(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 August 2019 (01.08.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/147799 A1

(51) International Patent Classification:
A23K 10/00 (2016.01) *A23K 50/70* (2016.01)
A23K 10/18 (2016.01) *A23K 50/75* (2016.01)
A23K 50/00 (2016.01)

(21) International Application Number:
PCT/US2019/014938

(22) International Filing Date:
24 January 2019 (24.01.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/621,196 24 January 2018 (24.01.2018) US

(71) Applicant: **OMNIGEN RESEARCH, LLC** [US/US]; 1767 N.W. Kings Blvd., Corvallis, OR 97330 (US).

(72) Inventors: **JOHNSON, A. Bruce**; Glenpointe Centre East, 3rd Floor, 300 Frank W. Burr Blvd., Suite 21, Teaneck, NJ 07666-6712 (US). **BAFUNDO, Kenneth W.**; Glenpointe Centre East, 3rd Floor, 300 Frank W. Burr Blvd., Suite 21, Teaneck, NJ 07666-6712 (US).

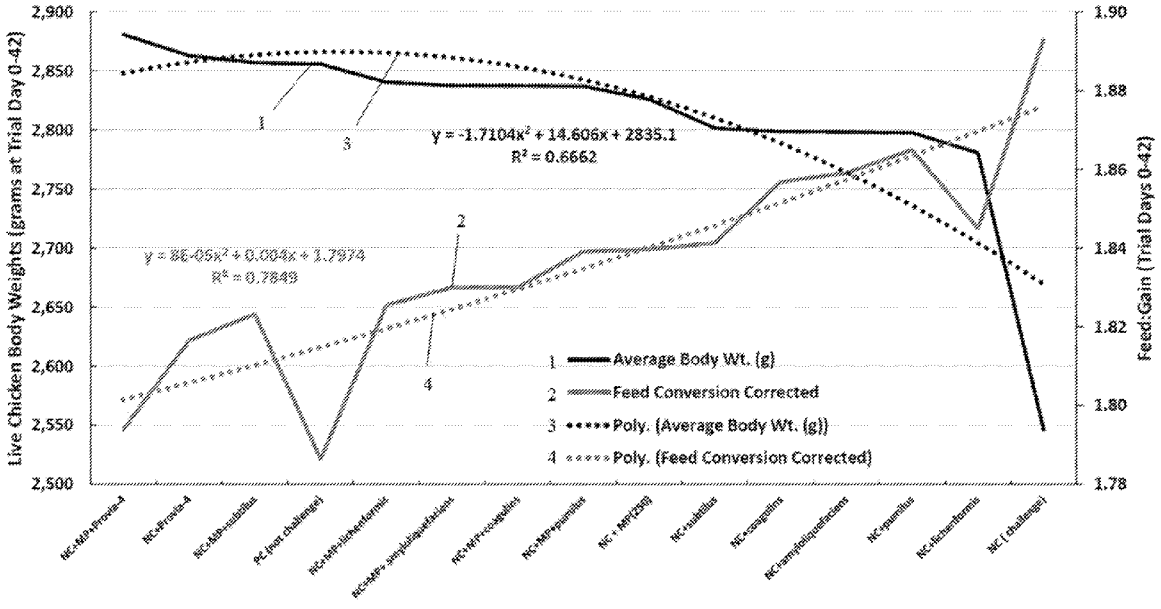
(74) Agent: **BURGESS, Steven J.**; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: *BACILLUS* COMBINATION FOR ADMINISTRATION TO ANIMALS

FIG. 17



(57) Abstract: Embodiments of a composition comprising three or four Bacillus species selected from *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus coagulans* are disclosed. The composition may further comprise, or be used in combination with, feed and/or feed supplements, including feed supplements comprising yucca, quillaja, silica, mineral clay, glucan, mannans, or combinations thereof. Also disclosed is a method of administering the composition and/or combination to animals, such as poultry.

[Continued on next page]

WO 2019/147799 A1

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

BACILLUS COMBINATION FOR ADMINISTRATION TO ANIMALS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 62/621,196, filed January 24, 2018, which is incorporated herein by reference in its entirety.

FIELD

The present application concerns a combination and/or composition comprising three or four Bacillus species selected from *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* for administration to animals, particularly avians.

BACKGROUND

The gastrointestinal (GI) tracts of mammals are colonized by a diverse community of microflora. The GI tract may include hundreds of different species, and this community profile may change over time based on age and health of the individual. A healthy microbiota community in a subject provides many benefits, such as resistance to pathogens, nutrient absorption, and immune system performance. Intestinal microbiota also play a significant role in mediating pathogenic infections of the gut, which significantly affect quality of life. The gastrointestinal microflora compositions of both humans and animals substantially depend upon ingested materials. Accordingly, direct-fed microbial (DFM) compositions are commonly administered to influence physiological health.

DFM products also are administered as an alternative to antibiotics in livestock species. DFMs can restrict adherence of pathogenic microbes to mucosal surfaces, can stimulate an immune response or proliferation of other endogenous beneficial microorganisms. Moreover, certain DFMs produce and secrete other beneficial compounds or compositions, such as antimicrobial substances. Similar results between feeding DFM products and prophylactic levels of antibiotics for growth have been demonstrated. As a result, DFM products may be used as an alternative to antibiotics, or perhaps in combination with antibiotics.

Despite these prior DFM compositions, a need still exists for DFM compositions that beneficially effect subjects receiving such compositions.

SUMMARY

Embodiments of a combination and/or composition comprising three or four Bacillus species selected from *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and

Bacillus coagulans are disclosed. The combination/composition is referred to herein as the Bacilli combination. In some embodiments, the composition comprises, consists essentially of, or consists of, *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*. In other embodiments, the composition comprises, consists essentially of, or consists of, *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens*, and *Bacillus coagulans*. In further embodiments, the composition comprises, consists essentially of, or consists of, *Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis.*

Also disclosed are embodiments of an admixed composition comprising a Bacilli combination and an additional component or composition. Exemplary admixed compositions comprise a first composition consisting essentially of, or consisting of, the Bacilli combination, and a second component or composition comprising one or more of a feed, yucca, quillaja, yucca and quillaja, a silica, mineral clay, a glucan and mannans mixture, a copper salt, a vitamin, an additional direct fed microbial, and any combination thereof.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a table illustrating the results from the study described in Example 3.

FIG. 2 is a table illustrating results from the study described in Example 4.

FIG. 3 is a table illustrating additional results from the study described in Example 4.

FIG. 4 is a table illustrating results from days 0-14 of the study described in Example 6 for treatment groups T1-T11.

FIG. 5 is a table illustrating results from days 0-14 of the study described in Example 6 for treatment groups T12-T15.

FIG. 6 is a table illustrating results from days 0-21 and 0-35 of the study described in Example 6 for treatment groups T1-T11.

FIG. 7 is a table illustrating results from days 0-21 and 0-35 of the study described in Example 6 for treatment groups T12-T15.

FIG. 8 is a table illustrating results from days 0-42 and 15-22 of the study described in Example 6 for treatment groups T1-T11.

FIG. 9 is a table illustrating results from days 0-42 and 15-22 of the study described in Example 6 for treatment groups T12-T15.

FIG. 10 is a table illustrating results from days 22-35 and 36-42 of the study described in Example 6 for treatment groups T1-T11, and also providing the lesion scores.

FIG. 11 is a table illustrating results from days 22-35 and 36-42 of the study described in Example 6 for treatment groups T12-T15, and also providing the lesion scores.

FIG. 12 is a table illustrating the intestinal bacterial data and processing data obtained from treatment groups T1-11 from the study described in Example 6.

FIG. 13 is a table illustrating the intestinal bacterial data and processing data obtained from treatment groups T12-T15 from the study described in Example 6.

FIG. 14 is a graph of live chicken body weights and feed:gain ratio, illustrating the effect of Magni-Phi® (250 ppm) alone, various DMFs alone, and Magni-Phi®/DFM combinations on body weight gain (0-14 days) and feed conversion (Feed:Gain 0-14 days) from the study described in Example 6, with the dotted lines indicating the polynomial trend line for the respective graphs.

FIG. 15 is a graph of live chicken body weights and feed:gain ratio, illustrating the effect of Magni-Phi® (250 ppm) alone, various DMFs alone, and Magni-Phi®/DFM combinations on body weight gain (0-21 days) and feed conversion (Feed:Gain 0-21 days) from the study described in Example 6, with the dotted lines indicating the polynomial trend line for the respective graphs.

FIG. 16 is a graph of live chicken body weights and feed:gain ratio, illustrating the effect of Magni-Phi® (250 ppm) alone, various DMFs alone, and Magni-Phi®/DFM combinations on body weight gain (0-35 days) and feed conversion (Feed:Gain 0-35 days) from the study described in Example 6, with the dotted lines indicating the polynomial trend line for the respective graphs.

FIG. 17 is a graph of live chicken body weights and feed:gain ratio, illustrating the effect of Magni-Phi® (250 ppm) alone, various DMFs alone, and Magni-Phi®/DFM combinations on body weight gain (0-42 days) and feed conversion (Feed:Gain 0-42 days) from the study described in Example 6, with the dotted lines indicating the polynomial trend line for the respective graphs.

FIG. 18 is a table of results from an *in vitro* culture test looking at pathogen growth inhibition.

FIG. 19 is a table of results from a second *in vitro* culture test looking at pathogen growth inhibition.

DETAILED DESCRIPTION

I. Terms

The following explanations of terms and abbreviations are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. As used herein, "comprising" means "including" and the singular forms "a" or "an" or

"the" include plural references unless the context clearly dictates otherwise. The term "or" refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise.

Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting. Other features of the disclosure are apparent from the following detailed description and the claims.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, percentages, temperatures, times, and so forth, as used in the specification or claims are to be understood as being modified by the term "about." Accordingly, unless otherwise indicated, implicitly or explicitly, the numerical parameters set forth are approximations that may depend on the desired properties sought and/or limits of detection under standard test conditions/methods. When directly and explicitly distinguishing embodiments from discussed prior art, the embodiment numbers are not approximates unless the word "about" is recited.

Administering: Administration by any route to a subject, such as poultry. In some embodiments, the route of administration is oral.

Antimicrobial: An agent that kills and/or inhibits the growth of microorganisms. As used herein, antimicrobials include antibiotics, antifungals, antivirals, and antiparasitics including anticoccidials, or combinations thereof.

Carrier: A substance that is used as an additive in (or with) a combination, composition, or component as disclosed herein. As used herein, a carrier may be incorporated within particles of a combination, composition, or component, or it may be physically mixed with particles of a combination, composition, or component. A carrier can be used, for example, to modify non-biological properties of a combination or composition, such as flowability, stability during storage, exposure to moisture, etc. Examples of carriers are included herein.

Colony forming units (CFU): "Colony forming units" refers to individual colonies of bacteria. A colony is a mass of individual bacteria growing together. For certain embodiments, a colony comprises substantially the same species, and may comprise, but does not necessarily comprise, substantially the same strain. CFU are a measure of the number of bacteria present in or on a surface of a sample. However, CFU is not necessarily a measure of individual cells or spores, as a colony may be formed from a single or a mass of cells or spores.

Combination: A combination includes two or more components that are administered such that the effective time period of at least one component overlaps with the effective time period of at least one other component. A combination, or a component thereof, may be a composition. In some embodiments, effective time periods of all components administered overlap with each other. In an exemplary embodiment of a combination comprising three components, the effective time period of the first component administered may overlap with the effective time periods of the second and third components, but the effective time periods of the second and third components independently may or may not overlap with one another. In another exemplary embodiment of a combination comprising three components, the effective time period of the first component administered overlaps with the effective time period of the second component, but not that of the third component; and the effective time period of the second component overlaps with those of the first and third components. A combination may be a composition comprising the components, a composition comprising one or more components and another separate component (or components) or composition(s) comprising the remaining component(s), or the combination may be two or more individual components. In some embodiments, the two or more components may comprise the same component administered at two or more different times, two or more different components administered substantially simultaneously or sequentially in any order, or a combination thereof.

Bacilli Combination: Refers to a combination, or a composition, of DFMs including only three or four *Bacillus* species selected from *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*. In some disclosed embodiments, "Bacilli combination" refers to a composition for administration to a subject, particularly to an animal, and even more particularly to an avian, such as chickens and turkeys, that consists of or consists essentially of any three or four of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*. In other embodiments, "Bacilli combination" refers to *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* administered in combination without any other DFMs. A person of ordinary skill in the art will understand that the Bacilli combination may include additional residual material that is carried over from the production of any or all of the three or four *Bacillus* species, such as a dry milk product, and/or a carrier that does not materially affect the structure, function, novel and/or basic features of the Bacillus species.

CSL Combination: Refers to a combination, or a composition, of DFMs including only *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis*. In some disclosed embodiments, "CSL combination" refers to a composition for administration to a subject, particularly to an animal, and even more particularly to an avian, such as chickens and turkeys, that consists of or consists essentially of *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis*. In other

embodiments, "CSL combination" refers to *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis* administered in combination without any other DFMs. A person of ordinary skill in the art will understand that the CSL combination may include additional residual material that is carried over from the production of any or all of the three *Bacillus* species, such as a dry milk product, and/or a carrier that does not materially affect the structure, function, novel and/or basic features of the three Bacillus species.

ASL Combination: Refers to a combination, or a composition, of DFMs including only *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis*. In some disclosed embodiments, "ASL combination" refers to a composition for administration to a subject, particularly to an animal, and even more particularly to an avian, such as chickens and turkeys, that consists of or consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis*. In other embodiments, "ASL combination" refers to *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis* administered in combination without any other DFMs. A person of ordinary skill in the art will understand that the ASL combination may include additional residual material that is carried over from the production of any or all of the three *Bacillus* species, such as a dry milk product, and/or a carrier that does not materially affect the structure, function, novel and/or basic features of the three Bacillus species.

ASLC Combination: Refers to a combination, or a composition, of DFMs including only *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*. In some disclosed embodiments, "ASLC combination" refers to a composition for administration to a subject, particularly to an animal, and even more particularly to an avian, such as chickens and turkeys, that consists of or consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*. In other embodiments, "ASLC combination" refers to *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* administered in combination without any other DFMs. A person of ordinary skill in the art will understand that the ASLC combination may include additional residual material that is carried over from the production of any or all of the four *Bacillus* species, such as a dry milk product, and/or a carrier that does not materially affect the structure, function, novel and/or basic features of the four Bacillus species.

Direct fed microbial: A composition that contains live and/or viable microorganisms, typically bacteria and/or yeast, that provides a beneficial effect on an animal, such as, but not limited to, an antimicrobial effect including decreased bacterial counts, improved feed conversion rate, improved weight gain, improved health parameters, reduced mortality rate, and/or improved lesion scores.

Feed conversion rate: A measure of the efficiency of an animal to convert feed mass into increased body mass. Typically, the feed conversion rate is calculated as pounds of feed divided by pounds of weight gain, and therefore may be expressed as a dimensionless number. The feed conversion rate is also known in the art as the feed conversion ratio, or feed efficiency.

Mannans: A class of polysaccharides including the sugar mannose. The mannans family includes pure mannans (*i.e.*, the polymer backbone comprises of mannose monomers), glucomannan (the polymer backbone comprises mannose and glucose), and galactomannan (mannans or glucomannan in which single galactose residues are linked to the polymer backbone). Mannans are found in cell walls of some plant species and yeasts, and may be provided as extracts of such plant species and/or yeasts.

Mineral clay: The term "mineral clay" refers to hydrous aluminum silicates. Mineral clays usually include minor amounts of impurities, such as potassium, sodium, calcium, magnesium, and/or iron.

Saponin: A class of chemical compounds, one of many secondary metabolites found in natural sources. Saponins are found in particular abundance in various plant species, such as quillaja and yucca. More specifically, saponins are amphipathic glycosides grouped, in terms of structure, by their composition. In certain embodiments, a saponin comprises one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or a triterpene derivative, a steroid or a steroidal derivative, or both.

Strain: A strain refers to two members of the same species having a discernible phenotypic and/or genetic difference.

Subject: Any animal or human, but particularly livestock (e.g., cows, sheep, goats, pigs, turkeys, and chickens) and household pets (e.g., dogs, cats, and rodents), and most typically "subject" refers herein to avians, including poultry, such as chickens and turkeys.

Effective amount: A quantity or concentration of a specified compound, composition or combination sufficient to achieve an effect in a subject.

Vitamin: Includes Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), vitamin C, vitamin D, vitamin E, vitamin K, K1 and K2 (i.e. MK-4, MK-7), folic acid and biotin, and derivative and analogs thereof.

Additional disclosure is provided by U.S. Patent Application No. 14/699,740, U.S. Patent Application No. 13/566,433, U.S. Patent Application No. 13/872,935, U.S. Patent Publication No.

2013/0017211, U.S. Patent Publication No. 2012/0156248, U.S. Patent Publication No. 2007/0253983, U.S. Patent Publication No. 2007/0202092, U.S. Patent Publication No. 2007/0238120, U.S. Patent Publication No. 2006/0239992, U.S. Patent Publication No. 2005/0220846, U.S. Patent Publication No. 2005/0180964, and Australian Patent Application No. 2011/201420, each of which is incorporated herein by reference in its entirety.

II. Bacilli Combination

A Bacilli combination is a combination or composition comprising three or four DFMs selected from *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*. A CSL combination is a combination or composition comprising the DFMs *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis* and no additional DFMs. An ASL combination is a combination or composition comprising the DFMs *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis*. In some embodiments, an ASL combination comprises, consists essentially of, or consists of *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis* and no additional DFMs. An ASLC combination is a combination or composition comprising the DFMs *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*. In some embodiments, an ASLC combination comprises, consists essentially of, or consists of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*, but no other additional DFMs.

Certain aspects of the present invention concern the discovery that administering a Bacilli combination, such as a CSL combination, an ASL combination, or an ASLC combination, to a subject provides a substantial benefit to the subject compared to a subject that is not administered the combination. The combination may be administered as a composition. With particular reference to poultry, a Bacilli combination provides a substantial benefit with respect to one or more of feed conversion rate, average body weight, average body weight gain, body weight coefficient of variation, bird mortality, lesion scores, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter relative to poultry fed none, one, or two of these bacilli in any combination.

In some embodiments, one or more of the bacillus in the Bacilli combination is provided as a bacillus spore, and in certain embodiments, all of the bacillus in the Bacilli combination are provided as spores.

In some embodiments, one or more of the bacillus in the Bacilli combination is dehydrated, such as by freeze drying or lyophilization, spray drying or other suitable dehydration techniques.

Dehydration, such as by freeze drying or spray drying may improve the stability and/or shelf-life of the bacteria. In certain embodiments, all the bacillus in the *Bacillus* combination are freeze dried.

A. *Bacillus* Strains

A person of ordinary skill in the art will appreciate that any strain, or combinations of strains, of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and/or *Bacillus amyloliquefaciens* can be used in the Bacilli combination. As used herein the terms "*Bacillus amyloliquefaciens*," "*Bacillus coagulans,*" "*Bacillus subtilis*" and "*Bacillus licheniformis*" independently may refer to a single strain of the respective *Bacillus* species, or to multiple strains, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more strains, of each respective *Bacillus* species. Solely by way of example and without limitation, certain acceptable exemplary strains of each *Bacillus* species are listed below. In certain embodiments, a *Bacillus* combination includes one or more of *Bacillus amyloliquefaciens* TOA5001, *Bacillus coagulans* GBI-30 strain, ATCC Designation Number PTA-6086, *Bacillus licheniformis* OBT618, and *Bacillus subtilis* strain OBT 1224, and in a particular embodiments, the *Bacillus* combination includes *Bacillus amyloliquefaciens* TOA5001, *Bacillus coagulans* GBI-30 strain, ATCC Designation Number PTA-6086, *Bacillus licheniformis* OBT618, and *Bacillus subtilis* strain OBT 1224.

Bacillus coagulans Strains

Bacillus coagulans Hammer ATCC® BAA-738™ strain LMG 17453, Logan B0934, NCTC 3992, Vitek #202384, *Bacillus coagulans* Hammer ATCC® 7050™ strain NRS 609, NCIB 9365, NCTC 10334, DSM 1, CCM 2013, WDCM 00002, *Bacillus coagulans* Hammer ATCC® 8038™ strain NCA 43P, NCIB 8080, NRS 770, DSM 2312 deposited with ATCC as *Bacillus thermoacidurans* by Berry, *Bacillus coagulans* Hammer ATCC® 10545™ strain NRS 784, NCIB 8041, DSM 2311, CCM 1082, deposited with ATCC as *Bacillus dextrolacticus* by Andersen and Werkman, *Bacillus coagulans* Hammer ATCC® 11014™ strain NRS T27, 78G, DSM 2383, *Bacillus coagulans* Hammer ATCC® 11369™ strain C, DSM 2384 deposited with ATCC as *Bacillus dextrolacticus* by Andersen and Werkman, *Bacillus coagulans* Hammer ATCC® 12245™ strain NCA 308, DSM 2308, NCIB 8870, *Bacillus coagulans* Hammer ATCC® 15949™ strain NCA 4259, DSM 2385, *Bacillus coagulans* Hammer ATCC® 23498™ strain M-39, DSM 2314, NCIB 10276 deposited with ATCC as *Bacillus racemilacticus* by Nakayama and Yanoshi, *Bacillus coagulans* Hammer ATCC® 31284™ deposited with ATCC as *Lactobacillus sporogenes* by Horowitz-Wiassowa and Nowotelnow, Ganeden Biotech Inc.'s *Bacillus coagulans* GBI-30 strain, ATCC Designation Number PTA-6086, *Bacillus coagulans* Hammer ATCC® 53595™ strain PM-

1000, *Bacillus coagulans* Hammer strain DSM 2350, NRRL-NRS 2012, *Bacillus coagulans* Hammer strain DSM 2356, NCIB 8523, N.R.Smith (NRS) 798, B. Hammer Iowa State College 200, *Bacillus coagulans* Hammer strain DSM 30760, *Bacillus coagulans* Hammer strain STI09070 (IMET), 1032-005, *Bacillus coagulans* Hammer strain STI09076 (IMET), 1141-003, *Bacillus coagulans* Hammer strain STI09080 (IMET), 1136-014, *Bacillus coagulans* Hammer strain STI09208 (IMET), 491-25, *Bacillus coagulans* Hammer strain STI09210 (IMET), 485-59, *Bacillus coagulans* Hammer strain NCIB 700460, Th1, *Bacillus coagulans* Hammer strain NCIB 701099, BG5, TH27 (205), *Bacillus coagulans* Hammer strain NCIB 701159, 254, and *Bacillus coagulans* Hammer strain NCIB 701164, 259.

Bacillus licheniformis Strains

Bacillus licheniformis (Weigmann) Chester ATCC® 6598™ strain NRS 745 deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 6634™ strain NRS 304, *Bacillus licheniformis* (Weigmann) Chester ATCC® 8480™ strain NRS 1128, *Bacillus licheniformis* (Weigmann) Chester ATCC® 9259™, *Bacillus licheniformis* (Weigmann) Chester ATCC® 9789™ strain AMNH 723, ATCC 102, ATCC 4527, ATCC 8243, ATCC 9800, NCTC 2586, NCTC 6346, NRS 243, NRS 978, W. Ford 1, DSM 8785, DSM 46308, BU 171, CCDB b-30, CCEB 631, CCM 2205, CN 1060, HNCMB 101012, IFO 12195, IFO 12196, IMET 11025, NBRC 12195, NBRC 12196, NCDO 735, NCDO 835, NCIB 6346, NCIB 8059, NCIB 8061, OUT 8367, OUT 8368, Smith 243, Smith 978, HankeyB13 deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 9945™ strain NRS 712, NCIB 8062 deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 9945a™ strain CD-2, NCIB 11709, *Bacillus licheniformis* (Weigmann) Chester ATCC® 10716™ strain ATCC 11944, BS 2181, Boots 1343, CCM 2181, FDA BT1, NCIB 8874, NRS 1330, Tracy I, DSM 603, IFO 12199, NBRC 12199, *Bacillus licheniformis* (Weigmann) Chester ATCC® 11945™ strain 1331, FDA BT3, *Bacillus licheniformis* (Weigmann) Chester ATCC® 11946™ strain 1333, B-1001, *Bacillus licheniformis* (Weigmann) Chester ATCC® 12139™ strain CSC deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 12713™ strain PRL B479, NRRL B-1001, *Bacillus licheniformis* (Weigmann) Chester ATCC® 12759™ strain ATCC 11560, Damodaron P-8, LMG 7560, NRS 1415, Vitek #200148, NCIB 8549, HankeyB133, P8, *Bacillus licheniformis* (Weigmann) Chester ATCC® 12759-MINI-PACK™ strain ATCC 11560, Damodaron P-8, LMG 7560, NRS 1415, Vitek #200148, *Bacillus licheniformis* (Weigmann) Chester ATCC® 13438™ Strain NCTC 8233, M. II strain, *Bacillus licheniformis* (Weigmann) Chester ATCC®

14409™ strain 620, NRS 1114, NCIB 1042, deposited with ATCC as *Bacillus abysseus* by ZoBell and Upham, *Bacillus licheniformis* (Weigmann) Chester ATCC® 14580™ strain (Gibson) 46, NCIB 9375, NCTC 10341, NRS 1264, DSM 13, CCM 2145, IFO 12200, NBRC 12200, WDCM 00068, *Bacillus licheniformis* (Weigmann) Chester ATCC® 14580D-5™ strain designation: Genomic DNA from *Bacillus licheniformis* Strain 46 [ATCC® 14580™], *Bacillus licheniformis* (Weigmann) Chester ATCC® 14594™, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21038™ strain L-065, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21039™, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21415™ strain NS 1 deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21417™ strain M deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21418™ deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21424™ strain DSM 1969, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21610™ strain B-201-7 deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21667™ strain FD 23612, *Bacillus licheniformis* (Weigmann) Chester ATCC® 21733™ strain DSM 1913 deposited with ATCC as *Bacillus subtilis* by (Ehrenberg) Cohn, *Bacillus licheniformis* (Weigmann) Chester ATCC® 25972™ strain 749/C, DSM 8782, DSM 46217, IMET10723, NCIB 9443, *Bacillus licheniformis* (Weigmann) Chester ATCC® 27326™ strain OM-81, *Bacillus licheniformis* (Weigmann) Chester ATCC® 27811™ strain 584, FERM-P 1038, *Bacillus licheniformis* (Weigmann) Chester ATCC® 31667™ strain DG 14, *Bacillus licheniformis* (Weigmann) Chester ATCC® 31972™ strain PM-3, *Bacillus licheniformis* (Weigmann) Chester ATCC® 33632™ strain (IOC) 2390, NCIB 11672, *Bacillus licheniformis* (Weigmann) Chester ATCC® 39326™, *Bacillus licheniformis* (Weigmann) Chester ATCC® 53757™ strain PWD-1, *Bacillus licheniformis* (Weigmann) Chester ATCC® 53926™ strain E312, *Bacillus licheniformis* (Weigmann) Chester ATCC® 55768™ strain O.W.U. 138B [OWU 138B], *Bacillus licheniformis* (Weigmann) Chester strain DSM 15, C, *Bacillus licheniformis* (Weigmann) Chester strain DSM 392, *Bacillus licheniformis* (Weigmann) Chester strain DSM 394, *Bacillus licheniformis* (Weigmann) Chester strain DSM 7259, NRRL-NRS 1263, *Bacillus licheniformis* (Weigmann) Chester strain DSM 7459, *Bacillus licheniformis* (Weigmann) Chester strain DSM 11258, *Bacillus licheniformis* (Weigmann) Chester strain DSM 11259, *Bacillus licheniformis* (Weigmann) Chester strain DSM 12369, *Bacillus licheniformis* (Weigmann) Chester strain DSM 12370, *Bacillus licheniformis* (Weigmann) Chester strain DSM 26543, *Bacillus licheniformis* (Weigmann) Chester strain DSM 28096, *Bacillus licheniformis* (Weigmann) Chester strain DSM 28591, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30523, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30535,

Bacillus licheniformis (Weigmann) Chester strain DSM 30542, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30585, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30615, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30620, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30624, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30643, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30654, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30724, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30766, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30769, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30778, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30779, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30865, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30926, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30959, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30960, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30961, *Bacillus licheniformis* (Weigmann) Chester strain DSM 30976, *Bacillus licheniformis* (Weigmann) Chester strain DSM 31019, *Bacillus licheniformis* (Weigmann) Chester strain DSM 100653, *Bacillus licheniformis* (Weigmann) Chester strain DSM 100655, *Bacillus licheniformis* (Weigmann) Chester strain DSM 103059, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 1525, 1229, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 6816, Glaxo 417, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 7224, Loos, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 8536, P1, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 8537, Ho, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 9536, Gibson 1319, NRS 1553, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 9667, 1, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 9668, 2, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 9669, 3, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 10689, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 11143, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 11643, YNS7712R, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 13497, *Bacillus licheniformis* (Weigmann) Chester strain NCIB 14014, DA33, *Bacillus licheniformis* B1 (NRRL Deposit Number B-50907), *Bacillus subtilis* B2 (Deposit Number B-50908), *Bacillus licheniformis* RW25 (NRRL Deposit Number B-50911), *Bacillus licheniformis* RW32 (NRRL Deposit Number B-50912), and *Bacillus licheniformis* RW41 (NRRL Deposit Number B-50913), *Bacillus licheniformis* BL21 (NRRL B-50134), *Bacillus licheniformis* 3-12a (NRRL B-50504), *Bacillus licheniformis* 4-2a (NRRL B-50506), *Bacillus licheniformis* 842 (NRRL B-50516), *Bacillus licheniformis* DSM 5749 (BioPlus® 2B, Chr. Hansen Bio Systems), and *Bacillus licheniformis* OBT618 (ATCC PTA-122188, Osprey Biotechnics).

Bacillus subtilis Strains

Bacillus subtilis (Ehrenberg) Cohn ATCC®82™ strain AMC, ATCC 8037, NRS 315, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®82D-5™ strain designation: Genomic DNA from *Bacillus subtilis* strain AMC [ATCC® 82™], *Bacillus subtilis* (Ehrenberg) Cohn ATCC®465™ strain NRS 743, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®4529™ strain 3, ATCC 8013, NCTC 2588, NRS 1004 deposited with ATCC as *Bacillus vulgatus* by Trevisan, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®4925™ strain NRS 740 deposited with ATCC as *Bacillus nigrificans* by Fabian and Nienhuis, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®4944™ strain NCTC, NRS 1106 deposited with ATCC as *Bacillus parvus*, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Cohn ATCC® 6051™ strain Marburg strain, ATCC 6051-U, CCM 2216, CCRC 10255, CCUG 163B, CFBP 4228, CIP 52.65, DSM 10, IAM 12118, IFO 12210, IFO 13719, IFO 16412, IMET 10758, JCM 1465, LMG 7135, NCAIM B.01095, NCCB 32009, NCCB 53016, NCCB 70064, NCFB 1769, NCIB 3610, NCTC 3610, NRRL B-4219, NRS 1315, NRS 744, VKM B-501, NBRC 13719 deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®6051a™ strain P31K6, *Bacillus subtilis* bacteriophage phi-e ATCC®6051-B1™ strain Phi-e deposited with ATCC as phi e, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®6460™ strain NRS 259 deposited with ATCC as *Bacillus aterrimus* by Lehmann and Neumann, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®6461™ strain NRS 275, CN 2192, NCIB 8055 deposited with ATCC as *Bacillus aterrimus* by Lehmann and Neumann, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. ATCC® 6633™ strain NRS 231, DSM 347, CCM 1999, IAM 1069, NCIB 8054, NCTC 10400, WDCM 00003 deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. ATCC® 6633D-5™ strain designation: Genomic DNA from *Bacillus subtilis* subspecies *spizizenii* strain NRS 231 [ATCC®6633™] deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. ATCC® CRM-6633™ strain NRS 231 deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. ATCC® 6633-MINI-PACK™ strain NRS 231 deposited with ATCC as *Bacillus subtilis* (Ehrenberg) Cohn, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®6984™ strain NRS 747 deposited with ATCC as *Bacillus vulgatus* subspecies *hydrolyticus*, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7003™ strain NRS 730, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7058™ strain NRS 351, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7059™ strain NRS 352, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7060™ strain NRS 659, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7067™ strain NRS 238, ATCC 7974, ATCC 8012, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®7480™ strain NRS 1107 deposited with ATCC as *Bacillus endoparasiticus* by (Benedek) Benedek, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®8188™ strain

ATCC 8450, NRS 773 deposited with ATCC as *Tyrothrix minimus*, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®8473™ strain NRS 762, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®9466™ strain designation: FDA strain PCI 220 [BUCSAV 170, NCIB 8159, NRRL B-558, NRS 1088], *Bacillus subtilis* (Ehrenberg) Cohn ATCC®9524™ strain 3R9675, NRS 1109, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®9799™ strain NCTC 6276, NRS 1125, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®9858™ strain NRS 237, NCIB 8063, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®9943™ strain NRS 979, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®10774™ strain BU169, NCIB 8872, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®10783™ strain NRRL B-543, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®11774™ strain NCTC 8236, DSM 2109, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®11838™ strain AMC 46-A-6 (strain I), NCIB 8850, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®12100™ strain NCA 1558, ND 957, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®12432™ strain MB 32, 56R188, ATCC 13597, NCIB 8993, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®12695™ strain 51-52, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®12711™ strain PRL B92, Ra, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®13542™, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®13933™ strain NRRL B-1471, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®13952™ strain 1346, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14410™ strain 625, NRS 1115 deposited with ATCC as *Bacillus borborokoites* by ZoBell and Upham, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14415™ strain 569, NRS 1120 deposited with ATCC as *Bacillus submarinus* by ZoBell and Upham, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14416™ strain 576, NRS 1121 deposited with ATCC as *Bacillus thalassokoites* by ZoBell and Upham, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14593™ strain IAM 1145, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14617™ strain A-1625, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14660™ strain C30-1, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14662™ strain C30-109, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®14807™ strain MB-155, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15040™ strain SX-67, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15041™ strain SX-92, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15134™ deposited with ATCC as *Bacillus uniflagellatus* by Mann, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15183™ strain 309, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15244™ strain 3369, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15245™ strain 3349, IAM 1-3 deposited with ATCC as *Bacillus natto* by Sawamura, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15476™ strain M-4-45, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15477™ strain M-24-1 deposited with ATCC as *Bacillus pumilus* by Meyer and Gottheil, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15561™ strain K-X-1, A-1, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15563™ strain Marburg, *Bacillus subtilis* bacteriophage SP8 ATCC®15563-B1™ strain SP8 deposited with ATCC as SP8 bacteriophage, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15575™ strain SB 19, *Bacillus*

subtilis (Ehrenberg) Cohn ATCC®15811™ strain 5380, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15818™ strain RIA 445, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15819™ strain RIA 447, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®15841™, *Bacillus subtilis* bacteriophage S-a ATCC®15841-B1™ strain S-a deposited with ATCC as S-a bacteriophage, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®19659™ strain PRD 66, IFO 13722, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®19659-MINI-PACK™ strain PRD 66, IFO 13722, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21008™ strain 182-H-86 deposited with ATCC as *Bacillus pumilus* by Meyer and Gottheil, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21183™ strain 5221, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21228™ strain SC 8548, SO-4, DSM 1970, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21331™ strain IFO 35, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21332™ strain IAM 1213, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21394™ strain 4-3-Ky, DSM 1971 deposited with ATCC as *Bacillus subtilis* subspecies *sakainensis*, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21555™ strain Y 13, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21556™, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21742™ strain AHr-5, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21770™ strain SP-3 deposited with ATCC as *Bacillus cereus* by Frankland and Frankland, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®21951™ strain 716, IFO 13322 deposited with ATCC as *Bacillus pumilus* by Meyer and Gottheil, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23059™ strain W23, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23856™ strain EMG 50, SB19, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23857™ strain 168, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23857D-5™ strain Designation: Genomic DNA from *Bacillus subtilis* strain 168 [ATCC® 23857™], *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23858™ strain EMG 52, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®23859™ strain EMG 53, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®25369™ strain 24028 deposited with ATCC as *Bacillus pulvifaciens* by Nakamura, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®27328™ strain C, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®27370™ strain 168 M, *Bacillus subtilis* bacteriophage SPO1 ATCC®27370-B1™ strain SPO1 deposited with ATCC as SPO1, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®27505™ strain K49, HER 1346 deposited with ATCC as *Bacillus subtilis* subspecies *amyloliquefaciens*, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®27689™ strain SB168 (trp-), *Bacillus subtilis* (Ehrenberg) Cohn ATCC®29056™ strain SB100, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®29233™ strain X6, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31002™ strain Ahr.AUr-9, FERM-1998, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31028™ strain FD 6404 deposited with ATCC as *Bacillus globigii* by Migula, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31091™ strain 1054, IFO 13586, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31094™ strain 1097, IFO 13621, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31098™ strain 1027, IFO 13585 deposited with ATCC as *Bacillus pumilus* by Meyer and Gottheil, *Bacillus*

subtilis subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®31578™ strain DSM 6223, RUB 331, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®31954™ strain MO7S-16/11, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®33234™ strain NCIB 10106, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®35021™ strain 5230, NRS 6, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®35854™ strain NRRL B-3411, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®35946™ strain OSU 75, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®37014™ strain DSM 6224, BD170, pSA2100, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®37015™ strain DSM 4514, BD170, NCIB 11624, pUB110, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®37108™ strain DSM 4873, BGSC 1E32, BR151, pPL608, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®37128™ strain DSM 4554, BGSC 1E18, pE194, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. ATCC®39090™ strain DSM 6198, BGSC 1S53, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®39320™ strain MB 4488, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®39374™ strain MB 3575, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®39706™ strain B1-20, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®43223™ strain ABM261, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®49343™ strain IMVS 0101, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®49760™ deposited with ATCC as *Bacillus globigii* by Migula, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®49822™ deposited with ATCC as *Bacillus globigii* by Migula, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55033™ strain SMS274, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55060™ strain MB 4974, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55405™ strain 300, *Bacillus subtilis* subspecies *inaquosorum* ATCC®55406™ strain DA33 deposited with ATCC as *Bacillus licheniformis* (Weigmann) Chester, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55422™ strain SC 15257, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55614™ strain 1.2, AQ153, *Bacillus subtilis* (Ehrenberg) Cohn ATCC®55675™ strain BP01, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 402, BRC 111470, NCIB 10106, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 618, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 1087, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 1088, IFO 13169, NBRC 13169, OUT 8353, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 1089, IFO 3026, NBRC 3026, OUT 8350, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 1090, OUT 8424 , *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 1091, OUT 8425, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 1092, IFO 3009, NBRC 3009, OUT 8235, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 3256, IAM 1213, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 3257, IAM 1259, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 3258, IAM 1260, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4181, NCA 72-52, SA 22, *Bacillus subtilis* subspecies *subtilis*

(Ehrenberg) Nakamura et al. strain DSM 4393, pC194, SB202, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4449, natto 3335 UM4, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4450, natto 3335 UM8, pLS20, pBC16, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4451 *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4515, DB163, pGR71, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 4608, BR157, pMW1, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 4750, 1E7, BGSC 1E7, pE194-cop6, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 4751, 1E34, BGSC 1E34, pAM77, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 4871, BD426, BGSC 1E21, pBD8, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 4872, BD466, BGSC 1E24, pBD10, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 4874, BGSC 1E38, pMK3, YB886, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 5213, BGSC 1A40, BR 151, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 5214, BD 393, BGSC 1A511, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 5545, BGSC 1A459/SU+III, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 5547, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 5552, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 5611, NRRL B-360, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 5660, NRRL B-362, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 6395, BGSC 2A2, W23 2A2, WB 672, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 6397, BGSC 1A2, SB 491, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 6399, BGSC 2A1, SB 623 *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 6405, BGSC 2A3, W23 SR, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 6887, BGSC 1A309, NP40, *Bacillus subtilis* subspecies *subtilis* (Ehrenberg) Nakamura et al. strain DSM 6889, 1A658, BGSC 1A658, DA 65 *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 8439, CCM 2268, IAM 12021, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 13019, SSI MK1, *Bacillus subtilis* subspecies *spizizenii* Nakamura et al. strain DSM 15029, NRRL B-23049, *Bacillus subtilis* subspecies *inaquosorum* Rooney et al. strain DSM 21200, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 21393, *Bacillus subtilis* subspecies *inaquosorum* Rooney et al. strain DSM 22148, KCTC 13429, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 23521, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 23778, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 25152, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 28592, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30512, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30529, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30533, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30534, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30540, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30541, *Bacillus subtilis*

(Ehrenberg) Cohn strain DSM 30551, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30558, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30562, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30570, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30581, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30597, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30642, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30651, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30652, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30671, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30676, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30677, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30682, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30711, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30723, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30801, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30924, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30925, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30927, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30928, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30929, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30941, D1, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 30942, D-FC1, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31008, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31009, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31010, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31020, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31021, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 31033, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 100605, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 100612, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 100613, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 100614, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 103044, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 103047, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 103051, *Bacillus subtilis* (Ehrenberg) Cohn strain DSM 103758, *Bacillus subtilis* AM0904 (NRRL Deposit Number B-50914), *Bacillus subtilis* AM0911 (NRRL Deposit Number B-50915), *Bacillus subtilis* NP122 (NRRL Deposit Number B-50910), *Bacillus subtilis* NP119B (NRRL Deposit Number B-50909), *Bacillus subtilis* BS18 (NRRL B-50633), *Bacillus subtilis* BS278 (NRRL 50634), *Bacillus subtilis* 4-7d (NRRL B-50505), *Bacillus subtilis* 3-5h (NRRL B-50507), *Bacillus subtilis* AGTP BS3BP5 (NRRL B-50510), *Bacillus subtilis* BS918 (NRRL B-50508), *Bacillus subtilis* AGTP BS1013 (NRRL-50509), *Bacillus subtilis* AGTP 944 (NRRL B-50548), *Bacillus subtilis* AGTP BS442 (NRRL B-50542), *Bacillus subtilis* AGTP BS1069 (NRRL B-50544), *Bacillus subtilis* AGTP BS521 (NRRL B-50545), *Bacillus subtilis* B27 (NRRL B-50105), *Bacillus subtilis* 3A-P4 (PTA-6506), *Bacillus subtilis* 22C-P1 (PTA-6508), *Bacillus subtilis* BL21 (NRRL B-50134), *Bacillus subtilis* strain GB03, *Bacillus subtilis* strain QST713, *Bacillus subtilis* DSM 5750 (BioPlus® 2B, Chr. Hansen Bio Systems), *Bacillus subtilis* strain OBT 1224 (Osprey Biotechnic).

***Bacillus amyloliquefaciens* Strains**

Bacillus amyloliquefaciens (Fukumoto) Priest et al. (ATCC® 23350™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 23842™), *Bacillus amyloliquefaciens* SB 3296 (PTA-7548), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 23843™), *Bacillus amyloliquefaciens* SB3297 (PTA-7549), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® BAA-390™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 23845™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 23844™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 31592™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 53495™), *Bacillus amyloliquefaciens* (Fukumoto) Priest et al. (ATCC® 49763™), *Bacillus amyloliquefaciens*: SB 3276 (PTA-7541), *Bacillus amyloliquefaciens*: PMBP-M7 (vial labeled BCRC PMBP-M7) (PTA-5819), *Bacillus amyloliquefaciens* SB 3284 (PTA-7545), *Bacillus amyloliquefaciens* SB 3288 (PTA-7546), *Bacillus amyloliquefaciens* MF215 (SB3446) (PTA-7790), *Bacillus amyloliquefaciens* SB 3283 (PTA-7544), *Bacillus amyloliquefaciens* MF 225 (SB 3448) (PTA-7791), *Bacillus* sp. (ATCC® 70038™, Deposited As *Bacillus amyloliquefaciens* (Fukumoto) Priest et al.), *Bacillus amyloliquefaciens* TOA5001 (NITE Patent Microorganisms Depositary Accession Number BP-01844).

III. Amounts of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and/or *Bacillus amyloliquefaciens* in Bacilli Combinations

The relative amounts of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and/or *Bacillus amyloliquefaciens* present in the Bacilli combination are selected to obtain a desired result. For certain embodiments, the Bacilli combination comprises from about 10^5 to about 10^{12} CFU/gram, and more typically from about 10^5 to 10^{10} or from 10^8 to 10^{10} CFU/gram of each of the *Bacillus* species in the Bacilli combination. In certain embodiments, the Bacillus combination is added to feed in an amount sufficient to provide from 5 x 10^5 to 8 x 10^5 CFU/gram of the feed/Bacillus combination. And in particular embodiments, 0.25 pounds/ton of feed is sufficient to provide 5 x 10^5 to 8 x 10^5 CFU/gram.

In some embodiments, the Bacilli combination may be administered to provide different CFU ratios of the *Bacillus* species included therein. In some embodiments, the ratio of *Bacillus subtilis:Bacillus licheniformis* in the Bacilli combination may be from 2:1 to 1:2, and typically is about 1:1, relative to each other. And with respect to other *Bacillus* species in the Bacilli combination, the total amount of *Bacillus subtilis* and *Bacillus licheniformis* (BSBL) relative to the other *Bacillus* species may be from greater than zero to 99%, such as from 10% to 90%, from 15%

to 85%, from 20% to 80%, from 25% to 75%, from 35% to 65%, from 45% to 55%, or substantially 50%, based on CFU.

In some embodiments, the ASL combination may comprise, consist essentially of, or consist of, in amounts relative to each other, from 25% or less to 75% or more *Bacillus amyloliquefaciens* (BA) and from 75% or more to 25% or less BSBL. In certain embodiments, the ratio of BA to BSBL in the ASL combination is from 25%:75% BA:BSBL to 75%:25% BA:BSBL, and may be about 50%:50% BA:BSBL.

In some embodiments, the ASLC combination may comprise, consist essentially of, or consist of, in amounts relative to each other, from 25% or less to 75% or more in total of *Bacillus amyloliquefaciens* (BA) and *Bacillus coagulans* (BC), and from 75% or more to 25% or less BSBL. In certain embodiments, the ratio of BA+BC to BSBL in the ASL combination is from 25%:75% BA+BC:BSBL to 75%:25% BA+BC:BSBL, and may be about 50%:50% BA+BC:BSBL. The amounts of BA and BC, relative to each other may be from greater than zero to 99% BA relative to BC, such as from 10% to 90%, from 15% to 85%, from 20% to 80%, from 25% to 75%, from 35% to 65%, from 45% to 55%, or substantially 50% BA relative to BC, based on CFU.

For example, the CSL combination may comprise from 3.5 x 10^9 to 10 x 10^9 CFU *Bacillus coagulans* per gram of the CSL combination, such as from 4.1 x 10^9 to 7.5 x 10^9, from 5 x 10^9 to 6.4 x 10^9 or from 5 x 10^9 to 6 x 10^9 CFU *Bacillus coagulans*/gram. The CSL combination may comprise from 5 x 10^8 to 10 x 10^8 CFU *Bacillus subtilis* per gram of the CSL combination, such as from 6 x 10^8 to 8.7 x 10^8, from 6.9 x 10^8 to 9 x 10^8, or 7.2 x 10^8 to 8 x 10^8 CFU *Bacillus subtilis*/per gram. And the CSL combination may comprise from 5 x 10^8 to 10 x 10^8 CFU *Bacillus licheniformis* per gram of the CSL combination, such as from 6 x 10^8 to 8.7 x 10^8, from 6.9 x 10^8 to 9 x 10^8, or 7.2 x 10^8 to 8 x 10^8 CFU *Bacillus licheniformis* per gram.

In certain embodiments, the CSL combination may be administered to provide different CFU ratios of the three *Bacillus* species. For example, in one embodiment, the CSL combination ratio provides from about 6 parts to about 10 parts *Bacillus coagulans* to 1 part to 2 parts *Bacillus subtilis,* and from about 1 part to about 2 parts *Bacillus licheniformis*. The ratio of *Bacillus subtilis:Bacillus licheniformis* in the CSL combination may be from 2:1 to 1:2, and typically is about 1:1. In certain embodiments, the CSL combination comprises about 5 x 10^9 *Bacillus coagulans*, about 8 x 10^8 *Bacillus subtilis*, and 8 x 10^8 *Bacillus licheniformis* per gram of the CSL combination.

In other embodiments, the *Bacillus* combination comprises, consists essentially of, or consists of, *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens* and *Bacillus coagulans*. The relative amounts of the Bacillus species in such embodiments are from 25% to

50% *Bacillus subtilis*, from 30% to 65% *Bacillus licheniformis*, from 5% to 30% *Bacillus amyloliquefaciens* and from greater than zero to 15% *Bacillus coagulans*, in amounts relative to each other, such as from 30% to 45% *Bacillus subtilis*, from 40% to 60% *Bacillus licheniformis*, from 10% to 25% *Bacillus amyloliquefaciens* and from 1% to 12% *Bacillus coagulans*. In certain embodiments, the *Bacillus* combination comprises, consists essentially of, or consists of, from 30% to 40% *Bacillus subtilis*, from 40% to 50% *Bacillus licheniformis*, from 10% to 20% *Bacillus amyloliquefaciens* and from 2% to 10% *Bacillus coagulans* in amounts relative to each other. In some embodiments, the amount of *Bacillus licheniformis* is greater than the amount of *Bacillus subtilis* in the *Bacillus* combination. In certain embodiments, such a composition can be a commercially available product, such as the composition sold as Provia Prime™ by Phibro Animal Health Corporation.

IV. Additional Component(s)

The Bacilli combination also can be administered in combination with one or more additional components or compositions. An additional component or composition may be any component or composition that can be administered to a subject, particularly an animal, such as an avian, including poultry, in combination with the *Bacilli* species in the Bacilli combination, such as *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis*, or *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis* and optionally *Bacillus coagulans*. Certain disclosed Bacilli combinations are particularly formulated for administration to poultry, and therefore can comprise the Bacilli combination in combination with any other component or composition now known or hereafter developed for administration to poultry. Exemplary additional components include a carrier, a vitamin, a copper salt, a feed supplement, an additional DFM, a feed, such as a poultry feed, or a combination thereof. The additional component(s) will comprise from 1 wt% to 99 wt% and the Bacilli combination will comprise from 99 wt% to 1 wt% of the total weight of the combination. Preferably the additional component(s) will comprise from 10 wt% to 90 wt% and the Bacilli combination will comprise from 90 wt% to 10 wt% of the total weight of the combination. Yet even more preferably, the additional component(s) will comprise 20 wt% to 80 wt% and the Bacilli combination will comprise from 80 wt% to 20 wt% of the total weight of the combination. The Bacilli combination may be administered with the other component(s), optionally in a mixture with the other component(s), such as poultry feed and/or a feed supplement, in an amount sufficient to provide the desired amounts of the respective *Bacillus* species in the particular combination. For example for the CSL combination, the amount administered may be sufficient to provide from 0.5 x 10^5 to 2 x 10^5 CFU *Bacillus coagulans* per gram of the mixture,

from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus subtilis* per gram of the mixture, and/or from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus licheniformis* per gram of the mixture. In some embodiments, the amount of *Bacillus coagulans* in the mixture is from 0.6 x 10^5 to 1.5 x 10^5 CFU, or from 0.9 x 10^5 to 1.2 x 10^5 CFU per gram of the mixture. Independently, the amount of each of the *Bacillus subtilis* and *Bacillus licheniformis* in the mixture may be from 1.5 x 10^5 to 3 x 10^5 or from 2.3 x 10^5 to 3 x 10^5 CFU per gram of the mixture. In certain embodiments, the CSL combination is administered with one or more additional components, such as feed and/or feed supplement, in an amount sufficient to provide 0.62 x 10^5, 0.93 x 10^5 or 1.2 x 10^5 CFU *Bacillus coagulans*, and independently 1.5 x 10^5, 2.3 x 10^5 or 3 x 10^5 CFU of each of the *Bacillus subtilis* and *Bacillus licheniformis*, per gram of the mixture. Exemplary additional components and/or compositions of a combination comprising the Bacilli combination are discussed in more detail below.

A. Carrier

In some embodiments, the Bacilli combination may be mixed with and/or dispersed in a carrier to form a dispersed composition. The carrier(s) may be selected to provide a non-biological benefit to the composition, compared to a Bacilli combination without a carrier, such as, but not limited to, achieving or improving a readily flowable state, and/or improving stability during storage and/or transport. Suitable carriers that may be used in combination with a Bacilli combination include, but are not limited to, plant material, such as beet pulp, ground corn, corn syrup solids, plant fiber, rice hulls, soluble plant fiber, wheat middlings, microcrystalline cellulose; carbonates, such as metal carbonates, such as calcium carbonate, potassium carbonate; sulfates, such as metal sulfates, such as potassium sulfate, sodium sulfate; lactates, including metal lactates, such as calcium lactate; oxides, including metal oxides, such as calcium oxide; propionates, including metal propionates, such as calcium propionate; stearates, including metal stearates, such as calcium stearate; phosphates, such as dicalcium phosphate dehydrate, monocalcium phosphate, sodium tripolyphosphate, or tetra sodium pyrophosphate; minerals, such as dolomite, silicon dioxide, silica, limestone, or vermiculite; clays, such as bentonite, montmorillonite, kaolin; sugars, such as glucose, sucrose, dextrose, fructose, or a combination thereof; maltodextrin; salt, such as sodium chloride; carrageenan; cellulose; guar gum; polyols; sodium alumino silicate; urea; animal protein products; forage products; grain products; plant protein products; processed grain products; roughage products; molasses products; or combinations thereof. In certain embodiments, the carrier is calcium carbonate.

Animal protein products may include, but are not limited to, blood meal; animal by-product meal; buttermilk, including condensed buttermilk and dried buttermilk; casein; dried hydrolyzed

casein; cheese rind; crab meal; fish products, including fish by-products, fish liver and glandular meal, fish meal, fish protein concentrates, fish residue meal, and dried and/or condensed fish solubles; fleshings hydrolysate; hydrolyzed hair; hydrolyzed leather meal; hydrolyzed poultry by-product aggregate; hydrolyzed poultry feathers; leather hydrolysate; meat and bone meal; meat and bone meal tankage; meat meal; meat meal tankage; dried meat solubles; dried lactalbumin; dried feed grade milk; dried milk protein; poultry by-products and/or by-products meal; poultry hatchery by-product; shrimp meal; skimmed milk, including condensed, condensed cultured, dried, or dried cultured skimmed milk; whey, including condensed, condensed cultured, condensed hydrolyzed, dried, or dried hydrolyzed whey; condensed and/or dried whey product; condensed and/or dried whey solubles; or a combination thereof.

Forage products may include, but are not limited to, alfalfa products, such as dehydrated meal, optionally in pellet form, ground hay, or suncured meal, optionally in pellet form; coastal bermudagrass hay; dehydrated corn plant; dehydrated silage; flax plant product; ground grass; lespedeza meal and/or stem meal; ground soybean hay; or combinations thereof.

Grain products may include, but are not limited to, barley, corn, grain sorghum, mixed feed oats, oats, triticale, wheat, ground brown rice, ground or ground paddy rough rice, broken or chipped rice, brewers rice, rye, or a combination thereof. The grain products may be in any suitable form, such as whole, ground, cracked, hulls, bran, screen cracked, flaked, kibbled, toasted, and/or heat processed.

Plant protein products may include, but are not limited to, dried beans; canola meal; coconut meal; cottonseed, such as flakes, cake, meal, low gossypol meal, and/or whole pressed cottonseed; guar meal; dried kelp; linseed meal; peanut meal; peas; potato protein; dried seaweed meal; safflower meal; soy protein concentrate; soybean feed; ground soybeans; soybean meal, optionally kibbled; heat processed soybeans; ground, extruded whole soybeans; soy flour; soy grits; sunflower meal, optionally dehulled; or a combination thereof.

The processed grain by-products may be aspirated grain fractions; brewers dried grains; buckwheat middlings; condensed distillers solubles; condensed fermented corn extracts; corn bran; corn flour; corn germ meal; corn gluten feed and/or meal; corn grits; distillers dried grains, optionally with solubles; distillers dried solubles, flour, grain sorghum germ cake, meal, grits, and/or mill feed; meal hominy feed; malt sprouts; oat groats; feeding oat meal; pearl barley by-product; peanut skins; rice bran; rice polishings; rye middlings; gelatinized or partially aspirated sorghum grain flour; wheat bran, flour, shorts, germ meal, defatted germ meal, middlings, mill run and/or red dog; or a combination thereof.

Roughage products may include, but are not limited to, almond hulls; dried apple pectin pulp; dried apple pomace; bagasse; barley hulls; barley mill by-product; dried, plain beet pulp; buckwheat hulls; dried citrus meal; dried citrus pulp; citrus seed meal; corn cob fractions; cottonseed hulls; flax straw by-product; ground corn cob; psyllium seed husk; malt hulls; clipped oat by-product; oat hulls; oat mill by-product; peanut hulls; rice hulls; rice mill by-product; rye mill run; soybean hulls, mill feed, and/or mill run; sunflower hulls; ground straw; dried tomato pomace; or a combination thereof.

Molasses products may be beet molasses; dried beet molasses product; dried beet pulp molasses; cane molasses; citrus molasses; molasses yeast condensed solubles; concentrated separator by-product; condensed molasses fermentation solubles; starch molasses; molasses distillers condensed solubles; molasses distillers dried solubles; or a combination thereof.

B. Copper species

The disclosed combination may be mixed with a copper species such as a copper species that provides a copper ion. The copper species may be a copper salt. Exemplary copper species that may be combined with the Bacilli combination include, but are not limited to, copper chloride, copper bromide, copper iodide, copper sulfate, copper sulfite, copper bisulfite, copper thiosulfate, copper phosphate, monobasic copper phosphate, dibasic copper phosphate, copper hypophosphite, copper dihydrogen pyrophosphate, copper tetraborate, copper borate, copper carbonate, copper bicarbonate, copper metasilicate, copper citrate, copper malate, copper methionate, copper succinate, copper lactate, copper formate, copper acetate, copper butyrate, copper propionate, copper benzoate, copper tartrate, copper ascorbate, copper gluconate, or a combination thereof, preferably copper sulfate, copper acetate, copper citrate, copper methionate, or a combination thereof. A copper species, such as a copper salt, may be provided separately, or individually, or it may be provided as part of a composition, such as a feed or a feed supplement. Certain disclosed embodiments comprise, consist essentially of, or consist of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and a copper species. Other particular embodiments, comprise, consist essentially of, or consist of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and a copper species. And other particular embodiments, comprise, consist essentially of, or consist of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* and a copper species. In any embodiments, the copper species may be a copper salt, such as a salt that can provide a copper ion, for example, copper sulfate.

C. Vitamin(s)

Exemplary vitamins include, but are not limited to, one or more of Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (including folic acid), Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), Vitamin C (ascorbic acid or a salt thereof, such as sodium ascorbate or calcium sorbate), Vitamin D (vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, vitamin D_5, 25-hydroxy vitamin D_3, 25-dihydroxy vitamin D_3, or combinations thereof), Vitamin E, Vitamin K (K1 and K2 (i.e. MK-4, MK-7)), and biotin, and derivatives, salts and/or analogs thereof.

D. Feed

The feed may be any feed suitable for administration to an animal. The Bacilli combination may be administered in combination with the feed, such as by forming a mixture of the Bacilli combination and the feed, or by administering the Bacilli combination and the feed sequentially, in any order. In certain disclosed embodiments the animal is a poultry, and the Bacilli combination is used in combination with, and may be admixed with, a poultry feed, such as a poultry basal diet. The feed may comprise corn, alfalfa, peas, soybean meal, soybean oil, wheat, oats, sorghum, barley, rye, rice hulls, canola, corn oil, limestone, salt (for example, sodium chloride), distillers dried grains with solubles (DDGS), dicalcium phosphate, sodium sesquicarbonate, methionine source, lysine source, L-threonine, mineral oil, biotin, folic acid, kelp, menadione dimethylpyrimidinol bisulfite, calcium aluminosilicate, or any combination thereof. The feed may also comprise one or more additional components. Additional components may be used for any desired purpose, such as a substantially biologically inert material added, for example, as a filler, or to provide a desired beneficial effect. For example, the feed may include a carbonate (including a metal carbonate such as calcium carbonate); a trace mineral, such as, but not limited to, chloride, fluoride, iodide, chromium, copper, zinc, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, sulfur, selenium, or a combination thereof; a bulking agent; a carrier; a colorant; a taste enhancer; a preservative; one or more vitamins; or a combination thereof. The preservative may be benzoic acid or a salt thereof, *e.g.* sodium benzoate; lactic acid or a salt thereof, *e.g.* sodium lactate, potassium lactate or calcium lactate; propionic acid or a salt thereof, *e.g.* sodium propionate; ascorbic acid or a salt thereof, *e.g.* sodium ascorbate; gallic acid or a salt thereof *e.g.* sodium gallate; sulfur dioxide and/or sulfites; nitrites; nitrates; choline, or a salt thereof, such as an anion salt of choline, *e.g.* choline halide, such as chloride, bromide, iodide, fluoride, or

choline hydroxide; or any combination thereof. The one or more vitamins may include vitamin A; vitamin B_1, such as thiamine mononitrate; vitamin B_2, such as riboflavin-5-phosphate; vitamin B_3, such as niacin or niacinamide; vitamin B_5, such as pantothenic acid or d-calcium pantothenate; vitamin B_6, such as pyridoxine or pyridoxine hydrochloride; vitamin B_{12}; vitamin C, such as ascorbic acid, sodium ascorbate, or calcium sorbate; vitamin D; vitamin E; vitamin K, or a combination thereof. Vitamin D may comprise vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, vitamin D_5, 25-hydroxy vitamin D_3, 25-dihydroxy vitamin D_3, or combinations thereof.

The feed, such as a poultry feed, may also include fats and/or oils, such as tallow, optionally derived from the rendering of beef offal; lard, optionally derived from the rendering of pork offal; poultry fat, optionally derived from poultry offal; feed grade animal fat, optionally derived from a mixture of rendered beef, pork, and/or poultry raw material; yellow grease, optionally derived from reprocessed restaurant grease and/or cooking oil; and/or blended animal-vegetable fat, which may include blends of different types and/or amounts of animal fats and vegetable oils from restaurant grease. Additionally, or alternatively, the feed may include protein sources, such as canola, fish meal, field peas, meat and bone meal, soybeans, and/or cereal by-products.

E. Feed Supplements

The Bacilli combination may be used in combination with one or more feed supplements. In some embodiments, the Bacilli combination is mixed with the feed supplement to form a mixture or composition comprising the Bacilli combination and the feed supplement(s). In other embodiments, the Bacilli combination is administered in combination with a feed supplement.

1. Yucca and/or Quillaja, or Extracts thereof

Additionally, or alternatively, a disclosed Bacilli combination can be administered in combination with yucca and/or quillaja plant material, or extracts thereof. Examples of yucca include, but are not limited to, *Yucca aloifolia, Yucca angustissima, Yucca arkansana, Yucca baccata, Yucca baileyi, Yucca brevifolia, Yucca campestris, Yucca capensis, Yucca carnerosana, Yucca cernua, Yucca coahuilensis, Yucca constricta, Yucca decipiens, Yucca declinata, Yucca de-smetiana, Yucca elata, Yucca endlichiana, Yucca faxoniana, Yucca filamentosa, Yucca filifera, Yucca flaccida, Yucca gigantean, Yucca glauca, Yucca gloriosa, Yucca grandiflora, Yucca harrimaniae, Yucca intermedia, Yucca jaliscensis, Yucca lacandonica, Yucca linearifolia, Yucca luminosa, Yucca madrensis, Yucca mixtecana, Yucca necopina, Yucca neomexicana, Yucca pallida, Yucca periculosa, Yucca potosina, Yucca queretaroensis, Yucca reverchonii, Yucca rostrata, Yucca rupicola, Yucca schidigera, Yucca schottii, Yucca sterilis, Yucca tenuistyla, Yucca thompsoniana,*

Yucca treculeana, Yucca utahensis, Yucca valida or combinations thereof. In certain embodiments, the Yucca is or comprises *Yucca schidigera.*

Examples of quillaja include, but are not limited to, *Quillaja brasiliensis, Quillaja lanceolata, Quillaja lancifolia, Quillaja molinae, Quillaja petiolaris, Quillaja poeppigii, Quillaja saponaria, Quillaja sellowiana, Quillaja smegmadermos* or combinations thereof. In certain embodiments, the quillaja is or comprises *Quillaja saponaria.*

A person of ordinary skill in the art will appreciate that, as used herein, a plant name may refer to the plant as a whole, or to any part of the plant, such as the roots, stem or trunk, bark, leaves, flower, flower stems, seeds, or a combination thereof. These plant parts may be used fresh, or dried, and may be whole, pulverized, or comminuted. The plant name may also refer to extracts from any part or parts of the plant, such as chemical extracts, or extracts obtained by pressing, or any other methods of concentrating or extracting oils or other extracts known to those in the art or that are hereafter discovered. Plant extracts may include compounds that are saponins, triterpenoids, polyphenols, antioxidants or resveratrol, or combinations thereof.

The combination may comprise a composition comprising yucca and/or quillaja that may also include carriers and binding agents suitable to formulate the yucca and/or quillaja for administration to an animal. In certain embodiments, such a composition can be a commercially available product, such as a composition comprising *Yucca schidigera* and *Quillaja saponaria*, sold under the trademark NUTRAFITO PLUS by Desert King International and/or MAGNI-PHI® by Phibro Animal Health Corporation. Such compositions may comprise from 99% or more *Quillaja saponaria* and 1% or less *Yucca schidigera* to 75% *Quillaja saponaria* and 25% *Yucca schidigera*, such as from 95% *Quillaja saponaria* and 5% *Yucca schidigera* to 80% *Quillaja saponaria* and 20% *Yucca schidigera*, and in certain embodiments, 85% *Quillaja saponaria* and 15% *Yucca schidigera,* or 90% *Quillaja saponaria* and 10% *Yucca schidigera.*

In some embodiments, a combination and/or composition comprises, consists essentially of, or consists of, yucca, quillaja, *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*, such as *Yucca schidigera, Quillaja saponaria, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens.* The combination and/or composition may comprise, consist essentially of, or consist of, from 100 to 500 ppm or more yucca and quillaja, and from 0.1 to 1 pounds of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*, per ton of a feed, such as from 200 to 500 ppm yucca and quillaja, and from 0.1 to 0.5 pounds of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*, and in certain embodiments, from 200 to 300 ppm

Yucca schidigera and *Quillaja saponaria*, and from 0.2 to 0.3 pounds of *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*.

2. Silica, Mineral Clay, Glucan and Mannans

Additionally, or alternatively, a Bacilli combination can be administered in combination with a feed supplement comprising silica, mineral clay, glucan and mannans. The feed supplement may further comprise an endoglucanohydrolase, either endogenously or as an affirmatively added ingredient. As used herein, weight% for endoglucanohydrolase is based on a 70,000 unit/gram endoglucanohydrolase product. The endoglucanohydrolase may be β-1,3 (4)-endoglucanohydrolase.

In any embodiments disclosed herein, the feed supplement may comprise, consist essentially of, or consist of, glucan (e.g., β-1,3 (4)glucan), silica, mineral clay and mannans. In some embodiments, the feed supplement comprises, consists essentially of, or consists of, glucan (e.g., β-1,3 (4)glucan), silica, mineral clay, mannans and endoglucanohydrolase. In any embodiments disclosed herein, the glucan and mannans may be provided, at least in part, by yeast cell wall or an extract thereof. Thus, in some embodiments, the feed supplement may comprise, consist essentially of, or consist of, silica, mineral clay and yeast cell wall or an extract thereof, or the feed supplement may comprise, consist essentially of, or consist of, silica, mineral clay, yeast cell wall or an extract thereof, and endoglucanohydrolase. Similarly, endoglucanohydrolase may, in certain disclosed embodiments, be provided by yeast cell wall or a yeast cell wall extract.

Suitable sources of silica include, but are not limited to, sand, diatomaceous earth, and synthetic silica. In one embodiment, quartz may be used. In certain embodiments, the mannans comprise glucomannan.

The components of the feed supplement are prepared by methods commonly known in the art and can be obtained from commercial sources. β-1,3 (4)-endoglucanohydrolase may be produced from submerged fermentation of a strain of *Trichoderma longibrachiatum*. Diatomaceous earth is available as a commercially-available product with from 70% to 95% silica (SiO_2) and with its remaining components not assayed but primarily ash (minerals) as defined by the Association of Analytical Chemists (AOAC, 2002). The mineral clays (e.g., aluminosilicates) used in this feed supplement may be any of a variety of commercially-available clays including, but not limited to, montmorillonite clay, bentonite and zeolite. Glucan, mannans, and/or endoglucanohydrolase can be obtained from plant cell walls, yeast or yeast cell wall or an extract thereof (e.g., *Saccharomyces cerevisiae*, *Candida utilis*), certain fungi (e.g., mushrooms), algae,

and bacteria. In certain embodiments, yeast can be administered affirmatively to provide glucan, mannans and endoglucanohydrolase endogenously.

In one embodiment, the feed supplement comprises, consists essentially of, or consists of, 1-40 wt% silica, 0.5-25 wt% glucan and mannans, and 40-92 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 5-40 wt% silica, 0.5-15 wt% glucan and mannans, and 40-80 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 20-40 wt% silica, 0.5-10 wt% glucan and mannans, and 50-70 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 15-40 wt% silica, greater than zero to 15 wt% glucans, greater than zero to 10 wt% mannans, and 50-81 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 15-40 wt% silica, 0.5-5.0 wt% glucans, 0.5-8.0 wt% mannans, and 50-81 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 20-30 wt% silica, 0.5-3.5 wt% glucans, 0.5-6.0 wt% mannans, and 60-70 wt% mineral clay, in amounts relative to each other.

In some embodiments, β-glucans and mannans are obtained from yeast or yeast cell wall or an extract thereof. The feed supplement may comprise, consist essentially of, or consist of, 1-40 wt% silica, 1-30 wt% yeast cell wall or an extract thereof, and 40-92 wt% mineral clay, in amounts relative to each other. In one embodiment, the feed supplement comprises, consists essentially of, or consists of, 10-40 wt% silica, 5-20 wt% yeast cell wall or an extract thereof, and 40-80 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 15-30 wt% silica, 5-15 wt% yeast cell wall or an extract thereof, and 50-70 wt% mineral clay, in amounts relative to each other.

In any of the above embodiments, the feed supplement may further comprise an endoglucanohydrolase, such as β-1,3 (4)-endoglucanohydrolase. The feed supplement may include from 0.025 wt% endoglucanohydrolase to 5 wt% endoglucanohydrolase or more, such as from 0.05 wt% to 3 wt% β-1,3 (4)-endoglucanohydrolase, relative to the amounts of silica, mineral clay, glucan, mannans, and/or yeast, yeast cell wall, or yeast cell wall extract present in the feed supplement. In one embodiment, the feed supplement comprises, consists essentially of, or consists of, 0.1-3 wt% β-1,3 (4)-endoglucanohydrolase, 20-40 wt% silica, 0.5-20 wt% glucan and mannans, and 50-70 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 0.1-3 wt%, β-1,3 (4)-endoglucanohydrolase, 20-40 wt% silica, 0.5-10 wt% glucan and mannans, and 50-70 wt% mineral

clay, in amounts relative to each other. Alternatively, the feed supplement may comprise, consist essentially of, or consist of, 0.1-3 wt% β-1,3 (4)-endoglucanohydrolase, 1-40 wt% silica, 5-30 wt% yeast cell wall or an extract thereof, and 40-92 wt% mineral clay, in amounts relative to each other. In one embodiment, the feed supplement comprises, consists essentially of, or consists of, 0.1-3 wt% β-1,3 (4)-endoglucanohydrolase, 10-40 wt% silica, 5-20 wt% yeast cell wall or an extract thereof, and 40-80 wt% mineral clay, in amounts relative to each other. In another embodiment, the feed supplement comprises, consists essentially of, or consists of, 0.1-3 wt% β-1,3 (4)-endoglucanohydrolase, 15-30 wt% silica, 5-15 wt% yeast cell wall or an extract thereof, and 50-70 wt% mineral clay, in amounts relative to each other.

In any of the above embodiments, the silica may be provided by diatomaceous earth. In any of the above embodiments, the glucans may be β-glucans. In some embodiments, the β-glucans can be obtained from yeast, or other materials, such as fungi, algae, bacteria, or the like. In any of the above embodiments, the mannans may comprise glucomannan.

The glucan and mannans (or yeast or yeast cell wall or an extract thereof) can be prepared by a method known to a person of ordinary skill in the art and as further disclosed by the patent documents incorporated herein by reference. Yeast cell wall or an extract thereof may have a feed supplement comprising 0-15% moisture and 85-100% dry matter. The dry matter may comprise 10-65 % protein, 0-25 % fats, 0-3% phosphorus, 5-30% β-glucan, 5-35% mannans, and 0-15% ash. In an independent embodiment, a commercial source of β-1,3 (4) glucan and glucomannan derived from primary inactivated yeast (*Saccharomyces cerevisiae*) with the following chemical feed supplement can be used: moisture 2-5%; proteins 40-50%; fats 3-8%; phosphorus 0-2%; mannans 10-16%; β-l,3-(4) glucan 10-20%; and ash 2-12%.

In another independent embodiment, the yeast cell wall or an extract thereof comprises moisture 1-7% and dry matter 93-99%, and the dry matter may comprise proteins 18-28%, fats 10-17%, phosphorus 0-2%, mannans 20-30%, β-l,3-(4) glucan 18-28%, and ash 2-5%.

In an independent embodiment of the feed supplement, silica, glucan and mannans, and mineral clay are combined at 1-40%, 0.5-25% and 40-92% by weight, respectively. In an independent embodiment of the feed supplement and/or combination, β-1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall or an extract thereof, and mineral clay are combined at 0.05-3%, 1-40%, 1-20% and 40-92% by weight, respectively. In an independent feed supplement and/or combination, β-1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall or an extract thereof, and mineral clay are combined at 0.1-3%, 5-40%, 2-15% and 40-80% by weight, respectively. In another independent embodiment of the feed supplement and/or combination, β-1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall or an extract

thereof, and mineral clay are combined at 0.1-3%, 30-40%, 4-15% and 50-65% by weight, respectively.

The feed supplement may further comprise one or more additional components. Additional components may be used for any desired purpose, such as a substantially biologically inert material added, for example, as a filler, or to provide a desired beneficial effect. For example, the feed supplement may include a carbonate (including a metal carbonate such as calcium carbonate); a trace mineral, such as, but not limited to, chloride, fluoride, iodide, chromium, copper, zinc, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, sulfur, selenium, or a combination thereof; a bulking agent; a micro tracer, such as iron particles coated with a dye; yeast; allicin; alliin; allinase; algae; a polyphenol or plant material comprising polyphenol; a carrier; a colorant; a taste enhancer; a preservative; an oil; a vitamin; a sorbic acid or a salt thereof; or a combination thereof. The yeast may be yeast culture, active yeast, a live yeast, a dead yeast, yeast extract, active dried yeast, brewers dried yeast, culture yeast, dried yeast, primary dried yeast, torula dried yeast, candida dried yeast, or a combination thereof. The preservative may be benzoic acid or a salt thereof, *e.g.* sodium benzoate; lactic acid or a salt thereof, *e.g.* sodium lactate, potassium lactate or calcium lactate; propionic acid or a salt thereof, *e.g.* sodium propionate; ascorbic acid or a salt thereof, *e.g.* sodium ascorbate; gallic acid or a salt thereof *e.g.* sodium gallate; sulfur dioxide and/or sulfites; nitrites; nitrates; choline, or a salt thereof, such as an anion salt of choline, *e.g.* choline halide, such as chloride, bromide, iodide, fluoride, or choline hydroxide; or any combination thereof. The oil may be mineral oil, corn oil, soybean oil, or a combination thereof. The sorbic acid or salt thereof may be potassium sorbate, sodium sorbate, ammonium sorbate, or a combination thereof. The vitamin may be vitamin A, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, vitamin K, or a combination thereof.

Allicin (diallyl thiosulfate; 2-Propene-1-sulfinothioic acid S-2-propenyl ester) is a compound found in garlic, such as raw garlic. Allicin is typically produced from alliin ((2*R*)-2-amino-3-[(*S*)-prop-2-enylsulfinyl]propanoic acid) in damaged garlic cells by the action of the enzyme alliinase. Allicin, alliin, and/or alliinase may be provided as whole garlic cloves or bulbs; crushed, mashed, or chopped garlic; a garlic extract; and/or as a synthesized or isolated compound.

The polyphenol may be provided by a plant extract from a polyphenol-containing plant material. The plant material also may include non-polyphenol compounds, including polyphenol degradation products, such as gallic acid and trans-caftaric acid. Degradation can occur, for example, through oxidative and/or biological processes. Both the polyphenols and the non-polyphenol compounds may have biological activity. The plant extract may be prepared from a

single plant material or from a combination of plant materials. Suitable plant materials from which a plant extract can be obtained include, but are not limited to, apples, blackberries, black chokeberries, black currants, black elderberries, blueberries, cherries, cranberries, grapes, green tea, hops, onions, quillaja, plums, pomegranates, raspberries, strawberries, and yucca.

In some embodiments, the plant extract is prepared from a pressed plant material, such as grape pomace, a dried plant material, such as tea, or a combination thereof. Pomace may be obtained substantially immediately post-pressing or as an ensiled product, *i.e.*, pomace collected and stored for up to several months post-pressing. Suitable plants have a plurality of polyphenols and/or other non-polyphenolic compounds including, but not limited to, non-polyphenolic organic acids (such as gallic acid and/or trans-caftaric acid), flavanols, gallate esters, flavanodiols, phloroglucinol, pyrogallol, and catechol. In some embodiments, the plant extract is prepared from Pinot noir pomace, Pinot gris pomace, or green tea.

In some embodiments, pressed or dried plant material is ground to a fine powder prior to, or during, extraction. Pressed plant materials may be frozen to facilitate grinding. Polyphenols and other non-polyphenolic compounds may be extracted for administration. For example, polyphenols and other non-polyphenolic compounds may be extracted from the powder using a solution comprising a polar solvent, such as water, an alcohol, an ester, or a combination thereof. In some embodiments, the solution comprises a water-miscible alcohol, ester, or combination thereof, such as a lower alkyl alcohol, lower alkyl ester, or a combination thereof. In some embodiments, the solution is water or an aqueous solution comprising 25-99% solvent, such as 25-95% solvent, 30-80% solvent, or 50-75% solvent, and water. In certain embodiments, the solution is an aqueous solution comprising methanol, ethanol, isopropanol, ethyl acetate, or a combination thereof. The solution may be acidified by addition of an acid. The acid may prevent or minimize oxidative degradation of biologically-active polyphenols and other non-polyphenolic compounds in the extract. The acid may be any suitable acid, such as a mineral acid (*e.g.*, hydrochloric acid), or an organic acid such as citric acid or acetic acid. In some embodiments, the solution comprises from 0.01% to 1% acid, such as 0.02-0.5%, 0.025-0.25%, or 0.05-0.15%. In some examples, the solution includes 0.1% hydrochloric acid.

Extraction may be performed at a temperature ranging from 0-100 °C. In some embodiments, extraction is performed at a temperature ranging from 20-70 °C, or at ambient temperature. Extraction may be performed for a duration ranging from several minutes to several days. To increase extraction efficiency, the plant material and solution may be mixed or agitated during extraction, such as by grinding the plant material during extraction, stirring the mixture, shaking the mixture, or homogenizing the mixture. In some embodiments, the extraction may be

repeated one or more times with fresh solution to increase recovery of polyphenols and other non-polyphenolic compounds from the plant material. The liquid phases from each extraction cycle are then combined for further processing.

The liquid phase can be recovered, and the residual solids, or pulp, are discarded. Recovering the liquid phase may comprise decanting the liquid from the remaining solids and/or filtering the liquid phase to remove residual solids. The solvent (alcohol, ester, or combination thereof) can be removed from the liquid solution by any suitable means, such as evaporation (*e.g.*, roto-evaporation), to produce an aqueous extract containing the biologically-active components in a mildly acidic solution.

In certain embodiments where the plant material includes a significant amount of oils, or lipids, an initial extraction of nonpolar components may be performed before extracting the polyphenols and other polar, non-polyphenolic compounds. Nonpolar components may be extracted by homogenizing the plant material in a nonpolar solvent, *e.g.*, hexanes, heptanes, or a combination thereof. The solvent layer including the extracted nonpolar components is separated from the plant material and discarded.

The aqueous plant extract may be further purified by suitable means, *e.g.*, extraction, chromatographic methods, distillation, etc., to remove non-polyphenolic compounds and/or to increase the concentration of polyphenols relative to other compounds in the extract.

The aqueous plant extract may be dried, for example by freeze-drying or other low-temperature drying methods, and ground to a powder to provide a dried plant extract. In some embodiments, the dried plant extract comprises 0.01 wt% to 25 wt% total polyphenols, such as 0.01 wt% to 10 wt%, 0.01 wt% to 5 wt%, 0.01 wt% to 2.5 wt%, 0.01 wt% to 1 wt%, 0.01 wt% to 0.5 wt%, 0.02 to 0.25 wt%, or 0.03-0.1 wt% total polyphenols. In certain embodiments, the dried plant extract further comprises non-polyphenolic compounds. For example, the dried plant extract may comprise 0.01-1 mg/g gallic acid, such as 0.05-0.5 mg/g or 0.09-0.25 mg/g gallic acid, and/or 0.001-0.1 mg/g trans-caftaric acid, such as 0.005-0.05 mg/g or 0.01-0.025 mg/g trans-caftaric acid.

The aqueous plant extract may be concentrated to a smaller volume, *e.g.*, by evaporation, and used as an aqueous plant extract. In other embodiments, the aqueous plant extract is mixed with a carrier before drying and grinding. Suitable carriers include, for example, diatomaceous earth, silica, maltodextrin, ground grain (*e.g.*, corn), meals (*e.g.*, soybean or cottonseed meal) by-products (*e.g.*, distiller's dried grains, rice hulls, wheat mill run), clays (*e.g.*, bentonite), and combination thereof. The plant extract may be combined with a carrier in a ratio ranging from 10:1 to 1:10 by weight, such as from 5:1 to 1:5. For example, the plant extract may be mixed with diatomaceous earth in a ratio of 3:1 by weight.

Additionally, or alternatively, the additional components may comprise corn, soybean meal, wheat, wheat fiber, barley, rye, rice hulls, canola, limestone, salt, distillers dried grains with solubles (DDGS), dicalcium phosphate, sodium sesquicarbonate, methionine source, lysine source, L-threonine, biotin, folic acid, kelp, menadione dimethylpyrimidinol bisulfite, calcium aluminosilicate, or any combination thereof.

Additional information concerning feed supplement and/or additional components can be found in PCT application No. PCT/US2015/053439, and U.S. application Nos. 15/359,342, 14/699,740, 14/606,862, and 62/449,959 each of which is incorporated herein by reference in its entirety.

In some embodiments, the feed supplement does not comprise additional components. In other embodiments, the feed supplement comprises from greater than zero to 40% or more by weight additional components, such as from 0.1% to 40% by weight, or from 0.2% to 35% by weight additional components. In certain embodiments, the feed supplement comprises from 0.1% to 5% by weight additional components, such as from 0.2% to 3% by weight. In other embodiments, the feed supplement comprises from 5% to 20% by weight additional components, such as from 10% to 15% by weight. And in further embodiments, the feed supplement comprises from 20% to 40% by weight additional components, such as from 30% to 35% by weight additional components.

In some embodiments, the feed supplement comprises, consists essentially of, or consists of, silica, mineral clay, glucan, mannans, and endoglucanohydrolase; silica, mineral clay, glucan, mannans, endoglucanohydrolase, micro tracers and mineral oil; silica, mineral clay, glucan, mannans, endoglucanohydrolase, micro tracers, mineral oil, and vitamins; silica, mineral clay, glucan, mannans, endoglucanohydrolase, micro tracers, mineral oil, vitamins, and potassium sorbate; silica, mineral clay, glucan, mannans, endoglucanohydrolase, vitamins, and active yeast; silica, mineral clay, glucan, mannans, endoglucanohydrolase, micro tracers, mineral oil, and active yeast; silica, mineral clay, glucan, mannans, endoglucanohydrolase, and mineral oil; silica, mineral clay, glucan, mannans, endoglucanohydrolase, vitamins, and calcium carbonate; silica, mineral clay, glucan, mannans, endoglucanohydrolase, micro tracers, and wheat fiber; or silica, mineral clay, glucan, mannans, endoglucanohydrolase, and micro tracers. In any of these embodiments, the glucan and mannans may be provided by yeast, yeast cell wall, or yeast cell wall extract.

In some embodiments, the feed supplement does not comprise a peroxide compound. In some embodiments, the feed supplement does not comprise hydrogen peroxide. In some embodiments, the feed supplement does not comprise carbamide peroxide. In some embodiments,

the feed supplement does not comprise urea. In some embodiments, the feed supplement does not comprise hydrogen peroxide and urea.

In certain embodiments, the feed supplement is a powdered supplement. In other embodiments, the feed supplement is a granulated supplement. The granulated feed supplement may comprise silica, mineral clay, glucan and/or mannans, and optionally endoglucanohydrolase as discussed above. The granulated feed supplement may have a bulk loose density of from 40 lb/ft^3 to 150 lb/ft^3. In some embodiments, each granule in the granular composition comprises silica, mineral clay, glucan and/or mannans, and optionally endoglucanohydrolase, in relative amounts substantially the same as a relative amount of each ingredient in the composition as whole. Each granule in the granular composition may comprise, consist essentially of, or consist of, silica, mineral clay, glucan, mannans and endoglucanohydrolase. Alternatively, or additionally, each granule may comprise a substantially homogenous blend of silica, mineral clay, glucan and mannans, and optionally endoglucanohydrolase. The composition may comprise greater than 40% by weight granules having at least one dimension between 0.149 mm (100 mesh, U.S. standard mesh size) and 4.76 mm (4 mesh), and in some embodiments, the composition comprises greater than 90% by weight granules having at least one dimension between 0.149 mm (100 mesh) and 2 mm (10 mesh). And/or the composition may comprise from greater than 0% to 100% granules by weight and from 0% to no more than 60%, such as no more than 10%, particles by weight, the granules having at least one dimension between 10 mesh (2.00 mm) and 100 mesh (0.149 mm), and the particles having at least one dimension of less than (i.e., smaller than) 100 mesh (0.149 mm). In any embodiments, the granular composition comprises plural granules, each granule comprising silica, mineral clay, glucan and mannans, the granules having a size that when administered to an animal increases expression of interleukin 10 receptor β (IL10RB) for a time period subsequent to administration, such as subsequent to the onset of administration, relative to an animal that does not receive the composition. In some embodiments the time period may be from the start of administration to from 28 days to at least 42 days. And/or the composition may have a mineral coefficient of variation of from 0% to 10%, or a proximate coefficient of variation of from 0% to 20%, or both. Additional information concerning the granular feed supplement can be found in U.S. application No. 62/449,959 which is incorporated herein by reference in its entirety.

In some embodiments, the feed supplement is administered daily to an animal at time intervals believed or determined to be effective for achieving a beneficial result. The feed supplement may be administered in a single dose daily or in divided doses throughout the day. The amount may be from greater than zero to 500 grams per animal per day, such as from 0.5 grams to 250 grams, from 5 grams to 200 grams, or from 10 grams to 70 grams per animal per day.

Alternatively, the feed supplement may be fed or administered in an amount of from greater than zero to 1000 mgs or more per kilogram of the animal's body weight per day, such as from greater than zero to 500 mgs per kilogram body weight. In other embodiments, the feed supplement is fed or administered per weight of animal feed. The feed supplement may be fed or administered in an amount of from greater than zero to 150 kg per ton (2000 pounds) of feed, such as from 0.1 kg to 100 kg per ton of feed. Alternatively, the feed supplement may be fed or administered in an amount of from greater than zero to 20 grams per kilogram of feed, such as from greater than zero to 10 grams of feed.

In some embodiments, a composition and/or combination comprises a first composition comprising, consisting essentially of, or consisting of, *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*, and a second composition comprising, consisting essentially of, or consisting of, one or more of yucca, quillaja, silica, mineral clay, glucan, mannans, and endoglucanohydrolase. The second composition may comprise, consist essentially of, or consist of, yucca and quillaja, such as *Yucca schidigera* and *Quillaja saponaria*. In other embodiments, the second composition comprises, consists essentially of, or consists of, silica, mineral clay, glucan and mannans, and optionally endoglucanohydrolase.

3. Additional *DFM(s)*

The disclosed Bacilli combination can be administered to an animal in combination with one or more additional DFMs. The additional DFM(s) may be any DFM suitable for administration to the particular animal. In some embodiments, the animal is a poultry, particularly a chicken or a turkey, and the additional DFM is a DFM that provides a benefit to the poultry. The additional DFM may be, by way of example and without limitation, an additional *Bacillus* species, *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Propionibacterium*, *Streptococcus*, *Pediococcus*, yeast, or a combination thereof.

Exemplary additional DFMs include, but are not limited to, *Bacillus alcalophilus, Bacillus alvei, Bacillus aminovorans, Bacillus aneurinolyticus, Bacillus anthracis, Bacillus aquaemaris, Bacillus atrophaeus, Bacillus boroniphilus, Bacillus brevis, Bacillus caldolyticus, Bacillus centrosporus, Bacillus cereus, Bacillus circulans, Bacillus firmus, Bacillus flavothermus, Bacillus fusiformis, Bacillus galliciensis, Bacillus globigii, Bacillus infernus, Bacillus larvae, Bacillus laterosporus, Bacillus lentus, Bacillus megaterium, Bacillus mesentericus, Bacillus mucilaginosus, Bacillus mycoides, Bacillus natto, Bacillus pantothenticus, Bacillus polymyxa, Bacillus pseudoanthracis, Bacillus pumilus, Bacillus schlegelii, Bacillus sphaericus, Bacillus sporothermodurans, Bacillus stearothermophilus, Bacillus thermoglucosidasius, Bacillus*

thuringiensis, Bacillus vulgatis, Bacillus weihenstephanensis, Lactobacillus acidophilis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus gallinarum, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus reuteri, Lactobacillus bulgaricus, Bifidobacterium pseudolongum, Bifidobacterium thermophilium, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium infantis, Streptococcus bovis, Streptococcus faecium, Enterococcus faecium, Enterococcus faecalis, Enterococcus diacetylactis, Saccharomyces cerevisiae, Saccharomyces boulardii Aspergillus oryzae, Aspergillus niger, Selenomonas ruminantium, Megasphaera elsdenii, Propionibacterium freudenreichii, Propionibacterium shermanii, Propionibacterium acidipropionici, Propionibacterium fensenii, Prevotella bryantii, Pediococcus acidilactici, Pediococcus cerevisiae, or a combination thereof. In certain embodiments, *Bacillus pumilus* may be administered in combination with the Bacilli combination.

V. Beneficial Results from Administering the Bacilli combination

Administering a Bacilli combination to an animal, such as poultry, has provided a substantial beneficial result when compared to administering each of the respective *Bacillus* species individually, or in combinations comprising only two species. These beneficial results are determined by considering, for example, feed conversion rate, average body weight, average body weight gain, body weight coefficient of variation, breast meat yield, bird mortality, lesion scores, necrotic enteritis incidence, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter at various times during chick rearing. With reference to Example 1, these benefits can be determined by comparing feed conversion rate (FCR) for birds fed a basal diet and challenged with CP relative to birds that receive the Bacilli combination, such as *Bacillus coagulans, Bacillus subtilis* and *Bacillus licheniformis* in combination, or *Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus licheniformis*, optionally with *Bacillus coagulans*.

Administration of a disclosed Bacilli combination to poultry may reduce *E. coli* in the poultry. The amount of *E. coli* reduction may be from greater than zero to 25% or more, such as a reduction of from 5% to 25%, or 10% to 22%, compared to an amount of *E. coli* present in poultry that are not administered the combination. The reduction may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may reduce Aerobic Plate Count *(APC)* in the poultry. The APC reduction may be from greater than zero to 20% or more, such as a reduction of from 5% to 20%, or 10% to 18%, compared to an amount of *APC* present in

poultry that are not administered the combination. The reduction may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may reduce *Salmonella* in the poultry. The *Salmonella* reduction may be from greater than zero to 65% or more, such as a reduction of from 25% to 65%, 35% to 65%, or from 45 to 65%, compared to an amount of *Salmonella* present in poultry that are not administered the combination. The reduction may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may reduce *Clostridium perfringens* in the poultry. The *Clostridium perfringens* reduction may be from greater than zero to 30% or more, such as a reduction of from 5% to 30%, 10% to 30%, or from 15% to 30%, compared to an amount of *Clostridium perfringens* present in poultry that are not administered the combination. The reduction may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may reduce fecal oocysts in the poultry. The oocysts reduction may be from greater than zero to 90% or more, such as a reduction of from 50% to 90%, or 75% to 90%, compared to an amount of oocysts present in poultry that are not administered the combination. The reduction may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may result in an improved lesion score in the poultry. The lesion score may be improved (i.e. lowered) by from greater than zero to 75% or more, such as from 25% to 75%, or from 30% to 75%, compared to a lesion score of poultry that are not administered the combination. The improvement may be identified at various times, such as at 21 and/or 42 days old for certain disclosed working embodiments.

Administration of a disclosed Bacilli combination to poultry may result in an improved feed conversion rate in the poultry. The feed conversion rate may be improved (i.e. lowered) by from greater than zero to 10% or more, such as from 2% to 8%, or from 4% to 8%, compared to a feed conversion rate of poultry that are not administered the combination. The improvement may be identified at various times, such as poultry at 14, 21 and/or 42 days old.

Administration of a disclosed Bacilli combination to poultry may result in a reduced poultry mortality rate. The mortality rate may be reduced by from greater than zero to 95% or more, such as from 50% to 95%, from 75% to 95% or from 80% to 95%, compared to a mortality rate of poultry that are not administered the combination. The improvement may be identified at various times, such as when the poultry are 14, 21 and/or 42 days old.

Administration of a disclosed Bacilli combination to poultry may result in an improved average weight gain. The weight gain may be increased by from greater than zero to 20% or more, such as from 5% to 15%, from 7% to 15% or from 10% to 15%, compared to an average weight gain of poultry that are not administered the combination. The improvement may be identified at various times, such as when the poultry are 14, 21 and/or 42 days old.

VI. Examples

The following examples are provided to illustrate certain features of exemplary working embodiments. A person of ordinary skill in the art will appreciate that the scope of the invention is not limited to the particular features of these examples.

Example 1

This example concerns the results of a field study to compare the efficacy of various combinations of direct fed microbials that are administered in the feed to control necrotic enteritis caused by *Clostridium perfringens* in broiler chickens. The goal of this study is comparative evaluations of poultry performance in a challenge model after administration of different strains or combinations of direct fed microbials. The study is performed as a battery trial using a challenge model wherein the birds are challenged with coccidia and *Clostridium perfringens*.

The study consists of 56 cages starting with 8 chicks each. The treatments are replicated in 8 blocks of 7 cages each, as shown in Table 1.

Table 1

Treatment		Coccidial Challenge	*Clostridium perfringens*	Cages/Trt
T1	Nonmedicated	DOT 15	No	8
T2	Nonmedicated	DOT 15	DOT 20,21,and 22	8
T3	Gallipro Max 10G, 10g/t	DOT 15	DOT 20,21,and 22	8
T4	BC1, 7.5 g/ t	DOT 15	DOT 20,21,and 22	8
T5	BL1, 113. 5 g/t + BS1, 113. 5 g/t	DOT 15	DOT 20,21,and 22	8
T6	BL1, 113. 5 g/t + BS1, 113. 5 g/t + BC1 6086, 7.5 g/t	DOT 15	DOT 20,21,and 22	8
T7	BL2 + BS2 200 g/t + BC2, 7.5 g/t	DOT 15	DOT 20,21,and 22	8

A. Materials and Methods

An un-medicated chicken starter compound is formulated, comprising feeds commonly used in the United States. The diet is representative of a local commercial formulation and calculated analyses meets or exceeds NRC broiler starter requirements. Experimental treatment feeds are prepared from this basal starter feed. Treatment feeds are mixed to assure a uniform distribution of respective test article. The mixer is flushed to prevent cross contamination. The feed is distributed among cages of the same treatment. This ration (in mash form) is fed during the study.

Day of hatch male broiler chicks are obtained from Cobb-Vantress, Cleveland, GA. The strain is Cobb 500. Breeder flock information is recorded. At the hatchery, the birds are sexed and receive routine vaccinations. Only healthy appearing chicks are used in the study.

B. Procedures

The study begins when the birds are placed (day of hatch) (DOT 0) at which time they are allocated to experimental cages. No birds are replaced during the course of the study. All birds are weighed on DOT 0, 15, 22, and 28. Feed and water are given *ad libitum*. Feed is weighed in on DOT 0 and remaining feed is weighed on DOT 15, 22, and 28.

On DOT 15, all birds are orally inoculated with about 5,000 oocysts of *E. maxima*. Starting on DOT 20, all birds except Treatment 1 are given a broth culture of *C. perfringens* about 10^8 CFU/ml. The birds are administered a fresh broth culture once daily for 3 days (on DOTs 20, 21, and 22). On DOT 22, three birds from each cage are selected, sacrificed, weighed, and examined for the degree of presence of Necrotic Enteritis lesions. The scoring is based on a 0 to 3 score, with 0 being normal and 3 being the most severe.

The cages are checked twice daily. Observations included are the availability of feed and water, temperature control, and any unusual conditions. The birds are watched closely for any abnormal reactions. When mortality birds are removed from cages, the cage number, date, weight of the bird, sex, and probable cause of death are recorded. Means for cage weight gain, feed consumption, feed conversion, lesion scores, and mortality are calculated.

C. Results

The study is expected to show that administration of the CSL combination provides an improved feed conversion rate and/or increased weight gain, compared to birds not administered any *Bacillus* DFMS, and compared to birds administered only one or two of the *Bacillus* species rather than the CSL combination.

Additionally, the results will demonstrate that birds that are administered the CVSL combination have reduced mortality when challenged with *C. perfringens*, compared to birds that are challenged but not administered the CSL combination, such as those not administered a *Bacillus* DFM and those administered only one or two *Bacillus* species.

Example 2

A field study is performed to compare the efficacy of various combinations of direct fed microbials that are administered in the feed to control necrotic enteritis caused by *Clostridium perfringens* in broiler chickens. The goal of this study is comparative evaluations of poultry performance in a challenge model after administration of different strains or combinations of direct fed microbials. The study is performed as a battery trial using a challenge model wherein the birds are challenged with coccidia and *Clostridium perfringens.*

The study consisted of 56 cages starting with 8 chicks each. The treatments are replicated in 8 blocks of 7 cages each, as shown in Table 2.

Table 2

Treatment		**Coccidial Challenge**	***Clostridium perfringens***	**Cages/Trt**
T1	Nonmedicated	DOT 14	No	8
T2	Nonmedicated	DOT 14	DOT 19, 20,and 21	8
T3	BC1, 7.5 g/ t	DOT 14	DOT 19, 20,and 21	8
T4	BC2, 46.875 g/t	DOT 14	DOT 19, 20,and 21	8
T5	BC2, 7.5 g/t	DOT 14	DOT 19, 20,and 21	8
T6	BL1,113. 5 g/t BL1 + BS1, 113. 5 g/t	DOT 14	DOT 19, 20,and 21	8
T7	BL2 + BS2 (200 g/t	DOT 14	DOT 19, 20,and 21	8

A. Materials and Methods

An un-medicated chicken starter compound is formulated with feeds commonly used in the United States. The diet is representative of a local commercial formulation and calculated analyses meets or exceeds NRC broiler starter requirements. Experimental treatment feeds are prepared from this basal starter feed. Treatment feeds are mixed to assure a uniform distribution of respective test article. The mixer is flushed to prevent cross contamination. This ration (in mash form) is fed during the study.

Day of hatch male broiler chicks are obtained from Cobb-Vantress, Cleveland, GA. The strain is Cobb 500. Breeder flock information is recorded. At the hatchery, the birds are sexed and receive routine vaccinations. Only healthy appearing chicks are used in the study.

B. Procedures

The study begins when the birds are placed (day of hatch) (DOT 0) at which time they are allocated to experimental cages. No birds are replaced during the course of the study. All birds are weighed on DOT 0, 14, 21, and 28. Feed is weighed in on DOT 0 and remaining feed is weighed on DOT 14, 21, and 28. Feed and water are given *ad libitum*.

On DOT 14, all birds are orally inoculated with about 5,000 oocysts of *E. maxima*. Starting on DOT 19, all birds except Treatment 1 are given a broth culture of *C. perfringens* about 10^8 CFU/ml. The birds are administered a fresh broth culture once daily for 3 days (on DOTs 19, 20, and 21).

On DOT 21, three birds from each cage are selected, sacrificed, weighed, and examined for the degree of presence of Necrotic Enteritis lesions. The scoring is based on a 0 to 3 score, with 0 being normal and 3 being the most severe.

The cages are checked twice daily. Observations included are the availability of feed and water, temperature control, and any unusual conditions. The birds are watched closely for any abnormal reactions. When mortality birds are removed from cages, the cage number, date, weight of the bird, sex, and probable cause of death are recorded. Means for cage weight gain, feed consumption, feed conversion, lesion scores, and mortality are calculated.

C. Results

The study is expected to show that administration of the CSL combination provides an improved feed conversion rate and/or increased weight gain, compared to birds not administered any *Bacillus* DFMS, and compared to birds administered only one or two of the *Bacillus* species rather than the CSL combination.

Additionally, the results will demonstrate that birds that are administered the CVSL combination have reduced mortality when challenged with *C. perfringens*, compared to birds that are challenged but not administered the CSL combination, such as those not administered a *Bacillus* DFM and those administered only one or two *Bacillus* species.

Example 3

A total of 4,992 chicks strain were housed at hatch (one day of age, or Trial Day 0) to begin the test feeding period. The chicks were fed as shown in Table 3.

Table 3

Ration Number	TEST MATERIAL (additives) [1, 2]
PHIBRO4-1	Control 1 (Clean)- Basal diet + No challenge
PHIBRO4-2	Control 2 (Challenged)- Basal diet + CP Challenge
PHIBRO4-3	Provia 6086 + CP Challenge
PHIBRO4-4	Osprey BL+ BS + CP Challenge
PHIBRO4-5	Osprey BL+ BS + Provia 6086 (recommended levels) + CP Challenge
PHIBRO4-6	Osprey BL+ BS + Provia 6086 (@half the recommended levels) + CP Challenge
PHIBRO4-7	Control 1 (Clean) + Osprey BL+BS+ Provia 6086 (recommended levels) + No Challenge
PHIBRO4-8	Calsporin + CP Challenge

[1] Each treatment was fed to 12 replicates of 52 mixed-sex broilers.

[2] A basal (with enough feed for the entire treatment) was mixed first and then each test material was added and remixed.

[4] Recommended levels:

Provia 6086 (full dose): 7.5 grams per ton of complete feed. Product contains 15 billion CFU of *Bacillus coagulans* per gram resulting in approximately 1.2 x 10^5 CFU per gram in final feed.

Osprey BL (full dose): 0.25 lbs per ton of complete feed. Product contains 2.4 x 10^9 CFU *Bacillus subtilis* per gram resulting in approximately 3 x 10^5 CFU per gram in final feed.

Osprey BS (full dose): 0.25 lbs per ton of complete feed. Product contains 2.4 x 10^9 CFU *Bacillus licheniformis* per gram resulting in approximately 3 x 10^5 CFU per gram in final feed.

CALSPORIN (N/C): 9.07 grams per ton of feed.

The results of this study are summarized in FIG. 1.

Example 4

This example concerns a field study. The protocol for this study was as shown in Table 4.

Table 4

Ration Number[1]	Treatment Identification Code	Test Material (Provia 6086 @ 7.5g/ton of feed)[2,3]	Test Material (Osprey BS &+ BL @ 113.4g/ton of feed, each)[2,3]
PHIBRO7-1	Treatment 1 (Control, No CP)	0	0
PHIBRO7-2	Treatment 2 (Control, CP)	0	0
PHIBRO7-3	Treatment 3 (BL&BS + BC, CP)	100%	100%
PHIBRO7-4	Treatment 4 (BL&BS + BC, CP)	75%	100%
PHIBRO7-5	Treatment 5 (BL&BS + BC, CP)	100%	75%
PHIBRO7-6	Treatment 6 (BL&BS + BC, CP)	100%	50%
PHIBRO7-7	Treatment 7 (BL&BS + BC, CP)	75%	50%
PHIBRO7-8	Treatment 8 (BL&BS + BC, CP)	75%	75%
PHIBRO7-9	Treatment 9 (Calsporin, CP)	0	0
PHIBRO7-10	Product Y1-1 (182 g/ton), CP	0	0
PHIBRO7-11	Product Y2-2 (182 g/ton), CP	0	0

[1] Each treatment was fed to 12 replicates of 52 mixed-sex broilers.

2 A basal feed (with enough feed for the entire treatment) was mixed first and then each test material was added and remixed.

[3] RECOMMENDED LEVELS:

Provia 6086: 7.5 grams per ton of complete feed. Product contains 15 billion CFU of *Bacillus coagulans* per gram resulting in approximately 1.2 x 105 CFU per gram in final feed.

Osprey BL (full dose): 0.25 lbs per ton of complete feed. Product contains 2.4 x 109 CFU Direct Fed Microbials per gram resulting in approximately 3 x 105 CFU per gram in final feed.

Osprey BS (full dose): Use 0.25 lbs per ton of complete feed. Product contains 2.4 x 109 CFU Direct Fed Microbials per gram resulting in approximately 3 x 105 CFU per gram in final feed.

CALSPORIN (N/C): 9.07 grams per ton of feed.

Product Y1-1 and Y2-2: 182 grams per ton of feed was administered for each of Treatments #10 and #11.

The results of this study are provided in FIGS. 2 and 3. With respect to FIGS. 2 and 3, "Significance (P<0.05)" indicates that averages within a row that are without a common superscript are significantly different (P<0.05) as determined by Least Significant Difference.

Example 5

This example concerns the results of a field study to compare the efficacy of various combinations of direct fed microbials that are administered in the feed to control necrotic enteritis caused by *Clostridium perfringens* in broiler chickens, and the effect of the direct fed microbials on feed conversion rates and weight gain in the chickens. The goal of this study is comparative evaluations of poultry performance in a challenge model after administration of different strains or combinations of direct fed microbials. The study is performed as a battery trial using a challenge model wherein the birds are challenged with coccidia and *Clostridium perfringens*.

The study consists of 56 cages starting with 8 chicks each. The treatments are replicated in 8 blocks of 7 cages each, as shown in Table 5.

Table 5

Treatment		Coccidial Challenge	*Clostridium perfringens*	Cages/Trt
T1	Nonmedicated	DOT 15	No	8
T2	Nonmedicated	DOT 15	DOT 20,21,and 22	8
T3	BA	DOT 15	DOT 20,21,and 22	8
T4	BSBL	DOT 15	DOT 20,21,and 22	8
T5	50%:50% BA:BSBL	DOT 15	DOT 20,21,and 22	8
T6	25%:75% BA:BSBL	DOT 15	DOT 20,21,and 22	8
T7	75%:25% BA:BSBL	DOT 15	DOT 20,21,and 22	8

BSBL: 1:1 mixture of *Bacillus subtilis* and *Bacillus licheniformis*.

BA: *Bacillus amyloliquefaciens*

A. Materials and Methods

An un-medicated chicken starter compound is formulated, comprising feeds commonly used in the United States. The diet is representative of a local commercial formulation and calculated analyses meets or exceeds NRC broiler starter requirements. Experimental treatment feeds are prepared from this basal starter feed. Treatment feeds are mixed to assure a uniform

distribution of respective test article. The mixer is flushed to prevent cross contamination. The feed is distributed among cages of the same treatment. This ration (in mash form) is fed during the study.

Day of hatch male broiler chicks are obtained from Cobb-Vantress, Cleveland, GA. The strain is Cobb 500. Breeder flock information is recorded. At the hatchery, the birds are sexed and receive routine vaccinations. Only healthy appearing chicks are used in the study.

B. Procedures

The study begins when the birds are placed (day of hatch) (DOT 0) at which time they are allocated to experimental cages. No birds are replaced during the course of the study. All birds are weighed on DOT 0, 15, 22, and 28. Feed and water are given *ad libitum*. Feed is weighed in on DOT 0 and remaining feed is weighed on DOT 15, 22, and 28.

On DOT 15, all birds are orally inoculated with about 5,000 oocysts of *E. maxima*. Starting on DOT 20, all birds except Treatment 1 are given a broth culture of *C. perfringens* about 10^8 CFU/ml. The birds are administered a fresh broth culture once daily for 3 days (on DOTs 20, 21, and 22). On DOT 22, three birds from each cage are selected, sacrificed, weighed, and examined for the degree of presence of Necrotic Enteritis lesions. The scoring is based on a 0 to 3 score, with 0 being normal and 3 being the most severe.

The cages are checked twice daily. Observations included are the availability of feed and water, temperature control, and any unusual conditions. The birds are watched closely for any abnormal reactions. When mortality birds are removed from cages, the cage number, date, weight of the bird, sex, and probable cause of death are recorded. Means for cage weight gain, feed consumption, feed conversion, lesion scores, and mortality are calculated.

C. Results

The study is expected to show that administration of the combination of *Bacillus amyloliquefaciens, Bacillus subtilis and Bacillus licheniformis* provides an improved feed conversion rate and/or increased weight gain, compared to birds not administered any *Bacillus* DFMS, and compared to birds administered only one or two of the *Bacillus* species rather than the combination of the three *Bacilli*.

Additionally, the results will demonstrate that birds that are administered the combination have reduced mortality when challenged with *C. perfringens*, compared to birds that are challenged but not administered the combination of *Bacillus amyloliquefaciens,*

Bacillus subtilis and Bacillus licheniformis, such as those not administered a *Bacillus* DFM and those administered only one or two *Bacillus* species.

Example 6

STUDY OVERVIEW

This study was conducted to determine the dose titration safety and efficacy of Magni-Phi® alone (fed at either 0 and 250 ppm) or in combination with a combination of *B. subtilis, B. amyloliquefaciens, B. coagulans* and *B. licheniformis* (administered as Provia Prime™) as well as other DFM test materials on broiler chicken live performance from hatch to 42 days of age. Provia Prime™ was fed one level at 0.25 pounds per ton. In particular, Provia Prime™ alone was tested to determine if maximum genetic potentials could be met or if feeding both Magni-Phi® at both levels plus Provia Prime™, as well as other single-strain candidates (*B. pumilus, B. subtilis, B. amyloliquefaciens, B. coagulans* or *B. licheniformis*) was beneficial.

The test period began on Trial Day 0 (day of hatch of chicks) and ended on Day 42. The chicks were fed a commercial-type mash feed. The chicks were randomly assigned to one of 15 test groups, which were either 11 rep groups (T1-T5) or 4 rep groups (T6-T15) of 52 mixed-sex broilers for each replication for a total number of 4,940 animals. Chicks were randomly assigned to treatments on Trial Day 0 (day of hatch) and were not replaced during the trial. The chicks were observed daily for signs of unusual grow-out patterns or health problems. Body weights and food consumption was measured on Trial Days 0, 14, 21, 35 and 42.

OBJECTIVE, INTRODUCTION AND STUDY OVERVIEW:

1. When broilers are subjected to normal live performance stresses the major objective is to determine the effect of poultry-related product test sources from various sources on live performance and processing factors under typical coccidial-clostridium challenge model stress points, when broilers are reared from hatch to 42 days of age in floor pens. The coccidial-clostridium challenge model comprises clostridium bacteria and coccidiosis challenges, and was administered to all of the birds within the scope of this trial. The test materials were compared to both each other, and to a negative control (NC), which was without test material administration but included the challenges.

2. The major objective included comparing different combined poultry-related product test sources, using levels normally fed in the USA chicken industry, and determining the effects on

broiler chicken market weight, mortality and feed conversion on live performance and other test parameters.

3. All birds in this trial were stress challenged by administering Clostridium and coccidia oocysts, along with other natural bacteria from build-up litter from a farm experiencing high mortality.

4. Intestinal bacteria were counted, including *Clostridium perfringen, E. coli* and APC (Aerobic Plate Count), and were measured in Log_{10} counts.

5. Particular to this trial, Salmonella spp. incidence was tested on a statistically sound number of birds (2M and 2F at 21 days of age per 52-bird pen and 5M and 5F per replicate at 42 days of age per 52-bird pen) to simulate counts required by USDA/FSIS at processing.

6. Dry Yield (%) and Parts Yield (%), were tested following 42-day body weights and live performance measurement.

7. Another objective was to determine if a combination of *B. subtilis, B. amyloliquefaciens, B. coagulans* and *B. licheniformis* (administered as Provia Prime™), as well as other Bacillus products, provided an added benefit toward ROI (Return-on-Investment) beyond feeding Magni-Phi® alone.

TEST SYSTEM

The commercial-simulated test stress model employed in this study used broiler breed chicks (*Gallus domesticus*) reared on a normal poultry industry Starter diet (0-21 days of age), Grower diet (22-35 days of age) and Finisher diet (36-42 days of age) at a floor space requirement (minimum of 0.85 ft^2 per bird), reared in pens with raised wire floors. Ration formulations (Table 6) were conducted via computer-generated linear regression program that simulates formulations conducted during practical poultry production techniques. Broilers were fed *ad libitum* and continuously dosed with their experimental diets from time of placement 0 (day of hatch) to up to 42 days of age, as outlined in Table 7. Treatments T1-T15 were fed to 11 reps (T1-T5) and 4 reps (T6-T15) of 52 mixed-sex broilers. A basal diet (with enough feed for the entire treatment) was mixed first and then each test material was added and remixed. Each treatments provided a minimum of 5×10^5 CFU per gram final feed. All birds was stressed by administering Clostridium and coccidia oocysts, along with other natural bacteria from build-up litter from a farm experiencing high mortality.

Table 6. Feed Formulation Parameters:

Nutrient	Starter 0-21 days	Grower 22-35 days	Finisher 36-42 days
Metabolizable Energy (kcal/ kg)	3,025	3,150	3,201
Metabolizable Energy (kcal/ #)	1,372	1,429	1,452
Protein (%)	22	20	19
Lysine (%)	1.28	1.15	1.02
Methionine (%) Min	0.512	0.46	0.408
Methionine + Cysteine (%)	0.947	0.851	0.755
Arginine (%) Min	1.36	1.22	1.08
Threonine (%) Min	0.858	0.771	0.68
Tryptophan (%) Min	0.19	0.17	0.15
Total Phosphorus (%) Min	0.70	0.65	0.60
Available Phosphorus (%)	0.5	0.45	0.42
Total Calcium (%)	1.05	0.9	0.85
Dietary Sodium (%)	0.20	0.20	0.20
Dietary Choline (%)	1.35	1.15	0.95

Table 7. Experimental Diets

Treatment Group	Magni-Phi® TEST MATERIAL (additives)	TEST MATERIAL (additives)
T1	POSITIVE CLEAN-LITTER CONTROL (PC, No additive, challenged)	NEGATIVE CONTROL (PC, No additive, challenged)
T2	NEGATIVE CHALLENGED LITTER CONTROL (NC, No additive, challenged)	NEGATIVE CHALLENGED LITTER CONTROL (NC, No additive, challenged)
T3	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone	None
T4	None	NC (Trt #2) + Provia Prime™ (0.25 Lbs. per ton level).
T5	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone	NC (Trt #2) + Provia Prime™. (0.25 Lbs. per ton level).
T6	None	NC (Trt #2) + *B. subtilis* OSPREY (0.25 Lbs. per ton level).
T7	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone	NC (Trt #2) + *B. subtilis* OSPREY (0.25 Lbs. per ton level).
T8	None	NC (Trt #2) + *B. lichenformis* OSPREY (0.25 Lbs. per ton level).
T9	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone.	NC (Trt #2) + *B. lichenformis* OSPREY (0.25 Lbs. per ton level).
T10	None	NC (Trt #2) + *B. coagulans* OSPREY (0.25 Lbs. per ton level).
T11	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone.	NC (Trt #2) + *B. coagulans* OSPREY (0.25 Lbs. per ton level).
T12	None	NC (Trt #2) + *B. amyloliquefaciens* OSPREY

		(0.25 Lbs. per ton level).
T13	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone.	NC (Trt #2) + *B. amyloliquefaciens* OSPREY (0.25 Lbs. per ton level).
T14	None	NC (Trt #2) + *B. pumilus*;OSPREY (0.25 Lbs. per ton level).
T15	NC (Trt #2) + Magni-Phi® 250 ppm (0.5 lbs. per ton) Alone.	NC (Trt #2) + *B. pumilus*;OSPREY (0.25 Lbs. per ton level).

Broiler chicks were randomized by individual weight on day of hatch and housed into each pen onto pens with raised wire floors. Each pen had sufficient floor, feeder and water space for each growout pen area for humane treatment of chickens up to 42 days of age. Following 42 days of growout, broilers were weighed, feed consumption determined, lesion score determined and feed conversion (feed consumed/body weight) calculated.

MATERIALS AND METHODS

Test Material Description

Provia Prime™, a combination of *B. subtilis, B. amyloliquefaciens, B. coagulans* and *B. licheniformis*, and Magni-Phi®, which is a composition comprising from 10% to 20% *Yucca schidigera* and from 80% to 90% *Quillaja saponaria*, were each presented in a premix "powder form". These were fed at various levels, continuously from Trial Days 0-42, as defined in the experimental section of this report.

One objective was to compare different test materials, including Magni-Phi® and Provia Prime™ and other Bacillus species or combinations thereof, against a Negative Control (providing no other test material and with a stress challenge) on broiler live performance and lesion scores when broilers were subjected to a sub-clinical challenge (built-up litter and moderate stress conditions via bacterial and coccidia challenge, when administered). The objective was to determine if Provia Prime™ provided an added benefit toward ROI (Return-on-Investment) beyond feeding Magni-Phi® (250 ppm) or the individual Bacillus species alone.

Description of Bacteria Challenge ("***Test Stress Model***")

The birds were grown on built-up litter sources that contained *Clostridium perfringens* and other pathogenic bacteria, in a "coccidial-clostridium challenge model." Layout time among flocks was <11 days. Based on previous trials, these organisms were expected to be present in the present study. Each pen was top-dressed a minimum of 5-million total oocysts (or 100,000-150,000 per bird) + sterile water and not incorporated into the litter. Coccidia oocysts were predominate *E. acervulina* and *E. maxima*. Each pen was "walked with plastic boot covers designed to pick up

litter" at a minimum of 3-times per day, for the purpose of spreading the litter from pen-to-pen and equalize the challenge.

Experimental Design

A total of 5,500mixed sex broiler chicks (a sufficient number to ensure availability of at least 4,940 healthy mixed-sex chicks for the conduct of the study) were obtained from a commercial hatchery on Trial Day 0 (same as hatch date). These were immediately transported to the research pen units under temperature-controlled conditions to assure bird comfort. After arrival at the research facility, broilers were immediately randomized. There were 52 healthy/viable broiler mixed-sexes per pen with either 11 replicates (Treatments 1-5) or 4 replicates (Treatment 6-15) per test group. Broilers were fed *ad libitum* their respective treatment from day of hatch (termed in this study as Trial Day 0) to 42 days of age.

Broiler Chick Description

Broilers were evaluated upon receipt for signs of disease or other complications that may have affected the outcome of the study. Following examination, broilers were weighed. Broilers were allocated to each pen and to treatment groups using a randomized block design. Weight distribution across the treatment groups were assessed prior to feeding by comparing the individual test and reference group standard deviations of the mean against that of the control group. Differences between control and test or reference groups were within one standard deviation, and as such, weight distribution across treatment groups were considered acceptable for this study.

Broiler chickens (at hatch to 1-day of age, called Day 0) were collected in early am (day of placement or Day 0) and randomly assigned to each experimental pen within 12 hours of hatch. Weak birds were humanely sacrificed. Birds were not replaced during the study.

Housing and Daily Observations

Each experimental test unit of broiler mixed-sex chicken pens were housed in separated pens, located in a room containing forced air heaters with a cross-house ventilation system, precision controlled by the operation manager. Broilers were placed in 5 ft x 10 ft pen floor area and space with a minimum of 0.85 ft^2 per bird (without feeder and waterer space) provided. At least two nipple drinkers per pen (*via* well water) provided water.

Two-chick trough feeders per pen were employed for the initial 0-7 days growout period and then larger trough feeders were employed for the remaining growout period.

Continuous (24 hr) use of incandescent lights (LED light system, that employs >10*fc*)

attraction around feeders/waterers for attraction to feeders/waterers), was used during the entire study. Full lighting of >10 *fc* (around waterers/feeders only) and >2 *fc* in remaining floor space was used the first week and then dimmed to <4 *fc* for the next two weeks of age.

Birds were observed at least three times daily for overall health, behavior and/or evidence of toxicity, and environmental conditions. Temperature was checked in the pen room three times daily. Drinking water and feed were provided *ad libitum*.

No type of medication (other than treatment group test material) was administered during the entire feeding period. Mortalities were recorded, refrigerated and, within an 8 hour period, were necropsy (both internal and external body mass) by examination examinations were performed on all broilers found dead or moribund.

Data and Observations

Live performance body weights and feed intakes were collected on Day 0 and 42 during the growing period. Weight gain, feed intake, feed:gain ratio (feed efficiency) were calculated for 0-42 days of age. Lesion Scores were collected at both 21 and 42 days of age. Differences between broilers fed control and test groups were statistically evaluated at $P<0.05$ in a typical ANOVA analysis of variance test model, employing Treatment x Replicate RCB (Randomized Complete Block). The control group was the NC CONTROL with no added test materials.

At the end of the study, all carcasses of necropsied broilers and all birds remaining at the end of the study were disposed of according to local regulations via on-farm composting techniques.

Diet Preparation

Diets were formulated to meet minimum nutrient requirements of a typical commercial broiler diet using formulations employed by qualified nutritionist with training in poultry feed formulations. Actual feed formulations are shown in Table 6, above.

Magni-Phi® and Provia Prime™ feed ingredient sources were added to each ration on an "*as is*" basis with first mixing small amount of feed with the basal and then adding the remaining test material to complete the mix formula. Dietary protein, lysine, methionine, methionine+cystine, arginine, threonine, tryptophan, total phosphorus, available phosphorus, total calcium, dietary sodium, and dietary choline were met by adjusting the concentrations of corn and soybean meal ingredients, as well as other minor ingredients commonly used in poultry production. Mixing equipment was flushed with ground corn prior to diet preparation. All diets were prepared using a paddle mixer. The mixer was cleaned between each diet using compressed air and vacuum; mixing

equipment was flushed with ground corn between each treatment group and flush material was retained for disposal.

Diet and Water Administration

Diets were fed in three feed phases: Starter diet (0-21 days of age), Grower diet (22-35 days of age) and Finisher diet (36-42 days of age). All diets were offered *ad libitum*. Fresh well water (from the research facility deep well) was provided *ad libitum*.

Table 8. Test Criteria Performed

Data/Sample Collected	When	Sample Size	Measurements
Feed Intake, BW, mortality & BW Uniformity	Weekly	Individual weights (14, 21, 35 & 42 days)	Feed Intake, BW, BWG Adjusted FCR, mortality, BW coefficient of variation.
Lesion Score	Day 21 and 42	Day 21 (4 birds) and 42 (10 birds per pen)	21-day Lesion scores from individual birds (2M and 2F birds). 42-day Lesion scores from individual birds (10M and 10F birds).
Intestinal Bacteria	Days 21 and 42	Day 21 (4 birds) and 42 (10 birds per pen)	Intestinal bacteria levels: Intestinal E. coli, APC (Aerobic Plate Count), Clostridium perfringens colonies were enumerated, and Salmonella Incidence determined.
Processing Data	Days 43 to 46	Day 21 (4 birds) and 42 (10 birds per pen)	Processing Dry Yield (%) Dry Yield (%) Breast meat yield (%) Processing Parts Yield (%) Major Parts Yield (%) Breast Meat Yield (% of chilled and live weights)

RESULTS AND DISCUSSION

Chicks were randomly assigned to treatments on Trial Day 0 (or day of hatch). At 42 days of age, live performance (growth weight gain, mortality and feed conversion) and other criteria were determined. Also at 42 days of age, lesion scores were measured, and intestinal fecal samples taken for further laboratory analyses. At 43-46 days of age, processing data were measured.

FIGS. 4-23 provide the data from the study. With respect to daily observations, each pen was closely monitored, at a minimum of three times per day, to determine overall health, bird behavior and/or evidence of toxicity, and environmental conditions. Temperature (both high and low temperature monitored each time period) was checked within the growing area employed for this study three times daily. Temperatures observed range from 86-91°F (Trial Days 0-7), 84-88°F (Days 8-14), 83-85°F (Days 15-21), 78-83°F (Days 22-28), 73-77°F (Days 29-35), and 70-74°F (Days 36-42).

Mortality in this study for the NC (challenged, no test materials added) ration was considered average to moderate for moderately bacteria built-up litter challenged, by strategy, and

on the upper quadrant of the Poultry Industry mortality standards (>6% for the NC Negative Control, with an average mortality in USA is <5%) throughout the growing period to 42 days of age, especially for a mild to moderate disease challenge model, and caused by bacteria stress (as targeted). Normal poultry industry mortality is typically and usually <5% when birds are grown on built-up litter bedding floors, as compared to <9% for a high disease challenge model. These data indicate that the mild to moderate disease challenge model functioned as intended.

Significant ($P > 0.05$) improvements in lower mortality rates (i.e., lower mortality) was found with all sources of Magni-Phi® and/or in combination with Provia Prime™, as well as other DFM sources. With the improvement in mortality with all Magni-Phi® (fed at 250 ppm), Provia Prime™ and the other DFM sources, these data indicate the safety of the product specifically for poultry and livability improvements that may be made. Furthermore, various significant differences ($P > 0.05$) were observed in body weight, feed conversion, and body weight gain (for 0-42 days of age) with treatments containing Magni-Phi® (fed at 250 ppm), Provia Prime™ and other DFMs, as compared to the control with no added test material but with a stress challenge, that affected live performance and mortality.

At 42 days of age, body weights (FIGS. 4-23) were significantly ($P>0.05$) increased and 0-42 day feed conversion significantly ($P>0.05$) decreased with either Magni-Phi® up to the 250 ppm, as well as other DFMs and in combination of Magni-Phi® feed additive level. However, when Provia Prime™ was combined with Magni-Phi® at 250 ppm, the combination of the two products appeared to statistically ($P<0.05$) to maximize live performance further and beyond just feeding Magni-Phi® alone. In most live performance cases in this trial, and with all ages of birds, there were statistical ($P<0.05$) improvements in 250 ppm Magni-Phi® vs. NC Control, as well as, feeding the combination of both Magni-Phi® and DFMs test materials, as compared to the Negative Control group of broiler chickens (without either Magni-Phi® or Provia Prime™. Other single Bacillus DFMs showed a similar trend but were of less significance than with Provia Prime™.

Intestinal bacteria levels (including Intestinal *E. coli*, APC or Aerobic Plate Count, *Clostridium perfringens* colonies were $\log_{10}$ enumerated and *Salmonella* Incidence (% of population) was determined at 21 and 42 days of age. Provia Prime™, in combination with Magni-Phi® fed at 250 ppm, appeared to statistically ($P<0.05$) maximize live performance, as well as, intestinal bacteria (Clostridium perfringens, E. coli, APC, Salmonella spp. levels) reductions levels at both 21 and 42 days of age. Additionally, breast meat (either hot carcass weights, chilled weights and compared as percentage of live weights) showed a similar trend statistical trend. Intestinal *E. coli, Clostridium perfringens* and *Salmonella* Incidence was significantly ($P<0.05$)

reduced with increasing levels of Magni-Phi®, as well as, the added addition of Provia Prime™ to these Magni-Phi® levels. The combination of the two test materials of Magni-Phi® and Provia Prime™, statistically (P<0.05) decreased *E. coli* and *Salmonella* incidence at both 250 ppm Magni-Phi® and adding in combination the 1X level of Provia Prime™.

Similar to live performance data (i.e., body weight and feed conversion), intestinal lesion scores on Day 21 and 42 (FIGS. 10 and 11) were significantly (P>0.05) improved with the use of either 250 ppm Magni-Phi®, with or without Provia Prime™.

Similar to live performance data, Dry Yield (%) and Breast Meat Yield (% of chilled and live weights) was statistically (P<0.05) increased with both Magni-Phi® and Provia Prime™ with the combination of the two boosting improvements by about >4% for each criterion. Of less significance were changes associated with Processing Parts Yield (%) due to the additions of either or both Magni-Phi® and Provia Prime™. Other single Bacillus DFMs showed a similar trend but were of less significance than with Provia Prime™.

The combination of both Magni-Phi® and Provia Prime™ resulted in most criteria from 14-42 days of age being at least numerical, if not statistically significantly (P<0.05) superior to either Magni-Phi® or Provia Prime™ alone. Additionally, in most cases from 21-42 days of age, the combination of both Magni-Phi® and Provia Prime™ resulted in at least numerical, if not statistically significantly (P<0.05) superior to PC (non-challenged, non-treated Control). Either Magni-Phi® or Provia Prime™ alone could make this claim. Remarkably, no single-strain Bacillus source (including: *B. pumilus, B. subtilis, B-amyloliquefaciens, B-coagulans or B-licheniformis*) resulted in improvements over the PC Control ration.

The European Production Efficiency Factor (EPEF) can be sued to standardize technical results, such as weight gained per day, survival rate and feed conversion. The EPEF is calculates as follows:

EPEF = (Average grams gained/day x % survival rate) / (Feed Conversion x 10).

The EPEF was found statistically (P<0.05) higher (or improved) with the combination 250 ppm Magni-Phi® and Provia Prime™, although closely behind individual treatment of either 250 ppm Magni-Phi® and Provia Prime™, with both appearing to be statistically (P<0.05) similar. In both Magni-Phi® addition levels of 250 ppm, Provia Prime™ further improved the genetic potential of the 3-criteria of live body weights, feed conversion and mortality, calculating EPEF, providing the best performance combination.

Growth and Feed Conversion graphs (FIGS. 16-23) for each test parameter and age group were used to determine potential most efficacious level, via the use of linear regression. In general, the following conclusions were made, based on these regression analyses curves and graph data:

1. When birds were reared to 21 days of age, maximum mean body weights were achieved with the combined use of 250 ppm Magni-Phi® and 1X Provia Prime™. The most efficient feed:gain values were found by feeding at least 250 ppm Magni-Phi® and Provia Prime™ in combination with 0.25 pounds per ton of Provia Prime™.

2. Remarkably, when birds were reared to 42 days of age, statistically ($P<0.05$) maximum mean body weights were achieved with the combined use of 250 ppm Magni-Phi® plus in combination with Provia Prime™ (at 0.25 pounds per ton), as compared to feeding either 250 ppm Magni-Phi® alone, Provia Prime™ alone or NC Control. However, either Magni-Phi® (at the 250 ppm Magni-Phi® level) or Provia Prime™ (at 0.25 pounds per ton) levels data were significantly ($P<0.05$) better than NC Control. In other words, the most efficient body weight and feed:gain values were found by feeding 250 ppm Magni-Phi® in combination with Provia Prime™, followed by 250 ppm Magni-Phi® alone, followed by Provia Prime™ alone.

3. With respect to % mortality during the entire trial, statistically ($P<0.05$) similar mean mortality improvements were achieved with the use of 250 ppm Magni-Phi® in combination with most all DFMs fed in the study (including: Provia Prime™ 4-way DFM combination, *B. pumilus, B. subtilis, B-amyloliquefaciens, B-coagulans* or *B-licheniformis*).

4. Both weight gains and feed efficiency values for 22-35 day of age period data were even more dramatically lower for the 250 ppm Magni-Phi® level plus Provia Prime™ level data than the NC Control but were relatively similar to the PC Control.

5. When birds were reared to 22-35 days or 36-42 days of age periods, higher mean market body weights were achieved with the use of the combination of Provia Prime™ and 250 ppm Magni-Phi®, as compared to either 250 ppm Magni-Phi® alone or in combinations with the other DFMs single strain doses.

6. Similar to both 22-35 and 36-42 days of age results, statistically ($P<0.05$) improved Body Weight Coefficient of Variation (or CV%) values were achieved by feeding Provia Prime™ in combination with 250 ppm Magni-Phi®.

7. Intestinal bacteria content *E. coli* and APC (Aerobic Plate Counts) were statistically ($P<0.05$) similar among all test feed articles; however, all treatments were superior to the Negative Control.

8. Intestinal *Clostridium perfringens* and Salmonella Incidence was significantly ($P<0.05$) reduced with the use of the combination of Magni-Phi® and Provia Prime™. The

combination of the two test materials, statistically (P<0.05) decreased *Clostridium perfringens* and Salmonella Incidence levels at 250 ppm Magni-Phi® and were even more dramatically reduced by adding in combination the 0.25 pounds per ton Provia Prime™ level.

9. Dry Yield (%) and Breast Meat Yield (% of chilled and live weights) was statistically (P<0.05) increased when both Magni-Phi® and Provia Prime™ were added, with the combination of the two boosting improvements in processing values.

CONCLUSIONS

Magni-Phi® (fed at 250 ppm) alone or the combination of Provia Prime™ (fed at 0.25 pounds per ton level), appeared to improve (at various efficiency) body weight, feed conversion, mortality, dry yield, breast meat yield and intestinal bacteria levels at all feed additive addition levels and combinations, as compared to feeding no ABF feed additive or NEGATIVE CONTROL. The true test in defining essential usage levels is to define ROI (Return-on-investment) and to determine the "*cost-effectiveness*" of feeding the combination of products. It appeared that feeding the combination (as compared to feeding just up to 21 or 35 days of age) of all products throughout the entire life-cycle of the broiler chicken, to 42-days of age and at market age, appeared to improve live performance and other critical criteria data.

Provia Prime™, as well as all other DFM single strain products, appeared to play a role in aiding Magni-Phi® in improving statistically (P<0.05) maximum live performance, lesion scores and processing characteristics (especially breast meat yield). The most discernible effects of significant improvements (body weight, feed conversion, mortality, breast meat yield and lesion scores at P<0.05) were shown with the use of 250 ppm Magni-Phi® level in combination with Provia Prime™, where significance at the 5% level of probability was found over the Negative Control Ration (infected and without other test materials), feeding either product alone, as well as, in most cases including Positive or PC Control Ration (non-infected and without other test materials). Single strain DFMs also had an effect, especially when combined with Magni-Phi®.

Remarkably, Magni-Phi®, regardless of DFM usage, appeared to independently improve growth rate and feed efficiency, all the way to market age. Both Magni-Phi® and Provia Prime™ appeared to be feed ingredients beneficial to the broiler chicken live performance and processing characteristics, but ultimately achieving maximize genetic potential, as it may relate to cost effectiveness when compared to PC Control or NC Control groups. Provia Prime™ appeared to be an important component to achieve this very high standard marketing goal, with advantages over single-strain Bacillus sources ((including: *B. pumilus, B. subtilis, B. amyloliquefaciens, B. coagulans or B. licheniformis*).

VII. Exemplary Embodiments

The following numbered statements illustrate exemplary embodiments of the disclosed technology.

Statement 1. A Bacilli combination, consisting essentially of three or four *Bacillus* species selected from *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*.

Statement 2. A composition, consisting essentially of *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*.

Statement 3. The composition of statement 2, consisting of *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens*.

Statement 4. A composition, consisting essentially of *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus coagulans*.

Statement 5. A composition, consisting of *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus coagulans*.

Statement 6. A composition, consisting essentially of *Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis*.

Statement 7. The composition of statement 6, consisting of *Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis*.

Statement 8. An admixed composition, comprising:

a Bacilli combination; and

an additional component.

Statement 9. The admixed composition of statement 8, wherein the additional component comprises a copper salt.

Statement 10. The composition of statement 9, wherein the copper salt is copper chloride, copper bromide, copper iodide, copper sulfate, copper sulfite, copper bisulfite, copper thiosulfate, copper phosphate, monobasic copper phosphate, dibasic copper phosphate, copper hypophosphite, copper dihydrogen pyrophosphate, copper tetraborate, copper borate, copper carbonate, copper bicarbonate, copper metasilicate, copper citrate, copper malate, copper methionate, copper succinate, copper lactate, copper formate, copper acetate, copper butyrate, copper propionate, copper benzoate, copper tartrate, copper ascorbate, copper gluconate, or a combination thereof.

Statement 11. The admixed composition of statement 8 wherein the additional component comprises a vitamin.

Statement 12. The admixed composition of any one of statements 5-6 where the composition further comprises a vitamin.

Statement 13. The composition of any one of statements 11-12, wherein the vitamin is vitamin A, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, vitamin K, or a combination thereof.

Statement 14. The admixed composition of statement 8 wherein the additional component comprises an additional direct fed microbial, or statements 9-13 where the composition further comprises an additional direct fed microbial.

Statement 15. The admixed composition of any one of statements 8-14, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis.*

Statement 16. The admixed composition of any one of statements 8-14, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans.*

Statement 17. A composition for administration to poultry, comprising:

a Bacilli combination; and

a poultry feed.

Statement 18. The composition of statement 17, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis.*

Statement 19. The composition of statement 17, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans.*

Statement 20. The composition of statements 17-19, wherein the poultry feed comprises plant material, a carbonate, sulfate, lactate, oxide, propionate, stearate, phosphate, mineral, copper species, sugar, salt, animal protein product, forage product, grain product, plant protein product, processed grain product, roughage product, molasses product, or combinations thereof

Statement 21. The composition of any one of statements 17-20, wherein the poultry feed comprises beet pulp, ground corn, corn syrup solids, plant fiber, rice hulls, soluble plant fiber, wheat middlings, microcrystalline cellulose, calcium carbonate, potassium carbonate, potassium sulfate, sodium sulfate, calcium lactate, calcium oxide, calcium propionate, calcium stearate, dicalcium phosphate dehydrate, monocalcium phosphate, sodium tripolyphosphate, tetra sodium pyrophosphate, dolomite, silicon dioxide, silica, limestone, vermiculite, bentonite, montmorillonite, kaolin, glucose, sucrose, dextrose, fructose, maltodextrin, sodium chloride, carrageenan, cellulose,

guar gum, polyols, sodium alumino silicate, urea, biotin, folic acid, sodium sesquicarbonate, methionine source, lysine source, L-threonine, or combinations thereof.

Statement 22. The composition of any one of statements 17-21, wherein the poultry feed comprises copper sulfate.

Statement 23. The composition of any one of statements 17-22, wherein the composition comprises from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus subtilis* per gram of feed.

Statement 24. The composition of any one of statements 17-23, wherein the composition comprises from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus licheniformis* per gram of feed.

Statement 25. An admixture composition, comprising:

a first composition consisting essentially of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis*; and

a second composition comprising one or more of a feed, yucca, quillaja, yucca and quillaja, a silica, mineral clay, glucan and mannans mixture, or a combination thereof.

Statement 26. A composition, comprising *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis* and one or more of (a) yucca, quillaja, or yucca and quillaja, (b) silica, mineral clay, glucan, and mannans, or (c) a combination thereof.

Statement 27. A composition formulated for administration to poultry, comprising:

Bacillus amyloliquefaciens, Bacillus subtilis, and *Bacillus licheniformis*; and

a feed supplement.

Statement 28. A package, comprising:

a composition consisting essentially of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis*; or

three separate package portions, one for each of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis*.

Statement 29. A method, comprising administering a Bacilli combination to a subject.

Statement 30. The method according to statement 29 wherein the subject is livestock.

Statement 31. The method according to statement 24 wherein the Bacilli combination is administered as an admixture comprising a composition consisting essentially of *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis* and at least one additional component.

Statement 32. A method, comprising administering a composition comprising *Bacillus amyloliquefaciens, Bacillus subtilis*, and *Bacillus licheniformis* to poultry.

Statement 33. The method of statement 32, wherein the composition consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis*, and *Bacillus licheniformis*.

Statement 34. The method of statement 32, wherein the composition is a composition according to any one of statements 1-27.

Statement 35. A method, comprising:

providing *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis*; and

forming a first composition comprising *Bacillus amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis* and a feed, a feed supplement, a direct fed microbial, a carrier, or a combination thereof.

Statement 36. The method of statement 35, wherein the feed supplement comprises:

yucca;

quillaja;

yucca and quillaja;

silica, mineral clay, glucan, and mannans; or

a combination thereof.

Statement 37. A composition made by the method of any one of statements 35-36.

Statement 38. A method of reducing bird mortality, lesion scores, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter, comprising administering to poultry an effective amount of a composition comprising *Bacillus amyloliquefaciens, Bacillus subtilis*, and *Bacillus licheniformis*.

Statement 39. The method according to statement 38, wherein the composition consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis*, and *Bacillus licheniformis*.

Statement 40. A composition for use in administration to poultry, comprising *Bacillus amyloliquefaciens, Bacillus subtilis*, and *Bacillus licheniformis*.

Statement 41. The composition of statement 40 for use in reducing bird mortality, lesion scores, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter.

Statement 42. The composition of any one of statements 40-41, for use in increasing breast meat yield.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A Bacilli combination, consisting essentially of three or four *Bacillus* species selected from *Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens.*

2. The Bacilli combination of claim 1, wherein the Bacilli combination is a composition consisting essentially of *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus coagulans.*

3. The Bacilli combination of claim 2, wherein the composition consists of *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus coagulans.*

4. The Bacillus combination of any of claims 1-3, wherein relative amounts of Bacillus species in the Bacillus combination are from 25% to 50% *Bacillus subtilis*, from 30% to 65% *Bacillus licheniformis*, from 5% to 30% *Bacillus amyloliquefaciens* and from greater than zero to 15% *Bacillus coagulans*, in amounts relative to each other.

5. The Bacilli combination of claim 1, wherein the Bacilli combination is a composition consisting essentially of *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens.*

6. The Bacilli combination of claim 5, wherein the composition consists of *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefaciens.*

7. The Bacilli combination of claim 1, wherein the Bacilli combination is a composition consisting essentially of *Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis.*

8. The Bacilli combination of claim 7, wherein the composition consists of *Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis.*

9. The Bacilli combination of any one of claims 1-8, wherein one or more bacillus species in the Bacilli combination is dehydrated.

10. The Bacilli combination of claim 9, wherein all bacillus species in the Bacilli combination are dehydrated.

11. The Bacilli combination of claim 9 or claim 10, wherein the bacillus species are freeze dried.

12. The Bacilli combination of claim 1, consisting essentially of from 30% to 45% *Bacillus subtilis,* from 40% to 60% *Bacillus licheniformis*, from 10% to 25% *Bacillus amyloliquefaciens* and from 1% to 12% *Bacillus coagulans.*

13. The Bacilli combination of claim 12, wherein each of *Bacillus subtilis, Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus coagulans* is freeze dried.

14. The Bacilli combination of any one of claims 1-2, 4-5, 7, or 9-13, further comprising a carrier.

15. The Bacilli combination of claim 14, wherein the carrier is a metal carbonate.

16. A combination and/or composition, comprising:
a Bacilli combination according to any one of claims 1-15; and
an additional component.

17. The combination and/or composition of claim 16, wherein the additional component comprises one or more of yucca, quillaja, silica, mineral clay, glucan, mannans, endoglucanohydrolase, a copper salt, a vitamin.

18. The combination and/or composition of claim 17, wherein the additional component comprises *Yucca schidigera, Quillaja saponaria*, or a combination thereof.

19. The combination and/or composition of claim 17 or claim 18, wherein the additional component comprises silica, mineral clay, glucan, and mannans.

20. The combination and/or composition of claim 19, wherein the additional component further comprises endoglucanohydrolase.

21. The combination and/or composition of anyone of claims 16-20, wherein the additional component comprises a copper salt.

22. The combination and/or composition of claim 21, wherein the copper salt is copper chloride, copper bromide, copper iodide, copper sulfate, copper sulfite, copper bisulfite, copper thiosulfate, copper phosphate, monobasic copper phosphate, dibasic copper phosphate, copper hypophosphite, copper dihydrogen pyrophosphate, copper tetraborate, copper borate, copper carbonate, copper bicarbonate, copper metasilicate, copper citrate, copper malate, copper methionate, copper succinate, copper lactate, copper formate, copper acetate, copper butyrate, copper propionate, copper benzoate, copper tartrate, copper ascorbate, copper gluconate, or a combination thereof.

23. The combination and/or composition of any one of claims 16-22 wherein the additional component comprises a vitamin.

24. The combination and/or composition of claim 23, wherein the vitamin is vitamin A, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, vitamin K, or a combination thereof.

25. The combination and/or composition of any one of claims 16-24, wherein the combination and/or composition comprises a first composition and a second composition, the first composition being the Bacilli combination according to any one of claims 1-6, and the second composition comprising the additional component.

26. The combination and/or composition of claim 25, wherein the second composition comprises *Yucca schidigera* and *Quillaja saponaria.*

27. The combination and/or composition of claim 25 or claim 26, wherein the second composition comprises silica, mineal clay, glucan, mannans, and optionally endoglucanohydrolase.

28. The combination and/or composition of any one of claims 25-27 wherein the second composition comprises a direct fed microbial.

29. The combination and/or composition of any one of claims 16-28, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*.

30. The combination and/or composition of claim 29, wherein amounts of the *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* in the combination and/or composition are from 25% to 50% *Bacillus subtilis*, from 30% to 65% *Bacillus licheniformis*, from 5% to 30% *Bacillus amyloliquefaciens* and from greater than zero to 15% *Bacillus coagulans*, in amounts relative to each other.

31. A composition for administration to poultry, comprising:
a Bacilli combination according to any one of claims 1-15; and
a poultry feed.

32. The composition of claim 31, wherein the Bacilli combination consists essentially of *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans*.

33. The composition of claim 32, wherein the Bacilli combination consists essentially of from 25% to 50% *Bacillus subtilis*, from 30% to 65% *Bacillus licheniformis*, from 5% to 30% *Bacillus amyloliquefaciens* and from greater than zero to 15% *Bacillus coagulans*, in amounts relative to each other.

34. The composition of claims 31-33, wherein the poultry feed comprises plant material, a carbonate, sulfate, lactate, oxide, propionate, stearate, phosphate, mineral, copper species, sugar, salt, animal protein product, forage product, grain product, plant protein product, processed grain product, roughage product, molasses product, or combinations thereof

35. The composition of any one of claims 31-34, wherein the poultry feed comprises beet pulp, ground corn, corn syrup solids, plant fiber, rice hulls, soluble plant fiber, wheat middlings, microcrystalline cellulose, calcium carbonate, potassium carbonate, potassium sulfate, sodium sulfate, calcium lactate, calcium oxide, calcium propionate, calcium stearate, dicalcium

phosphate dehydrate, monocalcium phosphate, sodium tripolyphosphate, tetra sodium pyrophosphate, dolomite, silicon dioxide, silica, limestone, vermiculite, bentonite, montmorillonite, kaolin, glucose, sucrose, dextrose, fructose, maltodextrin, sodium chloride, carrageenan, cellulose, guar gum, polyols, sodium alumino silicate, urea, biotin, folic acid, sodium sesquicarbonate, methionine source, lysine source, L-threonine, or combinations thereof.

36. The composition of any one of claims 31-35, wherein the poultry feed comprises copper sulfate.

37. The composition of any one of claims 31-36, wherein the composition comprises from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus subtilis* per gram of feed.

38. The composition of any one of claims 31-37, wherein the composition comprises from 1.2 x 10^5 to 4 x 10^5 CFU *Bacillus licheniformis* per gram of feed.

39. The composition of any one of claims 31-38, wherein the composition comprises from 0.1 pound to 1 pound of the Bacilli combination per ton of feed.

40. The composition of any one of claims 31-39, wherein the composition comprises from 0.1 pound to 0.5 pounds of the Bacilli combination per ton of feed.

41. The composition of any one of claims 31-40, wherein the composition further comprises yucca and/or quillaja.

42. The composition of any one of claims 31-41, wherein the composition comprises *Yucca schidigera* and *Quillaja saponaria.*

43. The composition of any one of claims 31-42, wherein the composition comprises from 100 ppm to 500 ppm of a composition comprising *Yucca schidigera* and *Quillaja saponaria*, per ton of feed.

44. A method, comprising administering a Bacilli combination according to any one of claims 1-15 to a subject.

45. The method according to claim 44 wherein the subject is livestock.

46. The method of claim 44, wherein the subject is poultry.

47. The method according to any one of claims 44-46 wherein the Bacilli combination is administered as a composition comprising a first composition consisting essentially of *Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus subtilis,* and *Bacillus licheniformis* and a second composition comprising at least one additional component.

48. The method of claim 39, wherein the composition is a composition according to any one of claims 25-28.

49. A method, comprising:

providing *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus coagulans,* and *Bacillus licheniformis*; and

forming a first composition comprising *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus coagulans,* and *Bacillus licheniformis* and a feed, a feed supplement, a direct fed microbial, a carrier, or a combination thereof.

50. The method of claim 49, wherein the feed supplement comprises:

yucca;

quillaja;

yucca and quillaja;

silica, mineral clay, glucan, and mannans; or

a combination thereof.

51. A composition made by the method of any one of claims 49-50.

52. A composition for use in administration to poultry, the composition comprising *Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus subtilis*, and *Bacillus licheniformis*.

53. The composition for the use of claim 52 wherein administration of the composition reduces bird mortality, lesion scores, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter.

54. The composition for the use of claim 52 or claim 53 wherein administration of the composition increases breast meat yield, increases weight gain, improves a feed conversion ratio, or a combination thereof.

55. The composition for the use of any one of claims 52-54, wherein the composition comprises *Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus subtilis*, and *Bacillus licheniformis* and no other direct-fed microbial or bacillus species.

56. The composition for the use of any one of claims 52-55, wherein the composition comprises from 25% to 50% *Bacillus subtilis*, from 30% to 65% *Bacillus licheniformis*, from 5% to 30% *Bacillus amyloliquefaciens* and from greater than zero to 15% *Bacillus coagulans*, in amounts relative to each other.

57. A method of reducing bird mortality, lesion scores, *Salmonella/E. Coli/Clostridium perfingens* (CP) incidence, and/or oocysts in fecal matter, comprising administering to poultry an effective amount of a composition comprising *Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus subtilis*, and *Bacillus licheniformis*.

58. The method according to claim 57, wherein the composition consists essentially of *Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus subtilis*, and *Bacillus licheniformis*.

Data	T1	T2	T3	T4	T5	T6	T7	T8
Average Body Wt. (g) Day 0	46.13	46.36	46.27	46.33	46.33	46.03	46.08	46.00
Significance (P<0.05)	a	a	a	a	a	a	a	a
Average Body Wt. (g) Day 10	268.00	248.37	254.50	260.61	263.60	254.48	270.37	260.87
Significance (P<0.05)	a	d	cd	bc	ab	cd	a	bc
Feed Conversion Corrected Day 0-10	1.086	1.115	1.111	1.081	1.068	1.093	1.054	1.082
Significance (P<0.05)	bc	d	cd	b	ab	bcd	a	b
Mortality % Day 0-10	1.76	3.69	2.88	1.60	1.44	2.72	1.28	1.92
Significance (P<0.05)	ab	d	cd	a	a	bcd	a	abc
Average Body Wt. Gain (g) Day 0-10	221.87	202.01	208.23	214.28	217.27	208.45	224.29	214.87
Significance (P<0.05)	ab	e	de	cd	bc	de	a	bcd
Average Body Wt. (g) Day 21	888.30	853.12	869.19	878.65	883.30	867.22	895.30	875.82
Significance (P<0.05)	ab	d	c	bc	ab	c	a	bc
Feed Conversion Corrected Day 0-21	1.372	1.401	1.388	1.361	1.355	1.375	1.338	1.352
Significance (P<0.05)	bcd	d	cd	abc	ab	bcd	a	ab
Mortality % Day 0-21	2.244	5.288	3.846	1.923	2.083	3.686	1.603	2.564
Significance (P<0.05)	a	c	b	a	a	b	a	ab
Average Body Wt. Gain (g) Day 0-21	842.17	806.76	822.91	832.32	836.96	821.19	849.21	829.82
Significance (P<0.05)	ab	d	c	bc	ab	c	a	bc
Body Weight Coefficient of Variation (period)	10.98	14.99	11.06	10.98	10.28	11.19	11.10	10.94
Significance (P<0.05)	b	c	b	b	a	b	b	b
Lesion Scores Day 21	1.042	2.125	1.375	1.146	0.854	1.396	0.250	1.375
Significance (P<0.05)	bc	e	d	cd	b	d	a	d
Average Body Wt. (g) Day 42	2651.302	2575.083	2589.927	2634.402	2672.351	2603.780	2684.210	2617.893
Significance (P<0.05)	ab	e	de	bc	a	cde	a	bcd
Feed Conversion Corrected Day 0-42	1.831	1.884	1.862	1.835	1.829	1.843	1.823	1.853
Significance (P<0.05)	ab	d	cd	ab	ab	abc	a	bc
Mortality % Day 0-42	2.604	6.250	4.340	2.083	2.257	4.340	1.736	2.951
Significance (P<0.05)	a	c	b	a	a	b	a	ab
Average Body Wt. Gain (g) Day 0-42	2605.172	2528.722	2543.654	2588.067	2626.016	2557.751	2638.127	2571.893
Significance (P<0.05)	ab	c	c	ab	a	bc	a	bc
Body Weight Coefficient of Variation (period)	10.276	12.726	10.287	10.322	10.466	10.226	10.262	10.129
Significance (P<0.05)	a	b	a	a	a	a	a	a
Average Body Wt. Gain (g) Day 22-42	1763.004	1721.960	1720.740	1755.751	1789.051	1736.558	1788.914	1742.069
Significance (P<0.05)	ab	c	c	ab	a	bc	a	bc
Feed Conversion Corrected Day 22-42	2.042	2.104	2.080	2.051	2.042	2.056	2.045	2.083
Significance (P<0.05)	a	d	bcd	ab	a	abc	a	cd
Mortality % Day 22-42	0.361	0.962	0.494	0.160	0.174	0.654	0.134	0.387
Significance (P<0.05)	ab	c	ab	a	a	bc	a	ab
Lesion Scores Day 42	0.483	1.283	0.842	0.625	0.458	0.875	0.275	0.783
Significance (P<0.05)	bc	f	e	cd	b	e	a	de
E. coli (log10) Day 21	6.011	6.693	6.006	6.010	5.800	5.984	5.447	6.016
Significance (P<0.05)	b	c	b	b	ab	b	a	b
E. coli (log10) Day 42	6.035	6.272	6.019	6.028	5.983	6.074	5.861	5.980
Significance (P<0.05)	a	b	a	a	a	ab	a	a
APC (log10) Day 21	7.936	8.569	8.619	7.924	7.976	7.993	7.408	7.705
Significance (P<0.05)	ab	c	c	ab	b	b	a	ab
APC (log10) Day 42	7.953	8.255	7.977	7.975	8.055	7.888	7.624	8.099
Significance (P<0.05)	ab	b	ab	ab	b	ab	a	b
Salmonella Incidence (%) Day 21	58.333	75.000	54.167	60.417	50.000	45.833	33.333	58.333
Significance (P<0.05)	bc	c	abc	bc	ab	ab	a	bc
Salmonella Incidence (%) Day 42	41.667	65.833	48.333	50.000	47.500	49.167	33.333	50.000
Significance (P<0.05)	ab	c	b	b	b	b	a	b
Clostridium perfringens (log10 per g) 21 days.	3.510	3.880	3.403	3.577	3.226	3.388	2.740	3.308
Significance (P<0.05)	bc	c	bc	bc	ab	bc	a	abc
Clostridium perfringens (log10 per g) 42 days.	3.539	3.801	3.299	3.429	3.411	3.463	2.819	3.484
Significance (P<0.05)	ab	c	ab	bc	bc	bc	a	bc
Oocyst Count per g of wet fecal Day 21	3009	257550	88471	59554	31926	76969	31977	56411
Significance (P<0.05)	a	e	d	c	b	cd	b	bc
Oocyst Count per g of wet fecal Day 42	8618	305996	97416	64825	43759	80335	53090	115414
Significance (P<0.05)	a	g	ef	cd	b	de	bc	f

NOTE: "Significance P<0.05)" refers to means within a row without a common superscript are significantly different (P<0.05) as determinedby Least Significant Difference.

FIG. 1

Data	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Average Body Wt. (g) Day 0	50.88	50.95	51.29	51.04	50.82	50.85	51.07	50.68	50.74	50.88	51.13
Significance (P<0.05)	a	a	a	a	a	a	a	a	a	a	a
Average Body Wt. (g) Day 14	387.92	361.45	384.36	382.11	381.13	381.98	378.22	379.98	379.85	376.60	375.91
Significance (P<0.05)	a	c	ab	ab	ab	ab	b	ab	ab	b	b
Feed Conversion Corrected Day 0-14	1.128	1.193	1.126	1.112	1.152	1.155	1.167	1.160	1.168	1.169	1.154
Significance (P<0.05)	abc	e	ab	a	bcd	cd	de	d	de	de	cd
Mortality % Day 0-14	0.32	2.72	0.32	0.32	0.64	1.12	0.96	0.80	0.80	1.12	1.28
Significance (P<0.05)	a	c	a	a	ab	ab	ab	ab	ab	ab	b
Average Body Wt. Gain (g) Day 0-14	337.03	310.50	333.07	331.07	330.32	331.13	327.15	329.30	329.11	325.73	324.78
Significance (P<0.05)	a	c	ab	ab	ab	ab	b	ab	ab	b	b
Average Body Wt. (g) Day 21	922.91	874.19	915.92	915.23	909.77	900.18	897.24	913.72	911.31	908.55	891.37
Significance (P<0.05)	a	f	ab	ab	abcd	cde	de	ab	abc	bcd	e
Feed Conversion Corrected Day 0-21	1.363	1.421	1.367	1.374	1.365	1.398	1.391	1.361	1.388	1.386	1.399
Significance (P<0.05)	a	d	ab	abc	a	bcd	bcd	a	abc	abc	cd
Mortality % Day 0-21	0.481	4.006	0.801	0.801	0.962	1.763	0.962	1.122	1.282	2.083	2.083
Significance (P<0.05)	a	d	ab	ab	ab	bc	ab	abc	abc	c	c
Average Body Wt. Gain (g) Day 0-21	872.03	823.24	864.63	864.19	858.96	849.33	846.17	863.04	860.56	857.67	840.24
Significance (P<0.05)	a	f	ab	ab	abcd	cde	de	ab	abc	bcd	e
Body Weight Coefficient of Variation (period)	8.28	16.27	10.01	9.55	11.11	13.04	12.37	11.15	11.16	15.72	15.86
Significance (P<0.05)	a	e	b	b	c	d	d	c	c	e	e
Lesion Scores Day 21	0.479	1.563	0.438	0.354	0.958	1.063	0.979	1.104	0.854	1.333	1.271
Significance (P<0.05)	a	d	a	a	b	bc	b	bc	b	cd	c
Average Body Wt. (g) Day 42	2540.31	2451.17	2535.54	2504.78	2504.50	2482.00	2500.75	2513.43	2519.48	2512.66	2514.91
Significance (P<0.05)	a	d	ab	abc	abc	cd	bc	abc	abc	abc	abc
Feed Conversion Corrected Day 0-42	1.763	1.902	1.781	1.809	1.806	1.843	1.837	1.824	1.829	1.820	1.828
Significance (P<0.05)	a	f	ab	bcd	bc	e	de	cde	cde	cde	cde
Mortality % Day 0-42	0.521	5.035	0.868	0.868	1.042	2.083	1.215	1.215	1.736	2.778	2.951
Significance (P<0.05)	a	f	ab	ab	abc	cde	abc	abc	bcd	de	e
Average Body Wt. Gain (g) Day 0-42	2489.42	2400.22	2484.25	2453.74	2453.68	2431.15	2449.69	2462.75	2468.74	2461.79	2463.78
Significance (P<0.05)	a	d	ab	abc	abc	cde	bc	abc	abc	abc	abc
Body Weight Coefficient of Variation (period)	8.431	15.567	9.678	9.622	11.119	12.760	12.749	11.290	11.615	15.219	15.791
Significance (P<0.05)	a	e	b	b	b	d	d	c	c	e	e
Average Body Wt. Gain (g) Day 22-42	1617.40	1576.98	1619.61	1589.55	1594.72	1581.82	1603.51	1599.71	1606.18	1604.12	1623.53
Significance (P<0.05)	ab	c	ab	abc	abc	bc	abc	abc	abc	abc	a
Feed Conversion Corrected Day 22-42	1.998	2.180	2.021	2.067	2.064	2.103	2.094	2.096	2.087	2.075	2.072
Significance (P<0.05)	a	c	a	b	b	b	b	b	b	b	b
Mortality % Day 22-42	0.040	1.028	0.067	0.067	0.080	0.321	0.254	0.093	0.454	0.694	0.868
Significance (P<0.05)	a	d	a	a	a	ab	ab	a	abc	bcd	cd

FIG. 2

Data	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Lesion Scores Day 42	0.358	1.875	0.717	0.783	1.000	1.075	1.025	1.083	0.992	1.258	1.392
Significance (P<0.05)	a	g	b	bc	cd	de	d	de	cd	ef	fg
E. coli (log10) Day 21	5.394	6.717	6.410	6.268	6.100	6.044	6.039	6.037	6.016	6.062	6.085
Significance (P<0.05)	a	c	a	a	b	b	b	b	b	b	b
E. coli (log10) Day 42	6.452	6.799	6.464	6.352	6.682	6.003	6.052	6.037	6.045	6.132	6.101
Significance (P<0.05)	a	d	a	a	b	c	c	c	c	c	c
APC (log10) Day 21	7.479	9.332	7.677	7.879	8.504	8.144	8.301	8.254	8.433	8.096	8.273
Significance (P<0.05)	a	d	ab	ab	c	bc	c	c	c	bc	c
APC (log10) Day 42	7.645	8.751	7.710	7.705	8.382	8.508	8.137	8.159	8.122	8.298	8.314
Significance (P<0.05)	a	d	a	a	bc	cd	b	b	b	bc	bc
Salmonella Incidence (%) Day 21	22.917	87.500	27.500	21.250	52.083	64.167	47.917	50.000	52.083	68.750	75.000
Significance (P<0.05)	a	e	abc	ab	cd	cd	bc	bcd	cd	de	e
Salmonella Incidence (%) Day 42	10.000	66.667	30.833	34.167	43.333	40.833	50.000	41.667	50.000	33.333	35.000
Significance (P<0.05)	a	d	b	b	bc	bc	c	bc	c	b	b
Clostridium perfringens (log10 per g) 21 days	3.415	4.746	3.715	3.378	4.083	3.896	3.918	4.085	3.928	3.907	3.886
Significance (P<0.05)	a	d	b	a	c	bc	bc	c	bc	bc	bc
Clostridium perfringens (log10 per g) 42 days	3.754	4.327	4.051	4.012	4.527	4.504	4.643	4.582	4.597	4.527	4.549
Significance (P<0.05)	a	d	b	b	c	c	c	c	c	c	c

FIG. 3

Data	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Average Body Wt. (g) Day 0	39.22	39.59	39.51	39.61	39.44	39.64	39.32	39.78	39.45	39.21	39.61
Significance (P<0.05)	b	a	a	a	a	a	a	a	a	a	a
Days 0-14											
Average Body Wt. (g)	460.99	418.09	457.38	462.86	466.52	461.56	465.24	457.00	461.88	455.22	462.45
Significance (P<0.05)	ab	c	b	ab	a	ab	ab	b	ab	b	ab
Feed Conversion Corrected	1.091	1.131	1.111	1.105	1.079	1.129	1.102	1.121	1.104	1.134	1.106
Significance (P<0.05)	ab	d	bcd	abcd	a	cd	abcd	bcd	abcd	d	abcd
Mortality %	0.17	3.32	0.87	0.87	0.87	0.96	1.44	0.96	0.96	1.44	0.48
Significance (P<0.05)	a	c	ab	ab	ab	ab	b	ab	ab	b	ab
Average Body Wt. Gain (g)	421.77	378.51	417.87	423.25	427.08	421.72	425.92	417.22	422.43	416.00	422.85
Significance (P<0.05)	ab	c	b	ab	a	ab	ab	b	b	ab	b
Body Weight Coefficient of Variation (period)	8.61	20.35	16.01	14.35	8.49	15.74	16.30	15.58	15.60	15.77	15.75
Significance (P<0.05)	a	c	bc	b	a	bc	bc	bc	bc	bc	bc
Feed Consumption (g per bird per day)	32.87	30.74	33.19	33.47	32.96	34.09	33.63	33.45	33.37	33.79	33.43
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average g gained/day for each period	32.93	29.86	32.67	33.06	33.32	32.97	33.23	32.64	32.99	32.52	33.03
Significance (P<0.05)	ab	c	ab	ab	a	ab	ab	ab	ab	b	ab
European Production Efficiency Factor (EPEF)	301.32	253.11	291.10	296.03	305.59	288.33	295.93	288.02	295.47	281.70	297.29
Significance (P<0.05)	ab	f	cde	abcd	a	cde	abcde	cde	abcde	e	abcd

FIG. 4

Data	T12	T13	T14	T15
Average Body Wt. (g) Day 0	39.20	39.54	39.94	39.27
Significance (P<0.05)	a	a	a	a
Days 0-14				
Average Body Wt. (g)	455.07	466.66	454.96	466.61
Significance (P<0.05)	b	ab	b	ab
Feed Conversion Corrected	1.128	1.092	1.128	1.096
Significance (P<0.05)	bcd	abc	bcd	abcd
Mortality %	1.44	0.96	1.44	1.44
Significance (P<0.05)	b	ab	b	b
Average Body Wt. Gain (g)	415.87	427.12	415.02	427.34
Significance (P<0.05)	b	ab	b	ab
Body Weight Coefficient of Variation (period)	15.77	14.91	15.57	14.93
Significance (P<0.05)	bc	bc	bc	bc
Feed Consumption (g per bird per day)	33.61	33.40	33.51	33.51
Significance (P<0.05)	a	a	a	a
Average g gained/day for each period	32.50	33.33	32.50	33.33
Significance (P<0.05)	b	ab	b	ab
European Production Efficiency Factor (EPEF)	282.87	301.47	283.14	298.90
Significance (P<0.05)	de	abc	de	abc

FIG. 5

FIG. 6

Days 0-21	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Average Body Wt. (g)	860.4	786.9	849.1	866.3	870.1	843.3	860.9	840.5	852.7	834.7	852.0
Significance (P<0.05)	abc	d	bc	ab	a	bc	abc	bc	abc	c	bc
Feed Conversion Corrected	1.324	1.387	1.352	1.343	1.317	1.373	1.336	1.360	1.333	1.375	1.359
Significance (P<0.05)	ab	cd	abcd	abc	a	bcd	abc	abcd	abc	bcd	d
Mortality %	0.52	5.24	1.22	1.40	1.22	1.92	1.92	0.96	1.44	1.92	0.96
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average Body Wt. Gain (g)	821.1	747.3	809.6	826.7	830.6	803.5	821.6	800.7	813.3	795.5	812.4
Significance (P<0.05)	abc	d	bc	ab	a	bc	abc	bc	abc	c	bc
Body Weight Coefficient of Variation (period)	14.14	15.63	14.17	13.61	8.55	15.95	15.92	15.81	15.49	15.31	16.18
Significance (P<0.05)	bc	d	bc	b	a	d	d	d	cd	cd	b
Feed Consumption (g per bird per day)	51.92	50.46	52.34	53.18	52.31	53.09	52.57	51.92	51.92	52.36	52.60
Significance (P<0.05)	ab	b	ab	a	ab	a	ab	ab	ab	ab	a
Average g gained/day for each period	40.97	37.47	40.44	41.25	41.43	40.16	41.00	40.02	40.61	39.75	40.57
Significance (P<0.05)	abc	d	abc	ab	a	abc	abc	bc	abc	c	abc
European Production Efficiency Factor (EPEF)	307.02	249.70	294.24	301.01	309.43	284.16	298.99	291.10	298.36	281.82	295.51
Significance (P<0.05)	a	f	bcd	ab	a	bc	abc	bcde	abc	e	de
Days 0-35											
Average Body Wt. (g)	2110.3	1911.7	2041.5	2054.5	2094.9	2044.6	2060.2	2013.6	2041.3	2011.0	2038.8
Significance (P<0.05)	a	e	d	bc	ab	bcd	abc	d	bcd	d	cd
Feed Conversion Corrected	1.534	1.611	1.573	1.553	1.506	1.568	1.528	1.552	1.531	1.558	1.549
Significance (P<0.05)	abc	c	bc	abc	a	abc	ab	abc	ab	abc	abc
Mortality %	0.758	6.818	1.515	1.894	1.705	2.083	2.604	1.042	1.563	2.604	1.042
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average Body Wt. Gain (g)	2071.1	1872.1	2002.0	2014.9	2055.5	2004.8	2020.9	1973.9	2001.9	1971.7	1999.2
Significance (P<0.05)	a	d	c	abc	ab	abc	abc	c	abc	c	bc
Body Weight Coefficient of Variation (period)	13.728	15.743	14.547	14.871	8.697	16.752	17.331	15.729	15.833	16.365	17.013
Significance (P<0.05)	b	cde	bc	cd	a	de	e	cde	cde	de	de
Feed Consumption (g per bird per day)	94.022	91.108	93.362	92.877	91.808	93.326	91.823	90.616	90.698	91.440	91.021
Significance (P<0.05)	a	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab
Average g gained/day for each period	60.295	54.620	58.329	58.700	59.855	58.417	58.864	57.533	58.323	57.456	57.901
Significance (P<0.05)	a	d	c	abc	ab	abc	abc	c	abc	c	bc
European Production Efficiency Factor (EPEF)	376.238	297.858	351.716	356.527	375.736	350.795	359.853	354.215	361.371	344.309	356.837
Significance (P<0.05)	a	c	b	b	a	b	ab	b	ab	b	ab

Days 0-21	T12	T13	T14	T15
Average Body Wt. (g)	834.7	856.7	833.6	856.7
Significance (P<0.05)	c	abc	c	abc
Feed Conversion Corrected	1.386	1.363	1.370	1.351
Significance (P<0.05)	cd	abcd	bcd	abcd
Mortality %	1.44	1.44	1.92	1.92
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	795.5	817.2	793.7	817.4
Significance (P<0.05)	c	abc	c	abc
Body Weight Coefficient of Variation (period)	16.18	15.70	15.58	15.12
Significance (P<0.05)	d	d	cd	cd
Feed Consumption (g per bird per day)	52.61	53.35	52.13	52.81
Significance (P<0.05)	ab	a	ab	a
Average g gained/day for each period	39.75	40.80	39.70	40.79
Significance (P<0.05)	c	abc	c	abc
European Production Efficiency Factor (EPEF)	282.48	293.30	282.46	294.50
Significance (P<0.05)	e	bcde	e	bcde
Days 0-35				
Average Body Wt. (g)	2010.6	2039.2	2011.5	2054.3
Significance (P<0.05)	d	bcd	d	abc
Feed Conversion Corrected	1.545	1.520	1.558	1.529
Significance (P<0.05)	abc	ab	abc	ab
Mortality %	1.563	2.083	2.083	2.083
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	1971.4	1999.6	1971.5	2015.1
Significance (P<0.05)	c	bc	c	abc
Body Weight Coefficient of Variation (period)	16.624	16.051	16.927	15.802
Significance (P<0.05)	de	cde	de	cde
Feed Consumption (g per bird per day)	90.154	90.384	91.080	91.263
Significance (P<0.05)	b	ab	ab	ab
Average g gained/day for each period	57.447	58.262	57.471	58.695
Significance (P<0.05)	c	bc	c	abc
European Production Efficiency Factor (EPEF)	352.933	359.862	347.315	361.853
Significance (P<0.05)	b	ab	b	ab

FIG. 7

Days 0-42	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Average Body Wt. (g)	2855.6	2546.1	2825.9	2852.9	2880.9	2801.6	2857.0	2780.6	2840.4	2798.4	2837.5
Significance (P<0.05)	ab	c	ab	ab	a	ab	ab	b	ab	ab	ab
Feed Conversion Corrected	1.786	1.893	1.840	1.817	1.794	1.841	1.823	1.845	1.825	1.857	1.830
Significance (P<0.05)	a	c	bc	abc	ab	abc	abc	abc	abc	bc	abc
Mortality %	0.947	7.197	1.705	2.083	2.083	2.604	2.604	1.042	1.563	2.604	0.521
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average Body Wt. Gain (g)	2816.6	2506.5	2786.4	2823.3	2841.5	2761.8	2817.6	2740.9	2801.0	2759.2	2797.9
Significance (P<0.05)	ab	c	ab	ab	a	ab	ab	b	ab	ab	ab
Body Weight Coefficient of Variation (period)	14.250	15.879	14.501	13.644	8.202	15.048	15.539	16.321	16.138	16.173	15.573
Significance (P<0.05)	bc	d	bc	b	a	cd	cd	d	d	d	cd
Feed Consumption (g per bird per day)	123.060	118.076	125.433	125.677	124.964	124.913	125.816	123.433	124.988	125.568	124.926
Significance (P<0.05)	a	b	a	a	a	a	a	ab	a	a	a
Average g gained/day for each period	67.996	60.621	67.284	68.163	68.593	66.705	68.023	66.206	67.629	66.629	67.560
Significance (P<0.05)	ab	c	ab	ab	a	ab	ab	b	ab	ab	ab
European Production Efficiency Factor (EPEF)	366.959	283.441	349.534	356.968	363.228	341.496	352.572	346.048	354.600	339.052	357.939
Significance (P<0.05)	a	d	bc	abc	ab	bc	abc	bc	abc	bc	abc
Days 15-22											
Average Body Wt. (g)	399.366	368.833	391.761	403.446	403.528	381.765	395.693	383.508	390.850	379.482	379.094
Significance (P<0.05)	a	b	a	a	a	ab	a	ab	a	ab	ab
Feed Conversion Corrected	1.470	1.452	1.477	1.505	1.463	1.601	1.500	1.509	1.474	1.549	1.600
Significance (P<0.05)	cd	d	bc	abcd	d	a	abcd	abcd	cd	abcd	a
Mortality %	0.350	1.923	0.350	0.524	0.350	0.962	0.481	0.000	0.481	0.481	0.000
Significance (P<0.05)	a	b	a	a	a	ab	a	a	a	a	a
Average Body Wt. Gain (g)	399.366	368.833	391.761	403.446	403.528	381.765	395.693	383.508	390.850	379.482	389.596
Significance (P<0.05)	a	b	a	a	a	ab	a	ab	a	ab	ab
Feed Consumption (g per bird per day)	83.964	75.970	84.689	87.066	84.620	86.432	84.672	83.639	83.256	84.245	87.574
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average g gained/day for each period	57.052	52.690	55.966	57.635	57.647	54.538	56.528	54.787	55.836	55.836	55.836
Significance (P<0.05)	a	b	a	a	a	ab	a	ab	a	ab	ab

FIG. 8

Days 0-42	T12	T13	T14	T15
Average Body Wt. (g)	2798.3	2837.7	2797.7	2837.1
Significance (P<0.05)	ab	ab	ab	ab
Feed Conversion Corrected	1.859	1.830	1.865	1.839
Significance (P<0.05)	bc	abc	bc	abc
Mortality %	1.563	2.083	2.604	2.083
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	2759.1	2798.1	2757.8	2797.8
Significance (P<0.05)	ab	ab	ab	ab
Body Weight Coefficient of Variation (period)	16.318	15.358	16.225	16.264
Significance (P<0.05)	d	cd	d	d
Feed Consumption (g per bird per day)	125.247	125.415	126.267	125.778
Significance (P<0.05)	a	a	a	a
Average g gained/day for each period	66.627	67.564	66.612	67.549
Significance (P<0.05)	ab	ab	ab	ab
European Production Efficiency Factor (EPEF)	343.527	351.016	337.467	349.950
Significance (P<0.05)	bc	abc	c	abc
Days 15-22				
Average Body Wt. (g)	379.681	390.080	378.643	390.049
Significance (P<0.05)	ab	a	ab	a
Feed Conversion Corrected	1.572	1.586	1.541	1.506
Significance (P<0.05)	abc	ab	abcd	abcd
Mortality %	0.000	0.481	0.481	0.481
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	379.681	390.080	378.643	390.049
Significance (P<0.05)	ab	a	ab	a
Feed Consumption (g per bird per day)	85.344	87.570	83.554	85.600
Significance (P<0.05)	a	a	a	a
Average g gained/day for each period	54.240	55.726	54.092	55.721
Significance (P<0.05)	ab	a	ab	a

FIG. 9

FIG. 10

Days 22-35	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
Average Body Wt. (g)	1250.0	1124.8	1192.4	1188.2	1224.9	1201.3	1199.3	1173.1	1188.6	1176.3	1197.2
Significance (P<0.05)	a	c	b	b	ab	ab	ab	bc	ab	b	ab
Feed Conversion Corrected	1.684	1.786	1.737	1.711	1.650	1.702	1.664	1.665	1.681	1.694	1.660
Significance (P<0.05)	bc	a	ab	abc	bc	bc	bc	bc	bc	bc	bc
Mortality %	0.233	1.573	0.291	0.495	0.481	0.160	0.681	0.080	0.120	0.681	0.040
Significance (P<0.05)	a	b	a	a	a	a	a	a	a	a	a
Average Body Wt. Gain (g)	1250.0	1124.8	1192.4	1188.2	1224.9	1201.3	1199.3	1173.1	1188.6	1176.3	1186.7
Significance (P<0.05)	a	c	b	b	ab	ab	ab	bc	ab	b	ab
Feed Consumption (g per bird per day)	150.476	144.368	148.008	145.319	144.079	146.806	143.519	142.097	142.274	142.900	142.855
Significance (P<0.05)	a	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab
Average g gained/day for each period	89.283	80.341	85.170	84.870	87.490	85.806	85.664	83.795	84.898	84.018	85.517
Significance (P<0.05)	a	c	b	b	ab	ab	ab	bc	ab	b	ab
Days 36-42											
Average Body Wt. (g)	745.5	634.4	784.4	808.4	786.0	757.0	796.7	767.0	799.1	787.5	798.7
Significance (P<0.05)	b	c	ab	a	ab	ab	ab	ab	ab	ab	ab
Feed Conversion Corrected	2.519	2.818	2.544	2.514	2.583	2.834	2.608	2.630	2.600	2.605	2.527
Significance (P<0.05)	a	b	a	a	a	ab	ab	ab	ab	ab	a
Mortality %	0.189	0.379	0.189	0.189	0.379	0.521	0.000	0.000	0.000	0.000	0.000
Significance (P<0.05)	a	a	a	a	a	a	a	a	a	a	a
Average Body Wt. Gain (g)	745.5	634.4	784.4	808.4	786.0	757.0	796.7	767.0	799.1	787.5	798.7
Significance (P<0.05)	b	c	ab	a	ab	ab	ab	ab	ab	ab	ab
Feed Consumption (g per bird per day)	267.266	251.012	284.894	288.772	286.861	280.327	296.783	287.523	296.439	296.211	291.154
Significance (P<0.05)	ab	b	a	a	a	ab	a	a	a	a	a
Average g gained/day for each period	106.499	90.628	112.056	115.482	112.284	108.142	113.820	109.571	114.158	112.495	114.109
Significance (P<0.05)	b	c	ab	a	ab	ab	ab	ab	ab	ab	ab
Lesion Scores											
Lesion Scores Day 21	0.205	1.795	0.373	0.500	0.364	0.250	0.563	0.188	0.500	0.188	0.313
Significance (P<0.05)	ab	c	ab	ab	ab	ab	ab	ab	ab	ab	ab
Lesion Scores Day 42	0.318	1.709	0.418	0.464	0.427	0.350	0.350	0.425	0.500	0.500	0.500
Significance (P<0.05)	a	c	ab	ab	ab	ab	ab	ab	ab	ab	a

Days 22-35	T12	T13	T14	T15
Average Body Wt. (g)	1175.9	1182.4	1177.9	1197.7
Significance (P<0.05)	b	b	b	ab
Feed Conversion Corrected	1.662	1.646	1.695	1.644
Significance (P<0.05)	bc	c	bc	c
Mortality %	0.120	0.641	0.160	0.160
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	1175.9	1182.4	1177.9	1197.7
Significance (P<0.05)	b	b	b	ab
Feed Consumption (g per bird per day)	139.776	138.684	142.846	142.192
Significance (P<0.05)	b	b	ab	ab
Average g gained/day for each period	83.992	84.460	84.135	85.548
Significance (P<0.05)	b	b	b	ab
Days 36-42				
Average Body Wt. (g)	787.7	798.5	786.2	782.7
Significance (P<0.05)	ab	ab	ab	ab
Feed Conversion Corrected	2.676	2.639	2.697	2.673
Significance (P<0.05)	ab	ab	ab	ab
Mortality %	0.000	0.000	0.521	0.000
Significance (P<0.05)	a	a	a	a
Average Body Wt. Gain (g)	787.7	798.5	786.2	782.7
Significance (P<0.05)	ab	ab	ab	ab
Feed Consumption (g per bird per day)	300.715	300.570	299.718	298.349
Significance (P<0.05)	a	a	a	a
Average g gained/day for each period	112.527	114.073	112.316	111.820
Significance (P<0.05)	ab	ab	ab	ab
Lesion Scores				
Lesion Scores Day 21	0.688	0.563	0.250	0.125
Significance (P<0.05)	b	ab	ab	a
Lesion Scores Day 42	0.475	0.550	0.475	0.575
Significance (P<0.05)	ab	b	ab	b

FIG. 11

FIG. 12

Intestinal Bacteria Data	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
E. coli (log10) Day 21	4.919	7.029	5.957	5.532	4.825	6.058	5.791	6.111	5.890	6.146	5.503
Significance (P<0.05)	a	f	de	c	a	de	cd	de	cd	de	bc
E. coli (log10) Day 42	5.147	6.773	5.745	5.436	5.108	6.270	6.008	6.149	5.724	6.518	5.744
Significance (P<0.05)	a	f	c	d	a	ef	cde	def	bc	f	bc
APC (log10) Day 21	6.004	6.365	5.950	6.213	5.988	5.934	5.972	5.900	5.906	6.094	6.094
Significance (P<0.05)	a	b	a	ab	a	a	a	a	a	ab	ab
APC (log10) Day 42	6.440	6.230	6.063	6.075	6.322	6.071	5.777	6.051	5.955	5.952	6.136
Significance (P<0.05)	e	bcde	abc	abcd	cde	abcde	a	abcd	abc	abc	abcde
Salmonella Incidence (%) Day 21	6.818	72.727	29.545	36.364	20.455	56.250	43.750	43.750	31.250	37.500	18.750
Significance (P<0.05)	a	e	abcd	bcd	ab	de	bcd	bcd	abcd	bcd	abc
Salmonella Incidence (%) Day 42	3.636	83.636	47.273	41.818	39.091	52.500	35.000	50.000	42.500	70.000	42.500
Significance (P<0.05)	a	e	b	b	b	bcd	b	bc	b	cde	b
Clostridium perfringens (log10) Day 21	4.640	6.758	5.542	5.776	5.101	5.853	5.133	5.672	5.811	5.655	4.955
Significance (P<0.05)	a	d	c	c	b	c	b	c	c	c	ab
Clostridium perfringens (log10) Day 42	4.749	6.738	5.732	5.787	5.125	5.683	5.842	5.685	5.811	5.802	5.852
Significance (P<0.05)	a	d	c	c	b	c	c	c	c	c	c
Processing Data											
Carcass Yield (NOT chilled) Day 42	71.569	67.652	68.394	70.325	71.604	70.361	71.922	70.244	71.190	70.958	72.131
Significance (P<0.05)	bc	a	ab	bc	bc	bc	bc	bc	bc	bc	bc
Carcass Yield (CHILLED) Day 42	72.943	69.048	69.781	71.742	73.004	71.767	73.400	71.608	72.585	72.306	73.540
Significance (P<0.05)	cd	a	ab	bc	cd	bcd	cd	bc	cd	cd	cd
Breast Yield (% live weight) Day 42	16.482	14.494	15.476	15.874	16.524	15.584	16.514	15.473	16.482	15.550	16.617
Significance (P<0.05)	d	a	b	c	d	b	d	b	d	b	d
Breast Yield (% pre-chill weight) Day 42	23.194	21.597	22.803	22.738	23.262	22.271	23.149	22.186	23.345	22.110	23.208
Significance (P<0.05)	ef	a	cdef	cdef	f	abcd	def	abcd	f	abc	ef
Breast Yield (% post-chill weight) Day 42	22.752	21.153	22.344	22.283	22.809	21.833	22.677	21.760	22.890	21.692	22.755
Significance (P<0.05)	e	a	bcde	bcde	e	abcd	cde	abc	e	abc	de

NOTE: "Significance P<0.05)" refers to means within a row without a common superscript are significantly different (P<0.05) as determinedby Least Significant Difference.

Intestinal Bacteria Data	T12	T13	T14	T15
E. coli (log10) Day 21	6.313	5.866	6.502	5.083
Significance (P<0.05)	de	cd	e	ab
E. coli (log10) Day 42	6.229	5.826	6.399	5.919
Significance (P<0.05)	ef	cd	f	cde
APC (log10) Day 21	5.849	5.942	6.296	6.058
Significance (P<0.05)	a	a	ab	ab
APC (log10) Day 42	6.386	6.029	5.882	6.112
Significance (P<0.05)	de	abcd	ab	abcde
Salmonella Incidence (%) Day 21	56.250	6.250	50.000	25.000
Significance (P<0.05)	de	a	cde	abc
Salmonella Incidence (%) Day 42	72.500	40.000	42.500	32.500
Significance (P<0.05)	de	b	b	b
Clostridium perfringens (log10) Day 21	5.708	4.984	5.678	5.037
Significance (P<0.05)	c	ab	c	ab
Clostridium perfringens (log10) Day 42	5.751	5.070	5.700	5.110
Significance (P<0.05)	c	b	c	b
Processing Data				
Carcass Yield (NOT chilled) Day 42	71.432	72.976	69.482	70.963
Significance (P<0.05)	bc	c	abc	bc
Carcass Yield (CHILLED) Day 42	72.822	74.427	70.914	72.317
Significance (P<0.05)	cd	d	abc	cd
Breast Yield (% live weight) Day 42	15.621	16.479	15.502	16.465
Significance (P<0.05)	b	d	b	d
Breast Yield (% pre-chill weight) Day 42	22.027	22.707	22.519	23.356
Significance (P<0.05)	ab	bcdef	bcde	f
Breast Yield (% post-chill weight) Day 42	21.601	22.260	22.056	22.916
Significance (P<0.05)	ab	bcde	bcde	e

NOTE: "Significance P<0.05)" refers to means within a row without a common superscript are significantly different (P<0.05) as determinedby Least Significant Difference.

FIG. 13

FIG. 14

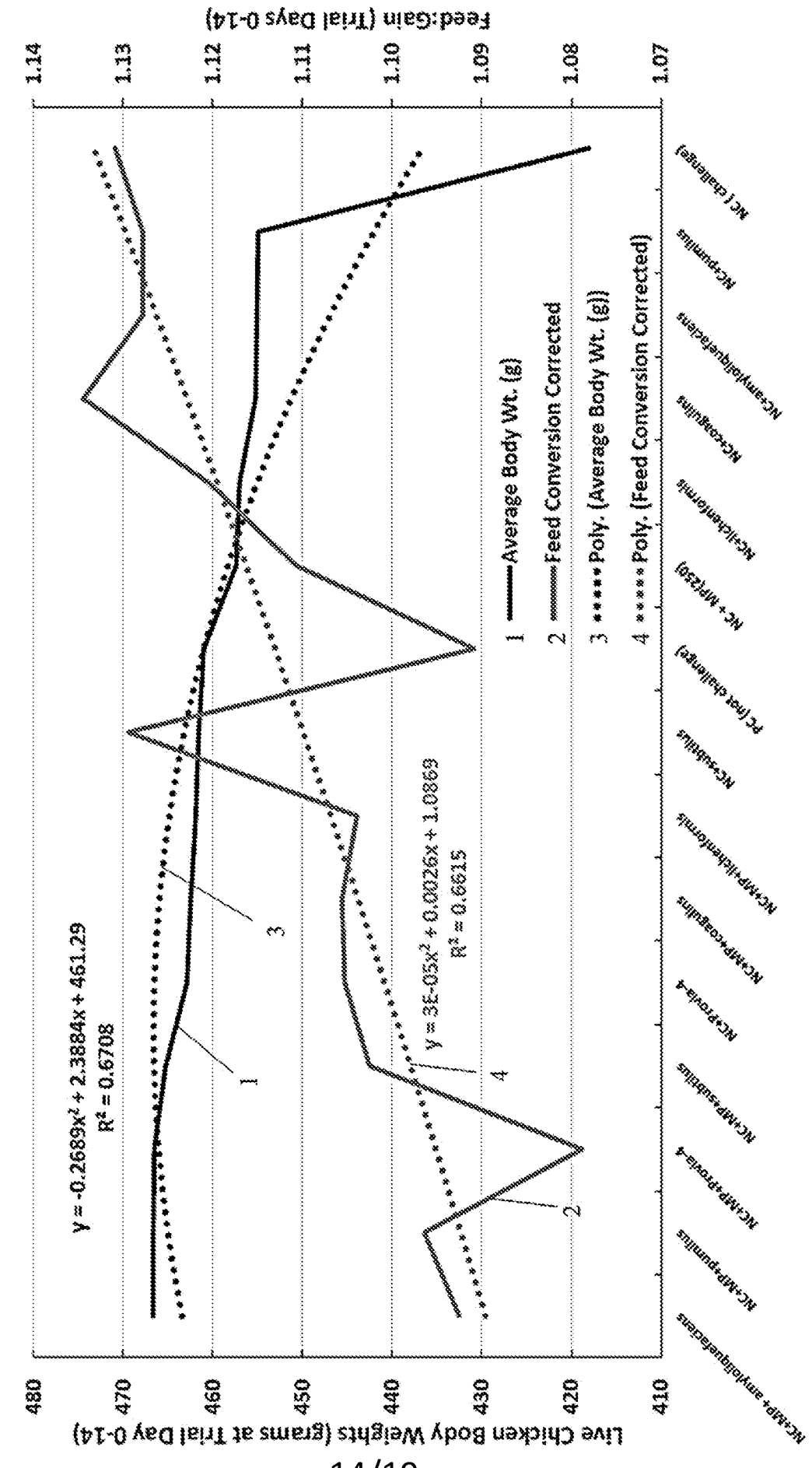

Live Chicken Body Weights (grams at Trial Day 0-14)
Feed:Gain (Trial Days 0-14)
480
470
460
450
440
430
420
410
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
y = -0.2689x² + 2.3884x + 461.29
R² = 0.6708
y = 3E-05x² + 0.0026x + 1.0869
R² = 0.6615
1 Average Body Wt. (g)
2 Feed Conversion Corrected
3 Poly. (Average Body Wt. (g))
4 Poly. (Feed Conversion Corrected)
NC+BP+ amylolliquefaciens
NC+BP+pumilus
NC+MP+Provia-4
NC+MP+subtilus
NC+Provia-4
NC+MP+coagulins
NC+MP+ilikenformis
NC+subtilus
PC (not challenge)
NC+ MP(250)
NC+ilikenformis
NC+coagulins
NC+amyloliquefaciens
NC+pumilus
NC (challenge)

FIG. 15

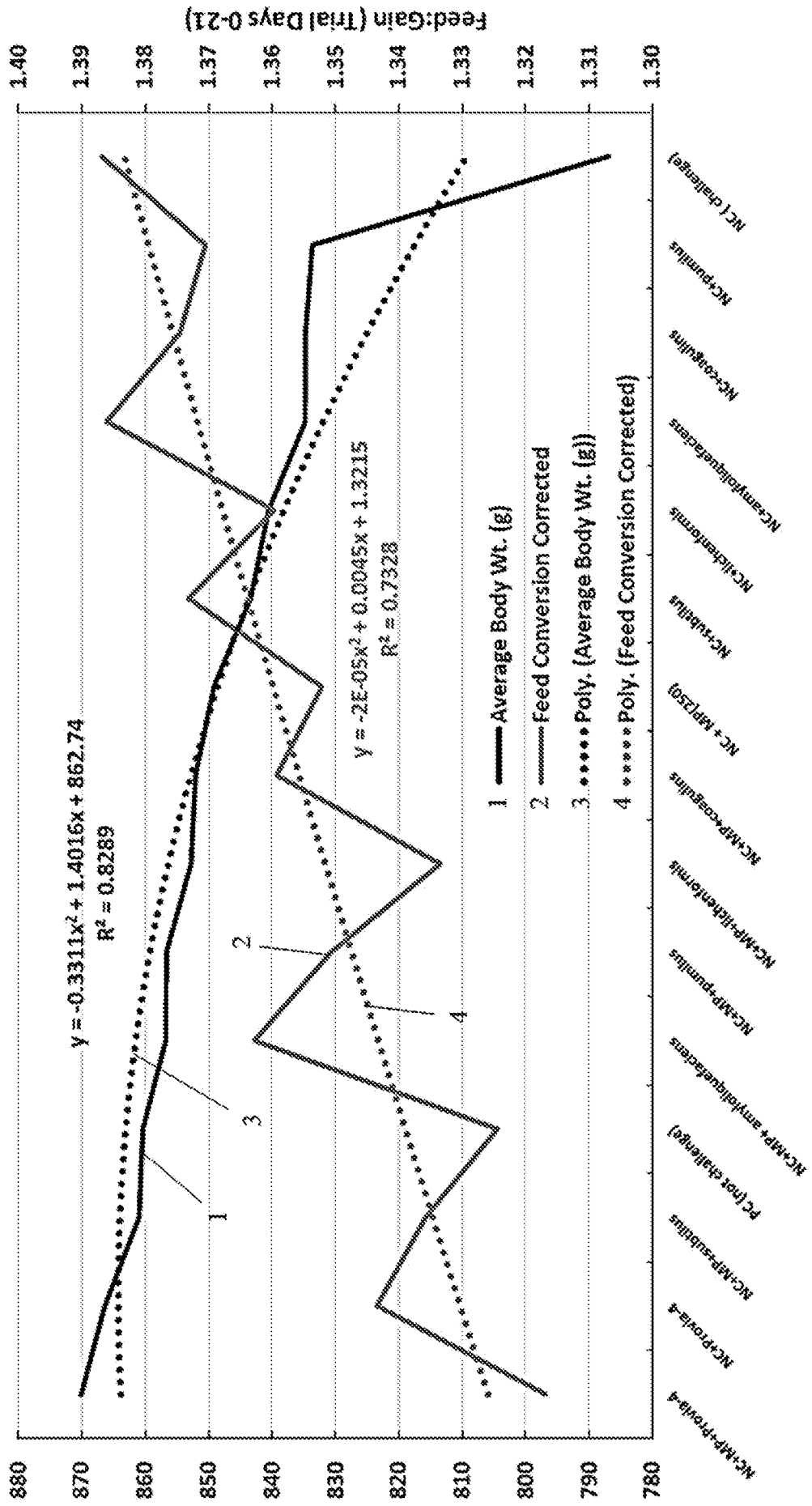

Live Chicken Body Weights (grams at Trial Day 0-21)
880
870
860
850
840
830
820
810
800
790
780
Feed:Gain (Trial Days 0-21)
1.40
1.39
1.38
1.37
1.36
1.35
1.34
1.33
1.32
1.31
1.30
y = -0.3311x² + 1.4016x + 862.74
R² = 0.8289
y = -2E-05x² + 0.0045x + 1.3215
R² = 0.7328
1 Average Body Wt. (g)
2 Feed Conversion Corrected
3 Poly. (Average Body Wt. (g))
4 Poly. (Feed Conversion Corrected)
NC+MP+Provia-4
NC+Provia-4
NC+MP+subtilus
PC (not challenge)
NC+MP+anerobiquefaciens
NC+MP+pumilus
NC+MP+lichenformis
NC+MP+coagulins
NC+ MP(250)
NC+subtilus
NC+lichenformis
NC+anerobiquefaciens
NC+coagulins
NC+pumilus
NC (challenge)

FIG. 16

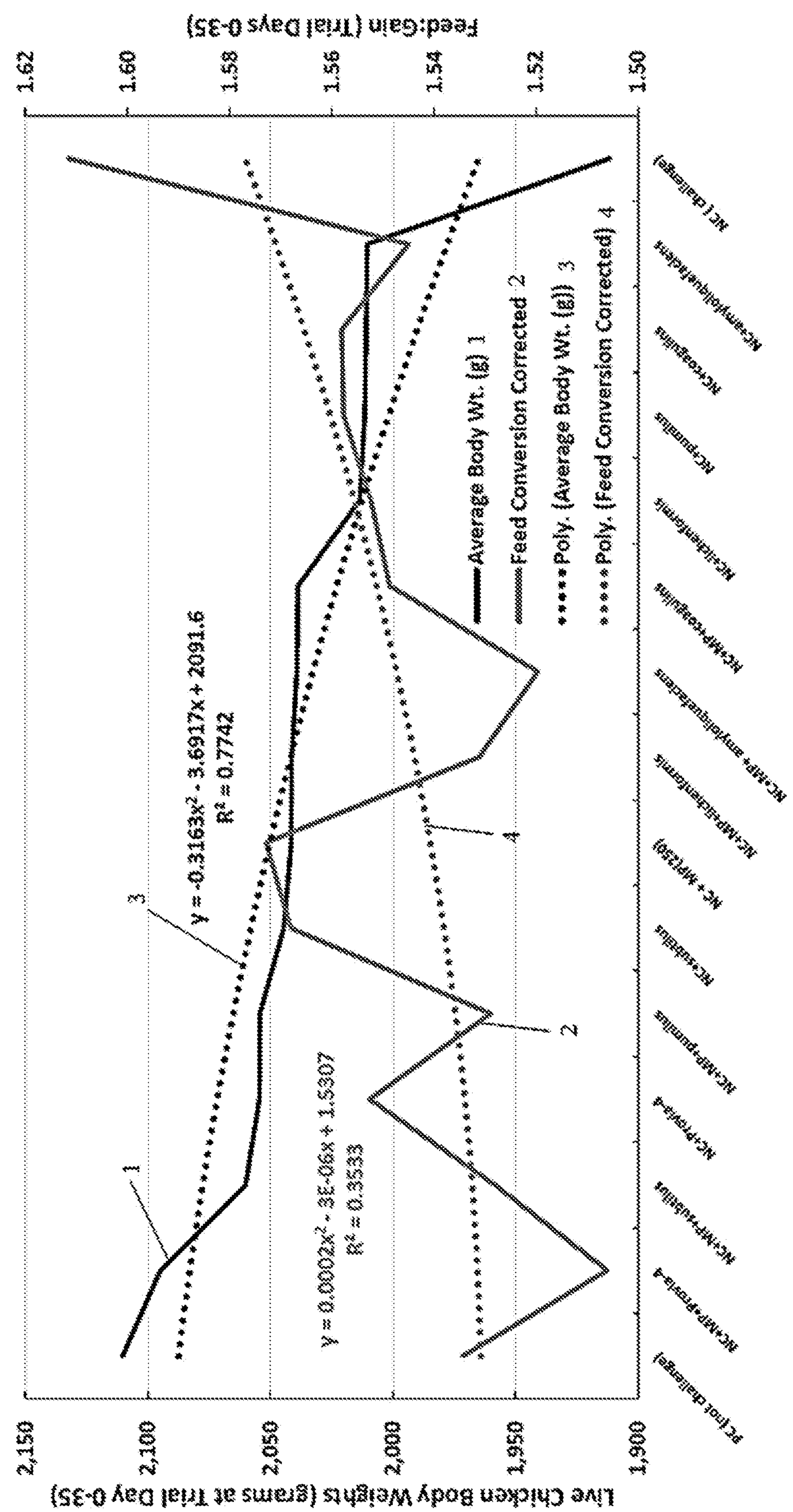
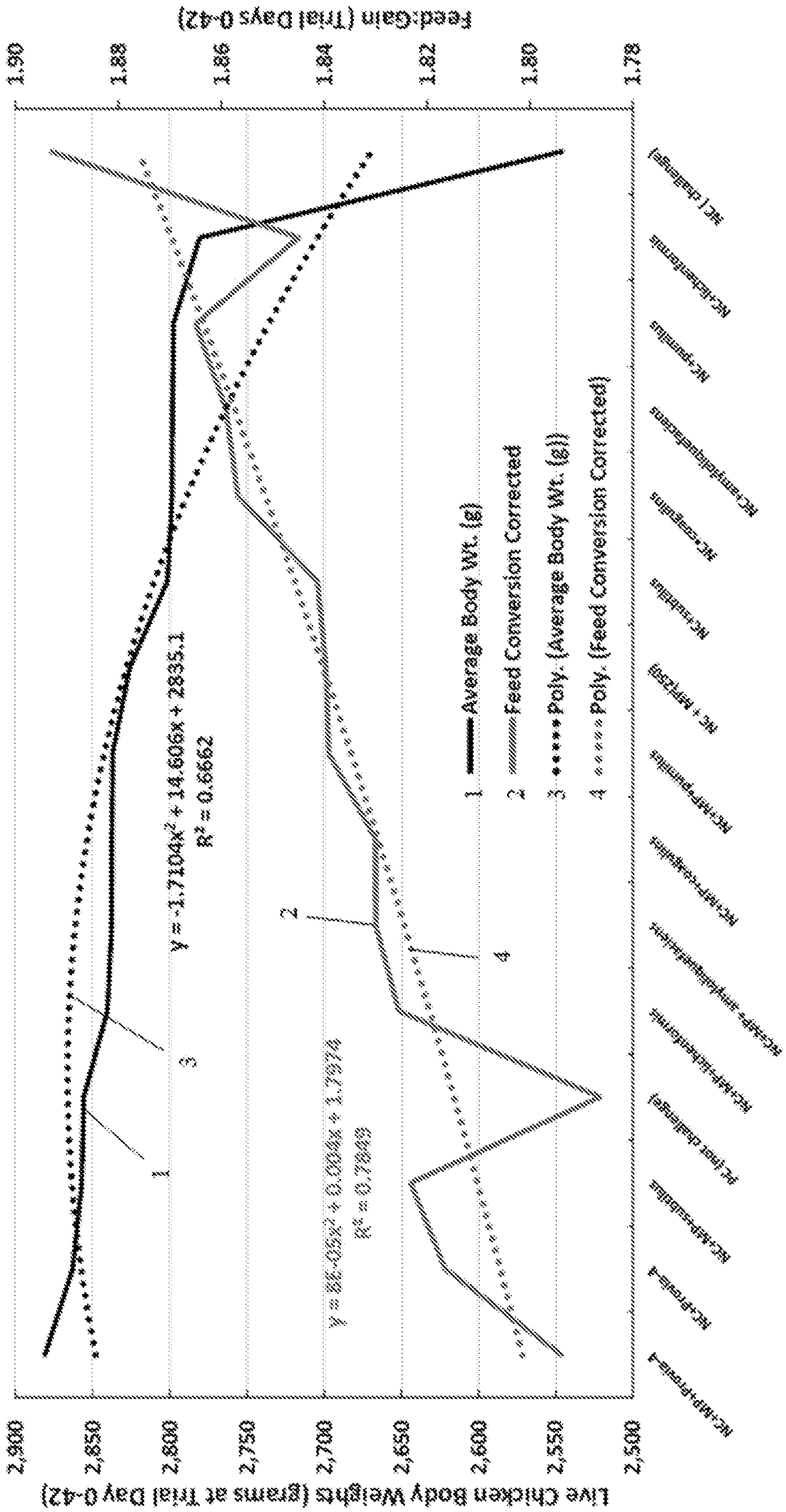


FIG. 17


Feed:Gain (Trial Days 0-42)
1.90
1.88
1.86
1.84
1.82
1.80
1.78
Live Chicken Body Weights (grams at Trial Day 0-42)
2,900
2,850
2,800
2,750
2,700
2,650
2,600
2,550
2,500
y = -1.7104x² + 14.606x + 2835.1
R² = 0.6662
y = 8E-05x² + 0.004x + 1.7974
R² = 0.7849
1 Average Body Wt. (g)
2 Feed Conversion Corrected
3 Poly. (Average Body Wt. (g))
4 Poly. (Feed Conversion Corrected)

	Pathogens	Salmonella Heidelberg	E.Coli O157	Enterococcus cecorum	Clostridium perfringens
	Original Pathogen levels	8.5	12	9.1	0.79
Bacillus coagulans	Provia 6086	9	16	4.6	0.66
	Pathogen Growth	6%	33%	-49%	-16%
Bacillus licheniformis	Osprey	5.1	14	9.5	0.7
	Pathogen Growth	-40%	17%	4%	-11%
Bacillus coagulans	Unique Biotech	10	9.9	12	0.45
	Pathogen Growth	18%	-18%	32%	-43%
Bacillus subtilis	Calsporin	4.9	4.1	5.4	0.27
	Pathogen Growth	-42%	-66%	-41%	-66%
Bacillus subtilis	Galipro	3.7	4.5	1.4	0.19
	Pathogen Growth	-56%	-63%	-85%	-76%
Bacillus licheniformis	Vland BL	2.5	3.6	1.5	0.87
	Pathogen Growth	-71%	-70%	-84%	10%
Bacillus subtilis	Vland BS	3.6	2.3	2.2	0.92
	Pathogen Growth	-58%	-81%	-76%	16%
Lactobacillus animalis 51	NPC LA 51	4.2	4.5	3.7	0.71
	Pathogen Growth	-51%	-63%	-59%	-10%

FIG. 18

	Pathogens	Salmonella Heidelberg	E.Coli O157	Enterococcus cecorum	Clostridium perfringens
	Original Pathogen levels	6.1	15	11	1.1
LR-01	Provia 6086	3.4	6.3	5	0.6
	Pathogen Growth	-44%	-58%	-55%	-45%
LR-02	Osprey	5.9	10	7.2	0.66
	Pathogen Growth	-3%	-33%	-35%	-40%
LR-03	Unique Biotech	4	9.7	6.1	0.49
	Pathogen Growth	-34%	-35%	-45%	-55%
LR-05	Galipro	2.2	7.4	3.4	0.41
	Pathogen Growth	-64%	-51%	-69%	-63%
Bacillus licheniformis	Vland BL	3.7	5.9	3.5	0.65
	Pathogen Growth	-39%	-61%	-68%	-41%
Bacillus subtilis	Vland BS	3.5	8.2	4.7	0.22
	Pathogen Growth	-43%	-45%	-57%	-80%
Osprey 1	BC	3.5	6.9	5.4	0.32
	Pathogen Growth	-43%	-54%	-51%	-71%
Osprey 2	BS	3.1	8.5	6.2	0.59
	Pathogen Growth	-49%	-43%	-44%	-46%

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/014938

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K10/00 A23K10/18 A23K50/00 A23K50/70 A23K50/75
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/083196 A1 (DUPONT NUTRITION BIOSCI APS [DK]; DU PONT [US]) 18 May 2017 (2017-05-18)	1-48
Y	* pages 25-27; claims 1-21 *	49-58

X	DATABASE WPI Week 201726 Thomson Scientific, London, GB; AN 2017-18400L XP002792011, & KR 2017 0021002 A (TAE YANG C & L CO LTD) 27 February 2017 (2017-02-27)	1-30
A	abstract	31-58

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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
12 June 2019	24/06/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Georgopoulos, N

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/014938

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2018/148563 A1 (MCLEAN DEREK [US]; CHAPMAN JAMES D [US] ET AL.) 16 August 2018 (2018-08-16) * claims 1-39 * -----	1,5,6, 16-19, 25-27, 44,45, 47,48
Y	US 2016/286833 A1 (YASUDA GENTARO [JP] ET AL) 6 October 2016 (2016-10-06)	49-58
A	* claims 1-27 * -----	1-48

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/014938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017083196 A1	18-05-2017	BR 112018009235 A2	06-11-2018
		CA 3004522 A1	18-05-2017
		CN 108366582 A	03-08-2018
		EP 3373741 A1	19-09-2018
		JP 2019500054 A	10-01-2019
		KR 20180067700 A	20-06-2018
		PH 12018500954 A1	19-11-2018
		WO 2017083196 A1	18-05-2017
KR 20170021002 A	27-02-2017	NONE	
WO 2018148563 A1	16-08-2018	NONE	
US 2016286833 A1	06-10-2016	BR 112017020691 A2	31-07-2018
		CN 107427028 A	01-12-2017
		EP 3281531 A1	14-02-2018
		JP WO2016163388 A1	01-02-2018
		TW 201701765 A	16-01-2017
		US 2016286833 A1	06-10-2016
		WO 2016163388 A1	13-10-2016