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(54) Title: COMPOSITION FOR USE IN THE TREATMENT OF PISCIRICKETTSIOSIS

(57) Abstract: The current invention provides a composition for use in the treatment, suppression and / or prevention of piscirickettsiosis, preferably in aquatic poikilotherms, wherein the composition comprises one or more medium-chain fatty acids (MCFAs) and / or derivatives thereof.



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COMPOSITION FOR USE IN THE TREATMENT OF PISCIRICKETTSIOSIS

5 TECHNICAL FIELD

This invention relates generally to the field of obligate intracellular bacteria, and in particular to agents of rickettsia type diseases, specifically *Piscirickettsia salmonis* in aquatic poikilotherms.

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BACKGROUND

Piscirickettsia salmonis was the first member of the order Rickettsiales to be isolated from an aquatic poikilothermic organism. *P. salmonis* is the etiological agent of salmonid rickettsial septicemia or piscirickettsiosis, and is an economically significant pathogen of salmonids that is responsible for extensive mortalities in the cold water aquaculture industry. *P. salmonis*, a gram-negative obligate intracellular bacterium, was first observed in 1989 in a diseased, moribund Coho salmon from a saltwater net pen site on the coast of Chile. It is now known that *P. salmonis* is geographically more widespread than was initially suspected. *P. salmonis* has been observed to infect a wide range of salmonid species and causes a systemic infection that targets the kidney, liver, spleen, heart, brain, intestine, ovary, and gills of salmonids.

25 Piscirickettsiosis is the main health challenge in the cold water aquaculture sector and the disease has a huge economic impact on the sector. Indeed, outbreaks of the disease in the past have resulted in losses worth approximately up to hundreds of millions of US dollars in the sector.

30 Currently, the sector has focused its control strategies for the disease on antimicrobial therapies and vaccines. Piscirickettsiosis has evolved over time where each new outbreak is increasingly insidious and refractory to treatments, and each has shown increased bacterial virulence, clinical and pathological severity and variable presentation under similar conditions of species, age and management measures.

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The use of antibiotics, both prophylactically and during early infection have been largely unsuccessful in stopping disease outbreaks. Similarly, commercial vaccines against *P. salmonis* have not proven to be of high efficacy.

- 5 JP283135B2 describes the use of an antimicrobial composition comprising a combination of a medium chain-fatty acid (MCFA), a monoglyceride and a diglyceride of the MCFA for the prevention of growth of a number of harmful bacteria. JP283135B2, however, does not describe antimicrobial activity of this composition towards *Piscirickettsia salmonis* or towards any other bacteria that can
10 cause piscirickettsiosis.

The current approaches therefore do not satisfy the needs of the cold water aquaculture sector.

- 15 The present invention aims to provide an alternative for the treatment and prevention of piscirickettsiosis.

SUMMARY OF THE INVENTION

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The invention thereto aims to provide a composition for use in the treatment, suppression and /or prevention of piscirickettsiosis according to claim 1. More in particular, the invention provides a composition comprising one or more medium-chain fatty acids (MCFAs) and / or derivatives thereof. In a further aspect, the
25 present invention provides a fish feed according to claim 15. More in particular, the invention provides a fish feed comprising a composition for use according to the invention wherein the dosage of the composition in the feed is is X ppm to X ppm by weight, based on the total weight of the fish feed.

- 30 The inventors have found that a composition according to the invention is surprisingly effective for the prevention, suppression, and/or treatment of piscirickettsiosis, thus allowing to significantly increase both the productivity and the profitability of aquaculture. In addition, the current invention also provides a considerable contribution to the reduction of animal suffering and the improvement
35 of animal welfare. Finally, the composition for use according to the invention also reduces the need for administering antibiotics to the animals during aquaculture thus helping to prevent the further upcoming spread of drug resistant pathogens.

In a third aspect, the present invention provides a method for enhancing the zootechnical performance of aquatic poikilotherms according to claim 16. More in particular, the invention provides a method comprising the step of administering a composition comprising one or more medium-chain fatty acids (MCFAs) and / or derivatives thereof to the aquatic poikilotherms. Administration of the composition of the invention to the aquatic poikilotherms, led to an unexpected increase in the productivity of the animals, as a consequence of their improved zootechnical performance. The method of the invention therefore results in increased revenues for the farmer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns a composition for use in the prevention, suppression and / or treatment of piscirickettsiosis in aquatic poikilotherms, characterized in that said composition comprises one or more medium-chain fatty acids (MCFAs) or derivatives thereof.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

As used herein, the following terms have the following meanings:

"A", "an", and "the" as used herein refers to both singular and plural referents unless the context clearly dictates otherwise. By way of example, "a compartment" refers to one or more than one compartment.

"About" as used herein referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/- 20% or less, preferably +/-10% or less, more preferably +/-5% or less, even more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, in so far such variations are appropriate to perform in the disclosed invention. However, it is to be understood that the value to which the modifier "about" refers is itself also specifically disclosed.

"Comprise", "comprising", and "comprises" and "comprised of" as used herein are synonymous with "include", "including", "includes" or "contain", "containing", "contains" and are inclusive or open-ended terms that specifies the presence of what follows e.g. component and do not exclude or preclude the presence of additional, non-recited components, features, element, members, steps, known in the art or disclosed therein.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within that range, as well as the recited endpoints.

The expression "% by weight", "weight percent", "%wt" or "wt%", here and throughout the description unless otherwise defined, refers to the relative weight of the respective component based on the overall weight of the formulation.

The term "medium-chain fatty acid" or "MCFA" as used herein, refers to fatty acids with a medium-chain length, wherein the fatty acids may be saturated or unsaturated. In the present invention, the longest continuous chain of an MCFA can consist of 5 to 12 carbon atoms, for example, valeric acid (C5), caproic acid (C6), caprylic acid (C8), capric acid (C10) or lauric acid (C12). The term MCFA also refers to medium-chain fatty acids that are chemically modified, and to medium-chain fatty acids that are provided with side-chains, such as, without limitation, one or more alkyl groups, preferably C1-C10 alkyl groups. As described herein, the term "medium-chain fatty acid (MCFA) derivative" refers to a fatty acid chain of which the carboxyl group is reversibly converted to a different group, preferably, but without limitation, to an amide, salt, ester or glyceride such as, for example, a mono-, di- or tri-glyceride.

The term "piscirickettsiosis" as used herein, refers to an infectious disease affecting aquatic poikilothermic organisms caused by *Piscirickettsia salmonis* (*P. salmonis*), *Piscirickettsia*-like organisms (PLO) and/or fish rickettsia-like organisms (RLO). The term piscirickettsiosis as used herein, also refers to the disease salmonid rickettsial septicemia (SRS).

"*Piscirickettsia*-like organisms" (PLO) and "fish rickettsia-like organisms" (RLO), as used herein are organisms whose developmental stages are morphologically similar to that of organisms belonging to the family of *Piscirickettsiaceae* and/or to that of organisms belonging to the order of *Rickettsiales* and which cause disease in aquatic

poikilothermic organisms resulting in symptoms similar to those caused by infection of salmonids with *P. salmonis*.

5 The term "aquatic poikilotherm" or "aquatic poikilothermic organism" as used herein, refers to aquatic animals whose internal temperature varies considerably, and most of the time, depends on the environment. Aquatic poikilotherms that can suffer from piscirickettsiosis include, but are not limited to salmonids, white seabass (*Atactoscion noblis*), black seabass (*Dicentrarchus sp.*), tilapia (*Oreochromis*, *Tilapia* and *Sarotherodon spp.*) and blue-eyed plecostomus (*Panaque suttoni*).

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The term "salmonid" refers to fish species belonging to the family of Salmonidae within the order of Salmoniformes. Salmonids include, but are not limited to, salmon, trout, char, freshwater whitefishes and graylings. Salmon include, but are not limited to, Atlantic salmon species such as Atlantic salmon (*Salmo salar*) and Pacific salmon species such as Chinook salmon (*Oncorhynchus tshawytscha*), Chum salmon (*O. keta*), Coho salmon (*O. kisutch*), Masu salmon (*O. masou*), Pink salmon (*O. gorbuscha*) and Sockeye salmon (*O. nerka*).

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20 The term "Feed Conversion Ratio" or "FCR" describes the efficiency with which the animals convert feed into the desired output. For animals raised for meat (such as beef cows, pigs, chickens and fish) the output is the flesh, that is, the body mass gained by the animal, represented either in the final mass of the animal or the mass of the dressed output.

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25 The term "minimum inhibitory concentration" or "MIC" as used herein refers to the lowest concentration of a compound or composition which prevents measurable growth of a bacterium.

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30 The term "minimum bactericidal concentration" or "MBC" as used herein refers to the lowest concentration of a compound or composition which results in microbial death as defined by the inability to re-culture bacteria.

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35 The term "colony-forming unit" (CFU) refers to a unit that is used to estimate the number of viable bacteria or fungal cells in a sample, that can be for example a cell culture, a feed additive or a feed composition. Viable is defined as the ability to multiply via binary fission under the controlled conditions.

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In a first aspect, the invention provides a composition for use in the prevention, suppression and / or treatment of piscirickettsiosis, preferably, in aquatic poikilotherms, characterized in that said composition comprises one or more medium-chain fatty acids (MCFAs) or derivatives thereof. MCFA(s) are fatty acids with a medium-chain length, wherein the fatty acids may be saturated or unsaturated. In the present invention, the longest continuous chain of an MCFA consists of 5 (C5) to 12 (C12) carbon atoms. The fatty acids that can be used in this invention include both fatty acids with an even and an odd number of carbon atoms. Preferably, the composition of the invention comprises one or more MCFAs chosen from C5 to C12 or derivatives thereof. The term MCFA also refers to medium-chain fatty acids that are chemically modified, and to medium-chain fatty acids that are provided with side-chains, such as, without limitation, one or more alkyl groups, preferably C1-C10 alkyl groups. MCFA derivatives include MCFAs of which the carboxyl group is reversibly converted to a different group such as but not limited to an amide, a salt, an ester or a glyceride such as, for example, a mono-, di- or tri-glyceride.

Piscirickettsiosis is the main health challenge in the cold water aquaculture sector, affecting both productivity and profitability, resulting in a huge economic impact of this disease on the aquaculture sector. The inventors have found that a composition comprising at least one MCFA or MCFA derivative is surprisingly effective for the prevention, suppression, and/or treatment of this disease, thus allowing to significantly increase both the productivity and profitability of aquaculture.

Furthermore, piscirickettsiosis is a cruel and nasty disease whose impact on animal well-being should not be underestimated. Fish suffering from the disease show, amongst other symptoms, extreme weight loss, skin lesions that progress to ulcers on flanks and head, a grey, swollen spleen and kidney diffuse hemorrhaging in the liver and diverse lesions resulting from granulomas and areas of tissue necrosis. Unfortunately, piscirickettsiosis infection in many cases eventually leads to the death of the animal. The current invention thus provides a composition that can make a considerable contribution to the reduction of animal suffering and the improvement of animal welfare.

In order to reduce the occurrence of diseases in cold water aquaculture systems, antibiotics are often administered to the animals. This strategy has proven largely unsuccessful in preventing or stopping piscirickettsiosis disease outbreaks. In addition, the problem of resistance development of pathogenic organisms and the

transfer of the antibiotic substances into animal-derived products makes it very desirable to reduce the use of antibiotics. Therefore, the current invention provides an efficient alternative for the administration of antibiotics and thus helps to prevent the upcoming spread of drug resistant pathogens. Finally, the current invention also has the advantage that the need for administering antibiotics to the animals is decreased.

The inventors have found that supplying specific MCFA in the range of C5-C12, their derivatives and / or mixtures thereof to piscirickettsiosis causing bacteria inhibits their further growth and the bacteria are eventually killed by the administered MCFA. In one embodiment, the composition comprises one or more MCFAs selected from the group comprising caproic acid (C6), caprylic acid (C8), capric acid (C10), lauric acid (C12) and derivatives thereof. The inventors have found that these MCFAs and derivatives thereof are especially efficient against piscirickettsiosis causing bacteria. In the present invention, preferably use is made of a composition comprising a mixture of specific different MCFAs, the individual MCFAs containing a different number of carbon atoms. The inventors have found that a composition comprising at least caprylic acid (C8), capric acid (C10) and lauric acid (C12) or a respective derivative is remarkably effective to treat, prevent and / or suppress piscirickettsiosis. Preferably, the composition of the invention comprises:

- Between 30% and 70% caprylic acid (C8) or a derivative thereof;
- Between 15% and 55% capric acid (C10) or a derivative thereof; and
- Between 1% and 35% lauric acid (C12) or a derivative thereof based on the total weight of the composition.

The inventors have found that a composition comprising a mixture of MCFAs or MCFA derivatives as described above shows optimal antimicrobial properties towards the bacteria responsible for causing the piscirickettsiosis disease.

Due to this outstanding effectiveness on prevention, suppression and/or treatment of piscirickettsiosis, smaller doses of the composition according to the invention need to be used to obtain the desired results on animal health and the concomitant positive effects on aquaculture productivity. Therefore, the current invention leads to higher revenues for the farmers while reducing the costs of disease prevention, treatment and/or suppression.

In a further embodiment, the total amount of MCFAs and/or MCFA derivatives is comprised between 1% and 100% based on the total weight of the composition,

preferably between 5% and 90%, more preferably between 15% and 80%, even more preferably between 30% and 80%, based on the total weight of the composition. The inventors have found that within the ranges mentioned above, the efficiency of the composition for use in the prevention, treatment and / or suppression of piscirickettsiosis is further increased.

The species *Piscirickettsia salmonis* was the first pathogen identified that causes piscirickettsiosis. The disease itself, as well as the pathogens responsible for it have, however, not been characterized well yet. Unfortunately, *P. salmonis* is not the only pathogen that causes piscirickettsiosis, and similar bacteria are an emerging threat to fish production. The inventors have found that bacteria which are similar to *P. salmonis* are also sensitive to the effects of the composition according to the invention. Accordingly, in another further embodiment, the composition is suited for use in the treatment, prevention and/or suppression of the piscirickettsiosis disease when caused by at least one obligate intracellular parasitic species, preferably when it is caused by at least one species belonging to the family of *Piscirickettsiaceae* and / or to the order of *Rickettsiales*, even more preferably by at least one species selected from the group comprising *P. salmonis*, *Piscirickettsia*-like organisms (PLOs) and fish *Rickettsia*-like organisms (RLOs). The composition does not target a species-specific property of the pathogen, but rather a disease-specific property of several related pathogen species. The composition therefore provides a broad spectrum efficiency for use in the treatment, suppression and / or prevention of piscirickettsiosis in aquatic poikilotherms.

The composition of the invention can be conveniently used in the treatment, suppression and / or prevention of piscirickettsiosis in aquatic poikilotherms, as it can be administered orally and / or topically to the animals.

Topical administration of the composition to aquatic poikilotherms is obtained, in a further embodiment, by dissolving the composition in the swimming water of the animals. Preferably, the volume of swimming water of the animals is first reduced to limit the total amount of composition to be used. Topical administration by providing the composition directly to the swimming water of the animals offers the opportunity to treat a large number of animals simultaneously. In addition, the animals can thereafter be removed from the swimming water comprising the composition and a next group of animals can be treated in the same swimming water, thus further reducing the quantity of the composition needed per animal. In a further embodiment the composition for topical administration to the animals

further comprises one or more surfactants. Without wishing to be bound by theory, surfactants are thought to improve contact between the composition and the body surface of the animal, thus optimizing the topical administration of the composition. Surfactants are selected from the group comprising non-ionic, cationic or anionic surfactants such as, but not limited to, coconut alkylbenzyl dimethylammonium chloride, dioctyl dimethylammonium chloride, tributyl tetradecylphosphonium chloride, lauryl dimethyl amine oxide, cocoamidopropyl betaine, lauramidopropyl betaine, cocamide propyl ammonium hydroxide, alkyl benzene sulphonic acids, alkyl sulphonates, carboxylic acid ethers and ethoxylated and propoxylated fatty alcohols.

Alternatively, the inventors have found that oral ingestion of the composition by the animals results in efficient treatment, suppression and/or prevention of piscirickettsiosis in those animals. Therefore, and in an alternative further embodiment, a therapeutically effective amount of the composition for use in the treatment, suppression and / or prevention of piscirickettsiosis in aquatic poikilotherms is administered orally to said aquatic poikilotherm. In a further embodiment, oral administration of the composition is attained by feeding the composition to the animals. This method of administration provides the advantage that a minimal amount of composition is used and, consequently, a minimal amount of composition is wasted or remains in the swimming water of the animals. In addition, this method is easy to perform and it does not impose the burden of large investments for novel equipment on the farmer.

In a further embodiment, the composition further comprises vitamins, minerals, trace elements or a combination thereof. Vitamins include but are not limited to Vitamin A, Vitamin D3, Vitamin E, Vitamin K3, Thiamin, Riboflavin, Panthothenic acid, Biotin, Folic acid, Vitamin B12, Niacin, Pyridoxine, Ascorbic acid, Inositol and Choline. Minerals and trace elements include but are not limited to magnesium, sodium, manganese, iron, zinc, copper, selenium, phosphorous, cobalt and iodine.

The addition of these components further contributes to a complete and healthy diet of the animals and positively influences their zootechnical performance. Inclusion of these components in the composition simplifies the steps needed for feeding of the animals as these components no longer need to be added separately to the feed, resulting in an improved efficiency of the feeding process. In addition, the inventors have found that the provision of MCFAs and/or MCFA derivatives in the animals' feed results in an increase in feed palatability, which in turn leads to animals that eat more, grow bigger, faster and / or healthier, which eventually leads to an increase in the profitability of aquaculture.

Although piscirickettsiosis was first observed in a Coho salmon (*Oncorhynchus kisutch*), the disease is now known to affect a wide range of aquatic poikilotherms including, but not limited to salmonids, white seabass (*Atactoscion nobilis*), black seabass (*Dicentrarchus* sp.), tilapia (*Oreochromis*, *Tilapia* and *Sarotherodon* spp.) and blue-eyed plecostomus (*Panaque suttoni*). The term salmonid refers to fish species belonging to the family of Salmonidae including, but are not limited to, salmon, trout, charr, freshwater whitefishes and graylings. Salmon include, but are not limited to, Atlantic salmon species such as Atlantic salmon (*Salmo salar*) and Pacific salmon species such as Chinook salmon (*Oncorhynchus tshawytscha*), Chum salmon (*O. keta*), Coho salmon (*O. kisutch*), Masu salmon (*O. masou*), Pink salmon (*O. gorbuscha*) and Sockeye salmon (*O. nerka*).

Because salmonids constitute the most important group of fish in aquaculture, treatment, suppression and / or prevention of piscirickettsiosis in this group of species has a prominent positive effect on the productivity and profitability of the aquaculture sector. Accordingly, in a further embodiment the composition of the invention is used in the treatment, suppression and / or prevention of piscirickettsiosis in salmonids, more in particular of salmon, trout, freshwater whitefishes and graylings.

In a further embodiment, the composition according to the invention as described herein, is formulated as a liquid or as a solid form. The term "solid form" means a powder, a granule or a pellet in particular. The term "liquid form", in particular, means a solution in water or means a solution in oil and includes a viscous paste and a non-viscous solution. The MCFA and / or MCFA derivatives as described above are oil-soluble and can be provided both as powder and as an oil-solution. In particular, the composition is suitable for oral administration. A fish feed or feed additive comprising the composition according to an embodiment of the invention is produced or manufactured by means known to a person skilled in the art. Preferably, the fish feed comprising the composition according to the invention is provided as a dry extruded feed pellet. This formulation allows a relatively long shelf life and also permits the packaging and storage of large amounts of feed.

Oral administration of the composition provides, in contrast to other methods of administration, the advantage that a minimal amount of composition is used and, consequently, a minimal amount of composition is wasted or remains in the swimming water of the animals. In addition oral administration is easy to perform

and it does not require large investments for novel equipment. Oral administration of the composition can be performed by admixing the animal feed with the composition of the invention prior to feeding, by feeding the animal with an animal feed already comprising the composition. Accordingly, and in a third aspect, the invention provides a fish feed comprising a composition for use as described above, wherein the dosage of said MCFA in said fish feed is 100 ppm to 5000 ppm, between 100 and 3000 ppm by weight, between 100 and 1000 ppm, more preferably 100 and 500 ppm, more preferably 100 and 300 ppm, such as 225 ppm based on the total weight of said fish feed.

In another embodiment, said composition is added to the water of said aquatic animals, for instance in the tank. Said composition is added such that the MCFA concentration in the water is at a concentration of 100 and 5000 ppm; between 100 and 3000 ppm, between 100 and 1000 ppm, more preferably 100 and 500 ppm, more preferably 100 and 300 ppm, such as 225 ppm.

In a second aspect, the invention provides a method for enhancing the zootechnical performance of aquatic poikilotherms comprising the step of administering a composition comprising one or more medium-chain fatty acids (MCFAs) and / or derivatives thereof to said poikilotherm. Preferably, the total amount of MCFAs and/or MCFA derivatives is comprised between 1% and 100% based on the total weight of the composition, more preferably between 5% and 90%, even more preferably between 10% and 80%, most preferably between 10% and 50%, more preferably between 10 and 30% such as 15% based on the total weight of the composition. The inventors have found that, independent of the presence or absence of piscirickettsiosis, administration of the composition of the invention to the aquatic poikilotherms, leads to an unexpected increase in the productivity of the animals, as a consequence of their improved zootechnical performance. This positive effect can be measured, for example, based on the animals' growth or based on the nutritional value of the animals and their derived products. Evidently, other methods to measure the zootechnical performance of the animals, which are known to the person skilled in the art can also be used in combination with the invention. For instance, growth can be measured at the end of the growing period as the animal's weight or the animal's size (such as fish length for example). Alternatively, the animal's growth can be measured over a determined period of time resulting in the growth rate of the animal over time. The zootechnical performance can furthermore also be measured as the swimming capacity of the animal. Finally, farmers often

evaluate the zootechnical performance of animals based on the measurement of their feed conversion ratio or FCR. The FCR describes the efficiency with which the animals convert feed into the desired output. For animals raised for meat (such as beef cows, pigs, chickens and fish) the output is the flesh, that is, the body mass gained by the animal, represented either in the final mass of the animal or the mass of the dressed output.

The method described in the current invention is applicable to all cultured aquatic poikilotherms, and is particularly well suited for salmonids. Notably, salmonids tend to have a lower FCR than other cultured aquatic poikilotherms, and thus are more efficient at converting feed into body mass. The inventors have found that members of this group of fish are especially sensitive to the effects on zootechnical performance upon oral administration of the composition, which is reflected by a significant further decrease in the FCR. Therefore, application of the method in salmonid farming results in a major increase in revenues for the farmer. Additionally, because salmonids are fish-eating fish, a further decrease in FCR also translates into a lower burden on the environment because feeding of a lower quantity of wild fish results in an unaffected salmonid yield.

In a further embodiment, the fish feed further comprises other well-known ingredients so as to provide a nutritionally balanced complete food, including, but not limited to, plant matter, e.g., flour, meal, starch or cracked or processed grain produced from a crop plant such as wheat or other cereals, alfalfa, corn, oats, potato, rice, soybeans or other legumes; cellulose in a form that may be obtained from wood pulp, grasses, plant leaves, and waste plant matter such as rice or soy bean hulls, or corn cobs; animal matter, e.g., fish or crustacean meal, oil, protein or solubles and extracts, krill, meat meal, bone meal, feather meal, blood meal, or cracklings; algal matter; yeast; bacteria; vitamins, minerals, and amino acids; organic binders or adhesives; and chelating agents and preservatives.

In another further embodiment, administration of the composition according to the invention is performed orally or by feeding with a feed according to the invention. Oral administration can be performed in any manner and according to any feeding schedule employed in fish or crustacean cultivation. Feeding rates typically vary according to abiotic factors, mainly seasonal such as temperature, and biotic, in particular the size and the developmental stage of the animal.

Fish development recognizes 5 periods that occur in the following order: embryonic period; larval period; juvenile period; adult period and senescent period. The larval period occurs once the embryo has hatched and has the ability to feed independently of the egg yolk (or mother in rare cases), organ systems develop morphologically and gain physiological function. The juvenile period is when all organ systems are fully formed and functional (except the gonads) and fish attain the appearance of miniature adults. The juvenile period lasts until the gonads become mature. Once the gonads mature the fish attain the adult period. Finally, the senescence period is attained when growth ceases and gonads cease to produce gametes.

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Juvenile fish are typically fed 5-10% of their body weight per day over about 4-6 feeds per day. Larger fish are typically fed at 2-5% of their body weight per day over about 1-2 feeds per day. Juvenile crustaceans may fed up to 5-10% of their body weight over about 6 feeds per day, while larger crustaceans may be fed a minimum of about 2% of their body weight per day over about 2-3 feeds per day. The fish or crustaceans may be allowed to feed to appetite.

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Accordingly, in a further embodiment of the method according to the invention, the dosage of the MCFAs and / or MCFA derivatives comprises from 100 ppm to 5000 ppm by weight, based on the total weight of said aquatic poikilotherms' feed, preferably between 100 and 5000 ppm; between 100 and 3000 ppm, between 100 and 1000 ppm, more preferably 100 and 500 ppm, more preferably 100 and 300 ppm, such as 225 ppm.

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Preferably, the animals are fed at least once per day, more preferably two or more times per day such as, for example, 2-6 or 4-6 times per day. It is preferred that any excess food be removed after the feeding period, e.g., by flushing out of a raceway system, or through removal out of the bottom of the sea-cage. Alternatively, a fish such as a catfish can be added to the fish population to consume any excess food.

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In another embodiment, said composition is provided to then environment of the aquatic animals, such as the swimming water or tank. Said composition is added such that the MCFA concentration in the water is at a concentration of 100 and 5000 ppm; between 100 and 3000 ppm, between 100 and 1000 ppm, more preferably 100 and 500 ppm, more preferably 100 and 300 ppm, such as 225 ppm.

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The invention is further described by the following non-limiting examples which further illustrate the invention, and are not intended to, nor should they be interpreted to, limit the scope of the invention.

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EXAMPLES

Example 1. In vitro inhibition of *Piscirickettsia salmonis* growth by a composition according to an embodiment of the invention.

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A naturally occurring strain of *P. salmonis* (field strain PM-15972) was used. Bacterial growth was evaluated based on M49-A of the CLSI/NCCLS standards.

Bacterial growth medium (minimum essential medium (according Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 10 mM HEPES, 90%; fetal bovine serum, 10%) containing increasing concentrations of the composition and a control (bacterial growth medium lacking MCFA or MCFA derivatives) were inoculated with an equal amount of *P. salmonis* (PM-15972). The mixtures were incubated, and the bacterial growth was scored: positive for the presence of bacterial growth; negative for the absence of bacterial growth. The results are shown in Table 1.

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Table 1. In vitro inhibition of *P. salmonis* growth in the presence of MCFAs.

Composition concentration	<i>P. salmonis</i> growth
Control (0ppm)	Positive
60 ppm	Negative/Positive
100 ppm	Negative
300 ppm	Negative
1000 ppm	Negative
5000 ppm	Negative
120 ppm	Negative
5000 ppm	Negative

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Although *P. salmonis* was still able to grow in the presence of 60 ppm of the composition, the minimum inhibitory concentration (MIC) was reached in vitro at 70

ppm of the composition, as no *P. salmonis* growth could be detected in the presence of 70 ppm or more of the composition in the medium.

5 Example 2: In vitro viability of *Piscirickettsia salmonis* in the presence of a composition according to an embodiment of the invention.

A naturally occurring strain of *P. salmonis* (field strain PM-15972) was used. Bacterial survival was evaluated based on protocol BS EN 1656:2009 of the British
10 Standards Institution (BSI).

An aqueous solution containing increasing concentrations of the composition were inoculated with an equal amount of *P. salmonis* (PM-15972). The mixtures were incubated at 18°C. After 3 hours the composition was neutralized by dilution and
15 the solution was plated on *P. salmonis* medium. The plates were incubated for 7 to 10 days at 18°C and the viability of the bacteria was calculated based on the colony forming units (CFU) /ml. The "CFU" is a unit that is used to estimate the number of viable bacteria in a sample. "Viable" is defined as the ability to multiply via binary fission under the controlled conditions. The results are shown in table 2.

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Table 2. In vitro survival of *P. salmonis* in the presence of MCFAs.

Composition concentration	<i>P. salmonis</i> viability ^a	<i>P. salmonis</i> kill off ^b
Control	5.53 x 10 ⁸	0%
60 ppm	1.20 x 10 ⁵	99.9%
70 ppm	5.07 x 10 ⁴	99.99%
80 ppm	2.30 x 10 ³	99.999%
90 ppm	0	100%
100 ppm	0	100%
110 ppm	0	100%
120 ppm	0	100%
3000 ppm	0	100%

^a *P. salmonis* viability is expressed as CFU/ml

^b *P. salmonis* kill off is expressed as the reduction in *P. salmonis* viability (compared to the control sample).

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From these results it can be concluded that the minimum bactericidal concentration or MBC, the lowest concentration of the composition which results in efficient *P.*

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salmonis kill off is 80 ppm according to the standard protocol (99,999%). Furthermore, this test shows that at a concentration of 90 ppm or more the composition was 100% efficient in reducing the viability of P. salmonis.

5 Example 3: In vivo experiment on zootechnical performance / piscirickettsiosis treatment, suppression and / or prevention

Set up: fish tanks with 50 salmon infected with P. salmonis strain LF-89 (ATCC VR-1361) were treated with either 225 ppm of an MCFA composition of 40% C8, 40% C10 and 5% C12 in the water volume of the tank, or a control agent without MCFA. Results of the treatment were evaluated after 10 days of treatment:

MCFA treated population	Control population
2 deaths, overall healthy looking population	25 deaths, population clearly sick

After 15 days, the amount of deaths in the control population rose to 34, while in the MCFA treated tank no further deaths were reported.

This experiment was repeated with infected trouts. Also here the lesser deaths in the MCFA treated population was observed compared to the control population. To conclude, MCFAs can be used as an effective treatment of piscirickettsiosis.

It is supposed that the present invention is not restricted to any form of realization described previously and that some modifications can be added to the presented example without reappraisal of the appended claims. For example, the present invention has been described referring to P. salmonis, but it is clear that the invention can be applied to piscirickettsiosis caused by Piscirickettsia-like organisms (PLO) or, for instance, fish rickettsia-like organisms (RLO).

CLAIMS

1. A composition for use in the treatment, suppression and / or prevention of piscirickettsiosis, preferably in aquatic poikilotherms, characterized in that said
5 composition comprises one or more medium-chain fatty acids (MCFAs) and / or derivatives thereof.
2. The composition for use according to the previous claim, characterized in that said MCFAs are chosen from the group comprising fatty acids with a longest continuous chain of 5 carbon atoms to 12 carbon atoms and derivatives thereof.
- 10 3. The composition for use according to any one of the previous claims, characterized in that said MCFAs are selected from the group comprising caproic acid (C6), caprylic acid (C8), capric acid (C10), lauric acid (C12), derivatives thereof or a combination thereof.
4. The composition for use according to any one of the previous claims,
15 characterized in that said composition comprises at least caprylic acid (C8), capric acid (C10) and lauric acid (C12) or a derivative thereof.
5. The composition for use according to claim 4, characterized in that said composition comprises
 between 30% and 70% caprylic acid (C8) or a derivative thereof;
20 between 15% and 55% capric acid (C10) or a derivative thereof; and
 between 1% and 35% lauric acid (C12) or a derivative thereof;
 based on the total weight of the composition.
6. The composition for use according to any of the previous claims, characterized in that the total amount of MCFAs in the composition is comprised between 1%
25 and 100%, more preferably between 5 and 50% based on the total weight or volume of the composition.
7. The composition for use according to any one of the previous claims, characterized in that said piscirickettsiosis is caused by one or more obligate intracellular parasitic species.
- 30 8. The composition for use according to any one of the previous claims, characterized in that said piscirickettsiosis is caused by at least one species belonging to the family of Piscirickettsiaceae and / or to the order of Rickettsiales.
9. The composition for use according to any one of the previous claims, characterized in that said piscirickettsiosis is caused by at least one species
35 selected from the group comprising *Piscirickettsia salmonis*, *Piscirickettsia*-like organisms and fish rickettsia-like organisms.

10. The composition for use according to any one of the previous claims, characterized in that a therapeutically effective amount of said composition is administered orally to said aquatic poikilotherm.
11. The composition for use according to any one of the previous claims,
5 characterized in that said composition further comprises vitamins, minerals, trace elements or a combination thereof.
12. The composition for use according to any one of the previous claims, characterized in that said composition is administered as a powder, a granule, a pellet, a liquid or a paste.
- 10 13. The composition for use according to any one of the previous claims, characterized in that said aquatic poikilotherm is a salmonid.
14. The composition for use according to any one of the previous claims, characterized in that said poikilotherm is selected from the group comprising salmonids, trouts, freshwater whitefishes and graylings.
- 15 15. A fish feed comprising a composition for use according to any of the previous claims 1-14, wherein the dosage of said composition in said fish feed is 100 ppm to 5000 ppm by weight, based on the total weight of said fish feed.
16. A method for enhancing the zootechnical performance of aquatic poikilotherms comprising the step of administering a composition comprising one or more
20 medium-chain fatty acids (MCFAs) and / or derivatives thereof to said poikilotherms.
17. The method according to previous claim 16, characterized in that the total amount of MCFA in the composition is comprised between 1% and 100% based on the total weight or volume of the composition.
- 25 18. The method according to any one of previous claims 16-17, characterized in that said aquatic poikilotherm is selected from the group of salmonids.
19. The method according to any one of previous claims 16-18, characterized in that the dosage of said MCFAs and / or MCFA derivatives comprises from 100 ppm to 5000 ppm by weight, based on the total weight of said aquatic
30 poikilotherms' feed.
20. The method according to any of the claims 15 to 16, wherein said composition is added to the water of said aquatic poikilotherms, and wherein the concentration of the MCFAs or derivatives thereof is between 100 and 5000 ppm in said water.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/053269

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/19 A61K31/20 A23K50/80 A01K61/13 A61P31/04 A61K45/06 ADD. According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K C11C A01K A23K A61P Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data														
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category*</th> <th style="width: 70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width: 20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>WO 2015/052672 A1 (NUTRITION SCIENCES N V [BE]) 16 April 2015 (2015-04-16) page 7, line 36 pages 15-20; claims -----</td> <td style="text-align: center; vertical-align: top;">15-20</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>DATABASE WPI Week 199228 Thomson Scientific, London, GB; AN 1992-231738 XP002793573, & JP H04 158750 A (KAO CORP) 1 June 1992 (1992-06-01) cited in the application abstract -----</td> <td style="text-align: center; vertical-align: top;">15-20</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td>EP 1 123 701 A1 (NIPPON SUISAN KAISHA LTD [JP]) 16 August 2001 (2001-08-16) paragraph [0093] ----- -/-</td> <td style="text-align: center; vertical-align: top;">1-20</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	WO 2015/052672 A1 (NUTRITION SCIENCES N V [BE]) 16 April 2015 (2015-04-16) page 7, line 36 pages 15-20; claims -----	15-20	X	DATABASE WPI Week 199228 Thomson Scientific, London, GB; AN 1992-231738 XP002793573, & JP H04 158750 A (KAO CORP) 1 June 1992 (1992-06-01) cited in the application abstract -----	15-20	X	EP 1 123 701 A1 (NIPPON SUISAN KAISHA LTD [JP]) 16 August 2001 (2001-08-16) paragraph [0093] ----- -/-	1-20
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.														
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Date of the actual completion of the international search 3 April 2020		Date of mailing of the international search report 15/04/2020												
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Steendijk, Martin												

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/053269

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	NO 20 171 171 A1 (SAPONIN RES CENTER S A [CL]) 13 July 2017 (2017-07-13) claims -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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