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Probiotic health and performance promoting food, feed and/or drinking water additive and its use

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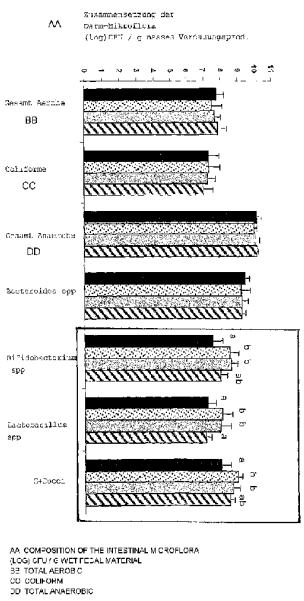
(81) Bestimmungsstaaten *(soweit nicht anders angegeben, für jede verfügbare nationale Schutzrechtsart)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,

[Fortsetzung auf der nächsten Seite]

(54) Title: PROBIOTIC HEALTH OR FITNESS PROMOTING HUMAN OR ANIMAL FOODSTUFF AND/OR DRINKING WATER ADDITIVE AND USE THEREOF

(54) Bezeichnung: PROBIOTISCHER, GESUNDHEITS- BZW. LEI STUNGS FÖRDERNDER NAHRUNGS-, FUTTERMITTEL UND/ODER TRINKWASSERZUSATZ SOWIE SEINE VERWENDUNG



(57) Abstract: The invention relates to a probiotic health or fitness promoting human or animal foodstuff and/or drinking water additive, comprising a mixture of microorganisms, selected from the group Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, Bifidobacterium animalis and DSM 16284. The invention further relates to a use of the human or animal foodstuff and/or drinking water additive, in particular for prevention of the harmful effect of a number of undesirable germs in the digestive system of animals and/or domestic birds.

(57) Zusammenfassung: Bei einem probiotischen, gesundheits- bzw. leistungsfördernden Nahrungs-, Futtermittel- und/oder Trinkwasserzusatz, welcher eine Mischung von Mikroorganismen enthält, ist vorgesehen, daß wenigstens zwei Mikroorganismen, gewählt aus der Gruppe Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, und Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, und Bif idobacterium animalis, DSM 16284, enthalten sind. Weiters wird eine Verwendung des Nahrungs-, Futtermittel- und/oder Trinkwasserzusatzes insbesondere zur Verhinderung der schädlichen Wirkung einer Vielzahl von unerwünschten Keimen im Verdauungssystem von Säugern und/oder Nutzvögeln zur Verfügung gestellt.

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KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Bestimmungsstaaten** *(soweit nicht anders angegeben, für jede verfügbare regionale Schutzrechtsart)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), eurasisches (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht:

— *mit internationalem Recherchenbericht*

— *mit Angaben über hinterlegtes biologisches Material, eingereicht gemäss Regel 13bis, getrennt von der Beschreibung*

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

PROBIOTIC HEALTH AND PERFORMANCE PROMOTING FOOD, FEED AND/OR DRINKING WATER ADDITIVE AND ITS USE

The present invention relates to a probiotic health and performance promoting food, feed and/or drinking water additive containing a mixture of microorganisms, and the use of such a probiotic health and performance promoting food, feed and/or drinking water additive.

Food, feed and/or drinking water additives containing mixtures of microorganisms are used to an increasing extent both in human and animal applications in order to prevent, as far as possible, infections by pathogenic germs such as, for instance, Salmonella or E. coli, Campylobacter, Clostridia etc. The use of such mixtures of microorganisms is based on what is called competitive exclusion (CE), by which it is attempted to suppress or eliminate "bad" bacteria, i.e. health-impairing or harmful bacteria, by so-called "good" bacteria. Thus, the growth of good bacteria inhibits that of bad bacteria, for instance by taking advantage from the environment of the digestive tract in terms of growth so as to proliferate more rapidly. Concretely, a special mixture of bacteria, which can also be isolated from the digestive tract, is used while trying to administer as broad a spectrum of intestinal bacteria as possible in order to achieve a wide field of application. However, the use of a spectrum as broad as possible, of intestinal bacteria which are not specified turned out to involve problems, since, on the one hand, possible problems of undefined mixtures such as, for instance, the transfer of antibiotic resistances or diseases can hardly be avoided and, on the other hand, the administration of undefined mixtures is limited by regional or international

regulations and legislations in order to prevent unexpected or undesired results from the administration of such mixtures. The use of defined mixtures and, in particular, defined probiotic cultures comprising one or several strains will, therefore, have to be resorted to in order to avoid unexpected effects and to comply with the legislations.

Bearing in mind the legal provisions and seeking to obtain results as concrete as possible, a plurality of publications have recently become known, among which US-A 5 372 810, for instance, describes a formulation and method for preventing and treating diarrhoea in farm animals by using sterilized bacterial cells, their homogenates or cell wall components, which were aerobically grown. The bacteria used in that case belong to the genera of Brevibacterium and/or Corynebacterium.

US Publication 2004-0115308 describes consumable products containing fresh, probiotic ingredients, said probiotics being comprised of the groups of yeasts, Aspergilla, Lactobacilli, Bifidobacteria, Streptococci, Enterococci and mixtures thereof. The bacteria used there are not dried or concentrated in any manner whatsoever, but are used as obtained, thus causing problems in terms of stability, applicability and the like.

From EP 1 037 964, a defined probiotic or formulation of anaerobic bacteria for controlling or inhibiting salmonellas in pigs has become known, said bacteria comprising Enterococcus faecalis, Streptococcus bovis, Clostridium clostridiforme, Clostridium symbiosum, Clostridium ramosum, Bacteroides fragilis, Bacteroides

distasonis, Bacteroides vulgatus, Bacteroides thetaiotamicron and Bacteroides caccae, wherein at least seven different groups of germs have to be contained in the product.

Finally, WO 03/054168 describes a method for preparing a formulation of intestinal germs that are capable of eliminating pathogenic germs. In that method, a microbial sample is obtained from the intestines of a particular animal species and exposed to aerobic conditions, subsequently frozen, and rethawed. The individual microbial groups are selectively cultivated and tested for their activities against pathogenic microbes in inhibition tests and finally fermented and used.

The methods described in the literature, however, involve problems in that only one activity against a special pathogenic germ will be obtained by using either individual specific bacteria or combinations thereof. An activity against the various pathogenic germs contained in the digestive tract is not achievable. Finally, the methods described in the literature mostly also suffer from that the administered spectrum of bacteria is not broad enough to achieve as complete an inhibition of pathogenic germs as possible. Other known products use bacteria that are not obtained from the intestines, thus providing not completely specific activities.

The present invention aims to provide a probiotic health and performance promoting food, feed and/or drinking water additive comprising a mixture of microorganisms, which displays its activity substantially in the entire digestive system of mammals and/or farm birds, thus preventing or at

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least reducing the harmful effects of a great number of undesired germs such as, e.g., Salmonella, E. coli, Clostridia etc.

To solve these problems, a probiotic health and performance promoting food, feed and/or drinking water additive containing a mixture of microorganisms is used according to the present invention, which comprises at least two microorganisms selected from the group consisting of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284. By using microorganisms from the group consisting of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, it is feasible, due to said microorganisms originating from the most diverse regions of the digestive tract such as, for instance, the appendix, jejunum, ileum or goiter, to substantially cover the entire digestive tract and, in particular, the gastro-intestinal region so as to successfully prevent pathogenic germs from working in the entire digestive tract.

With the exception of DSM 16284, Bifidobacterium animalis, which is supposed to constitute a new species, the deposited microorganisms are new strains of already known species, since they generally show more than 99% correspondence with known strains.

The individual sequences of the deposited strains are as follows:

Pediococcus acidilactici, DSM 16210

CCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTTCCGT
TAATTGATTATGACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAGTGGCGGACGG
GTGAGTAACACGTGGGTAACCTGCCCAGAAGCAGGGGATAACACCTGGAAACAGATGCTA
ATACCGTATAACAGAGAAAACCGCCTGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTT
CTGGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGAT
GCGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGTGCACGCCGCG
TGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTGAGAG
TAACTGTTCACCCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAG
GCGGTCTTTTAAGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACT
GGGAGACTTGAGTGCAGAAGAGGATAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGA
TATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGATT
ACTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGTAATCCGCC
TGGGGAGTACGACCGCAAGGTTGAAACTCAAAAGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTCTG
CCAACCTAAGAGATTAGGCGTTCCCTTCGGGGACAGAATGACAGGTGGTGCATGGTTGTC
GTCAGCTCGTGTCG

Sequencing primer:	341 forward, 530 reverse
Contig from 2 partial sequences:	341f530r
Sequence length:	1094 bases in total
Correspondence with known strain	99.6%

Lactobacillus reuteri, DSM 16350

GGATGAACGCCGGCGGTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTG
ATGGTGCTTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACGGGTGAGTAACAC
GTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAA
CAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGAC
CTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGA
GTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCACACCGCGTGAGTGAAGA
AGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCA
YGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTT
AGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTG
AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAG
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGG
GTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGGTGT
TGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA
CGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAAGCATG
TGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTT
AGAGATAAGGCGTTCCCTTCGGGGACGCAATGACAGGTGGTGCATG
GTCGTCGTCAGCTCGTG

Sequencing primer: 341 forward, 530 reverse

Contig from 2 partial sequences: 341f530r

Sequence length: 1083 bases in total

Correspondence with known strain 99.7%

Lactobacillus salivarius ssp. salivarius, DSM 16351

ACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAAACTTTCTTACACCGAATGCTTG
CATTCACTCGTAAGAAGTTGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTAA
AAGAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATATCTNTAAGGATCGCATGA
TCCTNAGATGAAAGATGGTTCTGCTATCGCTTNTAGATGGACCCGCGGCGTATTANCTAG
TTGGTGGGGTAACGGCNTACCAAGGNGATGATACGTAGCCGAACTGAGAGGNTGATCGGC
CACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCA
CAATGGACGCAAGTCTGATGGTGCCCGCCGCGAGAGTGAAGAAGGTCTTCGGATCGTAAA
ACTCTGTTGTTAGAGAAGAACACGAGTGAGAGTAACTGTTCATTCGATGACGGTATCTAA
CCAGCAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
GTCCGGATTTATTGGGCGTAAAGGGAACGCAGGCGGTCTTTTAAGTCTGATGTGAAAGCC
TTCGGCTTAACCGGAGTAGTGCATTGGAAACTGGAAGACTTGAGTGCAGAAGAGGAGAGT
GGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAAGC
GGCTCTCTGGTCTGTAACTGACGCTGAGGTTCGAAAGCGTGGGTAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTC
AGTGCCGCAGCTAACGCAATAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT
CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG
CGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACCTAAAAAATTAGGTTTCCCTTCG
GGGACAAAGTGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGT

Sequencing primer: 341 forward, 530 reverse

Contig from 2 partial sequences: 341f530r

Sequence length: 1066 bases in total

Correspondence with known strain 99.5%

Enterococcus faecium, DSM 16211

CCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGCTTCTTTT
TCCACCGGAGCTTGCTCCACCGGAAAAAGAGGAGTGGCGAACGGGTGAGTAACACGTGG
GTAACCTGCCCATCAGAAGGGGATAACACTTG
GAAACAGGTGCTAATACCGTATAACAATCAAAACCGCATGGTTTTGATTTGAAAGGCGC
TTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC
TCACCAAGGCCACGATGCATAGCCGACCTGAG
AGGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGT
AGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGG
TTTTCGGATCGTAAAACTCTGTTGTTAGAGAA
GAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG
GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGG

GGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTG
TAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTC
TGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAG
CTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTC

Sequencing primer: 341 forward, 530 reverse

Contig from 2 partial sequences: 341f530r

Sequence length: 1033 bases in total

Correspondence with known strain 99.9 %

Bifidobacterium animalis, DSM 16284

TTGCCATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGCGGGATGGAGGCCTTCGGGTTG
TAAACCGCTTTTGTTCAAGGGCAAGGCACGGTTTCGGGCCGTGTTGAGTGGATTGTTCGA
ATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTATC
CGGATTTATTGGGCGTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCAT
CGCCTAACGGTGGATCTGCGCCGGGTACGGGCGGGCTGGAGTGCGNTAAGGGAGACTGGA
ATTCCCGGTGTAACGNTGGAATGTGTANATATCGGGAAGAACACCAATGGNNAANGNAGG
TCTCTGGGCCGTTACTGACGCTGACGATNNAAAGACGTGAACCAGCGANCNCNATAANAT
ACCCTGACTACGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGATGCTGGATGT
GGGGCCCTTTCCACGGGTCCTGTGTCGGAGCCAACGCGTTAAGCATCCCGCCTGGGGAGT
ACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCGGAGCATG
CGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGTGCCGGATCGCCG
TGGAAACACGGTTTCCCTTCGGGGCCGGTTCACAGGTGGTGCATGGTCGTCGTCAGCTCG
TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCGCATGTTGCCAGC
GGGTGATGCCGGGAACTCATGTGGGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGATGA
CGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAA
CGCGATGCGACACGGTGACGTGGGGCGGATCGCTGAAAACCGGTCTCAGTTCGGATCGCA
GTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGGATCAGCAACGCCGCG
GTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGGTAGCACC
GGAAGCCGGTGGCCCGACCCTCGTGGGGCGGACCGTCTAATGGTGAGACTCGTGATTGG

Sequencing primer: 341 forward, 1492 reverse

Contig from 2 partial sequences: 341f1492r

Sequence length: 1139 bases in total

Correspondence with known strain 98.9% - new species

According to a further development of the invention, the food, feed and/or drinking water additive comprises at least three microorganisms, thus enabling the achievement

of a more complete coverage of the entire digestive tract and, in addition, the detection of a synergistic effect of the three used microorganisms against pathogenic germs.

A mixture comprising Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, has turned out to be a particularly preferred mixture for food, feed and/or drinking water additives. Such a mixture not only has proved to be beneficial for the inhibition of pathogenic germs, in particular E. coli bacteria, but, on account of its strong pH-lowering potential and high acid production rates, e.g. of lactic acid, also strongly restricts the milieu for pathogenic germs, thus, in addition, also strongly inhibiting the settlement of other, different pathogens.

Furthermore, a food, feed and/or drinking water additive comprising Enterococcus faecium, DSM 16211, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, is particularly preferred. Such a food, feed and/or drinking water additive, in particular, exhibits a uniformly enhanced inhibition of nearly all commonly present pathogenic germs and, in particular, E. coli, Salmonella choleraesuis, Campylobacter jejuni, Clostridium perfringens, which is increased relative to the single effects of the microorganisms contained in the mixture by such an extent as to provide a synergistic effect of the three microorganisms against a plurality of pathogenic germs. In addition, this mixture too has a strong pH-lowering potential, particularly on account of relatively high amounts of lactic acid and acetic acid forming, which

will again have a strongly adverse effect on the living conditions for pathogenic germs.

Due to the fact that, as in correspondence with a preferred further development of the present invention, nutritive substances and/or prebiotic substances additionally usable as carriers and selected from fructo-oligosaccharides, inulins, isomalto-oligosaccharides, lactitol, lactosucrose, lactulose, pyrodextrines, soy oligosaccharides, transgalacto-oligosaccharides, xylo-oligosaccharides, vitamins, in particular vitamin E, are contained in the food, feed and/or drinking water additive, the living conditions for the used microorganisms in the digestive tract will be markedly improved, and it will, in particular, be safeguarded that the used microorganisms will be able to rapidly and reliably propagate in the digestive tract, yet while, at the same time, impeding or preventing the propagation of pathogenic germs by competitive exclusion. The used nutritive substances and/or prebiotic acids provide growth advantages for the used microorganisms relative to pathogenic germs.

Since, as in correspondence with a preferred further development of the present invention, a further carrier selected from zeolites, calcium carbonate, magnesium carbonate, trehalose, chitosan, shellac, albumin, starch, skim-milk powder, swee-whey powder, maltodextrins, lactose, inulin, dextroses, vegetable oils, or a solvent selected from water or physiologic saline solution, is contained, it is feasible to obtain a uniform distribution of the microorganisms in the digestive tract, on the one hand, and to obtain further assistance of the competitive exclusion, on the other hand.

Since, as in correspondence with a preferred further development of the invention, a coating material selected from maltodextrins, guar seed flour, gum arabic, alginates, modified starch and starch derivates, dextrins, cellulose derivates like cellulose ester and ether, proteins like gelatine, albumin, casein, gluten, acacia gum, tragacanth, lipids like waxes, paraffin, stearic acid, mono- and diglycerides is contained, it is feasible to coat the microorganisms together with optionally contained carrier materials so as to ensure that the microorganisms will only develop their activities on the location of their purpose of use, i.e. in the digestive tract. Moreover, by using such coating materials, it is, for instance, possible to apply the food, feed and/or drinking water additive directly on animals to be treated therewith, such as day-old chicks, in their transport boxes by so-called gel or pellet application so as to provide a prophylaxis and therapeutic treatment of young animals against infections by pathogenic microbes. Such applications are of particular importance in modern animal breeding, since, because of the lacking direct contact of young animals with their mothers, the appropriate basis for an intact intestinal flora will no longer be passed on.

In order to achieve an even more complete activity of the food, feed and/or drinking water additive against pathogenic germs, the additive is preferably further developed to the effect that it additionally comprises at least one microorganism selected from the group of Bifidobacterium sp., Lactobacillus salivarius, Lactobacillus sp., Lactobacillus fermentum and Enterococcus faecalis. By admixing to the food, feed and/or drinking water additive a

further microorganism known per se, the activity against one or several pathogenic germ(s) to be expected in the respective animals will be selectively enhanced so as to ensure further reduction of the risk of infection.

According to a preferred further development, it has turned out to be particularly beneficial to use the food, feed and/or drinking water additive in suspended, powdery or encapsulated form with a maximum diameter of 2000 µm so as to enable the exact, selective release of the activity of the microorganisms as a function of the desired location of use such as, for instance, the goiter, the stomach, the small intestine and the like. In addition, it is, of course, also possible to use mixed forms of suspended and encapsulated food, feed and/or drinking water additives in order to be able to ensure an activity over the entire digestive system.

According to a preferred further development of the invention, a feed additive is provided, in particular for farm birds and/or pigs to increase the performance of said farm animals, which feed additive is comprised of 1×10^7 to 1×10^{14}, in particular 1×10^{10} to 1×10^{12}, CFU/kg feed additive Enterococcus faecium, DSM 16211, and 1×10^6 to 1×10^{14}, in particular 1×10^{10} to 1×10^{12}, CFU/kg feed additive Lactobacillus reuteri, DSM 16350. Such a selection of the amounts of used microorganisms, in particular, ensures the production of a feed additive displaying excellent activity against most of the E. coli germs.

According to a preferred further development of the invention, a feed additive is provided, in particular for farm birds and/or pigs to increase the performance of said

farm animals, which feed additive is comprised of 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Enterococcus faecium, DSM 16211, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Pediococcus acidilactici, DSM 16210, and 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Bifidobacterium animalis, DSM 16284. Such a feed additive, in particular, ensures to strongly, or uniformly strongly, inhibit the action of Salmonella choleraesuis and Clostridium perfringens as well as the action of E. coli bacteria and, in addition, to strongly lower the pH in the digestive tract of the thus fed animals on account of the high production of acids, namely acetic acid and lactic acid, by the microorganisms used, thus strongly impeding further pathogenic germs from growing.

To increase the activity of the feed additive, it is preferably further developed to the effect that it is comprised of 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Lactobacillus reuteri, DSM 16350, and 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Lactobacillus salivarius ssp. salivarius, DSM 16351. Such a selection of the amounts of used microorganisms, in particular, ensures the production of a feed additive displaying excellent activity against E. coli germs and, in addition, on account of the high pH lowering potential, providing strong inhibition of the growth of nearly all other pathogenic germs.

The feed additive intended, in particular, for farm birds and/or pigs to increase the performance of said farm

animals, according to a preferred further development of the present invention is comprised of 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Lactobacillus reuteri, DSM 16350, 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Pediococcus acidilactici, DSM 16210, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12}, CFU/kg feed additive Bifidobacterium animalis, DSM 16284, and 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{12} CFU/kg, feed additive Bifidobacterium sp. 2. Such a feed additive mixture, in particular, shows a markedly increased activity against the pathogenic germs Campylobacter jejuni as well as Salmonella choleraesuis. Moreover, also the activities against common E. coli bacteria are strongly enhanced and, in the main, largely exceed the activities of the individual strains. In addition, the metabolic reaction of such a feed additive has proved that a synergistic effect of the bacteria must be present. Thus, the present mixture of five microorganisms, *inter alia*, is able to convert xylitol, which cannot be done by a microorganism of the mixture alone. The reason for this is that, for instance, several enzymes are required for the degradation of xylitol, and it is only by the combination according to the invention of five microorganisms that the necessary enzymes are made available jointly so as to enable the conversion of a carbon hydrate that is so complicated to degrade.

According to a preferred further development, the feed additive additionally comprises 1% to 95%, in particular

20% to 98%, of a carrier or natural substance. By a carrier or natural substance being additionally contained, a further elimination of pathogenic germs from the gastrointestinal environment is feasible by supporting or promoting the selective growth of the microorganisms used.

Since, as in correspondence with a preferred further development of the invention, the aqueous bacterial suspension is applied on the feed or its pellets in an amount of from 0.01 g/kg to 10 g/kg feed, in particular 0.025 g/kg to 2.5 g/kg feed, it is ensured that, on the one hand, the feed additive will be readily taken up by the animals and, on the other hand, pathogenic germs already present on the surface of the feed prior to the feed intake will be combated or eliminated or degraded by the feed additive. In order to achieve a particularly good activity of the feed additive, the feed additive is preferably mixed into the feed in an amount of from 20 g/t to 20 kg/t, in particular 100 g/t to 2.5 kg/t, thus providing a sufficient amount of microorganisms, on the one hand, and ensuring the safe degradation or elimination of pathogenic germs from the gastrointestinal environment, on the other hand.

According to a preferred further development of the invention, the microorganisms, or mixture of microorganisms, is used as a drinking water additive, wherein, according to a preferred further development, the drinking water additive comprises 1×10^7 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive Enterococcus faecium, DSM 16211, and 1×10^6 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive Lactobacillus reuteri, DSM 16350. Such a drinking water additive is, in particular, beneficial for

the inhibition of pathogenic germs and, in particular, for the inhibition of E. coli. Moreover, such a mixture is able to strongly lower the pH in the gastrointestinal tract on account of the formation of lactic acid, acetic acid and propionic acid, thus creating unfavourable living conditions for pathogenic germs, and, on the other hand, the acids formed will also act bactericidally in their non-dissociated forms, as is generally known, so as to provide an altogether good action against a plurality of pathogenic germs.

According to a preferred further development, a drinking water additive is used, which is comprised of 1×10^7 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive Enterococcus faecium, DSM 16211, 1×10^6 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive Lactobacillus reuteri, DSM 16350, and 1×10^6 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive Lactobacillus salivarius ssp. salivarius, DSM 16351. Such a drinking water additive has turned out to be of particular benefit for the inhibition of E. coli as well as Salmonella choleraesuis and, in addition, also shows a synergistic effect in respect to the metabolic profile. This mixture is, thus, for instance, able to degrade D-tagatose, which cannot be done by any separate individual strain contained therein. A synergistic effect of these three strains in respect to the metabolic profile is, thus, detectable.

According to a preferred further development, the invention is further developed to the effect that the drinking water additive is comprised of 1×10^7 to 1×10^{14}, in particular 1×10^{10} to 1×10^{13}, CFU/kg drinking water additive

Enterococcus faecium, DSM 16211, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Pediococcus acidilactici, DSM 16210, and 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Bifidobacterium animalis, DSM 16284. Such a mixture of microorganisms has turned out to be of particular benefit for the inhibition of pathogenic germs of the group of Salmonella choleraesuis, Campylobacter jejuni and Clostridium perfringens and, due to the different origins of the microorganisms from the gastrointestinal tract, is, moreover, also able to develop its activity over the entire gastrointestinal tract so as to ensure an almost complete elimination of harmful, pathogenic germs from the entire gastrointestinal system.

According to a preferred further development, a drinking water additive is used, which is comprised of 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Lactobacillus reuteri, DSM 16350, 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Pediococcus acidilactici, DSM 16210, 1 x 10^7 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13}, CFU/kg drinking water additive Bifidobacterium animalis, DSM 16284, and 1 x 10^6 to 1 x 10^{14}, in particular 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Bifidobacterium sp. By such a drinking water additive, it is feasible to inhibit almost all frequently occurring pathogenic germs and, in particular, E. coli, Salmonella, Clostridia and Campylobacter to a degree

largely exceeding that of the cumulative action of the individual microorganisms, so that a massive synergistic effect against pathogenic germs will be achieved. In addition, it is feasible by such a microorganism to strongly and extremely rapidly lower the pH in the digestive tracts of animals so as to further enhance its action and, in particular, the inhibition of the growth of pathogenic germs.

In order to promote, in particular, the growth of the desired microorganisms in the gastrointestinal tract and further enhance the elimination effect, the drinking water additive according to a preferred further development additionally comprises 1% to 95%, in particular 20% to 92%, of a carrier or nutrient.

According to a further object, the present invention aims to selectively boost the immune systems of mammals and/or farm birds by the selective use of microorganisms, or a mixture of microorganisms, and thereby eliminate as completely as possible pathogenic germs from the digestive tract.

To solve these objects, the invention contemplates the use of a mixture of at least two microorganisms selected from the group consisting of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, as a probiotic health and performance promoting food, feed and/or drinking water additive. Such a use enables to almost completely eliminate pathogenic germs from the digestive tracts of mammals and farm animals by the so-

called competitive exclusion, since an extremely rapid and strong lowering of the pH in the digestive tract is, in particular, feasible by a selective mixture of said microorganisms, the use according to the invention enabling the selective inhibition of the development of pathogenic germs. Apart from that, none of the microorganisms exhibited an antibiotic resistance even at an extended, simultaneous administration of at least one antibiotic during such use.

According to a preferred further development, a mixture of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, is used according to the present invention. Such a use, in particular, ensures the inhibition of the action of pathogenic germs, namely E. coli, and, by the extremely rapid lowering of the pH in the digestive tract, to deteriorate the living conditions for pathogenic germs as such to such a degree that even the growth of any other harmful germs will be strongly impaired or inhibited so as to markedly reduce the susceptibility to diseases of mammals and/or farm birds supplied therewith.

According to a further development of the invention, a mixture of Enterococcus faecium, DSM 16211, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, is preferably used, which, in particular, renders feasible the inhibition of Salmonella choleraesuis, Campylobacter jejuni and Chlostridium perfringens, respectively, and, in addition, strongly deteriorates the living conditions for other pathogenic germs, so that, in the main, a probiotic effect of the used mixture largely

exceeding the cumulative effect when using the individual microorganisms can be demonstrated.

According to a preferred further development of the invention, a mixture comprised of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, is used along with other strains from these groups of Lactobacilli, Streptococci and/or Bifidobacteria, the additional use of strains from these groups of Lactobacilli, Streptococci and/or Bifidobacteria allowing the selective use or selective inhibition of special microorganisms in selective regions of the gastrointestinal tract so as to particularly inhibit the occurrence of a specific disease caused by pathogenic germs.

According to a preferred further development of the invention, the mixture of microorganisms is used to strengthen the immune defence. Such a use is, in particular, enabled by the selective choice of the microorganisms used and their origins from the most diverse regions of the digestive tract safeguarding that the microorganisms used will develop their activities over the entire length of the digestive system and, hence, will, in fact, markedly strengthen the immune defence of the thus treated mammal or farm bird against specific pathogenic germs in the entire digestive tract.

According to a preferred further development, the mixture of microorganisms is used along with at least one gelatinizing agent and/or pellet former or coating material selected from maltodextrins, guar seed flour, gum arabic, alginates, modified starch and starch derivates, dextrins, cellulose derivates, proteins, acacia gum, tragacanth,

lipids. By using the mixture of microorganisms together with a gel and/or pellet former, its use as a so-called pellet of gel application becomes feasible so as to allow, in particular, extremely young animals which can no longer be supplied with the appropriate immunoenhancing substances by their mothers to be safely and reliably supplied with the necessary microorganisms required to strengthen their immune defence. When using such a gel or pellet application, the mixture of microorganisms is covered with gelatinizing agents or encapsulated, and this mixture is directly sprayed or applied on the young animals to be supplied therewith, or, for instance, introduced into transport boxes in the event of young chicks, so as to safeguard the supply of the young animals with the microorganisms that are of essential importance to them. A similar, preferred use will be accomplished in that the mixture of microorganisms, along with a liquid carrier, as a spray suspension is directly applied on the young animals to be treated therewith. By spraying young animals with the mixture of microorganisms, a portion of this spray mixture will be taken up into the digestive tract by their own coat care or contact with other young animals, whereby the supply with the microorganisms that are essential for strengthening their immune defence will again be safeguarded.

In the following, the invention will be explained in more detail by way of exemplary embodiments. Therein, Example 1 illustrates the inhibition of pathogenic germs by isolated test germs in an agar plate assay model; Example 2 illustrates a feeding test in which day-old chicks are fed a feed and drinking water additive containing five, three or no microorganisms; Example 3 is a field study in which

day-old chicks are fed a drinking water additive containing five microorganisms; Example 4 illustrates a feeding test in which day-old chicks received a drinking water additive containing five microorganisms at defined times; Example 5 demonstrates Salmonella challenge in day-old chicks which were administered microorganisms either through drinking water or through fodder at defined times, with a mixture of three or five microorganisms having been used; and Example 6 shows a feeding test in weaned piglets.

Example 1

Inhibition of pathogenic germs by the isolated CE test germs (competitive exclusion test germs) according to the invention in an agar plate assay model

The assay was performed on MRS agar plates. The strains were applied on the agar plates by inoculation, the strains having been directly taken from cooling vials. The incubation of the plates took place for 48 hrs under anaerobic conditions. After the culturing of the pathogenic strains in the appropriate media, the plates were carefully poured with about 9 ml of semisolid medium containing the respective pathogenic strain. For E. coli and Salmonella chloeraesuis, semisolid VL medium, for Campylobacter jejuni, semisolid Brain Heart Infusion Medium, and for Clostridium perfringens, semisolid Reinforced Clostridial Medium, were used.

The plates were subsequently incubated under the respective incubation conditions of the pathogenic strains. The evaluation of the plates was effected by measuring the diameters of the test germs and inhibition zones and calculating therefrom the inhibition zone to test germ

ratios. Based on the inhibition zone/test germ ratios, an assessment of the inhibiting action of the test germs can be made. Table 1 below indicates the results for the strains used separately and in mixture.

Table 1

Isolate	Strain	E.coli	E.coli	Salmonella choleraesuis	Campylo-bacter jejuni	Clostri-dium per-fringens
DSM 16211	**Enterococcus faecium**	2.11	1.44	1.37	1.28	1.43
DSM 16284	**Bifidobacterium animalis**	0.45	0.85	0.84	0.33	1.85
DSM 16210	**Pediococcus acidilactici**	1.52	1.38	1.34	1.47	1.51
DSM 16351	**Lactobacillus salivarius**	1.49	2.26	1.46	1.50	2.05
DSM 16350	**Lactobacillus reuteri**	2.53	1.42	1.60	1.36	2.12
area		0.45 - 2.5	0.85 - 2.26	0.84 - 1.37	0.33 - 1.5	1.01 - 2.12
NCIMB 10415	*Enterococcus faecium*	*1.37*	*1.35*	*1.20*	*1.22*	*1.31*
DSM 20104	*Bifidobacterium animalis*	*0.41*	*0.79*	*0.65*	*0.31*	*1.42*
DSM 20284	*Pediococcus acidilactici*	*1.41*	*1.20*	*1.10*	*1.32*	*1.35*
DSM 20555	*Lactobacillus salivarius*	*1.40*	*2.19*	*1.09*	*1.41*	*1.63*
DSM 20016	*Lactobacillus reuteri*	*2.14*	*1.30*	*1.20*	*1.35*	*1.54*
Mean activity of individual strains		1.48	1.56	1.11	0.92	1.57
DSM 16211, DSM 16210, DSM 16284		1.70	1.70	1.90	1.30	1.60
DSM 16211, DSM 16350, DSM 16351		2.50	1.80	1.60	1.30	1.80
DSM 16210, DSM 16211, DSM 16350, DSM 16351, DSM 16284		2.60	1.90	2.10	1.70	1.90

From a comparison of the deposited microorganisms (written in bold letters) with related strains (written in cursive letters), it is clearly apparent that the deposited microorganisms (written in bold letters) are able to inhibit pathogenic germs at considerably higher degrees than already known strains (cursively written).

Example 2

Feeding test in day-old chicks which were administered either a mixture of all five deposited microorganisms or a mixture of three deposited microorganisms, the mixtures having been administered via drinking water in the starting period and, additionally, via the feed during the whole test period.

Test parameters:

600 day-old chicks (race: Ross 308) were divided into three groups:

Group 1: control without additives

Group 2: five microorganisms; days 1-21 in drinking water; days 1-42 via feed

Group 3: DSM 16210, DSM 16211, DSM 16284; day 1-21 in drinking water; days 1-42 via feed

Water and feed were available ad libitum for intake by the animals.

Recorded performance parameters:

Live weight, daily weight gains, feed intake, feed conversion ratio (= FCR), mortality, European Production Efficiency Factor (EPEF).

Table 2: Summary of performance data in feeding test

Group	Live weight (g)	Daily gain (g)	Feed intake (g/animal)	FCR	Mortality	EPEF
Control	2588[a]	60.0	91.0	1.68	6.4	343
DSM 16210, DSM 16211, DSM 16284	2657 [ab]	62.4	91.3	1.68	5.0	357
DSM 16211, DSM 16284, DSM 16210, DSM 16351, DSM 16350	2689[b]	63.1	92.3	1.69	3.3	366

a, b sign. difference ($P < 0,05$)
* EPEF (European Production Efficiency Factor)

The use of the products allowed significant improvements in the live weights of the animals in the test groups. The feed intake could be slightly increased in the product group containing all five microorganisms, no difference was, however, observed in respect to the feed conversion ratio. The mortality was high in general, since the test was performed in midsummer and the animals were confronted with very high ambient temperatures. Despite this additional stress factor, a marked increase in the performance of the product groups could, however, be observed.

Example 3

In a field test, a mixture of all five microorganisms was tested in day-old chicks under practical conditions and administered in drinking water over the entire period.

Test parameters:

About 53,000 day-old chicks were equally divided between two storeys.

Ground floor: control without additives

1st floor: all five microorganisms in drinking water

Water and feed were available ad libitum to the animals.

Recorded performance parameters:

Live weight, mortality

As is apparent from Table 3 below, the performances of the animals could be markedly increased, above all also in comparison to preceding production cycles listed in Table 4, with the previously occurring E. coli problems having been resolved.

Table 3: Summary of performance data

	Live weight (g)	Mortality %
Ground floor	2282	3.38
1st floor	2328	3.03

Table 4: Performances of the farm's last six production cycles

	Number of animals	Mortality (%)	Live weight (g)
1	51000	5.55	2109
2	49990	6.46	2228
3	49000	2.65	2043
4	50592	5.41	1627
5	48450	5.09	1785
6*	53244	3.20	2305

* ... with all five microorganisms

Example 4

In a feeding test, day-old chicks were administered a mixture of all five microorganisms via drinking water at defined times.

Test parameters:

400 day-old chicks, race: Ross 308, were divided between two groups according to the principle of contingency:

Group 1: control; without additives

Group 2: five microorganisms in drinking water on days 1, 2, 3, 7, 8, 9 and 14, 15, 16

Water and feed were available ad libitum for intake by the animals.

Recorded performance parameters:

Live weight gains, feed intake, feed conversion ratio (= FCR), mortality, EPEF (European Production Efficiency Factor).

The results are summarized in Table 5.

Table 5: Summary of performance data

	Control	Five microorganis-ms
Live weight (g)	2320.66a	2392.19b
Weight gain (g)	54.17	55.87
Feed consumption (g)	4365	4482
Feed conversion ratio (= FCR)	1.92	1.90
Mortality (%)	1	1.5
EPEF*	285	295

a, b sign. differences ($P < 0,05$)
* EPEF (European Production Efficiency Factor)

Due to the addition of the microorganisms, the live weights of the animals could be significantly increased. In terms of feed intake and feed conversion ratio, the two groups were comparable with each other. The productivity mass number (= EPEF) was clearly higher in the product group than in the control group.

Example 5

Salmonella challenge in day-old chicks

A product comprised of all five microorganisms, and a product comprised of three microorganisms, were

administered in feed over the entire test period and via drinking water in the starting phase.

Test parameters:

304 day-old chicks, race: Ross 308, were divided among four groups:

Group 1: negative control; without additives and without Salmonella challenge

Group 2: positive control; without additives; with Salmonella challenge

Group 3: with Salmonella challenge + product of five microorganisms: days 1-21 in drinking water; days 1-42 via feed

Group 4: with Salmonella challenge + product of three microorganisms (DSM 16211, DSM 16210, DSM 16284): days 1-21 in drinking water; days 1-42 via feed

Water and feed were available ad libitum for intake by the animals.

On day 5, all animals of groups 2, 3 and 4 were orally inoculated with a dose of 6 x 10^5 cfu Salmonella enteritidis. The negative control was carried along for the control of the normal performance data.

Recorded performance parameters:

Live weight gain, feed intake, feed conversion ratio (= FCR), morbidity (measured by the number of diarrhoeas), mortality, EPEF (European Production Efficiency Factor).

The results are summarized in Table 6.

Table 6: Summary of performance data

	1	2	3	4
Total weight gain (g)	2847.29[a]	2632.39[b]	2716.49[ab]	2702.18
Feed consumption (g)	4790.07[a]	4559.11[b]	4534.30[b]	4527.02
Feed conversion ratio (= FCR)	1.68	1.73	1.67	1.67
% Morbidity	6.34[a]	19.14[b]	15.65[b]	16.79[b]
% Mortality	0.96	0.47	0.72	0.669
EPEF*	422	385	416	412

a, b sign. differences ($P < 0,05$)
* EPEF (European Production Efficiency Factor)

In regard to the total weight gain, notable performance losses occurred due to the infection with Salmonella enteritidis as compared to the control group, which could be improved by the addition of the products according to the invention. It was shown that marked improvements could be reached by the addition of both the product comprising three microorganisms and that comprising five micro-organisms. Also the feed intake had been adversely affected by the infection, yet the feed conversion ratio could be markedly improved by the addition of the products according to the invention. Even the morbidity, measured by the number of diarrhoeas observed, could be markedly reduced by the addition of the products as compared to the untreated group.

Example 6

Feeding test in day-old chicks - Salmonella challenge

Three groups each consisting of 36 day-old chicks were subjected to a feeding test using Salmonella challenge. A first group was administered none of the products, a second group was administered a mixture of all five deposited

strains, and a third group received a commercially available probiotic product comprising the following microorganisms: Lactobacillus sp., Lactobacillus sp., Bifidobacterium sp und Enterococcus faecium.

Test approach:

The day-old chicks were kept in cages in an isolated room. The probiotic products were administered on life days 1, 2 and 3 via the drinking water, which was supplied ad libitum to the chicks. On the third day of life, all chicks were orally administered 1.0 ml of a suspension of Salmonella enteriditis. The concentration of the salmonella solution was 1.0×10^6 CFU/ml. All chicks additionally received feed and water ad libitum. The concentration of salmonellas in the animals' excrements was measured.

Evaluation:

Group	Concentration of salmonellas in excrements (CFU log 10)
Commercially available probiotic product	2.43 [b]
Product mixture	n.d. (<2,00)[a]
Control	3.2

The results demonstrate that only the combination of the five deposited microorganisms was able to significantly reduce the growth of salmonellas to such an extent that the number of salmonella germs fell below the detection limit.

Example 7

Feeding test in weaned piglets, using a mixture comprising five microorganisms and a mixture comprising three microorganisms, respectively.

In a feeding test, the microorganisms according to the present invention were tested for their effects on the performances of weaned piglets. The products were administered via the feed over the entire test period of 47 days.

Test parameters:

101 weaned piglets, race: ÖHYB, were divided among three groups:

Group 1: control without additives

Group 2: five microorganisms (C5); days 1-47 via feed;

Group 3: three microorganisms (C3) comprising DSM 16211, DSM 16350, DSM 16351; days 1-47 via feed

Water and feed were available ad libitum for intake by the animals.

Recorded performance parameters:

Live weight, daily weight gains, feed intake, feed conversion ratio (= FCR), mortality

The results are summarized in Table 7.

Table 7: Summary of performance data

	Control	C5	Difference between C5 and control	C3	Differencebetween C3 and control
Number of animals	34	33		33	
Initial weight kg)	7.75	7.76		7.75	
Weight day 13 (kg)	11.89	12.26	+ 3.1 %	12.09	+ 1.7 %
Final weight (kg)	31.67	33.00	+ 4.2 %	32.5	+ 2.6 %
Daily gain days 1-13 (g)	321	350	+ 9.0 %	334	+ 4.0 %
Daily gain days 14-47 (g)	582	610	+ 4.8 %	600	+ 3.09 %
Daily gain days 1-47 (g)	509	537	+ 5.5 %	527	+ 3.5 %
Mortality (number)	2	2			

The use of the products allowed significant improvements in the live weights of the animals in the test groups, namely by 3% and 1.7%, respectively, after the first 13 days, and by 4% and 2.6%, respectively, until the end of the test. A marked increase by the addition of the products could also be obtained in terms of daily weight gains.

In Table 8 below, the feed data are summarized.

Table 8: Summary of feed data

	Control	C5	Difference	C3	Difference
Feed consumption (g)					
Days 1-13	495	511	+ 3.2 %	504	+ 1.8
Days 14-47	1155	1193	+ 3.3 %	1180	+ 2.2
Days 1-47	972	1033	+ 6.3	1012	+ 4.1
FCR (kg/kg)					
Days 1-13	1.76	1.66		1.68	
Days 14-47	1.98	2.02		2.00	
Days 1-47	1.95	1.96		1.95	

The feed intakes could be slightly increased in the product groups, in terms of feed conversion ratios an improvement was above all noticed in the first test period. In the further course, no differences in the feed conversion ratios could, however, be determined.

List pursuant to Rule 13bis, para 4, of the Regulations Under the Patent Cooperation Treaty.

All of the microorganisms mentioned in the present application were deposited at the German Collection of Microorganisms and Cell Cultures GmbH - DSMZ, Mascheroder Weg 1b, 38124 Braunschweig, Germany (DE).

Accession number	Accession date
DSM 16284	10.03.2004
DSM 16211	06.02.2004
DSM 16210	06.02.2004
DSM 16350	15.04.2004
DSM 16351	15.04.2004

Example 8

Positive shift of intestinal flora in broiler chickens

Four groups of broiler chickens were subjected to a feeding test, 2 groups having been administered a product mixture

comprised of five deposited microorganisms, one group having been a negative control without any additive, one group having been a positive control with the addition of an antibiotic performance promoter, namely avialmycin, one group having received the mixture of five microorganisms via drinking water, and one group having been administered the mixture of microorganisms via the feed.

In the course of examination, it turned out that there were no statistically significant differences among the individual groups in terms of the total count of aerobic germs, coliformes, the total count of anaerobic germs and bacteroids. The groups that had received the mixture of five microorganisms showed significantly higher germ numbers in respect to Bifidobacterium sp, Lactobacillus spp and Gram+cocci (e.g. Enterococcus, Pediococcus) when compared to the positive and negative controls (NK, PK).

A flow chart of this comparison is illustrated in the Figure.

The flow chart of the Figure, in the black-framed part, shows that the groups having received the mixture of five microorganisms via drinking water or feed, respectively, exhibits considerably higher numbers of "good" germs. In the diagram of the Figure, the negative control group is represented as a black bar, the positive control group is represented as a hatched bar, the group that was administered the five microorganisms via the feed is indicated by a dotted bar, and the group that was administered the mixture of five microorganisms via the drinking water is illustrated as a plain grey bar.

Example 9

Feeding test in broiler chickens

4,500 broiler chickens (Ross 308) of mixed sexes were divided between two groups, each of the two groups having been subdivided into three further subgroups to facilitate observation. Both groups received a standard feedstuff, and the test group received a mixture of the five deposited microorganisms at a dose of $1*10^9$ CFU/kg feed. The control group was administered a commercially available probiotic product at the same dose. The administration of the substances was effected via the feedstuff.

After 38 days, it was found that the performance parameters of the test group were markedly improved both in terms of live weight and daily weight gains and feed conversion ratios. In addition, the mortality had significantly dropped relative to the comparative group. The results are indicated in Table 9 below.

Table 9

Results after 38 days

	Commercially available probitic product	C5 product mixture
Number of animals	2250	2250
Live weight(g)	1875	2034
Daily weight gains (g)	52	56.5
Feed conversion ratio	1.66	1.61
Mortality (%)	1.15	0.40

Example 10

In a laboratory test, the antibiotic resistances of the five deposited microorganisms were investigated and, where available, compared with the antibiograms of related bifidobacteria. It was only with the Bifidobakterium animalis strain that no comparative strain could be found in the literature or secured data could be obtained for comparative strains on account of difficult cultivation conditions. The results of the resistance tests in respect to common antibiotics, along with comparative strains where available, are indicated in the Tables below.

	Pediococcus acidilacti, DSM 16210	Pediococcus acidilacti, reference strain
β-Lactams - ampicillin	S	S
Aminoglycodsides		
Streptomycin	S	R
Kanamycin	S	R
Neomycin	S	R
Gentamicin	S	R
Amphenicol - chloroamphenicol	S	S
Macrolid - erythromycin	S	S
Ansamycin - rifampin	S	S
Streptogramin - chinu/dalfopristin	S	R
Fluorochinolone - enrofloxacin	S	n.a.
Oxazolidinone - linezolid	S	R

	Enterococcus faecium, DSM 16211	Enterococcus faecium, reference strain 1	Enterococcus faecium, reference strain 2
β-Lactame - ampicillin	S	S	S
Aminoglycosides			
Streptomycin	S	R	S
Kanamycin	S	R	R
Neomycin	S	R	R
Gentamicin	S	R	S
Amphenicol - chloroamphenicol	S	S	S
Tetracyclin	S	R	S
Macrolide - erythromycin	S	R	R
Ansamycin - rifampin	S	S	S
Streptogramin- cinu/dalfopristin	S	R	n.a.
Oxazolidinone- linezolid	S	S	n.a.
Folate inhibitor- trimethoprim	S	R	n.a.
Glycopeptide - vancomycin	S	S	S

	Lactobacillus saliv. ssp. Saliv., DSM 16351	Lactobacillus salivarius, reference strain 1	Lactobacillus salivarius, reference strain 2
β-Lactame - ampicillin	S	S	S
Amphenicol - chloroamphenicol	S	S	S
Tetracyclin	S	S	R
Macrolid - erythromycin	S	S	S
Ansamycin - rifampin	S	S	S
Streptogramin - chinu/dalfopristin	S	R	S
Oxazolidinone - linezolid	S	S	S
Folate inhibitor - trimethoprim	S	R	R

	Lactobacillus reuteri, DSM 16350	Lactobacillus reuteri, reference strain
Amphenicol - chloramphenicol	S	S
Mcrolid - erythromycin	S	R
Ansamycin - rifampin	S	S
Streptogramin - chinu/dalfopristin	S	R
Oxazolidinone - linezolid	S	S

	Bifidobacterium animalis, DSM 16284	
β-Lactame - ampicillin	S	
Aminoglycosides		
Neomycin	S	
Gentamicin	S	
Amphenicol - chloroamphenicol	S	
Tetraciclin	S	
Macrolid - erythromycin	S	
Ansamycin - rifampin	S	
Strptogramin - chinu/dalfopristin	S	
Fluorochinolone- enrofloxacin	S	
Oxazolidinone- linezolid	S	
Folate inhibitor - trimethoprim	S	
Glycopeptid- vancomycin	S	

S ... sensitive, R ... resistent

From these comparative tests results that none of the deposited microorganisms became resistant to any of the known antibiotics tested, they all remained sensitive and susceptible.

Annex to PCT application
Applicant: Erber Aktiengesellschaft et al.

List pursuant to Rule 13bis, para 4, of the Regulations Under the Patent Cooperation Treaty.

All of the microorganisms mentioned in the present application were deposited at the German Collection of Microorganisms and Cell Cultures GmbH - DSMZ, Mascheroder Weg 1b, 38124 Braunschweig, Germany (DE).

Accession number	Accession date
DSM 16284	10.03.2004
DSM 16211	06.02.2004
DSM 16210	06.02.2004
DSM 16350	15.04.2004
DSM 16351	15.04.2004

Throughout this specification and the claims, unless the context requires otherwise, the word "comprise" and its variations, such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that prior art forms part of the common general knowledge in Australia.

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C l a i m s :

1. A probiotic health and performance promoting food, feed and/or drinking water additive containing a mixture of microorganisms, characterized in that it comprises at least two microorganisms selected from the group consisting of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284.

2. The food, feed and/or drinking water additive according to claim 1, characterized in that it comprises at least three microorganisms.

3. The food, feed and/or drinking water additive according to claim 1 or 2, characterized in that it comprises Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351.

4. The food, feed and/or drinking water additive according to claim 1 or 2, characterized in that it comprises Enterococcus faecium, DSM 16211, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284.

5. The food, feed and/or drinking water additive according to any one of claims 1 to 4, characterized in that it comprises nutritive substances and/or prebiotic substances additionally usable as carriers and selected from the group comprising fructo-oligosaccharides, inulins, isomalto-oligosaccharides, lactitol, lactosucrose, lactulose,

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pyrodextrines, soy oligosaccharides, transgalacto-oligosaccharides, xylo-oligosaccharides, and vitamins, including vitamin E.

6. The food, feed and/or drinking water additive according to any one of claims 1 to 5, characterized in that it comprises a further carrier selected from the group comprising zeolites, calcium carbonate, magnesium carbonate, trehalose, chitosan, shellac, albumin, starch, skim-milk powder, swee-whey powder, maltodextrin, lactose, inulin, dextroses, vegetable oils, and a solvent selected from water or physiologic saline solution.

7. The food, feed and/or drinking water additive according to any one of claims 1 to 6, characterized in that it additionally comprises a coating material selected from the group comprising maltodextrins, guar seed flour, gum arabic, alginates, modified starch and starch derivates, dextrins, cellulose derivates including cellulose ester and ether, proteins including gelatine, albumin, casein, gluten, acacia gum, tragacanth, lipids including waxes, paraffin, stearic acid, and mono- and diglycerides.

8. The food, feed and/or drinking water additive according to any one of claims 1 to 7, characterized in that it additionally comprises at least one microorganism selected from the group consisting of Bifidobacterium sp., Lactobacillus salivarius, Lactobacillus sp., Lactobacillus fermentum and Enterococcus faecalis.

9. The food, feed and/or drinking water additive according to any one of claims 1 to 8, characterized in that the

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microorganisms are contained in suspended, powdery or encapsulated form with a maximum diameter of 2000 µm.

10. A feed additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that said feed additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Enterococcus faecium, DSM 16211, and 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350.

11. The feed additive according to claim 10, characterized in that said feed additive comprises 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Enterococcus faecium, DSM 16211, and 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350.

12. A feed additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that said feed additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Pediococcus acidilactici, DSM 16210, and 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Bifidobacterium animalis, DSM 16284.

13. The feed additive according to claim 12, characterized in that said feed additive comprises 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Pediococcus acidilactici, DSM 16210, and 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Bifidobacterium animalis, DSM 16284.

14. A feed additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that said feed additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350, and 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive of Lactobacillus salivarius ssp. salivarius, DSM 16351.

15. The feed additive according to claim 14, characterized in that said feed additive comprises 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350, and 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Lactobacillus salivarius ssp. salivarius, DSM 16351.

16. A feed additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that said feed additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350, 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Pediococcus acidilactici, DSM 16210, 1 x 10^7 to 1 x 10^{14} CFU/kg feed additive of Bifidobacterium animalis, DSM 16284, and 1 x 10^6 to 1 x 10^{14} CFU/kg feed additive of Bifidobacterium sp. 2.

17. The feed additive according to claim 16, characterized in that said feed additive comprises 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Enterococcus faecium, DSM 16211, 1

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x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Lactobacillus reuteri, DSM 16350, 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Pediococcus acidilactici, DSM 16210, 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Bifidobacterium animalis, DSM 16284, and 1 x 10^{10} to 1 x 10^{12} CFU/kg feed additive of Bifidobacterium sp. 2.

18. A feed additive according to any one of claims 12 to 17, characterized in that the feed additive additionally comprises 1% to 95%of a carrier or natural substance.

19. The feed additive according to claim 18, characterized in that the feed additive additionally comprises 20% to 98% of a carrier or natural substance.

20. A feed additive according to any one of claims 12 to 17, characterized in that the feed additive is applied on the feed as an aqueous suspension in an amount of from 0.01 g/kg to 10 g/kg feed.

21. The feed additive according to claim 20, characterized in that the feed additive is applied on the feed as an aqueous suspension in an amount of from 0.025 g/kg to 2.5 g/kg feed.

22. A feed additive according to any one of claims 10 to 21, characterized in that the feed additive is mixed into the feed in an amount of from 20 g/t to 20 kg/t.

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23. The feed additive according to claim 22, characterized in that the feed additive is mixed into the feed in an amount of from 100 g/t to 2.5 kg/t.

24. A drinking water additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that the drinking water additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, and 1 x 10^6 to 1 x 10^{14} CFU/kg drinking water additive of Lactobacillus reuteri, DSM 16350.

25. The drinking water additive according to claim 24 characterized in that the drinking water additive comprises 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, and 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Lactobacillus reuteri, DSM 16350.

26. A drinking water additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that the drinking water additive comprises 1 x 10^7 to 1 x 10^{14} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, 1 x 10^6 to 1 x 10^{14} CFU/kg drinking water additive of Lactobacillus reuteri, DSM 16350, and 1 x 10^6 to 1 x 10^{14} CFU/kg drinking water additive of Lactobacillus salivarius ssp. salivarius, DSM 16351.

27. The drinking water additive according to claim 26 characterized in that the drinking water additive comprises 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, 1 x 10^{10} to 1 x 10^{13} CFU/kg

drinking water additive of Lactobacillus reuteri, DSM 16350, and 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Lactobacillus salivarius ssp. salivarius, DSM 16351.

28. A drinking water additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that the drinking water additive comprises 1 x 10^{7} to 1 x 10^{14} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, 1 x 10^{7} to 1 x 10^{14} CFU/kg drinking water additive of Pediococcus acidilactici, DSM 16210, and 1 x 10^{7} to 1 x 10^{14} CFU/kg drinking water additive of Bifidobacterium animalis, DSM 16284.

29. The drinking water additive according to claim 28 characterized in that the drinking water additive comprises 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive of Pediococcus acidilactici, DSM 16210, and 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Bifidobacterium animalis, DSM 16284.

30. A drinking water additive for farm birds and/or pigs to increase the performance of said farm animals, according to any one of claims 1 to 9, characterized in that the drinking water additive comprises 1 x 10^{7} to 1 x 10^{14} CFU/kg drinking water additive of Enterococcus faecium, DSM 16211, 1 x 10^{6} to 1 x 10^{14} CFU/kg drinking water additive Lactobacillus reuteri, DSM 16350, 1 x 10^{6} to 1 x 10^{14} CFU/kg drinking water additive Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^{7} to 1 x 10^{14} CFU/kg drinking water additive Pediococcus acidilactici, DSM 16210, 1 x 10^{7}

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to 1 x 10^{14} CFU/kg drinking water additive Bifidobacterium animalis, DSM 16284, and 1 x 10^{6} to 1 x 10^{14} CFU/kg drinking water additive Bifidobacterium sp. 2.

31. The drinking water additive according to claim 30 characterized in that the drinking water additive comprises 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Enterococcus faecium, DSM 16211, 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Lactobacillus reuteri, DSM 16350, 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Lactobacillus salivarius ssp. salivarius, DSM 16351, 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Pediococcus acidilactici, DSM 16210, 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Bifidobacterium animalis, DSM 16284, and 1 x 10^{10} to 1 x 10^{13} CFU/kg drinking water additive Bifidobacterium sp. 2.

32. A drinking water additive according to any one of claims 24 to 31, characterized in that the drinking water additive additionally comprises 1% to 95% of a carrier or nutrient.

33. The drinking water additive according to claim 32 characterized in that the drinking water additive additionally comprises 20% to 92% of a carrier or nutrient.

34. Use of a mixture of at least two microorganisms selected from the group consisting of Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, as a probiotic health and performance promoting food, feed and/or drinking water additive.

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35. The use according to claim 34, characterized in that Enterococcus faecium, DSM 16211, Lactobacillus reuteri, DSM 16350, and Lactobacillus salivarius ssp. salivarius, DSM 16351, are used.

36. The use according to claim 34, characterized in that Enterococcus faecium, DSM 16211, Pediococcus acidilactici, DSM 16210, and Bifidobacterium animalis, DSM 16284, are used.

37. The use according to claim 34, characterized in that Enterococcus faecium, DSM 16211, and Lactobacillus reuteri, DSM 16350, are used with other strains of Lactobacilli, Streptococci and/or Bifidobacteria.

38. The use according to any one of claims 34 to 37, characterized in that the mixture of microorganisms is used to increase immune defence.

39. The use according to any one of claims 34 to 38, characterized in that the mixture of microorganisms is used with at least one gelatinizing agent selected from the group comprising maltodextrins, guar seed flour, gum arabic, alginates, modified starch and starch derivates, dextrins, cellulose derivates, proteins, acacia gum, tragacanth, and lipids.

40. The use according to any one of claims 34 to 39, characterized in that the mixture of microorganisms, along with a liquid carrier, is used as a spray suspension.

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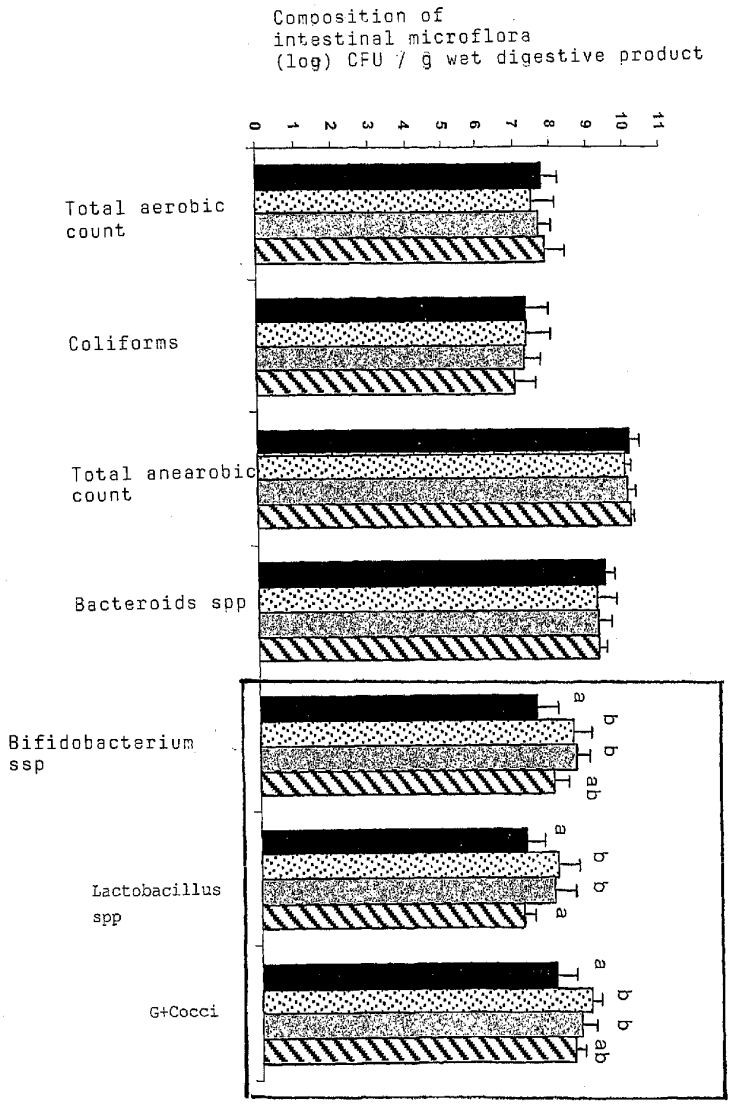
41. A probiotic health and performance promoting food, feed and/or drinking water additive containing a mixture of microorganisms substantially as herein described with reference to any one or more of the examples, excluding comparative examples.

42. A feed additive for farm birds and/or pigs to increase the performance of said farm animals substantially as herein described with reference to any one or more of the examples, excluding comparative examples.

43. A drinking water additive for farm birds and/or pigs to increase the performance of said farm animals substantially as herein described with reference to any one or more of the examples, excluding comparative examples.

44. Use of a mixture of at least two microorganisms as a probiotic health and performance promoting food, feed and/or drinking water additive substantially as herein described with reference to any one or more of the examples, excluding comparative examples.


Composition of
intestinal microflora
(log) CFU / g wet digestive product
0 1 2 3 4 5 6 7 8 9 10 11
Total aerobic count
Coliforms
Total anearobic count
Bacteroids spp
Bifidobacterium ssp
a b b ab
Lactobacillus spp
a b b a
G+Cocci
a b b ab

FIGURE