte} was the amount of analyte as determined by HPLC using the calibration curves, and A_{dpm} was the amount of radioactivity in the corresponding HPLC eluate fractions.

5 Fish

Rainbow trout (*Oncorhynchus mykiss*), average weight 500 g, equal amounts of male and female were selected from a 1000 L fiberglass aquarium. Fish were housed individually in 75 L rectangular glass aquaria that were operated in a flow through state at a constant 15 ± 1 °C temperature. A photoperiod of 12 h light and 12 h dark was maintained. Fish were fed daily with Rangen soft-moist trout feed (Rangen Feed, Buhl, ID) at a rate of 0.5 or 1 % body weight up to the day before medication or surgical experimentation.

Example 1

15 Methyltestosterone feed preparation for oral dosing

The amount of active substance that was introduced into the feed was calculated in such a manner that the daily dose was included in a fraction of the daily feed ration.

In a 1 L round bottom flask 1.9970 g 17α -methyltestosterone (Research Plus Inc. Bayonne, NJ) were dissolved in 300 mL acetone. For radiolabeled feed preparation, 3.69 mg of 17α -methyl [$4\text{-}^{14}\text{C}$]-methyltestosterone (Amersham International Plc Buckinghamshire, UK) were added.

105-110 g of Rangen moist trout feed (4 mm) was sieved through a 2 mm x 1 mm copper wire screen. This was done primarily to separate the fine food particles that the fish would be unlikely to ingest. 100 g of the sieved food was added to the solution. The round bottom flask was placed onto the Buchi rotavapor and rotated for 20 minutes slowly (ca. 60 rpm) under atmospheric pressure. The water bath was kept at 35-40 °C. After 20 minutes a vacuum was connected for complete evaporation of the solvent. The round-bottom flask was removed from the rotavapor thereafter.

Example 2**Methyltestosterone feed preparation for oral dosing with additional drying**

Medicated feed pellets were prepared according to Example 1. After
5 removal of the round-bottom flask from the rotavapor, nitrogen was purged
through the content of the flask.

The weight of the methyltestosterone feed product was 96.64 g.

Example 3**10 Analysis of the medicated feed pellets****a. Methyltestosterone content by HPLC**

Three 1 g aliquots of the prepared food were extracted each with 20 mL
methanol in screw cap vials using a Branson 2200 sonication bath. Sonication
was continued for 60 minutes or until the pellets were totally suspended. The
15 water temperature was maintained below 40 °C with ice. The supernatants
were filtered through 0.22 micrometer nylon microcentrifuge filters and
diluted with the sample solvent (a mixture of 1 volume methanol with 1
volume water). The samples were injected onto the HPLC column.

Using calibration curves, the methyltestosterone content was evaluated.
20 The average of the three samples was 20.02 ± 0.604 mg methyltestosterone/g
feed (CV = 3.02%).

**b. ¹⁴C-Methyltestosterone content by liquid scintillation counting
from the methanol extract**

25 The methanol extracts of from 3a. were directly measured for total
radioactivity, 100 µL aliquots from each extract. The average of the three
extracts as related to the feed weight was $16.52 \times 10^6 \pm 0.270 \times 10^6$ dpm/g feed
(CV = 1.63%)

30 c. Recovery

Recovery of methyltestosterone was calculated based on the total used
2000.7 mg methyltestosterone (labeled and unlabeled in Example 1) and the

amount in the product, i.e. $96.64 \text{ g} \times 20.02 \text{ mg/g} = 1934.7 \text{ mg}$. Thus 96.7% of the used drug was recovered.

Example 4

5 Testing the leaching of methyltestosterone from the medicated feed

1 g of the food prepared in Experiment 1 with 20.02 mg total methyltestosterone content was placed in an Erlenmeyer flask containing 20 mL of 15 °C distilled water. The water was stirred and kept at 15 °C in a water bath. Water samples were taken at the following times after the pellets
 10 were placed into the water: 5, 15, 30 and 60 minutes. 0.1 mL samples were injected onto the HPLC column and the concentration determined. The amounts of methyltestosterone that were found in the water were expressed as a ratio (%) to the amount in the food. Data are shown in *Table 1*.

15 *Table 1: Leaching of methyltestosterone from the medicated feed*

Time elapsed after food was dropped Into the water (min)	Amount leached %
5	0,208
15	0,416
30	0,832
60	1,71

It is apparent from the data shown in *Table 1* that after one hour only a minimal amount of the active substance has leached from the medicated
 20 formulation into the environment.

Example 5

Feeding the fish with the medicated feed

On each day of the dosing regimen, the medicated feed was
 25 administered prior the non-medicated feed.

The amount of daily medicated feed (MF) was calculated as:

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$$MF (g) = \text{daily dose (mg/kg)} \times \text{body weight (kg)} / C_M,$$

wherein

$$C_M = \text{concentration of medication in the feed (mg/g)}.$$

5

The total daily feed (TF) ration was:

$$TF (g) = N \times \text{fish body weight (g)}$$

wherein

$$10 \quad N = 0.005 \text{ or } 0.01.$$

The amount of non-medicated feed (NF) was:

$$15 \quad NF (g) = TF - MF.$$

Fish ingested the medicated feed readily, usually within 5–10 minutes after dropping the medicated feed into the aquaria. The non-medicated feed was offered thereafter.

20 Rainbow trout received a dose of 30 mg 17α -methyltestosterone per kg body weight.

Example 6

Bioavailability studies

25 To study the bioavailability of 17α -methyltestosterone via the medicated feed delivery system five fish received a single oral dose of 30 mg methyltestosterone per kg of fish body weight. Fish ate the medicated feed voluntarily within five minutes of administration. After dosing, serial blood samples were withdrawn from the fish via previously inserted cannules at 30 min, 1, 2, 4, 8, 12 h, 1, 2, 4 and 6 days post-dosage.

30 For intraarterial injection, methyltestosterone was dissolved in a mixture of 40 volumes of ethanol and 60 volumes of water. Five fish received a single intraarterial injection of 20 mg methyltestosterone per kg body

weight. Serial blood samples were collected at 15 min, 1, 2, 4, 8, 12 h, 1, 2, 4 and 6 days post dosage.

An equal volume of a modified Teleost saline was injected intraarterially into the trout after each blood sample.

5 The oral bioavailability of methyltestosterone (73.1%) was determined using the areas under the respective intraarterial (i.a.) and oral plasma (p.o.) concentration-time curves as described by the equation

$$F (\%) = 100 \times \text{AUC(p.o.)} \times \text{Dose(i.a.)} / \text{AUC(i.a.)} \times \text{Dose(p.o.)},$$

10

where AUC(p.o.) and AUC(i.a.) are the average values (n = 5) from calculated concentration-time profile curves.

Example 7

15

Storage stability of the methyltestosterone feed

Methyltestosterone feed prepared according to Example 1 was tested for storage stability. Aliquots were placed into three tared crystallization dishes, covered and stored at 4°C. The contents of two dishes were used for controlling the loss of weight during storage at 4°C. Weight loss was checked
 20 at the following times after medicated feed preparation: 3 days, 1, 3, 4, 9 weeks, 3, 7 months. The losses of weights were 0.123% and 0.091% respectively after 7 months of storage. The methyltestosterone concentration in the feed was checked in the freshly prepared feed and at different times thereafter. Three one-gram samples were measured at each time the feed
 25 consistency was checked as described in Example 3. *Table 2* shows the average of these three samples.

Table 2: Storage stability of the methyltestosterone feed

Time elapsed after feed preparation	Methyltestosterone conc. (mg/g)
0	20.02 ± 0.604
4 weeks	19.91 ± 0.652
3 months	18.46 ± 0.795
7 months	18.72 ± 0.748

The methyltestosterone concentration after 7 months storage was measured as 1.30 mg/g or 6.49 % less than on the day of the feed preparation. Comparing these values with the average fluctuations of the daily measurements, the decrease is about twice the average daily sample-to-sample fluctuation.

Example 8

Flumequine feed preparation

10 For flumequine feed preparation the procedures in Examples 1 and 2 were followed. Unlabeled flumequine was from Sigma Chemical Co. St. Luis, MO; {2-¹⁴C}-flumequine was supplied by Amersham International Plc Buckinghamshire, UK. 100 g Rangen moist trout feed (4 mm) was treated with a total of 0.9004 g of labeled and unlabeled flumequine that was
15 dissolved in 300 mL acetone. The concentration of flumequine in the product was 8.70 ± 0.219 mg flumequine/g feed.

Example 9

Oral administration of flumequine feed

20 Rainbow trout received 12 mg flumequine/kg body weight. As in Experiment 5, on each day of the dosing regimen, the medicated feed was administered prior the non-medicated feed. Fish ingested the flumequine feed readily within 5-10 minutes.

25 Advantageous features of the present invention over the prior art are as follows.

Veterinary drugs and feed additives (medication) are usually added to the feed either directly during the preparation of the feed; or the medication is
30 added to the feed product in a later step.

When medication is mixed with feed ingredients during production the drugs/feed additives may degrade during mixing, kneading, extrusion, pelleting and other processes as a result of the applied or formed heat. The

present invention provides a drug delivery system where the medication is preferably uniformly distributed through the porous feed in a stable form; a gentle treatment of the porous carrier with the solution of the medication allows for at least 90-97% recovery of the drug/feed additive.

5 Another disadvantage of adding the medication to the feed ingredients during feed preparation is that the whole production line is contaminated with the drug/feed additive. This includes such apparatuses that are difficult to clean, e.g. mixers, extruders, and other pelleting machines. According to the present invention when the feed is treated with the drug solution in a separate
10 process, only designated equipment get in contact with the active ingredient(s). Moreover, the cleaning of a vacuum evaporator glass bottle or the drum of a vacuum dryer is a relatively simple procedure.

Additional disadvantage of medicating feed during feed preparation is that there is no flexibility in the quality and quantity of the drug delivery
15 product once it is prepared. The present method allows for great flexibility: as demand occurs, the appropriate type and amount of medication can be added to the desired carrier using a fast and simple method. The drug delivery system with preferably uniformly sorbed medication in a feed carrier can be prepared on farms, in feed mills or by premix and drug manufacturers.

20 In prior technologies when medication is added to the ready-made feed, the product is a drug delivery system that is composed of a feed core coated with the medication. Usually, weak, unhealthy, sick animals are reluctant to ingest such drug-tasting feed. Therefore, tedious and more expensive delivery systems (injections, patches) are the alternative. The application of such
25 methods is often restricted; e.g. in animal farming, or because of their side effects. According to the present invention, the medication is not accumulated on the surface; the animals are offered a palatable drug delivery system with the usual feed taste. If it is offered as the initial part of the feeding regime, the animal will most probably consume at least those initial feed portions that
30 are medicated.

Other disadvantages of coating the feed with medication is that the outer layer is easily damaged mechanically, thus the dosage is hard to control. In aquaculture the surface coating easily results in leaching of the medication into the water. This not only causes a decrease of the administered drug

dosage but also is a source of water pollution. In order to avoid ecosystem contamination by drugs that can be also harmful to humans, introduction of novel fish medications has been a solution. Also nets have been applied in fish farms to collect unconsumed drugs. In another case re-floating of the drug was
5 achieved. Re-floating alone does not ensure increased intake of drug and does not protect against environmental contamination. According to the present invention the leaching of the drug from the feed carrier is negligible, thus the product is environmentally friendly. If appropriate, nets can be used for collecting unconsumed medicated feed.

10 Previous methods for adding medication to feed include mixing the feed and the drug in open mixers, e.g. cement mixers, or spraying the feed with the drug solutions or suspensions. Both methods are hazards for the environment and for the operating personnel. The present invention offers a method that is carried out in a closed system thus being environmentally and occupationally
15 safe. The importance of a closed system is even more pronounced when radiolabeled compounds are used e.g. for experimental purposes.

Using spraying for coating the feed with medication does not ensure uniform distribution of the drug. The present method results in preferably uniform sorption of the drug into the carrier.

20 Scale up from a small-scale rotary evaporator is readily accomplished, e.g. by using a Buchi vacuum evaporator from 20 mL to 50 L volume, and for larger batches a vacuum drum dryer.

From the foregoing, it will be appreciated that, although specific
25 embodiments of the invention have been described herein for purposes of illustration, various modifications may be without deviation from the spirit and scope of invention. Accordingly, the invention is not limited by the disclosed embodiments or examples except as by the appended claims.

What is claimed is:

1. Oral veterinary drug delivery system and/or feed additive delivery system comprising at least one biologically active substance sorbed at least to
5 a substantial extent into the bulk of a carrier, which is obtainable by the steps:

a) making available the solution of the biologically active substance and the carrier in a vessel;

b) mixing the content of the vessel for a time period sufficient for the
10 sorption of at least a substantial part of the biologically active substance into the carrier, preferably for 10 minutes to 2 hours, more preferably 20 to 30 minutes to promote sorption of the solution of the biologically active substance by the carrier; and

c) evaporating the solvent while maintaining mixing until dryness.

15 2. The oral veterinary drug delivery system and/or feed additive delivery system according to Claim 1, wherein the biologically active substance is uniformly sorbed into the carrier.

3. The oral veterinary drug delivery system and/or feed additive delivery system according to Claim 1 and 2, wherein the solvent is selected
20 from the group consisting of acetone, methanol, ethanol, propanol, isopropanol, ethyl acetate, halogenated hydrocarbons, water and any solvent of the active substance having a boiling point of less or equal to 120 °C, and their mixtures.

4. The oral veterinary drug delivery system and/or feed additive
25 delivery system according to Claims 1 to 3, wherein the solvent is evaporated in vacuum and/or in an air or nitrogen stream.

5. The oral veterinary drug delivery system and/or feed additive delivery system according to Claims 1 to 4, wherein mixing is carried out in a vessel selected from the group consisting of rotary evaporator, mixing vessel,
30 agitated vessel, drum dryer, vacuum drum dryer and fluid bed.

6. The oral veterinary drug delivery system and/or feed additive delivery system according to Claims 1 to 5, wherein steps a) to c) are performed in a closed system.

7. The oral veterinary drug delivery system and/or feed additive delivery system according to Claims 1 to 6, wherein the carrier is a porous material.

8. The oral veterinary drug delivery system and/or feed additive
5 delivery system according to Claims 1 to 7, wherein the carrier is a formulated animal feed selected from the group of feed pellets, granules, extrudates, tablets, flakes and other particulate feed in dry, semi-moist or moist state.

9. The oral veterinary drug delivery system and/or feed additive
10 delivery system according to Claims 1 to 8, wherein the carrier is a palatable substance for animals.

10. The oral veterinary drug delivery system and/or feed additive delivery system according to Claims 1 to 9, wherein the biologically active substance is selected from the group consisting of parasiticides,
15 anthelmintics, antibacterial agents, antistress agents, animal growth promoters, anti-inflammatory agents, antibiotics, hormones, hormone analogues, oral vaccines, vitamins and any other drugs, and any pharmaceutically acceptable combinations thereof.

11. The oral veterinary drug delivery system and/or feed additive
20 delivery system according to Claim 10, wherein the biologically active substance is selected from the group consisting of 17-alpha-methyl-testosterone, 17-alpha-ethyltestosterone, 17-alpha-methyldihydrotestosterone, 17-beta-estradiol, flumequine, oxolinic acid, sarafloxacin, oxytetracycline, sulfadimethoxine, sulfamonomethoxine, ormetoprim, sulfamerazine,
25 ampicillin, penicillin, enrofloxacin, buserelin, chloramphenicol, furazolidone, gentamycin, ivermectin, hexaflumuron, levamisole, metronidazole, nalidixic acid, nitroxynil, nitrofurazone, piromidic acid, streptomycin, sulfadiazine, trimethoprim, teflubenzuron, diflubenzuron, tetracycline, tobicillin, albendazole, bicozamycin, bicomycin, dimetridazole, doxycycline,
30 flubendazole, josamycin, kanamycin, leucomycin, lincomycin, novobiocin, praziquantel, rifampicin, spiramycin, tiamulin, emamectin, erythromycin, florfenicol, fumagillin, simazine, ofloxacin, norfloxacin, enoxacin, ciprofloxacin, tosufloxacin, benofloxacin, danofloxacin, enrofloxacin,

orbifloxacin and any of their pharmaceutically active salts, esters and other derivatives and pharmaceutically acceptable combinations thereof.

12. The oral veterinary drug delivery system and/or feed additive delivery system according to Claims 1 to 11, wherein the biologically active substance is a hazardous material for the environment and/or the operating personnel.

13. The oral veterinary drug delivery system and/or feed additive delivery system according to Claim 12, wherein the biologically active substance is radiolabeled.

14. The oral veterinary drug delivery system and/or feed additive delivery system according to any of Claims 1 to 13 that has good storage stability.

15. The oral veterinary drug delivery system and/or feed additive delivery system according to any of Claims 1 to 14, with no or low leaching of the active substance.

16. The oral veterinary drug delivery system and/or feed additive delivery system according to any of Claims 1 to 15 that is bioavailable.

17. A process for the preparation of the oral veterinary drug delivery system and/or feed additive delivery system according to any of Claims 1 to 16 comprising the steps of

- a) making available the solution of the biologically active substance and the carrier in a vessel;
- b) mixing the content of the vessel for a time period sufficient for the sorption of at least a substantial part of the biologically active substance into the carrier, preferably for 10 minutes to 2 hours, more preferably 20 to 30 minutes to promote sorption of the solution of the biologically active substance by the carrier; and
- c) evaporating the solvent while maintaining mixing until dryness.

18. The process according to Claim 17, wherein the solvent is selected from the group consisting of acetone, methanol, ethanol, propanol, isopropanol, ethyl acetate, halogenated hydrocarbons, water and any solvent of the active substance having a boiling point of less or equal to 120 °C, and their mixtures.

19. The process according to Claims 17 and 18, wherein the solvent is evaporated in vacuum and/or in an air or nitrogen stream.

20. The process according to Claims 17 to 19, wherein mixing is carried out in a vessel selected from the group consisting of rotary evaporator, mixing vessel, agitated vessel, drum dryer, vacuum drum dryer and fluid bed.

21. The process according to Claims 17 to 20, wherein steps a) to c) are performed in a closed system.

22. The oral veterinary drug delivery system and/or feed additive delivery system according to any of Claims 1 to 16 for use as a pharmaceutical or feed additive.

23. The oral veterinary drug delivery system and/or feed additive delivery system according to Claim 22 for use in aquaculture, especially farming of fish, farming of poultry, swine, cattle and for pet animals.

What is claimed is:

1. A process for the preparation of an oral veterinary drug delivery system and/or feed additive delivery system wherein the carrier material is a formulated animal feed selected from the group of feed pellets, granules, extrudates, tablets, flakes and other particulate feed in dry, semi-moist or moist state, further, wherein the carrier is a porous material and at least one biologically active substance is sorbed at least to a substantial extent into the bulk of a carrier, comprising the steps of

a) making available the solution of a biologically active substance and the carrier in a vessel;

b) mixing the content of the vessel for a time period sufficient for the sorption of at least a substantial part of the biologically active substance into the carrier to promote sorption of the solution of the biologically active substance by the carrier; and

c) evaporating the solvent while maintaining mixing until dryness.

2. A process according to Claim 1, wherein at least one biologically active substance is sorbed into the bulk of a carrier comprising the steps of

a) making available the solution of a biologically active substance and the carrier in a vessel;

b) mixing the content of the vessel for a time period of 10 minutes to 2 hours, preferably 20 to 30 minutes to promote sorption of the solution of the biologically active substance by the carrier; and

c) evaporating the solvent while maintaining mixing until dryness.

3. The process according to Claims 1 to 2, wherein the solvent is selected from the group consisting of acetone, methanol, ethanol, propanol, isopropanol, ethyl acetate, halogenated hydrocarbons, water and any solvent

of the active substance having a boiling point of less or equal to 120 °C, and their mixtures.

4. The process according to Claims 1 to 3, wherein the solvent is evaporated in vacuum and/or in an air or nitrogen stream.

5. The process according to Claims 1 to 4, wherein mixing is carried out in a vessel selected from the group consisting of rotary evaporator, mixing vessel, agitated vessel, drum dryer, vacuum drum dryer and fluid bed.

6. The process according to Claims 1 to 5, wherein steps a) to c) are performed in a closed system.

7. The process according to Claims 1 to 6, wherein the biologically active substance is selected from the group consisting of parasiticides, anthelmintics, antibacterial agents, antistress agents, animal growth promoters, anti-inflammatory agents, antibiotics, hormones, hormone analogues, oral vaccines, vitamins and any other drugs, and any pharmaceutically acceptable combinations thereof.

8. The process according to Claims 1 to 7, wherein the biologically active substance is selected from the group consisting of 17-alpha-methyltestosterone, 17-alpha-ethyltestosterone, 17-alpha-methyldihydrotestosterone, 17-beta-estradiol, flumequine, oxolinic acid, sarafloxacin, oxytetracycline, sulfadimethoxine, sulfamonomethoxine, ormetoprim, sulfamerazine, ampicillin, penicillin, enrofloxacin, buserelin, chloramphenicol, furazolidone, gentamycin, ivermectin, hexaflumuron, levamisole, metronidazole, nalidixic acid, nitroxynil, nitrofurazone, piromidic acid, streptomycin, sulfadiazine, trimethoprim, teflubenzuron, diflubenzuron, tetracycline, tobramycin, albendazole, bicozamycin, bicomycin, dimetridazole, doxycycline, flubendazole, josamycin, kanamycin, leucomycin, lincomycin,

novoblocin, praziquantel, rifampicin, spiramycin, tiamulin, emamectin, erythromycin, florfenicol, fumagillin, simazine, ofloxacin, norfloxacin, enoxacin, ciprofloxacin, tosufloxacin, benofloxacin, danofloxacin, enrofloxacin, orbifloxacin and any of their pharmaceutically active salts, esters and other derivatives and pharmaceutically acceptable combinations thereof.

9. The process according to Claims 1 to 8, wherein the biologically active substance is a hazardous material for the environment and/or the operating personnel.

10. The process according to Claims 1 to 9, wherein the biologically active substance is radiolabeled.