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(54) **Title**: COMPOSITIONS AND METHODS FOR CONTROLLING DISEASE IN ANIMALS

(57) **Abstract**: Disclosed herein are compositions for the treatment of a disease in an animal including yeast extract of Saccharomyces cerevisiae, *Bacillus licheniformis* or *Bacillus subtilis* spores, and a carrier. Also included are animal feed compositions including the composition for the prevention, control and/or treatment of a disease in an animal and an animal's food/feed. The compositions are useful to prevent, control, and treat diseases such as necrotic enteritis in poultry when used in combination with an anticoccidal ionophore or coccidiosis vaccine.

COMPOSITIONS AND METHODS FOR CONTROLLING DISEASES IN ANIMALS

This application claims benefit under 35 USC § 119(e) of U.S. Provisional patent Application Serial No. 61/187,316 filed June 3, 2009, U.S. Provisional patent Application Serial No. 61/156,902 filed March 3, 2009 and U.S. Provisional patent Application Serial No. 61/121,258 filed December 10, 2008.

BACKGROUND

[0001] Pigs and poultry, especially those which are intensively reared or reared in large-scale operations, have a tendency to suffer from or risk catching a variety of diseases and infections, for example, *Mycoplasma* diseases in pigs and poultry, *Lawsonia* infections (ileitis) and swine dysentery in pigs and necrotic enteritis in poultry. Medicaments have been proposed or used for the treatment of individual diseases or infections of these types.

[0002] *L. intracellularis,* the causative agent of porcine proliferative enteropathy (PPE; also called ileitis in swine), affects virtually all animals, including: rabbits, ferrets, hamsters, fox, horses, and other animals as diverse as ostriches and emus. PPE is a common diarrheal disease of growing-finishing and young breeding pigs characterized by hyperplasia and inflammation of the ileum and colon. It often is mild and self-limiting but sometimes causes persistent diarrhea, severe necrotic enteritis, or hemorrhagic enteritis with high mortality.

[0003] Necrotic enteritis (NE) in poultry is caused by a gram-positive, anaerobic bacteria *Clostridium perfringens*. The disease is an acute enterotoxemia condition primarily affecting 2-5 week old broiler chickens and 7-12 week old turkeys. The duration of the illness is very short, and typically the only sign of the disease is a sudden increase in mortality of the birds.

[0004] *C. perfringens* is a nearly ubiquitous bacteria found in soil, dust, feces, feed, and used poultry litter, and is also an inhabitant of the intestines of healthy chickens. The enterotoxemia that causes the necrotic enteritis occurs either following an alteration in the intestinal microflora or from a condition that results in damage to the intestinal mucosa (e.g., coccidiosis, mycotoxicosis, salmonellosis, ascarid larvae). High dietary levels of animal byproducts, wheat, barley, oats, or rye predispose birds to the

disease. Anything that promotes excessive bacterial growth and toxin production or slows feed passage rate in the small intestine could promote the occurrence of necrotic enteritis.

[0005] Diagnosis of necrotic enteritis is based on gross lesions and a gram-stained smear of a mucosal scraping that exhibits large, gram-positive rods. The gross lesions are primarily found in the small intestine, which may be ballooned, friable, and contain a foul-smelling, brown fluid. The mucosa is usually covered with a tan to yellow pseudomembrane often referred to as a "Turkish towel" appearance. This pseudomembrane may extend throughout the small intestine or be only in a localized area. The disease persists in a flock for 5-10 days, and mortality is 2-50%. Conventional products for preventing necrotic enteritis in poultry are medicated feeds containing virginiamycin (20 g/ton feed), bacitracin (50 g/ton), and lincomycin (2 g/ton). Medicated feeds containing anticoccidial compounds in the ionophore class have also been helpful in preventing necrotic enteritis. Treatment for necrotic enteritis is typically by administering bacitracin, penicillin, and lincomycin in the drinking water for 5-7 days. NE has been identified as a disease condition that may be prevented or controlled by use of Direct-Fed Microbials (DFM) products because they act on the intestinal microflora.

[0006] Coccidiosis causes considerable economic loss in the poultry industry. The disease is caused by several species of *Eimeria* including *E. tenella, E. necatrix, E. acervulina, E. brunetti,* and *E. maxima.* Stages of coccidiosis in chicken appear both within the host as well as outside. The developmental stages in the chicken give rise to a microscopic egg (called an oocyst) that is passed out in the droppings. Normally, most birds pass small numbers of oocysts in their droppings without ill effects, but intensive rearing of domestic chickens provides conditions which permit the build-up of infective oocysts in the environment thereby increasing the possibility of coccidiosis infections. Coccidiosis infections can become noticeable by the third day of infection. Symptoms include chickens drooping, stopping feeding and huddling together. Blood starts to appear in the droppings by day four and by day eight the chickens are either dead or have started to recover.

[0007] Prevention of coccidiosis includes mixing anticoccidial drugs with feed. Ionophores such as salinomycin are the most commonly-used drugs in the US for

coccidiosis prevention. Salinomycin and salts thereof are typically added to animal feed at a concentration of about 40 to about 60 grams per ton of animal feed (0.0044% to 0.0066%). Salimomycin sodium is the preferred form of salinomycin used in the United States. Narasin is the most commonly-reported ionophore promoted as an aid in NE prevention. Coccidiosis vaccines (Coccivac®-B) can also be used particularly by those wishing to market antibiotic-free chickens. Coccivac-B is a non-attenuated, live sporulated oocyst coccidiosis vaccine containing *E. acervulina, E. mivati, E. maxima* and *E. tenella.*

[0008] A need exists for compositions and methods to treat multiple diseases in animals, particularly diseases of bacterial origin. These compositions should be able to be used in combination with other treatments and/or compositions used for treating other diseases. Optimally the compositions for treatment of diseases would have a synergistic effect when combined either with other compositions or other treatment regimens.

BRIEF SUMMARY

[0009] The present invention is related to compositions and methods for treatment of disease in animals, particularly poultry. One embodiment of the invention is directed to a composition for the treatment of a disease in an animal that includes a yeast extract from *Saccharomyces cerevisiae*, *Bacillus licheniformis* spores, and optionally a carrier. An embodiment of the invention is also directed a method for preventing a disease in an animal that includes administering to an animal a composition containing yeast extract from *Saccharomyces cerevisiae*, *Bacillus licheniformis* spores, and optionally a carrier.

[0010] The present invention is also directed to a composition for the prevention and/or control of a disease in an animal which includes a yeast extract from *Saccharomyces cerevisiae* and QST-713 strain of *Bacillus subtilis*, and optionally a carrier. An embodiment of the invention is also directed to a method for preventing and/or controlling a disease in an animal that includes administering to the animal an effective amount of a composition containing a yeast extract from *Saccharomyces cerevisiae* and QST-713 strain of *Bacillus subtilis*, and optionally a carrier.

[0011] The present invention is further related to a composition containing about 50 wt% to about 90 wt% of the cell wall fraction from *Saccharomyces cerevisiae*; about 5

wt.% to about 50 wt.% of *Bacillus licheniformis* spores, comprising about $4.5x10^9$ to about $2.5x10^{10}$ spores per gram; and an anticoccidal ionophore. An embodiment of the invention is also directed to a method for preventing disease in an animal that includes vaccinating the animal with a coccidiosis vaccine and administering to the animal a composition containing about 50 wt.% to about 90 wt.% of the cell wall fraction from *Saccharomyces cerevisiae*; about 5 wt.% to about 50 wt.% of the *Bacillus licheniformis* spores, comprising about $4.5x10^9$ to about $2.5x10^{10}$ spores per gram; and about 0 wt.% to 45 wt.% of a carrier. An embodiment of the invention is additionally related to a method for decreasing the mortality in an animal challenged with *C. perfringens* that includes vaccinating the animal with a coccidiosis vaccine and administering to the animal a composition containing about 50 wt.% to about 90 wt.% of the cell wall fraction from *Saccharomyces cerevisiae*; about 5 wt.% to about 50 wt.% of the *Bacillus licheniformis* spores, comprising about $4.5x10^9$ to about $2.5x10^{10}$ spores per gram; and about 0 wt.% to 45 wt.% of a carrier. An embodiment of the invention is further directed to a method for decreasing the necrotic enteritis lesions in an animal challenged with *C. perfringens* which includes vaccinating the animal with a coccidiosis vaccine and administering to the animal a composition comprising about 50 wt.% to about 90 wt.% of the cell wall fraction from *Saccharomyces cerevisiae*; about 5 wt.% to about 50 wt.% of the *Bacillus licheniformis* spores, comprising about $4.5x10^9$ to about $2.5x10^{10}$ spores per gram; and about 0 wt.% to 45 wt.% of a carrier.

DETAILED DESCRIPTION

[0012] Disclosed herein are unexpectedly synergistic combinations of probiotic bacteria such as *B. licheniformis* and immune-enhancing yeast cell wall extracts comprising beta-glucans and mannans used for the prevention, control, and treatment of necrotic enteritis in combination with a coccidiostat or coccidiosis vaccine. These compositions (otherwise called an animal feed additive) suitable to treat diseases in an animal are particularly useful to treat diseases caused by infection with a bacterium such as *L. intracellularis* or *C. perfringens*.

[0013] Direct-Fed Microbials (DFM) are living microorganisms that are fed to animals to improve production by modulating the intestinal environment in the animal

and by improving digestion. They help maintain the proper balance of normal gut flora in the gastrointestinal tract. Although scientists do not fully understand the mechanism of action of DFMs, it is believed that DFMs may act by attaching themselves to gut surfaces, reducing or preventing harmful organisms from attaching to and colonizing the gut surface; stimulating intestinal immunity that in turn provides protection against disease; releasing enzymes that aid in digestion; producing organic acids that alter the pH of the gut and stimulate beneficial organisms; and producing nutrients, such as vitamins. AlCare™ (Alpharma Inc. of Bridgewater, New Jersey) is a DFM containing a modified strain of *B. licheniformis* (NCTC 13123). AlCare™ contains 1 X 10^{10} spores of *B. licheniformis* per gram in 70 wt% of a calcium carbonate carrier. One pound of AlCare™ per ton of feed provides 2.2 x 10^{9} colony forming units (cfu) *B. licheniformis* per pound of feed.

[0014] Brewer's yeast is a type of fungus known as *S. cerevisiae*. Alphamune® (Alpharma Inc. of Bridgewater, New Jersey) is a cross-linked natural polysaccharide fiber produced from the cell walls of food grade brewer's yeast. The ingredients in Alphamune® are also made under the name of Beta Mos® (Alpharma Inc. of Bridgewater, New Jersey), which is the cell wall fraction of *S. cerevisiae* after solubilizing and extracting the biological active materials from within the cell after autolysis. Alphamune® is a combination of beta-glucans and mannans. The beta-glucans present in Alphamune® have side chains (1,3-1,6) resulting in a specific complex structure which cannot be broken by glucanase. Beta-glucans have a strong immune enhancing effect in animals, binding to macrophages which are activated and secrete cytokines. The *S. cerevisiae* mannans are polysaccharide-polypeptide complexes which contain partially phosphorylated D-mannose residues. Mannans have been shown to have immunostimulatory effects. Alphamune® generally contains greater than or equal to about 24 wt.% of beta glucans and less than or equal to about 15 wt.% mannans.

[0015] Surprisingly, a combination, and compositions thereof, of QST-713 strain of *Bacillus subtilis*, sold as a biofungicide, and a yeast extract such as that from *Saccharomyces cerevisiae* containing beta-glucans and mannans, i.e., brewer's yeast, may be employed to prevent and/or control diseases, such as infections in animals caused by a bacterium, e.g., *L. intracellularis* or *Clostridium perfringens*.

[0016] As described herein above, the present invention further provides a composition comprising QST-713 strain of *Bacillus subtilis* and a yeast extract such as that from *Saccharomyces cerevisiae*, and optionally a carrier, for the prevention and/or control of diseases in animals, such as infections, in particular, necrotic enteritis in poultry and swine ileitis in pigs.

[0017] QST-713 strain of *Bacillus subtilis* is commercially available as a biofungicide under the trade name Serenade® MAX (AgraQuest Inc. of Davis, California). Serenade® MAX is a microbial pesticide based on a naturally occurring strain QST-713 of *Bacillus subtilis*. It produces three groups of lipopeptides: iturins, agrastatins/plipastatins and surfactins that act synergistically to inhibit germ tubes, mycelium and bacterial cells. Serenade® MAX contains 10-15 wt-% of dried *Bacillus subtilis* QST-715 in a carrier comprising a mixture of inert, non-reactive ingredients and provides a minimum of 7.3×10^9 cfu of *Bacillus subtilis* QST-715 *per* gram of the mixture. Serenade® MAX can be formulated as a wettable powder, a wettable granule and an aqueous suspension that are applied like any other foliar fungicide. Serenade® MAX is not toxic to non-target and beneficial organisms. Due to Serenade's complex mode of action, environmental friendliness, and broad spectrum control, it is well suited for use in Integrated Pest Management.

[0018] As indicated herein above, the composition of the present invention comprises QST-713 strain of *Bacillus subtilis* and a yeast extract such as that from *Saccharomyces cerevisiae*, and optionally a carrier. Preferably, the composition of the present invention comprises the QST-713 strain of *Bacillus subtilis* in an amount ranging from about 5 wt% to about 50 wt%, and more preferably, from about 10 wt% to about 15 wt%, based on the total weight of the composition. Preferably, the QST-713 strain of *Bacillus subtilis* component of the composition of the present invention contains from about 4.5×10^9 to about 2.5×10^{10} cfu of QST-713 strain of *Bacillus subtilis per* gram and, more preferably, a minimum of about 7.3×10^9 cfu of QST-713 strain of *Bacillus subtilis per* gram. As described herein above, the present invention further provides an animal feed composition comprising a combination of QST-713 strain of *Bacillus subtilis* and a yeast extract such as that from *Saccharomyces cerevisiae* for the prevention and/or

control of diseases such as infections in animals, in particular, necrotic enteritis in poultry and swine ileitis in pigs.

[0019] One embodiment of the invention includes a composition for the prevention of disease in an animal comprising a yeast extract from yeast such as *S. cerevisiae*, a probiotic bacterium and an anticoccidal ionophore. Anticoccidal ionophores include, but are not limited to salinomycin, monensin, nigericin, narasin, laidlomycin, noboritomycin, grisorixin, mutalomycin, alborixin, lonomycin, lasalocid maduramicin ammonium, semduramicin, and lysecellin. A preferred embodiment of the invention includes salinomycin (Bio-Cox®; Alpharma Inc. of Bridgewater, New Jersey). The anticoccidal ionophone is present in an amount ranging from about 0.0033 wt.% to about 0.0099 wt.%, preferably from about 0.0044 wt.% to about 0.0066 wt.%.

[0020] An embodiment of the invention is directed to a method for preventing disease in an animal which includes vaccinating the animal with a coccidiosis vaccine and administering to the animal a composition that contains yeast such as *S. Cerevisiae*, a probiotic bacterium, and a carrier. A preferred coccidiosis vaccine is Coccivac®-B (Schering Plough Animal Health Corporation), a non-attenuated, live sporulated oocyst coccidiosis vaccine containing *E. acervuline, E. mivati, E. maxima and E. tenella.*

[0021] The composition optionally comprises a carrier. A number of suitable dry carriers may be organic or inorganic. Exemplary inorganic carriers include, salts such as calcium carbonate, calcium sulfate, and the like. Suitable organic carriers include lactose and the like. Small amounts of other flow control agents, for example silica, may also be used. Mixtures comprising one or more of the foregoing may also be used.

[0022] The composition comprises a yeast extract such as one from *S. cerevisiae*. Specifically, the cell wall fraction comprises beta-glucans and mannans. The cell wall fraction is produced, for example, by mechanically ruptured cells. The yeast extract is present in an amount of about 50 wt.% to about 90 wt.%, specifically about 50 wt.% to about 85 wt.% and more specifically about 50 wt.% to about 55 wt.%, based on the total weight of the composition. In one embodiment, the beta-glucan has (1,3-1,6) side chains

and is resistant to cleavage by glucanase. In another embodiment, the mannans are polysaccharide-polypeptide complexes, partially phosphorylated D-mannose residues. The yeast extract comprises about 40 wt.% to about 85 wt.% cell walls, specifically about 40 wt.% to about 45 wt.% cell walls.

[0023] *B. licheniformis* is present in an amount of about 5 wt.% to about 50 wt.%, and specifically about 7.5 wt.% to about 12.5 wt.%, based on the total weight of the composition. The *B. licheniformis* component comprises about $4.5x10^9$ to about $2.5x10^{10}$ cfu per gram, and specifically about $5x10^9$ to about $1.25x10^{10}$ cfu per gram, based on the total weight of the composition.

[0024] The carrier is present in an amount of 0 wt.% to 45 wt.%, specifically about 5 wt.% to about 40 wt.%, and more specifically about 37.5 wt.% to about 42.5 wt.%, based on the total weight of the composition.

[0025] In one embodiment, the composition suitable for treatment of a disease in an animal is administered in an animal foodstuff. Manufactured foodstuffs for animals such as cattle, pigs, and fowl are usually provided in the form of pellets or similar particulate material. Pellets are typically manufactured by combining a cereal base with ingredients such as oil and protein, steam conditioning the mixture (for example at 70°C for 5 minutes), extruding the mixture through a circular die (typically between 2 mm and 15 mm in diameter), cutting into appropriately sized lengths (e.g., 5-20 mm), and drying. The finished pellets are generally cylindrically shaped and have a relatively smooth surface.

[0026] In one embodiment, an animal feed composition is prepared by adding an animal feed additive composition to an animal foodstuff. The animal feed additive composition may be added to the food in a number of ways. The animal feed additive composition containing a given quantity of active ingredients may be added to a given quantity of feed and mixed or blended to provide a substantially homogeneous medicated feed composition. Large feed lots may be prepared in this manner for treating a large

number of animals. Alternatively, feed batches containing feed for a single animal or single meal may be prepared either by mixing a predetermined quantity of animal feed additive composition with the animal feed or by adding a predetermined quantity of premix to an animal's feed as a top dressing.

[0027] In another embodiment, an animal feed composition comprises a composition suitable for treatment of a disease in an animal and an animal foodstuff, wherein the composition suitable for treatment of a disease in an animal is present in an amount of about 0.5 pounds to about 10 pounds, specifically about 1 pound to about 5 pounds, more specifically about 1.5 pounds to about 2.5 pounds, and most specifically 2 pounds of the composition suitable for treatment of a disease in an animal to a ton of animal foodstuff (mash or pellet forms). In one embodiment, the animal foodstuff may be a poultry foodstuff. In another embodiment, the animal foodstuff may be a swine foodstuff.

[0028] The invention is further illustrated by the following non-limiting examples.

Examples

[0029] Example 1.

[0030] One-day-old healthy broiler male chicks (Cobb X Cobb) chicks were obtained from Cobb-Vantress Hatchery of Cleveland, Georgia. Upon arrival, the chicks were raised in Petersime battery cages. Eight birds were placed in each cage. A thermostatically controlled gas furnace/air conditioner maintained uniform temperature and even continuous illumination was provided. Water was available *ad libitum* through the trial. The chicks were divided into groups containing 48 chicks. Two control treatment groups were fed an unmedicated commercial type chicken starter ration compounded with feedstuffs commonly used in the United States. One group (designated AlCare 0.5 Group) was fed AlCare™ (Alpharma of Bridgewater, New Jersey), a DFM product containing 1.0×10^{10} spores of *B. Licheniformis* per gram, at a rate of 0.5 pound per ton. Another group (AlCare 1 Group) was fed AlCare™ at a rate of 1.0 pound per ton. Two separate groups were fed Alphamune® (Alpharma of Bridgewater, New

Jersey), a brewer's yeast (*Saccharomyces cerevisiae*) extract for use in animal feeds, at a rate of 1 pound per ton (Alphamune 1 Group) and 2 pounds per ton (Alphamune 2 Group), respectively. Further groups were fed both AlCare and Alphamune at rates of AlCare 0.5 pound per ton plus Alphamune 1 pound per ton, AlCare 1 pound per ton plus Alphamune 1 pound per ton, AlCare 0.5 pound per ton plus Alphamune 2 pounds per ton, and AlCare 1 pound per ton plus Alphamune 2 pounds per ton.

[0031] The chicks were fed for 28 days. All dead birds dying during the study were weighed and necropsied for the presence of necrotic enteritis (NE) lesions. All groups were challenged orally with *Eimeria maxima* (5,000 oocysts per bird) on Day 14. All treatments except a control (NC) were challenged with *C. perfringens* (broth culture of *C. perfringens* 10^8 cfu/ml) on days 19, 20, and 21. On Day 22, three birds from each cage were lesion scored for NE. The trail was terminated on day 28. The results of the trials are shown in Table 1.

Table 1.

Treatments	**Necrotic Enteritis Lesion Score**	**Mortality % Necrotic Enteritis**
Control	0	0
Challenged Control	1.11	20.83
AlCare 0.5	0.56	6.25
AlCare 1	0.56	6.25
Alphamune 1	0.72	10.42
Alphamune 2	0.94	12.50
AlCare 0.5 + Alphamune 1	0.44	12.50
AlCare 1 + Alphamune 1	0.28	2.08
AlCare 0.5 + Alphamune 2	0.72	10.42
AlCare 1 + Alphamune 2	0.56	6.25

[0032] Example 2.

[0033] One-day-old healthy broiler male chicks (Cobb X Cobb) chicks were obtained from Cobb-Vantress Hatchery of Cleveland, Georgia. Upon arrival, fifty birds were allocated to each treatment pen by blocks. For environmental control there were ambient humidity and twenty-four hour lighting. Gas heaters were the primary heat source with a heat lamp in each pen for supplemental heat as required during the

brooding period. Fans and sidewall curtains manipulation were used for ventilation and to cool the birds. Water was available *ad libitum* through the trial. Two control treatment groups were fed a non-medicated commercial type chicken starter, grower and finisher rations compounded with foodstuffs commonly used in the United States. One group, designated as SM Group, was fed Serenade® MAX at a rate of 91 g *per* ton of foodstuff. Another group, designated as SM + Alphamune® (AM) Group, was fed both Serenade® MAX at a rate of 91 g *per* ton of foodstuff and Alphamune® at a rate of 1 lb *per* ton of foodstuff. The treatments were replicated in six blocks and randomized.

[0034] The chicks were fed *ad libitum* from the date of chick arrival until Day 42 of the study. All dead birds dying during the study were weighed and necropsied for the presence of necrotic enteritis (NE) lesions. All groups were challenged orally with *Eimeria maxima* (5,000 oocysts *per* bird) on Day 14. All treatment groups except one of the control groups (NC) were challenged with *C. perfringens* (CP, broth culture of *C. perfringens* 10^8 cfu/mL) on days 19, 20 and 21. On Day 22, five birds from each pen were lesion scored for NE. The trial was terminated on day 42, and the results are shown in Table 2.

Table 2

Treatments	**Necrotic Enteritis Lesion Score**	**Mortality % Necrotic Enteritis**
Control (NC)	0.17	1.00
Challenged Control (CP)	1.30	30.33
Serenade® MAX (SM); CP	0.77	21.00
SM + Alphamune® (AM); CP	0.50	17.00

[0035] Example 3.

[0036] One-day-old healthy male broiler chicks (Cobb X Cobb) were obtained from a hatchery where they were sexed and received routine vaccination (HVTSB1). Only healthy-appearing chicks were used in the study. No birds were replaced during the course of the study. Upon arrival, chicks were raised in litter floor pens. There were 50 birds placed per pen. Pens were blocked by location in the house. Each treatment

appeared once within each block. Thermostatically-controlled gas furnaces maintained uniform temperature. Water was available *ad libitum* throughout the trial.

[0037] All birds were weighed by pen on days 0, 21, and 42. Starter, grower, and finisher feed consumptions were measured from days 0 to 21, 21 to 35, and 35 to 42, respectively. The study was terminated on day 42. On Day 22, five birds from each pen were lesion scored for NE. The scoring was based on a 0 to 3 score, with 0 being normal and 3 being the most severe. The scoring was as follows: 0 for normal intestines, 1 for slight mucus covering and loss of tone, 2 for severe necrotizing enteritis, and 3 for extreme necrotizing enteritis with presence of blood in the lumen. Birds dying during the study were weighed and necropsied for the presence of NE. All birds from the study were disposed of at the research facility. Mortality was summarized as total and those resulting from NE.

[0038] A total of eight different regimens were tested. All groups were challenged orally with *Eimeria maxima* (5,000 oocysts per bird) on Day 14. All treatments except a control (NC) and a treatment group with AlCare and Alphamune (Group AA-NC) were challenged with *C. perfringens* (broth culture of *C. perfringens* 10^8 cfu/ml) on days 19, 20, and 21. Control feeds did not contain AlCare™ (AC), Alphamune® (AM), or Salinomycin (S). Control groups were not spray vaccinated using a Spraycox® (Schering Plough Animal Health Corporation) machine with the label recommended dosage on day of hatch. The groups were designated as follows:

[0039] Group NC (unchallenged control): Group NC was fed an unmedicated commercial type chicken starter ration compounded with feedstuffs commonly used in the United States and was unchallenged by *C. perfringens*.

[0040] Group CP (challenged control): Group CP was fed an unmedicated commercial type chicken starter ration compounded with feedstuffs commonly used in the United States and was challenged by *C. perfringens* as described above.

[0041] Group AA, NC (AlCare™ and Alphamune® fed; unchallenged): Group AA, NC was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus AlCare™ at a rate of 1.0 pound per ton and Alphamune® at a rate of 1 pound per ton. This group was not challenged.

[0042] Group AA, CP (AlCare™ and Alphamune® fed; challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus AlCare™ at a rate of 1.0 pound per ton and Alphamune® at a rate of 1 pound per ton. This group was challenged as described above.

[0043] Group S, CP (salinomycin treated, challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus salinomycin (66 ppm) and was challenged as described above.

[0044] Group S, AA, CP(salinomycin treated, AlCare™ and Alphamune® fed; challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus AlCare™ at a rate of 1.0 pound per ton and Alphamune® at a rate of 1 pound per ton plus salinomycin (66 ppm). This group was challenged as described above.

[0045] Group CV, CP (Coccivac®-B treated; challenged) was spray vaccinated using a Spraycox® machine (Schering-Plough Animal Health Corporation) with the label recommended dosage on day of hatch and fed was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States. This group was challenged as described above.

[0046] Group CV, AA, CP (Coccivac®-B treated; challenged) was spray vaccinated using a Spraycox® machine with the label recommended dosage on day of hatch and fed was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus AlCare™ at a rate of 1.0 pound per

ton and Alphamune® at a rate of 1 pound per ton. This group was challenged as described above.

[0047] Statistics were carried out using SAS® (SAS Institute, Inc., Cary, NC, USA, 2002) in the analyses of the data. Additional analyses were performed comparing AA with the anticoccidial program with and without AA.

[0048] Calculations of pen data used in the statistical analyses were performed using SAS. Average bird weight gains were calculated for the 0 to 21, 0 to 42, and 21 to 42-days periods. For the NE lesion scores, the pen average of the five birds selected for NE lesion scoring on Day 22 were calculated. The feed conversion ratio (FCR) was calculated on a pen basis for the 0 to 21, 0 to 42, and 21 to 42 days periods with adjustments made for dead and removed birds. The percentages of total mortality and those mortalities caused by NE were calculated. Any reference made to the performance of the chickens would be referring to effects on weight gain and/or FCR.

[0049] This study was conducted using a randomized complete block design. There were 6 blocks. The pen of birds was the experimental unit. The model included block and treatment. Block was a random effect. Treatment was a fixed effect, so the model was mixed.

[0050] The summary variables include performance data and data related to the NE challenge for each pen. A mixed model analysis was performed on the data using the PROC MIXED procedure of SAS.

[0051] The results of the trials are shown in Tables 3-8. In the tables the following abbreviations are used:

AA = AlCare + Alphamune

S = salinomycin

CV = Coccivac®-B

CP = Challenged

NC = Not Challenged

Table 3. Effects of dietary AlCare plus Alphamune on weight gains of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

Treatments	Average weight (kg) Day 0	Average Weight Gain (kg)		
		Days 0 to 21	Days 0 to 42	Days 21 to 42
Control (NC)	.044	.515	1.892	1.377
Challenge Control (CP)	.044	.469	1.704	1.235
AA, NC	.044	.497	1.899	1.403
AA, CP	.044	.488	1.861	1.373
S, CP	.044	.511	1.993	1.482
S, AA, CP	.044	.556	2.085	1.529
CV, CP	.044	.479	1.923	1.443
CV, AA, CP	.044	.501	2.017	1.516

Table 4. Effects of dietary AlCare plus Alphamune on feed conversion and necrotic enteritis lesions of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

Treatments	Feed Conversion Ratio (feed to gain ratio)			Necrotic Enteritis Lesion Score
	Days 0 to 21	Days 0 to 42	Days 21 to 42	
Control (NC)	1.723	1.943	2.055	0.17
Challenged Control (CP)	1.893	2.051	2.183	1.30
AA, NC	1.759	1.896	1.962	0.20
AA, CP	1.763	1.921	2.016	0.60
S, CP	1.688	1.876	1.963	0.07
S, AA, CP	1.647	1.824	1.910	0.17
CV, CP	1.792	1.893	1.950	0.30
CV, AA, CP	1.737	1.823	1.867	0.33

Table 5. Effects of dietary AlCare plus Alphamune on mortality of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

Treatments	Mortality (%)		
	All Causes		Necrotic Enteritis
	Days 0 to 21	Days 0 to 42	Days 0 to 42
Control (NC)	3.00	9.00	1.00
Challenged Control (CP)	5.33	35.67	30.33
AA, NC	4.00	9.33	2.67
AA, CP	2.33	18.67	11.67

S, CP	3.33	6.00	1.6
S, AA, CP	4.33	7.67	1.00
CV, CP	4.33	25.00	19.67
CV, AA, CP	4.00	13.67	5.67

Table 6. Effects of dietary AlCare plus Alphamune on weight gains of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Average weight (kg) Day 0	Average Weight Gain (kg)		
Treatments		Days 0 to 21	Days 0 to 42	Days 21 to 42
Control (NC)	.044	.515	1.892	1.377
Challenged Control (CP)	.044	.469	1.704	1.235
S, CP	.044	.511	1.993	1.482
S, AA, CP	.044	.556	2.085	1.529
CV, CP	.044	.479	1.923	1.443
CV, AA, CP	.044	.501	2.017	1.516

Table 7. Effects of dietary AlCare plus Alphamune on feed conversion and necrotic enteritis lesions of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Feed Conversion Ratio (feed to gain ratio)			Necrotic Enteritis Lesion Score
Treatments	Days 0 to 21	Days 0 to 42	Days 21 to 42	
Control (NC)	1.723	1.943	2.055	0.17
Challenged Control (CP)	1.893	2.051	2.183	1.30
S, CP	1.688	1.876	1.963	0.07
S, AA, CP	1.647	1.824	1.910	0.17
CV, CP	1.792	1.893	1.950	0.30
CV, AA, CP	1.737	1.823	1.867	0.33

Table 8. Effects of dietary AlCare plus Alphamune on mortality of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Mortality (%)		
	All Causes		Necrotic Enteritis
Treatments	Days 0 to 21	Days 0 to 42	Days 0 to 42
Control (NC)	3.00	9.00	1.00
Challenged Control (CP)	5.33	35.67	30.33
S, CP	3.33	6.00	1.67

S, AA, CP	4.33	7.67	1.00
CV, CP	4.33	25.00	19.67
CV, AA, CP	4.00	13.67	5.67

[0052] Data in Tables 3, 4, and 5 are the comparisons of between the AACP treatments and the S and CV treatments with and without AA, respectively. The combination of S + AA generally provided the best performance of all the treatments. The combination of S + AA provided greater weight gain from 0 to 21, 0 to 42, 21 to 42 days and better FCR from 0 to 21, 0 to 42, and 21 to 42 days, lower NE mortality compared to both the S and the AACP treatments. The combination of S + AA also provided lower NE lesion scores compared to the AACP treatment.

[0053] The combination of CV + AA provided greater weight gain from days 0-21. 0 to 42, 21 to 42 and better FCR from 0 to 21, 0 to 42, and 21 to42 days compared to both the CV and the AACP treatments (Tables 3 and 4). The combination of CV + AA provided lower total mortality from days 0 to 42 and lower NE mortality compared to the CV and AACP treatments (Table 5).

[0054] Birds medicated with salinomycin with and without AA generally had the best performance and lower NE lesion scores and mortality comparable to or better than the NC. The addition of AA to the feeds of birds fed salinomycin tended to improve performance but did not further reduce NE lesion scores or mortality. Generally, the addition of AA to the feeds of birds treated with CV improved performance and reduced mortality compared to those treated with CV alone. Birds challenged with NE and treated with a conventional coccidiostat program or a coccidiosis vaccine program can benefit from the addition of AA to their feeds. Data in Tables 6, 7, and 8 allow for direct comparisons of the coccidiosis control programs to each other (with and without AA) and to the controls (challenged and unchallenged). During the 0 to 21 days period, birds fed S + AA had greater weight gains compared to the other treatments (Table 6). During the 0 to 21 days period, birds fed S had weight gains that were comparable to the NC but greater than the CP. During the 0 to 21 days period, birds vaccinated with CV had

weight gains that were not different than the NC or CP birds. During the 0 to 42 and 21 to 42 days periods, birds treated with CV had greater weight gains compared to the CP birds.

[0055] During the 0 to 21 days period, birds fed S + AA had FCR that were better than the NC and CP birds (Table 7). During the 0 to 21 days period, birds medicated with S, CV, and CV + AA had FCR that were better than the CP birds. During the 0 to 42 and 21 to 42 days periods, birds treated with S, S + AA, CV, and CV + AA had FCR that were better than the NC and CP birds (Table 7). During the 0 to 42 days period, the addition of AA to the diets of birds treated with S and CV improved FCR compared to S and CV alone, respectively. During the 21 to 42 days periods the addition of AA to the diets of birds vaccinated with CV improved FCR compared to CV alone.

[0056] NE lesion scores of S and CV birds (with and without AA) were comparable to the NC but lower than the CP birds (Table 8). The treatments did not affect mortality during the 0 to 21 days period (Table 8). Mortality (total and NE) of the S, S + AA, and CV + AA birds were comparable to the NC but lower than the CP birds (Table 6). Mortality (total and NE) of CV birds was lower than the CP birds but higher than the NC birds. Mortality (total and NE) of birds treated with CV +AA was lower than with CV alone.

[0057] Example 4.

[0058] One-day-old healthy male broiler chicks (Cobb X Cobb) were obtained from a hatchery where they were sexed and received routine vaccination (HVTSB1). Only healthy-appearing chicks were used in the study. No birds were replaced during the course of the study. Upon arrival, chicks were raised in litter floor pens. There were 50 birds placed per pen. Pens were blocked by location in the house. Each treatment appeared once within each block. Thermostatically-controlled gas furnaces maintained uniform temperature. Water was available *ad libitum* throughout the trial.

[0059] All birds were weighed by pen on days 0, 21, and 42. Starter, grower, and finisher feed consumptions were measured from days 0 to 21, 21 to 35, and 35 to 42, respectively. The study was terminated on day 42. On Day 22, five birds from each pen were lesion scored for NE. The scoring was based on a 0 to 3 score, with 0 being normal and 3 being the most severe. The scoring was as follows: 0 for normal intestines, 1 for slight mucus covering and loss of tone, 2 for severe necrotizing enteritis, and 3 for extreme necrotizing enteritis with presence of blood in the lumen. Birds dying during the study were weighed and necropsied for the presence of NE. All birds from the study were disposed of at the research facility. Mortality was summarized as total and those resulting from NE.

[0060] A total of nine different regimens were tested. All groups were challenged orally with *Eimeria maxima* (5,000 oocysts per bird) on Day 14. All treatments except a non-challenged control (NC) were challenged with *C. perfringens* (broth culture of *C. perfringens* 10^8 cfu/ml) on days 19, 20, and 21. Control feeds did not contain Serenade® MAX (QST), Alphamune® (AM), or Salinomycin (S). Control groups were not spray vaccinated using a Spraycox® machine with the label recommended dosage on day of hatch. The groups were designated as follows:

[0061] Group NC (unchallenged control): Group NC was fed an unmedicated commercial type chicken starter ration compounded with feedstuffs commonly used in the United States and was unchallenged by *C. perfringens*.

[0062] Group CP (challenged control): Group CP was fed an unmedicated commercial type chicken starter ration compounded with feedstuffs commonly used in the United States and was challenged by *C. perfringens* as described above.

[0063] Group AM, CP (Alphamune® fed; challenged): Group AM, CP was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus Alphamune® at a rate of 1 pound per ton. This group was challenged.

[0064] Group QST, CP (Serenade® MAX fed; challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus Serenade® MAX at a rate of 0.25 pound per ton. This group was challenged as described above.

[0065] Group AQST, CP (Alphamune® and Serenade® MAX fed, challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus Alphamune® at a rate of 1 pound per ton and Serenade® MAX at a rate 0f 0.25 pound per ton, and was challenged as described above.

[0066] Group S, CP (salinomycin treated; challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus salinomycin (66 ppm). This group was challenged as described above.

[0067] Group S, AQST, CP (salinomycin treated, Alphamune® and Serenade® MAX fed; challenged) was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus Alphamune® and Serenade® MAX at a rate of 1.25 pounds per ton and treated with salinomycin (66 ppm) . This group was challenged as described above.

[0068] Group CV, CP (Coccivac®-B treated; challenged) was spray vaccinated using a Spraycox® machine with the label recommended dosage on day of hatch and fed was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States. This group was challenged as described above.

[0069] Group CV, AQST, CP (Coccivac®-B treated, Alphamune® and Serenade® MAX fed; challenged) was spray vaccinated using a Spraycox® machine with the label recommended dosage on day of hatch and fed was fed a commercial type chicken starter ration compounded with feedstuffs commonly used in the United States plus

Alphamune® and Serenade® MAX at a rate of 1.25 pounds per ton. This group was challenged as described above.

[0070] Statistics were carried out using SAS® (SAS Institute, Inc., Cary, NC, USA, 2002) in the analyses of the data. Additional analyses were performed comparing AA with the anticoccidial program with and without AA.

[0071] Calculations of pen data used in the statistical analyses were performed using SAS. Average bird weight gains were calculated for the 0 to 21, 0 to 42, and 21 to 42-days periods. For the NE lesion scores, the pen average of the five birds selected for NE lesion scoring on Day 22 were calculated. The feed conversion ratio (FCR) was calculated on a pen basis for the 0 to 21, 0 to 42, and 21 to 42 days periods with adjustments made for dead and removed birds. The percentages of total mortality and those mortalities caused by NE were calculated. Any reference made to the performance of the chickens would be referring to effects on weight gain and/or FCR.

[0072] This study was conducted using a randomized complete block design. There were 6 blocks. The pen of birds was the experimental unit. The model included block and treatment. Block was a random effect. Treatment was a fixed effect, so the model was mixed.

[0073] The summary variables include performance data and data related to the NE challenge for each pen. A mixed model analysis was performed on the data using the PROC MIXED procedure of SAS.

[0074] The results of the trials are shown in Tables 9-17. In the tables the following abbreviations are used:

AM = Alphamune®

QST= Serenade® MAX

AQST= Alphamune plus Serenade® MAX

S = salinomycin

CV = Coccivac®-B

CP = Challenged

NC = Not Challenged

Table 9. Effects of dietary Alphamune plus QST on weight gains of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

Treatments[1]	Average weight (kg) Day 0	Average Weight Gain (kg)		
		Days 0 to 21	Days 0 to 42	Days 21 to 42
Control (NC)	.044	.615	1.829	1.214
Challenged Control (CP)	.044	.514	1.753	1.238
AM, CP	.045	.579	1.840	1.261
QST, CP	.044	.595	1.825	1.230
AQST, CP	.044	.619	1.891	1.273
S,CP	.044	.592	1.856	1.264
S, AQST, CP	.044	.605	1.872	1.266
CV, CP	.044	.554	1.840	1.286
CV, AQST, CP	.045	.574	1.883	1.310

Table 10. Effects of dietary Alphamune plus QST on feed conversion and necrotic enteritis lesions of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

Treatments	Feed Conversion Ratio (feed to gain ratio)			Necrotic Enteritis Lesion Score
	Days 0 to 21	Days 0 to 42	Days 21 to 42	
Control (NC)	1.573	1.996	2.261	0.13
Challenged Control (CP)	1.818	2.025	2.156	0.90
AM, CP	1.624	1.983	2.196	0.20
QST, CP	1.612	1.991	2.234	0.30
AQST, CP	1.566	1.953	2.192	0.13
S,CP	1.550	1.914	2.126	0.10
S, AQST, CP	1.551	1.903	2.111	0.17
CV, CP	1.710	1.957	2.094	0.20
CV, AQST, CP	1.611	1.918	2.094	0.07

Table 11. Effects of dietary Alphamune plus QST on mortality of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Mortality (%)

	All Causes		Necrotic Enteritis
Treatments	Days 0 to 21	Days 0 to 42	Days 0 to 42
Control (NC)	1.33	3.33	0.33
Challenged Control (CP)	6.67	18.33	15.33
AM, CP	2.00	6.00	3.00
QST, CP	1.67	6.67	5.33
AQST, CP	0.67	5.33	3.00
S,CP	1.67	4.67	1.33
S, AQST, CP	1.33	3.33	1.00
CV, CP	4.67	8.6	5.33
CV, AQST, CP	2.67	7.33	3.00

Table 12. Effects of dietary Alphamune plus QST on weight gains of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Average weight (kg) Day 0	Average Weight Gain (kg)		
Treatments		Days 0 to 21	Days 0 to 42	Days 21 to 42
Control (NC)	.044	.615	1.829	1.214
Challenged Control (CP)	.044	.514	1.753	1.238
AM, CP	.045	.579	1.840	1.261
QST, CP	.044	.595	1.825	1.230
AQST, CP	.044	.619	1.891	1.273

Table 13. Effects of dietary Alphamune plus QST on feed conversion and necrotic enteritis lesions of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Feed Conversion Ratio (feed to gain ratio)			Necrotic Enteritis Lesion Score
Treatments	Days 0 to 21	Days 0 to 42	Days 21 to 42	
Control (NC)	1.573	1.996	2.261	0.13
Challenged Control (CP)	1.818	2.025	2.156	0.90
AM, CP	1.624	1.983	2.196	0.20
QST, CP	1.612	1.991	2.234	0.30
AQST, CP	1.566	1.953	2.192	0.13

Table 14. Effects of dietary Alphamune plus QST on mortality of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Mortality (%)		
	All Causes		Necrotic Enteritis
Treatments	Days 0 to 21	Days 0 to 42	Days 0 to 42
Control (NC)	1.33	3.33	0.33
Challenged Control (CP)	6.67	18.33	15.33
AM, CP	2.00	6.00	3.00
QST, CP	1.67	6.67	5.33
AQST, CP	0.67	5.33	3.00

Table 15. Effects of dietary Alphamune plus QST on weight gains of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Average weight (kg) Day 0	Average Weight Gain (kg)		
Treatments		Days 0 to 21	Days 0 to 42	Days 21 to 42
Control (NC)	.044	.615	1.829	1.214
Challenged Control (CP)	.044	.514	1.753	1.238
AQST, CP	.044	.619	1.891	1.273
S,CP	.044	.592	1.856	1.264
S, AQST, CP	.044	.605	1.872	1.266
CV, CP	.044	.554	1.840	1.286
CV, AQST, CP	.045	.574	1.883	1.310

Table 16. Effects of dietary Alphamune plus QST on feed conversion and necrotic enteritis lesions of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Feed Conversion Ratio (feed to gain ratio)			Necrotic Enteritis Lesion Score
Treatments	Days 0 to 21	Days 0 to 42	Days 21 to 42	
Control (NC)	1.573	1.996	2.261	0.13
Challenged Control (CP)	1.818	2.025	2.156	0.90
AQST, CP	1.566	1.953	2.192	0.13
S,CP	1.550	1.914	2.126	0.10
S, AQST, CP	1.551	1.903	2.111	0.17
CV, CP	1.710	1.957	2.094	0.20
CV, AQST, CP	1.611	1.918	2.094	0.07

Table 17. Effects of dietary Alphamune plus QST on mortality of broiler chickens in a *Clostridium perfringens* challenge model in litter floor pens.

	Mortality (%)		
	All Causes		Necrotic Enteritis
Treatments	Days 0 to 21	Days 0 to 42	Days 0 to 42
Control (NC)	1.33	3.33	0.33
Challenged Control (CP)	6.67	18.33	15.33
AQST, CP	0.67	5.33	3.00
S,CP	1.67	4.67	1.33
S, AQST, CP	1.33	3.33	1.00
CV, CP	4.67	8.67	5.33
CV, AQST, CP	2.67	7.33	3.00

The addition of AM, QST, and AQST to the feeds of broiler chickens improved performance compared to the CC. Birds medicated with salinomycin with and without AQST generally had the best performance and lower NE lesions and mortality comparable to or better than the NC. Generally, the addition of AQST to the feeds of birds treated with Coccivac improved performance and reduced mortality compared to those treated with Coccivac alone.

CLAIMS:

1. A composition for the treatment of a disease in an animal comprising: yeast extract from *Saccharomyces cerevisiae*, *Bacillus licheniformis* spores, and optionally a carrier.

2. The composition of claim 1, comprising

(a) about 50 wt.% to about 90 wt.% of the cell wall fraction from *Saccharomyces cerevisiae*,

(b) about 5 wt.% to about 50 wt.% of the *Bacillus licheniformis* spores, comprising about $4.5x10^9$ to about $2.5x10^{10}$ spores per gram, and

(c) about 0 wt.% to about 45 wt.% of the carrier.

3. The composition of claim 1, comprising

(a) 50 wt.% of the cell wall fraction from *Saccharomyces cerevisiae*,

(b) 10 wt.% of the *Bacillus licheniformis* spores, comprising about $5x10^9$ to about $1.25x10^{10}$ spores per gram, and

(c) 40 wt.% of the carrier.

4. The composition of claim 1 wherein the carrier comprises calcium carbonate, calcium sulfate, lactose, or a combination thereof.

5. An animal feed composition comprising the composition for the treatment of a disease in an animal of any one of claims 1-4, and an animal foodstuff, wherein the composition for the treatment of a disease in an animal is in an amount of about 0.5 pounds to about 10 pounds per ton of the animal foodstuff.

6. The animal feed composition of claim 5, wherein the composition for the treatment of a disease in an animal is in an amount of about 1 pound to about 5 pounds per ton of the animal foodstuff.

7. The animal feed composition of claim 6, wherein the composition for the treatment of a disease in an animal is in an amount of about 2 pounds per ton of the animal foodstuff.

8. The animal feed composition of claim 7, wherein animal foodstuff is a poultry foodstuff or a swine foodstuff.

9. A method for preventing a disease in an animal, comprising administering to the animal the composition for the treatment of a disease in an animal of any one of claims 1-8.

10. The method of claim 9, wherein the animal is poultry and the disease is necrotic enteritis.

11. The method of claim 9, wherein the animal is swine and the disease is swine ileitis.

12. A composition for the prevention and/or control of a disease in an animal comprising a yeast extract from *Saccharomyces cerevisiae* and QST-713 strain of *Bacillus subtilis*, and optionally a carrier.

13. The composition according to claim 12, comprising from about 75 wt-% to about 99 wt-% of the yeast extract from *Saccharomyces cerevisiae*; from about 1 wt-% to about 3 wt-% of the QST-713 strain of *Bacillus subtilis*; and from about 0 wt-% to about 24 wt-% of the carrier.

14. The composition according to claim 12, comprising 80 wt-% of the cell wall fraction from *Saccharomyces cerevisiae*; 2.5 wt-% of QST-713 strain of *Bacillus subtilis*; and 17.5 wt-% of the carrier.

15. The composition according to claim 12, wherein the carrier comprises calcium carbonate, calcium sulfate, lactose, or a combination thereof.

16. An animal feed composition comprising a composition according to any one of claims 12 to 15, and an animal foodstuff, wherein the composition for the prevention and/or control of a disease in an animal is present in an amount ranging from about 0.5 pounds to about 10 pounds per ton of the animal foodstuff.

17. The animal feed composition according to claim 16, wherein the composition for the prevention and/or control of a disease in an animal is in an amount of about 1.25 pounds per ton of the animal foodstuff.

18. The animal feed composition according to claim 16, wherein animal foodstuff is a poultry foodstuff or a swine foodstuff.

19. A method for preventing and/or controlling a disease in an animal comprising administering to the animal an effective amount of a composition according to any one of claims 12 to 18.

20. The method according to claim 19, wherein the animal is poultry and the disease is necrotic enteritis.

21. The method according to claim 19, wherein the animal is swine and the disease is swine ileitis.

22. A composition comprising the composition of any one of claims 1 to 8 further comprising an anticoccidal ionophore.

23. The composition of claim 22, wherein the anticoccidal ionophore is salinomycin.

24. The composition of claim 23, wherein the salinomycin is present at a level of about 66 ppm of the composition.

25. An animal feed composition comprising the composition for the treatment of necrotic enteritis in an animal of any one of claims 22 to 24, and an animal foodstuff, wherein the composition for the treatment of a disease in an animal is in an amount of about 0.5 pounds to about 10 pounds per ton of the animal foodstuff.

26. A method for improving the performance of an ionophore in the treatment of necrotic enteritis in an animal comprising administering to the animal the composition of any one of claims 22 to 25.

27. The method of claim 26 wherein the ionophore is salinomycin.

28. A method for preventing disease in an animal, comprising
vaccinating the animal with a coccidiosis vaccine; and
administering to the animal a composition comprising the composition of any one of claims 1 to 8.

29. The method of claim 28, wherein the animal is poultry and the disease is necrotic enteritis.

30. The method of claim 28, wherein the vaccine comprises a non-attenuated, live sporulated oocyst coccidiosis vaccine.

31. The method of claim 28, wherein the coccidiosis vaccine is Coccivac®.

32. A method for decreasing the mortality in an animal challenged with *C. perfringens*, comprising vaccinating the animal with a coccidiosis vaccine; and administering to the animal a composition comprising the composition of any one of claims 1 to 8.

33. The method of claim 32 wherein the animal is poultry.

34. The method of claim 33, wherein the vaccine comprises a non-attenuated, live sporulated oocyst coccidiosis vaccine.

35. The method of claim 34, wherein the coccidiosis vaccine is Coccivac®.

36. A method for decreasing the necrotic enteritis lesions in an animal challenged with *C. perfringens*, comprising vaccinating the animal with a coccidiosis vaccine; and administering to the animal a composition comprising the composition of any one of claims 1 to 8.

37. The method of claim 36 wherein the animal is poultry.

38. The method of claim 36, wherein the vaccine comprises a non-attenuated, live sporulated oocyst coccidiosis vaccine.

39. A composition comprising the composition of any one of claims 12 to 18 further comprising an anticoccidal ionophore.

40. The composition of claim 39, wherein the anticoccidal ionophore is salinomycin.

41. The composition of claim 40, wherein the salinomycin is present at a level of about 66 ppm of the composition.

42. An animal feed composition comprising the composition for the treatment of necrotic enteritis in an animal of any one of claims 39 to 41, and an animal foodstuff, wherein the composition for the treatment of a disease in an animal is in an amount of about 0.5 pound to about 10 pounds per ton of the animal foodstuff.

43. A method for improving the performance of an ionophore in the treatment of necrotic enteritis in an animal comprising administering to the animal the composition of any one of claims 39 to 41.

44. The method of claim 43 wherein the ionophore is salinomycin.

45. A method for preventing disease in an animal, comprising
vaccinating the animal with a coccidiosis vaccine; and
administering to the animal a composition comprising the composition of any one of claims 12 to 18.

46. The method of claim 45, wherein the animal is poultry and the disease is necrotic enteritis.

47. The method of claim 45, wherein the vaccine comprises a non-attenuated, live sporulated oocyst coccidiosis vaccine.

48. The method of claim 45, wherein the coccidiosis vaccine is Coccivac®.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/05303

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 1/00, C12N 9/54, C12N 9/56 (2009.01)
USPC - 435/255.1, 435/255.2; 514/221, 514/222
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)
USPC: 435/255.1-255.2; 514/221-222

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/93.51, 424/195.16, 424/246.1; 435/252.5, 435/252.31, 435/254.21, 435/836, 435/942 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); DialogPRO--Chemical Engineering and Biotechnology Abstracts, INSPEC, NTIS (National Technical Information Service), PASCAL, Current Contents Search, MEDLIN
Search Terms: disease, animal, feed, yeast extract, Saccharomyces cerevisiae, Bacillus licheniformis, cell wall, spores,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HUYGHEBAERT. Alternatives for Antibiotics in Poultry, in Proceedings of the 3rd Mid-Atlantic Nutrition Conference (2005), Zimmermann ed., Published by the Maryland Feed Industry Council, pg 38-57, [retrieved on 2009-11-01]. <URL: http://manc.umd.edu/2005_Complete_Proceedings.pdf>, pg 38, para 2, ln 7; pg 39, para 3, ln 7; pg 41, ln 21-22; pg 41, para 3, ln 1; pg 44, ln 8-9; pg 46, para 3, ln 5; pg 46, para 4, ln 1-2; pg 49, ln 10; pg 50, para 6, ln 5-6; pg51, para 3, ln 19; table 3, ln 4; title	1, 4 --- 2-3, 5(1-4), 6-8, 12-18
Y	US 4,649,143 A (Kantor et al.) 10 March 1987 (10.03.1987) col 3, ln 53-55	5(1-4), 6-8, 16-18
Y	US 2006/0276339 A1 (Windsor et al.) 07 December 2006 (07.12.2006) table 1, pg 34, ln 3.	12-18
Y	US 6,214,337 B1 (Hayen et al.) 10 April 2001 (10.04.2001) col 2, ln 48-49; col 3, ln 2-3, ln 14	2-3, 5-8(2,3),13-14
Y	US 2005/0271643 A1 (Sorokulova et al.) 08 December 2005 (08.12.2005) para [0137], [0166], [0172], [0179]	2-3, 5-8(2,3), 13-14

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 November 2009 (02.11.2009)	02 APR 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/05303

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-11, 19-48
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.