

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 199669269 B2**
(10) Patent No. **718898**

(54) Title
Antistress agents for aquatic animals

(51)⁶ International Patent Classification(s)
A23K 001/18 A61K 031/715
A23K 001/16

(21) Application No: **199669269**

(22) Application Date: **1996 .08 .22**

(87) WIPO No: **WO97/08960**

(30) Priority Data

(31) Number	(32) Date	(33) Country
19532682	1995 .09 .05	DE

(43) Publication Date : **1997 .03 .27**

(43) Publication Journal Date : **1997 .05 .22**

(44) Accepted Journal Date : **2000 .04 .20**

(71) Applicant(s)
Tetra Werke Dr. Rer. Nat. Ulrich Baensch GmbH

(72) Inventor(s)
Hubert Kurzinger

(74) Agent/Attorney
CALLINAN LAWRIE, Private Bag 7, KEW VIC 3101

(56) Related Art
DE 4335454
FR 2674755
JP 2-250832

OPI DATE 27/03/97 APPLN. ID 69269/96
AQJP DATE 22/05/97 PCT NUMBER PCT/EP96/03689



AU9669269

(51) Internationale Patentklassifikation ^o: A23K 1/18, 1/16, A61K 31/715		A1	(11) Internationale Veröffentlichungsnummer: WO 97/08960
		(43) Internationales Veröffentlichungsdatum:	13. März 1997 (13.03.97)
(21) Internationales Aktenzeichen: PCT/EP96/03689 (22) Internationales Anmeldedatum: 22. August 1996 (22.08.96) (30) Prioritätsdaten: 195 32 682.2 5. September 1995 (05.09.95) DE (71) Anmelder (für alle Bestimmungsstaaten ausser US): TETRAWERKE DR. RER. NAT. ULRICH BAENSCH GMBH [DE/DE]; Herrenteich 78, D-49324 Melle (DE). (72) Erfinder; und (75) Erfinder/Anmelder (nur für US): KÜRZINGER, Hubert [DE/DE]; Jeankamp 8, D-49324 Melle (DE). (74) Anwälte: MANSMANN, Ivo usw.; Gödecke AG, Patentwesen, Mooswaldallee 1, D-79090 Freiburg (DE).		(81) Bestimmungsstaaten: AL, AM, AU, AZ, BY, CA, CN, CZ, EE, GE, HU, IL, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MN, NZ, RU, SG, SI, SK, TJ, TM, UA, US, UZ, VN, eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Veröffentlicht <i>Mit internationalem Recherchenbericht.</i> <i>Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist. Veröffentlichung wird wiederholt falls Änderungen eintreffen.</i>	
(54) Title: ANTISTRESS AGENTS FOR AQUATIC ANIMALS (54) Bezeichnung: ANTISTRESSMITTEL FÜR WASSERTIERE (57) Abstract <p>The invention pertains to antistress agents to improve the resistance of aquatic animals, in particular fish, shrimp and invertebrates in fresh water and saltwater, to stresses of all kinds; these can also be used as antistress agents for warm- and cold-water toy fish and contain a vitamin or a combination of vitamins in megadoses and one or more immunostimulants</p> (57) Zusammenfassung <p>Die Erfindung betrifft Antistressmittel zur Verbesserung der Widerstandsfähigkeit von Wassertieren, insbesondere Fische, Shrimps und Invertebraten im Süß- und Seewasser, bei Belastungen aller Art, einsetzbar auch als Antistressmittel für Warm- und Kaltwasser-Zierfische enthaltend ein Vitamin oder eine Kombination von Vitaminen in Megadosen und einem oder mehreren Immunstimulatoren.</p>			

ABSTRACT

The present invention concerns anti-stress agents for improving the resistance of aquatic animals and especially of fish, shrimps and invertebrates in fresh and sea water in the case of strains of all kinds, which can also be used as anti-stress agents for warm and cold water decorative fish, said agents containing a vitamin or a combination of vitamins in megadoses and one or more immune stimulators.



ANTI-STRESS AGENTS FOR AQUATIC ANIMALS

The present invention is concerned with feed for the improvement of the resistance of aquatic animals, especially of fish, shrimps and invertebrates, in fresh and sea water, in the case of stresses of all kinds, which are also usable for warm and cold water decorative fish, said agents containing a vitamin or a combination of vitamins in megadoses and one or more immune stimulators.

The use is known from AU 92 10 574 of immune stimulators, for example glucan, for protection against bacterial and viral infectious diseases in shrimps. Administration takes place via the feed in a dosing of 0.001 - 10%. As in JP 22 18 615, in EP-A-0 466 037 is described the increasing of the resistance of fish and shrimps toward pathogens. The dry feed contains 5 - 100 mg glucan per kg of feed. In EP-A-0 384 323 is described the synergistic effect of a vaccination against *Aeromonas* in the case of fish by simultaneous administration of 15 - 20 mg glucan via the feed per kg of body weight. Furthermore, from EP-A-0 559 450 is known the use of glucan as a binding agent in fish feed.

The vitamin contents in the natural raw materials usually employed which are worked up to mixed feeds for aquatic animals are mostly not sufficient in order to prevent deficiency symptoms. To the most frequent nutritionally caused diseases belong vitamin deficiency phenomena. For this reason, vitamins are usually added to feedstuffs for fish and shrimps in amounts covering the requirements.

Commercially usual dosagings are described, for example in NRC, Nutrient Requirements of Warmwater Fishes and Shellfishes, 1983. The recommended commercially usual value requirements for vitamins for fish, for example *Cyprinus carpio* L., amount, per kg of feed, to 10000 IU of vitamin A, 500 to 1000 IU of vitamin D, 30 mg of Vitamin E, 1 mg of vitamin B₁, 9 mg of vitamin B₂, 3 mg of vitamin B₆, 60 mg of vitamin C, 10 to 20 mg of pantothenic acid and 14 mg of nicotinic acid. Statements of need for vitamin B₁₂, vitamin K, inositol, choline, folic acid and vitamin C equivalent from long-stable vitamin C phosphate are not documented.

The effects of overdosages of vitamins by the use of megadoses of vitamins have been described in the scientific literature (see, for example, Steffens: Grundlagen der Fischernährung, 1985). In the case of the investigations, it was primarily observed whether hypervitaminoses, such as increased mortality, reduced growth, unfavourable feed utilisation, strong defects caused by overdosages of vitamins, occurred, for example by the feeding of 3.75×10^6 IU of vitamin D₃/kg of feed; 2×10^6 IU of vitamin A/kg of feed; 5 g of vitamin E/kg of feed; 10 g of nicotinic acid/kg of feed. However, the examined megadoses showed no negative effects.

Surprisingly, we have now found that especially feed materials for the introduction into the living water, containing an overdosage of one or more vitamins in combination with one or more immune stimulators, are outstandingly suitable for increasing the resistance of aquatic animals in the case of strains of all kinds but especially due to stress.

Stress situations occur almost permanently in the case of aquatic animals and lead to a strong straining of the animals. Examples of stress situations are hunger, high occupation density, water change, changes of the water parameters, territorial battles, aggressive behaviour, handling, medicinal therapy, transport and diseases.

Therefore, the subject of the present invention is feed in the form of mixtures, flakes, extrudates, pellets or tablets in dry, moist or semi-moist condition for aquatic animals, especially fish, shrimps and invertebrates, in fresh and sea water, containing at least one immuno stimulating agent and at least one vitamin or a vitamin combination in an overdosing amount (megadose).

Preferred immune stimulators include poly-saccharides, for example glucan, zymosan, mannan, lichenan, postulan, lentinan, schizophyllan, scleroglucan, M-glucan, yeast glucan, muramyl dipeptide and chitin. Further immune stimulators which can be used according to the present invention include lactoferrin, lactoperoxidases, glycyrrhizins, diaminopimelic acid peptide derivatives, for example N-[(R)-6-carboxy-N²-[N-(1-oxoheptyl)-D-gamma-glutamyl]-L-lysyl]-D-alanine, levamisole, inosiplex, 4-methyluracil, tilorone, dipyridamole and azimexone.

Furthermore, plant and bacterial extracts can also be used, for example extracts of *Phytolacca*, *Bryonia*, *Baptisia*, aloes, *aristolochia*, *arnica*, mistletoe, *echinacea*, palmetto, *Eleutherococcus senticosus*, *Rosa roxburghii*, *Artemisiae argyi folium*, *Brassica oleracea* var. *capitata*, sterilised *Clostridium butyricum* *miyairi* and

5 *Saccharomyces cerevisiae*.

The immuno stimulating agent is present in the feed in an amount of from 0.0001 to 10% by weight and preferably of 0.1% by weight. The preferred immuno stimulating agent is β -glucan.

- 10 The vitamins are used in an overdosing of 2.5 fold up to 5000 fold of the recommended requirement values. The water- and/or fat-soluble vitamins are preferably dosed in the following ranges (per kg of feedstuff):



vitamin A	$3 \times 10^4 - 2 \times 10^6$ IU
vitamin D	$1.5 \times 10^3 - 1 \times 10^6$ IU
vitamin E	90 mg - 10 g
vitamin B ₁	3 mg - 5 g
vitamin B ₂	27 mg - 10 g
vitamin B ₆	9 mg - 5 g
vitamin B ₁₂	0.1 μ g - 5 mg
Vitamin C	180 mg - 50 g
Vitamin K	20 mg - 5 g
pantothenic acid	30 mg - 5 g
nicotinic acid	42 mg - 50 g
inositol	1 g - 50 g
choline	200 mg - 50 g
folic acid	0.1 mg - 5 g
vitamin C equivalent	0.1 mg - 5 g

Especially preferred is an agent containing, per kg of feed:

vitamin A	2.8×10^5 IU
vitamin D	2.5×10^3 IU
vitamin E	1.9 g
vitamin B ₁	330 mg
vitamin B ₂	950 mg
vitamin B ₆	190 mg
vitamin B ₁₂	820 μ g
vitamin C	6.35 g
vitamin K	96 mg
pantothenic acid	940 mg
nicotinic acid	4.7 g
inositol	7.3 g
choline	1.13 g
folic acid	96 mg
vitamin C equivalent	4.9
β -glucan	1 g



If chitin is to be used as immune stimulator, then this is preferably prepared according to JP 6271470. For example, 10 g of chitin PSH are mixed with 150 ml 36% hydrochloric acid and stirred at ambient temperature for 2 hours. The reaction is ended by the addition of 1 l of distilled water and the product is filtered off and washed with distilled water.

The novel anti-stress agent for aquatic animals was tested via the feed on territorial, territory-forming decorative fish (*Cichlasoma nicaraguense*) regarding stress behaviour in comparison with the control (without megadoses of vitamins and without immune stimulators), in each case with three parallels. Besides the permanently present social stress, in addition, in each aquarium stress was induced daily by stirring the pond several times with a fish collecting net.

The high losses of 19% in the control group are to be attributed to the increased stress due to the aggressive behaviour and, above all, to the increased stress due to the treatment with the collecting net. Animals of all sizes were thereby affected. Perch fed with the experimental feed showed, on the basis of the feed which contained megadoses of vitamins and an immune stimulator, almost no deaths (1% mortality).

The anti-stress agent according to the present invention can be administered via the feed as flocks, extrudates, pellets and tablets in a dry, moist or semi-moist state or in liquid form into the water. The use can take place prophylactically or also in the case of acute strains of the aquatic animals.

The administration of the anti-stress agent takes place in a combination of megadoses of one or more vitamins and one or more immune stimulators.



Example 1

The following trials were carried out as blind trials with the indicated fixed combination of vitamins in overdosages and varied β -glucan amounts of from zero to one % as shown in the table below. For comparative reasons the table contains also the ranges of the instant invention according to claim 1. The feed was very well tolerated by the fishes and no losses caused by any asserted toxicity of high dosages of vitamins have been observed.

10

Feed supplements	Range of claim 1	Türnau experiments	Units
vitamin A	30000-2000000	38400	IU/kg
vitamin D	1500-1000000	5600	IU/kg
vitamin E	90-10000	192	mg/kg
menadione	20-5000	51	mg/kg
vitamin B6	9-5000	45	mg/kg
folic acid	0.1-5000	15	mg/kg
vitamin B12	0.1-5000	250	μ g/kg
vitamin B1	3-5000	205	mg/kg
vitamin C	180-50000	1807	mg/kg
choline	200-50000	7168	mg/kg
inositol	1000-50000	2048	mg/kg
vitamin C equivalent	0.1-5000	35000	mg/kg
glucan	0.0001-10	0-1	%

The claims defining the invention are as follows:

1. Feed for aquatic animals in the form of mixtures, flakes, extrudates, pellets or tablets in dry, moist or semi-moist condition containing:
 - a) at least an immuno stimulating agent in an amount of 0,0001 – 10% by weight, preferred 0,1% by weight, selected from the group glucan zymosan, mannan, lichenan, pustulan, lentinan, schizophyllan, scleroglucan, M-glucan, yeast glucan, muramyl dipeptide, chitin, lactoferrin, lactoperoxidases, glycyrrhizins, diamino pimelic acid peptide derivatives, N-[(R)-6-carboxy-N2-[N-(1-oxoheptyl)-D-.gamma.-glutamyl]-L-lysyl]-D-alanine, levamisole, inosiplex, 4-methyluracil, tilorone, dipyridamole, azimexone, phytolacca, bryonia, baptisia, aloes, aristolochia, arnica, misteltoe, echinacea, palmetto, eleutherococcus senticosus, rosa roxburghii, artemisiae argyi folium, brassica oleracea var. capitata, sterilised clostridium butyricum miyairi, and/or saccharomyces cerevisiae; and
 - b) per kg an overdosage of at least a vitamin selected from 3×10^4 – 2×10^6 IU vitamin A, $1,5 \times 10^3$ – 1×10^6 IU vitamin D, 90 mg – 10 g vitamin E, 3 mg – 5 g vitamin B₁, 27 mg – 10 g vitamin B₂, 9 mg – 5 g vitamin B₆, 0,1 µg – 5 mg vitamin B₁₂, 180 mg – 50 g vitamin C, 20 mg – 5 g vitamin K, 30 mg – 5 g pantothenic acid, 42 mg – 50 g nicotinic acid, 1 g – 50 g inositol, 200 mg – 50 g choline, 0,1 mg – 5 g folic acid and/or 0,1 mg – 5 g vitamin C equivalent for the improvement of aquatic animals in case of strains of all kinds but especially of stress.
2. Feed according to claim 1, wherein it contains per kg
 - a) 1 g β-glucance and
 - b) $2,8 \times 10^5$ IU vitamin A, $2,5 \times 10^3$ IU vitamin D, 1,9 g vitamin E, 330 mg vitamin B₁, 950 mg vitamin B₂, 190 mg vitamin B₆, 820 µg vitamin B₁₂, 6,35 g vitamin C, 96 mg vitamin K, 940 mg pantothenic

acid, 4,7 g nicotinic acid, 7,3 g inositol, 1,13 mg choline, 96 mg folic acid and 4,9 g vitamin C equivalent.

3. Use of a combination of one or more immuno stimulating agents together with at least one vitamin or a mixture of vitamins in overdosage according to claims 1 or 2 for the manufacturing of feed for the improvement of the resistance and prevention of losses in the keeping of aquatic animals.

4. Feed according to claim 1 substantially as hereinbefore described according to any one of the Examples.

10 Dated this 9th day of December, 1999

TETRAWERKE DR. RER. NAT. ULRICH BAENSCH GmbH

By their Patent Attorneys:

CALLINAN LAWRIE

