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(54) Title: METHODS AND COMPOSITIONS FOR IMPROVING GROWTH AND PERFORMANCE

(57) Abstract: Compositions including a bacterial species or a combination of bacterial species and methods of using those compositions are described. In some embodiments, use of the compositions prevents disease and/or promotes growth in an animal. In some embodiments, the animal is a turkey. In some embodiments, the composition is antibiotic-free.

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METHODS AND COMPOSITIONS FOR IMPROVING GROWTH AND PERFORMANCE

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CONTINUING APPLICATION DATA

This application claims the benefit of U.S. Provisional Application Serial No. 62/572,732, filed October 16, 2017, which is incorporated by reference herein.

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SEQUENCE LISTING

This application contains a Sequence Listing electronically submitted to the United States Patent and Trademark Office via EFS-Web as an ASCII text file entitled "Sequence-Listing-110.05240201_ST25.txt" having a size of 56 kilobytes and created on October 12, 2018. Due to the electronic filing of the Sequence Listing, the electronically submitted Sequence Listing serves as both the paper copy required by 37 CFR § 1.821(c) and the CRF required by § 1.821(e). The information contained in the Sequence Listing is incorporated by reference herein.

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BACKGROUND

Because of concerns related to the use of antibiotics in animal agriculture, antibiotic-free alternatives are needed to prevent disease and promote animal growth. One of the current challenges facing commercial turkey production is declining performance as a result of reducing antimicrobial use.

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SUMMARY OF THE INVENTION

This disclosure describes methods and compositions for improving animal growth and performance. In one embodiment, this disclosure describes compositions including a bacterial species or a combination of bacterial species and methods of using those compositions. In some embodiments, use of those compositions prevents disease and/or promotes growth in an animal. In some embodiments, the animal is a turkey. In some embodiments, the composition is antibiotic-free.

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In one aspect, this disclosure describes a composition that includes at least 4 bacterial species or strains selected from the following bacterial species: *Clostridium bartlettii*; *Lactobacillus acidophilus*; *Lactobacillus aviarius*; *Lactobacillus crispatus*; *Lactobacillus gallinarum*; *Lactobacillus helveticus*; *Lactobacillus ingluviei*; *Lactobacillus johnsonii*; *Lactobacillus reuteri*;

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Lactobacillus salivarius; *Lactobacillus vaginalis*; and *Pediococcus acidolactici*. In some embodiments, at least one of the bacterial species or strains is an avian-sourced strain. In some embodiments, at least one of the bacterial species or strains is a turkey-sourced strain.

In another aspect, this disclosure describes a method that includes administering the composition to an animal. In some embodiments, the animal is a turkey. In some embodiments, the method further comprises administering a prebiotic to the animal.

The words “preferred” and “preferably” refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

The terms “comprises” and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

Unless otherwise specified, “a,” “an,” “the,” and “at least one” are used interchangeably and mean one or more than one.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (for example, 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows a phylogenetic tree of *Lactobacillus johnsonii* isolates. Turkey-source isolates are shown by gray lines, chicken-source isolates are shown by dotted lines, pig-source isolates are shown by dashed lines, and human/rodent-source isolates are shown by dotted/dashed lines. Turkey-source (gray lines) isolates were further subdivided into clades 1-8 based upon their single-nucleotide polymorphism (SNP) and gene content differences. The scale bar in the lower left indicates a scale of 4000 SNPs.

FIG. 2 shows body weights in grams (y-axis) of day-of-hatch turkeys inoculated with five different *L. johnsonii* isolates, compared with a saline (negative control) inoculum. Days are presented in age of turkeys. Error bars depict standard error of means. Letters depict groups based on statistical significance using ANOVA with Tukey's adjustment ($P < 0.05$); "a" is significantly different from "b." For each treatment group, five replicate cages were used with 10 birds per cage ($n=50$ per treatment group).

FIG. 3 shows a phylogenetic tree of *Lactobacillus aviarius* isolates. Turkey-source isolates of *L. aviarius* subsp. *aviarius* are shown by gray lines, and chicken-source isolates of *L. aviarius* subsp. *aviarius* and *L. aviarius* subsp. *araffinosus* are shown by dotted lines.

FIG. 4A and FIG. 4B show average body weights in grams (y-axis) for day-of-hatch turkeys treated with a 4-strain probiotic blend, a 10-strain (derived from birds aged 3 weeks) probiotic blend, and a 10-strain (derived from birds aged 6 weeks) probiotic blend, as described in Example 2, compared to body weights for day-of-hatch turkeys treated with a saline negative control and day-of-hatch turkeys treated with an existing commercial probiotic derived from chickens (FM-B11). FIG. 4A. Turkeys were weighed on day 0 (that is, day-of-hatch), day 7, day 10, and day 14. Error bars depict standard error of means. Letters depict groups based on statistical significance

using ANOVA ($P < 0.05$); "a" is significantly different from "b," and "ab" is not significantly different from either "a" or "b". FIG. 4B shows average body weights in grams (y-axis) for day 14 turkeys with an adjusted y-axis. For each treatment group, five replicate cages were used with 10 birds per cage ($n=50$ per treatment group).

5 FIG. 5A and FIG. 5B show average body weights in grams (y-axis) for day-of-hatch turkeys treated with a 4-strain probiotic blend inoculation group, day-of-hatch turkeys treated with an existing commercial probiotic derived from chickens (FM-B11), and day-of-hatch turkeys treated with a low-dose antibiotic (bacitracin methylene disalicylate or BMD), as described in Example 2, compared to day-of-hatch turkeys treated with a saline negative control. FIG. 5A. Turkeys were
10 weighed on day 0 (that is, day-of-hatch), day 3, day 6, and day 13. Error bars depict standard error of means. Letters depict groups based on statistical significance using ANOVA with Tukey's adjustment ($P < 0.05$); "a" is significantly different from "b," and "ab" is not significantly different from either "a" or "b". FIG. 5B shows average body weights in grams (y-axis) for day 13 turkeys with an adjusted y-axis. For each treatment group, eight replicate cages were used with 10 birds per
15 cage ($n=80$ per treatment group).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

This disclosure describes compositions including a bacterial species or a combination of bacterial species and methods of using those compositions. Alternatives to antimicrobials such as
20 antibiotics exist for the broiler chicken industry, but such alternatives are lacking for the commercial turkey industry. Moreover, many alternatives to antimicrobials are aimed at pathogen reduction and not performance. In some embodiments, the compositions disclosed herein prevent disease and/or promote growth in an animal.

In some embodiments, the animal is preferably a bird. In some embodiments, the animal is
25 preferably a turkey.

Compositions

The compositions of the present disclosure include a bacterial species or a combination of bacterial species. In some embodiments, as further described below, a composition includes a
30 combination of specific bacterial species or strains.

In some embodiments, the composition is preferably antibiotic-free.

Bacterial species and/or strains in the composition

In some embodiments, the composition includes at least 4 bacterial species or strains, at least 5 bacterial species or strains, at least 6 bacterial species or strains, at least 7 bacterial species or strains, at least 8 bacterial species or strains, at least 9 bacterial species or strains, at least 10 bacterial species or strains, at least 11 bacterial species or strains, or at least 12 bacterial species or strains selected from the following bacterial species: *Clostridium (Intestinibacter) bartlettii*; *Lactobacillus acidophilus*; *Lactobacillus aviarius*; *Lactobacillus crispatus*; *Lactobacillus gallinarum*; *Lactobacillus helveticus*; *Lactobacillus ingluviei*; *Lactobacillus johnsonii*; *Lactobacillus reuteri*; *Lactobacillus salivarius*; *Lactobacillus vaginalis*; and *Pediococcus acidilactici*.

In some embodiments, at least one of the bacterial species or strains may have been isolated from an avian source (that is, is avian-sourced). In some embodiments, at least 2 bacterial species or strains, at least 3 bacterial species or strains, at least 4 bacterial species or strains, or all of the bacterial species or strains are from an avian source.

In some embodiments, at least one of the bacterial species or strains may have been isolated from a turkey (that is, is turkey-sourced). In some embodiments, at least 2 bacterial species or strains, at least 3 bacterial species or strains, at least 4 bacterial species or strains, or all of the bacterial species or strains are from a turkey.

In some embodiments, the composition includes a strain of *Clostridium bartlettii*. In some embodiments, the strain of *Clostridium bartlettii* includes *Clostridium bartlettii* DSM 16795 (deposited in the NCBI database under GENBANK accession number FUXV000000000.1).

In some embodiments, the composition includes a strain of *Lactobacillus acidophilus*. In some embodiments, a strain of *Lactobacillus acidophilus* includes *Lactobacillus acidophilus* strain UMNPBX11 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYZ000000000).

In some embodiments, the composition includes a strain of *Lactobacillus aviaries*. In some embodiments, a strain of *Lactobacillus aviarius* includes at least one of *Lactobacillus aviarius* subspecies araffinosus and *Lactobacillus aviarius* subspecies aviaries. In some embodiments, a strain of *Lactobacillus acidophilus* includes at least one of *Lactobacillus aviarius* strain UMNLav12 (deposited in the NCBI database under GENBANK accession number NZ_LWUE000000000.1); *Lactobacillus aviarius* strain UMNLav13 deposited in the NCBI database under GENBANK accession number NZ_LWUF000000000.1); *Lactobacillus aviarius* strain UMNLA76 (deposited in

the NCBI database under GENBANK accession number PCZQ000000000); *Lactobacillus aviarius* strain UMNlav97 (deposited in the NCBI database under GENBANK accession number PCZP000000000); *Lactobacillus aviarius* strain UMNlav98 (deposited in the NCBI database under GENBANK accession number PCZO000000000); *Lactobacillus aviarius* strain DSM 20653 (deposited in the NCBI database under GENBANK accession number NZ_AYYZ000000000.1); and *Lactobacillus aviarius* strain DSM 20655 (deposited in the NCBI database under GENBANK accession number NZ_AYZA000000000.1).

In some embodiments, the composition includes a strain of *Lactobacillus crispatus*. In some embodiments, a strain of *Lactobacillus crispatus* includes at least one of *Lactobacillus crispatus* strain UMNPBX1 (deposited in the NCBI database under GENBANK accession number PCZI000000000); *Lactobacillus crispatus* strain UMNPBX8 (deposited in the NCBI database under GENBANK accession number PCZB000000000); *Lactobacillus crispatus* strain UMNPBX12 (deposited in the NCBI database under GENBANK accession number PCYY000000000); *Lactobacillus crispatus* strain UMNPBX15 (deposited in the NCBI database under GENBANK accession number PCYV000000000); and *Lactobacillus crispatus* strain UMNPBX16 (deposited in the NCBI database under GENBANK accession number PCYU000000000).

In some embodiments, the composition includes a strain of *Lactobacillus gallinarum*. In some embodiments, a strain of *Lactobacillus gallinarum* includes at least one of *Lactobacillus gallinarum* strain UMNPBX4 (deposited in the NCBI database under GENBANK accession number PCZF000000000); *Lactobacillus gallinarum* strain UMNPBX14 (deposited in the NCBI database under GENBANK accession number PCYW000000000); and *Lactobacillus gallinarum* strain UMNPBX17 (deposited in NCBI databases under GENBANK accession number PCYT000000000).

In some embodiments, the composition includes a strain of *Lactobacillus helveticus*. In some embodiments, a strain of *Lactobacillus helveticus* includes *Lactobacillus helveticus* strain UMNPBX6 (deposited in the NCBI database under GENBANK accession number PCZD000000000).

In some embodiments, the composition includes *Lactobacillus ingluviei*. In some embodiments, a strain of *Lactobacillus ingluviei* includes *Lactobacillus ingluviei* strain UMNPBX19 (deposited in the NCBI database under GENBANK accession number PCYR000000000).

In some embodiments, the composition includes a strain of *Lactobacillus johnsonii*. In some embodiments, a strain of *Lactobacillus johnsonii* includes at least one of *Lactobacillus johnsonii* strain UMNJ21 (deposited in the NCBI database under GENBANK accession numbers CP021701, CP021702, and CP021703); *Lactobacillus johnsonii* strain UMNJ94 (deposited in the NCBI database under GENBANK accession number PCZK000000000); and *Lactobacillus johnsonii* strain UMNJ113 (deposited in the NCBI database under GENBANK accession number PCZJ000000000).

In some embodiments, the composition includes a strain of *Lactobacillus reuteri*. In some embodiments, a strain of *Lactobacillus reuteri* includes at least one of *Lactobacillus reuteri* strain UMNPBX3 (deposited in the NCBI database under GENBANK accession number PCZG000000000); *Lactobacillus reuteri* strain UMNPBX7 (deposited in the NCBI database under GENBANK accession number PCZC000000000); *Lactobacillus reuteri* strain UMNPBX10 (deposited in the NCBI database under GENBANK accession number PCZL000000000); and *Lactobacillus reuteri* strain UMNPBX18 (deposited in the NCBI database under GENBANK accession number PCYS000000000).

In some embodiments, the composition includes a strain of *Lactobacillus salivarius*. In some embodiments, a strain of *Lactobacillus salivarius* includes at least one of *Lactobacillus salivarius* strain UMNPBX2 (deposited in the NCBI database under GENBANK accession number PCZH000000000); and *Lactobacillus salivarius* strain UMNPBX9 (deposited in the NCBI database under GENBANK accession number PCZA000000000).

In some embodiments, the composition includes a strain of *Lactobacillus vaginalis*. In some embodiments, a strain of *Lactobacillus vaginalis* includes at least one of *Lactobacillus vaginalis* strain UMNPBX5 (deposited in the NCBI database GENBANK accession number PCZE000000000); and *Lactobacillus vaginalis* strain UMNPBX13 (deposited in the NCBI database under GENBANK accession number PCYX000000000).

In some embodiments, the composition includes a strain of *Pediococcus acidolactici*. In some embodiments, a strain of *Pediococcus acidolactici* includes *Pediococcus acidolactici* UMNPBX20 (deposited in the NCBI database under GENBANK accession number PCYQ000000000).

Although the strains described herein are, in some embodiments, described by reference to particular sequences, a person having skill in the art will recognize that a sequence having some genetic variations from a sequence provided for the strain described herein will still describe the

same strain. In some embodiments, a sequence differing by fewer than 20,000 SNPs, fewer than 10,000 SNPs, fewer than 5,000 SNPs, fewer than 1,000 SNPs, fewer than 750 SNPs, fewer than 500 SNPs, fewer than 400 SNPs, fewer than 300 SNPs, fewer than 200 SNPs, or fewer than 100 SNPs from the sequence of a strain described herein is defined as describing the same strain. In some
5 embodiments, a sequence differing by less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, or less than 0.1% from the sequence of a strain described herein is defined as describing the same strain. Percent identity between two sequences (for example, nucleic acid sequences or amino acids sequences) may be determined in any one of numerous ways known to a person having skill in the art including, for example, using publicly available computer software such as the Smith-
10 Waterman algorithm, BESTFIT alignment, BLAST, FASTA, CLUSTALW, etc.

Concentration of the bacteria in the composition

In some embodiments, the composition includes at least 1×10^5 colony-forming units (CFU) of each of the bacterial species or strains, at least 1×10^6 CFU of each of the species or strains, at
15 least 1×10^7 CFU of each of the species or strains, or at least 1×10^8 CFU of each of the species or strains. In some embodiments, the composition includes up to 1×10^6 CFU of each of the species or strains, up to 1×10^7 CFU each of the species or strains, up to 1×10^8 CFU each of the species or strains, up to 1×10^9 CFU each of the species or strains, up to 1×10^{10} CFU each of the species or strains, or up to 1×10^{11} CFU each of the species or strains. For example, in some embodiments, a
20 composition may include at least 1×10^7 CFU of each of the species or strains and up to 1×10^9 CFU of each of the species or strains. In some embodiments, a composition may include 1×10^8 CFU of each of the species or strains.

In some embodiments, a viability count may be used to determine the number of CFU of the composition.

Methods of Using the Compositions

The compositions described herein may be administered to an animal using any suitable method. In some embodiments, the composition may be administered as a feed additive. In some
30 embodiments, the composition may be administered by oral gavage. In some embodiments, the composition may be administered in water and/or in a nutrient gel. In some embodiments, the composition may be micro-encapsulated.

In some embodiments, the composition may be administered multiple times. For example, the composition may be administered at least twice, at least three times, or at least four times. In some embodiments, the composition may be administered up to four times, up to five times, up to 10 times, up to 15 times, or up to 18 times. In some embodiments, the composition may be administered on multiple days. In some embodiments the composition may be administered weekly. In some embodiments, the composition may be administered daily and/or continuously, for example, in feed. In some embodiments, the administration of the composition may be timed to coincide with an event in the animal's development or treatment. For example, the composition may be administered in combination with one or more of the following events: before and/or after vaccination, before and/or after a move of the animal; before and/or after a diet change, before and/or after diagnosis with a disease, and before and/or after treatment for a disease.

In some embodiments, the composition may be administered to an animal that is less than a week old. For example, in some embodiments, the composition may be administered to a day-of-hatch bird. In some embodiments, the animal may be at least one week old, at least two weeks old, at least three weeks old, or at least four weeks old.

In some embodiments, the composition may be administered to an animal in combination with a prebiotic. The prebiotic may be administered by any suitable method. In some embodiments, a prebiotic may be administered at the same time – including, for example, in the composition that includes a combination of bacterial species or strains – or at a different time or different times from the composition. In some embodiments, a prebiotic may be administered continuously to the animal. In some embodiments, as described, for example, in Example 3, the prebiotic may be included in the animal's feed. The prebiotic may include any suitable prebiotic including, for example, one or more of a disaccharide, an oligosaccharide, and a polysaccharide. For example, a prebiotic may include one or more of lactose, lactulose, mannan-oligosaccharide (MOS), inulin, soy Fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and a β -glucan. In some embodiments, the prebiotic may preferably include lactose. In some embodiments, the prebiotic may preferably include a mannan-oligosaccharide, and/or a β -glucan. In some embodiments, the prebiotic may include SAFMANNAN (available from Phileo Lesaffre Animal Care, Marcq-En-Baroeul Cedex, France).

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLES

Example 1

Approximately 100 different hybrid turkeys ranging in age from 0-12 weeks were used to collect bacterial strains. Upon humane euthanization, the ileum was aseptically removed from each turkey and contents from each ileum section were homogenized using a stomacher. Ten-fold serial dilutions of each homogenized sample were performed in phosphate-buffered saline. Samples were then plated onto three types of media: 1) *Lactobacillus* selection (LBS) agar (BD Diagnostics Systems, Hunt Valley, MD), 2) Lactobacilli MRS agar (BD Diagnostic Systems, Hunt Valley, MD), and 3) Trypticase Soy Agar with 5% sheep blood (BD Diagnostic Systems, Hunt Valley, MD). Plates were incubated both aerobically and anaerobically overnight at 37°C. Isolated colonies were then selected from each plate and stored for further use in 20 percent weight-volume (% w:v) glycerol. In total, 1,267 isolates were obtained.

Each isolate was identified at the bacterial species level using sequencing of the full length bacterial 16S rRNA, as previously described (Lane DJ, 1991, 16S/23S rRNA sequencing. In: E. Stackebrandt, M. Goodfellow. Nucleic acid techniques in bacterial systematics. New York, N.Y., John Wiley & Sons, Inc., pp. 115-176.). Following species-level identification of these isolates, representative isolates from each bacterial species were subjected to whole genome sequencing using Illumina MiSeq at a depth of at least 50x coverage (Illumina, Inc., San Diego, CA).

For *L. johnsonii* and *L. aviarius*, each isolate was mapped to a reference genome matching its respective bacterial species using CLC Genomics Workbench (Qiagen, N.V., Hilden, Germany). Following mapping, single nucleotide polymorphisms (SNPs) were identified using variant calling in CLC Genomics Workbench. SNPs were then combined for each bacterial species of interest, and subsequently analyzed. Using Maximum Parsimony methods (MEGA, available at on the world wide web at megasoftware.net, version 6.06), a phylogenetic tree was constructed to group isolates based upon genetic similarity and to establish clades within each bacterial species. Finally, a pangenome analysis was performed for every isolate within each clade of a species, to establish core and unique genes belonging to each subset; an exemplary process is described in Example 1A.

For other bacterial species, strains were selected based upon their dominance in high-performing turkey flocks and upon the isolation of the strains from commercial turkeys of certain age groups.

Example 1A – Selection of *Lactobacillus johnsonii* Strain UMNJ21

One hundred seventeen *L. johnsonii* isolates were analyzed as described above. SNPs were used for phylogenetic analysis including all *L. johnsonii* isolates in the NCBI database (February 2017). Using Maximum Parsimony methods (MEGA, available at on the world wide web at megasoftware.net, version 6.06) a phylogenetic tree was constructed using the following non-turkey species sequences: chicken strain FI9785, GENBANK accession NC_013504; human strain NCC 533, GENBANK accession NC_005362; pig strain BS15, GENBANK accession NZ_CP016400; pig strain DPC 6026, GENBANK accession NC_017477; human strain N6.2, GENBANK accession NC_022909. Isolates were separated into clades based upon their host source of isolation (human, rodent, pig, chicken, or turkey). Furthermore, eight distinct clades were identified among the 117 turkey-source isolates (Figure 1). These clades were used for further study.

Strains were selected from each major clade and tested for their ability to enhance turkey growth. In this experiment, day-of-hatch turkey poults (n=360 total) in a caged trial with 6 replicate cages per treatment group (n=60 per treatment) were inoculated via oral gavage with either a negative saline control (negative control) or 1×10^9 colony forming units (CFU) of *L. johnsonii* belonging to one of five clades (Clades 1, 2, 4, 7, and 8, see Figure 1). Birds were weighed at four time points. Only the Clade 1 isolate was able to significantly enhance body weight in this experiment ($P < 0.01$). Results are shown in Figure 2 and Table 1. These results indicated that Clade 1 isolates have the propensity to enhance performance in commercial turkeys.

Table 1. Average body weights for turkey poults administered single strain *L. johnsonii* combinations. SEM = standard error of means.

	Day 0 (g)	Day0 SEM	Day 3 (g)	Day3 SEM	Day 7 (g)	Day7 SEM	Day 14 (g)	Day14 SEM
Negative control	59.2	1.3	81.3	2.5	141.1	2.3	326.5	10.9
Clade 1	57.7	1.3	84.8	2.5	148.1	2.3	366.5	10.9
Clade 2	59.3	1.3	84.4	2.5	144.4	2.3	334.7	10.9
Clade 4	60.1	1.3	84.6	2.5	143.2	2.3	324	10.9
Clade 7	57.2	1.3	82	2.5	143.3	2.3	333.5	10.9
Clade 8	56.7	1.3	81.5	2.5	135.5	2.3	318.6	10.9

Pangenome comparisons identified genes that were unique to Clade 1 isolates. A total of 49 genes were identified that were conserved across Clade 1 isolates, but unique to Clade 1 isolates compared to all other *L. johnsonii*. A list of the genes and their predicted functions are provided in Table 2; corresponding sequences of the genes of Table 2 are provided as SEQ ID NOs:1-49. These

genes included those predicted to encode for ABC transport systems (predicted for novel sugar utilization), CRISPR/Cas system (for bacterial defense against foreign DNA), mucus-binding proteins (for colonization), exopolysaccharide production (for survival and colonization), and restriction modification systems (for bacterial defense against foreign DNA). Thus, this gene subset includes unique proteins that may encode for the beneficial growth properties conferred in commercial turkeys.

The genome sequence of this strain is available in the NCBI database under accession numbers CP021701, CP021702, and CP021703. Clade 1 isolates differ from their closest clade (clade 2) by at least 2,921 SNPs, and differ from a chicken-source isolate described in the literature for its probiotic properties in broiler chickens (PMID Nos. 28318296, 19767436, 14962040) by 20,509 SNPs.

Table 2. List of unique genes present in clade 1 of *L. johnsonii* and their predicted functions.

Gene locus	Sequence	SEQ ID NO
79	YSIRK signal domain/LPXTG anchor domain surface protein	SEQ ID NO:1
100	hypothetical protein	SEQ ID NO:2
113	Type II restriction/modification system, DNA methylase subunit YeeA	SEQ ID NO:3
269	DNA (cytosine-5-)-methyltransferase	SEQ ID NO:4
386	putative antibiotic resistance protein	SEQ ID NO:5
417	ABC transporter, ATP-binding cassette protein	SEQ ID NO:6
517	Type I restriction modification DNA specificity protein	SEQ ID NO:7
584	putative polysaccharide biosynthesis protein	SEQ ID NO:8
609	hypothetical protein	SEQ ID NO:9
877	putative glycosyltransferase	SEQ ID NO:10
921	putative DNA-binding protein	SEQ ID NO:11
946	Type II restriction enzyme HphI	SEQ ID NO:12
968	putative glycosyltransferase	SEQ ID NO:13
979	putative polysaccharide biosynthesis protein	SEQ ID NO:14
1088	cell division protein FtsK	SEQ ID NO:15
1104	putative replication initiation protein	SEQ ID NO:16
1133	cell division protein	SEQ ID NO:17
1134	hypothetical protein	SEQ ID NO:18
1152	putative glycosyltransferase	SEQ ID NO:19
1160	site-specific integrase	SEQ ID NO:20
1180	putative sulfotransferase	SEQ ID NO:21
1350	putative glycosyltransferase	SEQ ID NO:22

1537	hypothetical protein	SEQ ID NO:23
1569	DNA methyltransferase	SEQ ID NO:24
1638	hypothetical protein	SEQ ID NO:25
1696	hypothetical protein	SEQ ID NO:26
1726	putative glycosyltransferase	SEQ ID NO:27
2099	hypothetical protein	SEQ ID NO:28
2228	hypothetical protein	SEQ ID NO:29
2447	type II-A CRISPR-associated protein Csn2	SEQ ID NO:30
2469	hypothetical protein	SEQ ID NO:31
2499	putative membrane protein	SEQ ID NO:32
2502	IS110 transposase	SEQ ID NO:33
2529	ISL3 transposase	SEQ ID NO:34
3071	hypothetical protein	SEQ ID NO:35
3106	bacteriophage infection resistance protein	SEQ ID NO:36
3140	hypothetical protein	SEQ ID NO:37
3220	hypothetical protein	SEQ ID NO:38
3641	hypothetical protein	SEQ ID NO:39
3725	hypothetical protein	SEQ ID NO:40
3836	hypothetical protein	SEQ ID NO:41
3879	hypothetical protein	SEQ ID NO:42
3880	transposase	SEQ ID NO:43
3963	hypothetical protein	SEQ ID NO:44
3979	hypothetical protein	SEQ ID NO:45
4005	hypothetical protein	SEQ ID NO:46
4021	hypothetical protein	SEQ ID NO:47
4040	hypothetical protein	SEQ ID NO:48
4044	hypothetical protein	SEQ ID NO:49

Example 1B - *Lactobacillus aviarius*

One hundred and four *L. aviarius* isolates were analyzed as described above. SNPs were used for phylogenetic analysis including all *L. aviarius* isolates in the NCBI database (February 2017). Using Maximum Parsimony methods, a phylogenetic tree was constructed. Isolates were separated into clades based upon their host source of isolation (chicken or turkey). Furthermore, distinct clades were identified among the 104 turkey-source isolates differentiating *L. aviarius* subsp. *aviarius* isolates (Figure 3).

Two strains from clades 1 and 4 were selected for further study (see Example 2 (4-strain probiotic blend)). Strains selected included UMNlav12 (NCBI Biosample SAMN04573032) and UMNlav13 (NCBI Biosample AMN04573033). These strains differ from chicken source

L. aviarius by at least 42,318 SNPs. Also, each clade-associated strain within turkeys differs from strains of other clades by at least 31,264 SNPs.

Example 1C – *Clostridium bartlettii* (or *Intestinibacter bartlettii*)

5 A strain of *Clostridium bartlettii* was isolated from a 6-week-old commercial turkey.

Example 2

10 The following combinations of bacterial species and/or strains were tested. Additional information about the strains in each blend is provided in Tables 3A, 3B, 3C, and 3D, respectively.

4-strain probiotic blend:

- *Clostridium bartlettii* strain DSM 16795 (deposited in the NCBI database under Bioproject accession number PRJNA245632 and GENBANK accession number FUXV000000000.1)
- 15 • *Lactobacillus aviarius* strain UMNlav12 (deposited in the NCBI database under Bioproject accession number PRJNA316009 and GENBANK accession number NZ_LWUE000000000.1)
- *Lactobacillus aviarius* strain UMNlav13 (deposited in the NCBI database under Bioproject accession number and GENBANK accession number NZ_LWUF000000000.1)
- 20 • *Lactobacillus johnsonii* strain UMNlj21 (deposited in the NCBI database under Bioproject accession number PRJNA316010 and GENBANK accession numbers CP021701, CP021702, and CP021703)

10-strain (3 week) probiotic blend:

- *Lactobacillus crispatus* strain UMNpbx1 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZI000000000)
- 25 • *Lactobacillus crispatus* strain UMNpbx8 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZB000000000)
- *Lactobacillus salivarius* strain UMNpbx2 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZH000000000)
- 30

- *Lactobacillus reuteri* strain UMNPBX3 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZG000000000)
- *Lactobacillus gallinarum* strain UMNPBX4 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZF000000000)
- *Lactobacillus vaginalis* strain UMNPBX5 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZE000000000)
- *Lactobacillus helveticus* strain UMNPBX6 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZD000000000)
- *Lactobacillus johnsonii* strain UMNLJ113 (deposited in the NCBI database under Bioproject accession number PRJNA316010 and GENBANK accession number PCZJ000000000)
- *Lactobacillus aviarius* strain UMNLAv76 (deposited in the NCBI database under Bioproject accession number PRJNA316009 and GENBANK accession number PCZQ000000000)
- *Lactobacillus reuteri* strain UMNPBX7 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZC000000000)

10-strain (6 week) probiotic blend:

- *Lactobacillus salivarius* strain UMNPBX9 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZA000000000)
- *Lactobacillus reuteri* strain UMNPBX10 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCZL000000000)
- *Lactobacillus acidophilus* strain UMNPBX11 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYZ000000000)
- *Lactobacillus crispatus* strain UMNPBX12 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYY000000000)

- *Lactobacillus vaginalis* strain UMNPBX13 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYX000000000)
- 5 • *Lactobacillus gallinarum* strain UMNPBX14 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYW000000000)
- *Lactobacillus crispatus* strain UMNPBX15 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYV000000000)
- 10 • *Lactobacillus johnsonii* strain UMNLJ21 (deposited in the NCBI database under Bioproject accession number PRJNA316010 and GENBANK accession numbers CP021701, CP021702, and CP021703)
- *Lactobacillus aviarius* strain UMNLav97 (deposited in the NCBI database under Bioproject accession number PRJNA316009 and GENBANK accession number PCZP000000000)
- 15 • *Lactobacillus aviarius* strain UMNLav98 (deposited in the NCBI database under Bioproject accession number PRJNA316009 and GENBANK accession number PCZO000000000)

12-strain (wild card) probiotic blend:

- 20 • *Clostridium bartlettii* strain DSM 16795 (deposited in NCBI databases under Bioproject accession number PRJNA245632 and GENBANK accession number FUXV000000000.1)
- *Lactobacillus aviarius* strain UMNLAv97 (deposited in NCBI databases under Bioproject accession number PRJNA316009 and GENBANK accession number PCZP000000000)
- *Lactobacillus aviarius* strain UMNLAv98 (deposited in NCBI databases under Bioproject accession number PRJNA316009 and GENBANK accession number PCZO000000000)
- 25 • *Lactobacillus aviarius* strain DSM 20653 (deposited in NCBI databases under Bioproject accession number PRJNA222257 and GENBANK accession number NZ_AYYZ000000000.1)
- *Lactobacillus aviarius* strain DSM 20655 (deposited in NCBI databases under Bioproject accession number PRJNA222257 and GENBANK accession number NZ_AYZA000000000.1)
- 30

- *Lactobacillus crispatus* strain UMNPBX16 (deposited in NCBI databases under Bioproject accession number PRJNA412075 and GENBANK accession number PCYU000000000)
- *Lactobacillus gallinarum* strain UMNPBX17 (deposited in NCBI databases under Bioproject accession number PRJNA412075 and GENBANK accession number PCYT000000000)
- *Lactobacillus ingluviei* strain UMNPBX19 (deposited in NCBI databases under Bioproject accession number PRJNA412075 and GENBANK accession number PCYR000000000)
- *Lactobacillus johnsonii* strain UMNLJ94 (deposited in NCBI databases under Bioproject accession number PRJNA316010 and GENBANK accession number PCZK000000000)
- *Lactobacillus johnsonii* strain UMNLJ21 (deposited in NCBI databases under Bioproject accession number PRJNA316010 and GENBANK accession numbers CP021701, CP021702, and CP021703)
- *Lactobacillus reuteri* strain UMNPBX18 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYS000000000)
- *Pediococcus acidilactici* strain UMNPBX20 (deposited in the NCBI database under Bioproject accession number PRJNA412075 and GENBANK accession number PCYQ000000000)

Table 3A. 4-strain combination

Target Strain	Host source	Strain name	Strain ID (internal)	Bioproject	Biosample	Genbank Accession
Lactobacillus johnsonii	Turkey	UMNLJ21	MO-F2-W2-B3-C5	PRJNA316010	SAMN04573145	CP021701, CP021702, and CP021703
Clostridium bartlettii	Human	DSM 16795	ATCC Strain	PRJNA245632	SAMN02745147	FUXV000000000.1
Lactobacillus aviarus	Turkey	UMNLAV12	SNF2W2B1L1	PRJNA316009	SAMN04573032	NZ_LWUE000000000.1
Lactobacillus aviarus	Turkey	UMNLAV13	SNF2W2B1L7	PRJNA316009	SAMN04573033	NZ_LWUF000000000.1

Table 3B. 10-strain combination from 3-wk-old birds

Target Strain	Host source	Strain name	Strain ID (internal)	Bioproject	Biosample	Genbank Accession
Lactobacillus crispatus	Turkey	UMNPBX1	MO-F2-W3-B1-T2	PRJNA412075	SAMN07702352	PCZI000000000
Lactobacillus salivarius	Turkey	UMNPBX2	MO-F2-W3-B1-T3	PRJNA412075	SAMN07702353	PCZH000000000
Lactobacillus reuteri	Turkey	UMNPBX3	MO-F2-W3-B1-C8	PRJNA412075	SAMN07702354	PCZG000000000
Lactobacillus gallinarum	Turkey	UMNPBX4	MO-F2-W3-B5-T3	PRJNA412075	SAMN07702355	PCZF000000000
Lactobacillus vaginalis	Turkey	UMNPBX5	SNF2W3B1L7	PRJNA412075	SAMN07702356	PCZE000000000
Lactobacillus helveticus	Turkey	UMNPBX6	SNF2W3B1L8	PRJNA412075	SAMN07702357	PCZD000000000
Lactobacillus johnsonii	Turkey	UMNLJ113	SNF2W3B3L6	PRJNA316010	SAMN04573237	PCZJ000000000
Lactobacillus aviarus	Turkey	UMNLAV76	SNF2W3B1M8	PRJNA316009	SAMN04573096	PCZQ000000000
Lactobacillus reuteri	Turkey	UMNPBX7	SNF2W3B2M2	PRJNA412075	SAMN07702358	PCZC000000000
Lactobacillus crispatus	Turkey	UMNPBX8	SNF2W3B2M1	PRJNA412075	SAMN07702359	PCZB000000000

Table 3C. 10-strain combination from 6-wk-old birds

Target Strain	Host source	Strain name	Strain ID (internal)	Bioproject	Biosample	Genbank Accession
<i>Lactobacillus salivarius</i>	Turkey	UMNPBX9	MO-F2-W6-B4-C3	PRJNA412075	SAMN07702360	PCZA000000000
<i>Lactobacillus johnsonii</i>	Turkey	UMNLJ21	MO-F2-W2-B3-C5	PRJNA316010	SAMN04573145	CP021701, CP021702, and CP021703
<i>Lactobacillus reuteri</i>	Turkey	UMNPBX10	MO-F2-W6-B4-C7	PRJNA412075	SAMN07702361	PCZL000000000
<i>Lactobacillus acidophilus</i>	Turkey	UMNPBX11	MO-F2-W6-B4-C8	PRJNA412075	SAMN07702362	PCYZ000000000
<i>Lactobacillus crispatus</i>	Turkey	UMNPBX12	MO-F2-W6-B4-C10	PRJNA412075	SAMN07702363	PCYY000000000
<i>Lactobacillus aviarius</i>	Turkey	UMNLAv97	SNF2W6B3L1	PRJNA316009	SAMN04573117	PCZP000000000
<i>Lactobacillus aviarius</i>	Turkey	UMNLAv98	SNF2W6B3L3	PRJNA316009	SAMN04573118	PCZO000000000
<i>Lactobacillus vaginalis</i>	Turkey	UMNPBX13	SNF2W6B1M2	PRJNA412075	SAMN07702364	PCYX000000000
<i>Lactobacillus gallinarum</i>	Turkey	UMNPBX14	SNF2W6B4L1	PRJNA412075	SAMN07702365	PCYW000000000
<i>Lactobacillus crispatus</i>	Turkey	UMNPBX15	SNF2W6B5L2	PRJNA412075	SAMN07702366	PCYV000000000

Table 3D. Wild card combination

Target Strain	Host source	Strain name	Strain ID (internal)	Bioproject	Biosample	Genbank Accession
<i>Lactobacillus crispatus</i>	Turkey	UMNPBX16	SNF2W6B1L3	PRJNA412075	SAMN07702367	PCYU000000000
<i>Lactobacillus gallinarum</i>	Turkey	UMNPBX17	Lacto-spp-9-G9	PRJNA412075	SAMN07702368	PCYT000000000
<i>Lactobacillus reuteri</i>	Turkey	UMNPBX18	SNF2W5B2M2	PRJNA412075	SAMN07702369	PCYS000000000
<i>Lactobacillus ingluviei</i>	Turkey	UMNPBX19	MOF2W5B3T10	PRJNA412075	SAMN07702370	PCYR000000000
<i>Pediococcus acidilactici</i>	Turkey	UMNPBX20	MOF2W5B3T3	PRJNA412075	SAMN07702371	PCYQ000000000
<i>Lactobacillus johnsonii</i>	Turkey	UMNLJ94	SNF2W1B5L4	PRJNA316010	SAMN04573218	PCZK000000000
<i>Lactobacillus johnsonii</i>	Turkey	UMNLJ21	MO-F2-W2-B3-C5	PRJNA316010	SAMN04573145	CP021701, CP021702, and CP021703
<i>Lactobacillus aviarius</i>	Turkey	UMNLAV97	SNF2W6B3L1	PRJNA316009	SAMN04573117	PCZP000000000
<i>Lactobacillus aviarius</i>	Turkey	UMNLAV98	SNF2W6B3L3	PRJNA316009	SAMN04573118	PCZO000000000
<i>Lactobacillus aviarius</i>	Human	DSM 20655	ATCC #1	PRJNA222257	SAMN02369392	NZ_AYZA000000000.1
<i>Lactobacillus aviarius</i>	Human	DSM 20653	ATCC #2	PRJNA222257	SAMN02369423	NZ_AYYZ000000000.1
<i>Clostridium bartlettii</i>	Human	DSM 16795	ATCC Strain	PRJNA245632	SAMN02745147	FUXV000000000.1

Testing and Results

The 4-strain probiotic blend, 10-strain (3 week) probiotic blend, and 10-strain (6 week) probiotic blend (described above) were tested for their ability to enhance turkey performance in two separate trials.

In the first trial, day-of-hatch turkey poults in a caged trial with 5 replicate cages per treatment group (n=50 per treatment) were inoculated via oral gavage with either a negative saline control (Negative control), a commercially available probiotic derived from chicken isolates (FM-B11, applied according to manufacturer's instructions), or 1×10^8 CFU of each of the strains described above for the 4-strain combination and the two 10-strain combinations. Birds were weighed at four time points. Feeds were weighed back at each sampling time point to calculate feed conversion rate. In this study, all of the novel turkey-source strain combination groups had significantly higher final body weights than the saline control and the commercial chicken source probiotic ($P < 0.05$).

Results are shown in Figure 4 and Table 4. These results indicate that the strain combinations performed equally well at enhancing turkey poult body weights over the course of 14 days in cages.

Table 4. Average body weights for turkey poults administered various probiotic combinations. SEM = standard error of means.

	Day 0 (g)	Day0 SEM	Day 7 (g)	Day7 SEM	Day 10 (g)	Day10 SEM	Day 14 (g)	Day14 SEM
Negative control	66.1	0.3	149.7	4.4	219.5	7.9	338.6	16.1
FM-B11	66.1	0.3	147.7	4.4	209.7	7.9	336.5	16.1
4-strain combination	66.3	0.3	146.5	4.4	223	7.9	364.8	16.1
10-strain combination from 3-wk-old birds	66	0.3	153	4.4	226.5	7.9	375.9	16.1
10-strain combination from 6-wk-old birds	66.6	0.3	157.8	4.4	230.2	7.9	365.6	16.1

Feed conversion was also assessed over the course of the experiment. Average feed conversion rate was reduced in all treatment groups from 1.35 (Control) to 1.29 (FM-B11), 1.31 (4-strain combination), 1.29 (10-strain combination from 3-wk-old birds), and 1.32 (10-strain combination from 6-wk-old birds). All groups except the 10-strain combination from 6-wk-old birds were significantly different from the negative control group.

A second trial was performed to repeat the 4-strain combination inoculation and compare it again with a negative saline control, an existing commercial probiotic derived from chickens (FM-B11), and a low-dose antibiotic (bacitracin methylene disalicylate or BMD) administered continuously at 50g/ton in the feed. In this trial, the 4-strain combination again displayed significantly higher final body weights than the saline control and the commercial FM-B11 probiotic ($P < 0.05$). Results are shown in Figure 5 and Table 5 and indicate that there is repeatability in the ability of this 4-strain combination to enhance turkey performance related to weight gain.

Table 5. Average body weights for turkey poult s administered various probiotic combinations. SEM = standard error of means.

	Day 0 (g)	Day 0 SEM	Day 3 (g)	Day3 SEM	Day 6 (g)	Day 6 SEM	Day 13 (g)	Day13 SEM
Negative control	60.1	--	80.7	1.3	125.7	2.4	309.1	6.8
FM-B11	60.1	--	83.5	1.3	127.8	2.4	306.9	6.8
4-strain combination	60.1	--	86.9	1.3	132.8	2.4	318.2	6.8
BMD Antibiotic	60.1	--	76.8	1.3	125.5	2.4	314.3	6.8

Example 3

Pen trial experiments were performed using the 4-strain probiotic blend and 10-strain (3 week) probiotic blend described in Example 2. The effect of each combination was assessed alone and in combination with one of two prebiotics, Lactose 1% in feed or 0.5 lbs SAFMANNAN per ton of feed (Phileo Lesaffre Animal Care, Marcq-En-Baroeul Cedex, France). The prebiotic was administered continuously in feed. The experimental design is shown in Table 6.

Table 6. Experimental design for pen trials.

Group	Pens per group	Birds per group
Control	7	168
SAFMANNAN	7	168
Lactose 1%	7	168
4-strain probiotic blend	7	168
10-strain probiotic blend	7	168
SAFMANNAN + 4-strain blend	7	168
SAFMANNAN + 10-strain blend	7	168
Lactose 1% + 4-strain blend	7	168
Lactose 1% + 10-strain blend	7	168
Total	63	1512

This Experiment started in December 2017 and lasted 15 weeks. Poult were housed in pens through 6 weeks of age then moved to a common area. They remained on experimental feed through 6 weeks; then were all given control feed. Probiotic inoculations were performed at day of hatch and 3 weeks of age.

5 Prior to shared housing, the combination of prebiotic with probiotic consistently resulted in the most enhanced bird weights, as compared to the control group or prebiotic or probiotic alone. Significant body weight enhancements in birds treated with the combination of prebiotic with probiotic compared to control birds and, in some cases, in birds treated with probiotic alone were observed at weeks 2 and 3 of age, and body weight trended higher than controls throughout the
10 experiment (Table 7). The best enhancements in body weights from control ranged from 2% to 6% across these time points.

Table 7. Body weights of turkey poult fed different combinations of prebiotics and probiotics.

Treatment	Total body weight average (grams)				
	Day 0	Week 1	Week 2	Week 3	Week 6
Control	64.2	171.5	343.0	693.0	2420.0
SAFMANNAN	64.6	172.0	352.1	712.0	2424.0
Lactose 1%	65.0	169.9	350.7	704.0	2471.0
4-strain probiotic blend	64.8	168.7	347.6	709.0	2440.0
10-strain probiotic blend	65.0	167.9	342.6	704.0	2463.0
SAFMANNAN + 4-strain blend	65.0	177.0	359.3	730.0	2473.0
SAFMANNAN + 10-strain blend	64.8	173.2	357.1	723.0	2474.0
Lactose 1% + 4-strain blend	65.0	174.0	361.7	728.0	2469.0
Lactose 1% + 10-strain blend	64.9	175.4	352.6	712.0	2476.0
Standard error of means	0.4	2.3	4.0	10.0	20.0
P value Diet	0.54	0.27	0.01	0.04	0.52
P value Probiotic	0.84	0.53	0.09	0.03	0.33

15 A significant positive effect on bird uniformity was observed with treatment at week 6 of age and probiotic administration at week 3 of age (Table 8). These enhancements trended throughout the experiment, particularly with prebiotic + probiotic administration. Feed conversion was significantly enhanced from 0-2 weeks of age, with a 0.03-0.10 enhancement with prebiotic + probiotic treatment groups compared to control (Table 9). This trend was not
20 observed across the entire experiment.

Overall, this experiment demonstrated that the probiotic blends tested had the ability to enhance performance in pen trials mimicking real life conditions, resulting in higher body weights, improved feed conversion, and increased bird uniformity. The addition of prebiotics further enhanced these effects of the probiotic blends.

5

Table 8. Coefficient of variation as a measure of bird uniformity at weeks 3 and 6 of age.

Treatment	Coefficient of variation	
	Week 3	Week 6
Control	10.426	9.752
SAFMANNAN	11.696	8.5166
Lactose 1%	10.304	9.3181
4-strain probiotic blend	9.5075	9.9281
10-strain probiotic blend	9.6519	9.4035
SAFMANNAN + 4-strain blend	8.4479	7.9967
SAFMANNAN + 10-strain blend	9.1229	8.1494
Lactose 1% + 4-strain blend	9.2001	9.0704
Lactose 1% + 10-strain blend	8.8312	8.8141
Standard error of means	0.63	0.67
P value Diet	0.81	0.06
P value Probiotic	0.02	0.97

Table 9. Feed conversion ratios from 0-2 and 0-6 weeks of age.

Treatment	Feed conversion ratio	
	0-2 weeks	0-6 weeks
Control	1.43	1.71
SAFMANNAN	1.38	1.74
Lactose 1%	1.40	1.71
4-strain probiotic blend	1.42	1.72
10-strain probiotic blend	1.43	1.71
SAFMANNAN + 4-strain blend	1.36	1.72
SAFMANNAN + 10-strain blend	1.40	1.72
Lactose 1% + 4-strain blend	1.34	1.71
Lactose 1% + 10-strain blend	1.40	1.71
Standard error of means	0.01	0.01
P value Diet	0.00	0.37
P value Probiotic	0.01	0.87

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, for example, GenBank and RefSeq, and amino acid sequence submissions in, for example, SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited
5 herein are incorporated by reference. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to
10 be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

What is claimed is:

1. A composition comprising at least 4 bacterial species or strains selected from the following bacterial species:

Clostridium bartlettii;
Lactobacillus acidophilus;
Lactobacillus aviarius;
Lactobacillus crispatus;
Lactobacillus gallinarum;
Lactobacillus helveticus;
Lactobacillus ingluviei;
Lactobacillus johnsonii;
Lactobacillus reuteri;
Lactobacillus salivarius;
Lactobacillus vaginalis; and
Pediococcus acidolactici.

2. The composition of claim 1, wherein at least one of the bacterial species or strains is an avian-sourced strain.

3. The composition of claim 1 or claim 2, wherein at least one of the bacterial species or strains is a turkey-sourced strain.

4. The composition of any one of the previous claims, wherein the bacterial species or strains comprise *Clostridium bartlettii* strain DSM 16795 (deposited in the NCBI database under GENBANK accession number FUXV000000000.1).

5. The composition of any one of the previous claims, wherein the bacterial species or strains comprise *Lactobacillus acidophilus* strain UMNPBX11 (deposited in the NCBI database under GENBANK accession number PCYZ000000000).

6. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of *Lactobacillus aviarius* subspecies araffinosus and *Lactobacillus aviarius* subspecies aviarius.

7. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus aviarius strain UMNlav12 (deposited in the NCBI database under GENBANK accession number NZ_LWUE000000000.1);

Lactobacillus aviarius strain UMNlav13 deposited in the NCBI database under GENBANK accession number NZ_LWUF000000000.1);

Lactobacillus aviarius strain UMNlav76 (deposited in the NCBI database under GENBANK accession number PCZQ000000000);

Lactobacillus aviarius strain UMNlav97 (deposited in the NCBI database under GENBANK accession number PCZP000000000);

Lactobacillus aviarius strain UMNlav98 (deposited in the NCBI database under GENBANK accession number PCZO000000000);

Lactobacillus aviarius strain DSM 20653 (deposited in the NCBI database under GENBANK accession number NZ_AYYZ000000000.1); and

Lactobacillus aviarius strain DSM 20655 (deposited in the NCBI database under GENBANK accession number NZ_AYZA000000000.1).

8. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus crispatus strain UMNPBX1 (deposited in the NCBI database under GENBANK accession number PCZI000000000);

Lactobacillus crispatus strain UMNPBX8 (deposited in the NCBI database under GENBANK accession number PCZB000000000);

Lactobacillus crispatus strain UMNPBX12 (deposited in the NCBI database under GENBANK accession number PCYY000000000);

Lactobacillus crispatus strain UMNPBX15 (deposited in the NCBI database under GENBANK accession number PCYV000000000); and

Lactobacillus crispatus strain UMNPBX16 (deposited in the NCBI database under GENBANK accession number PCYU000000000).

9. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus gallinarum strain UMNPBX4 (deposited in the NCBI database under GENBANK accession number PCZF000000000);

Lactobacillus gallinarum strain UMNPBX14 (deposited in the NCBI database under GENBANK accession number PCYW000000000); and

Lactobacillus gallinarum strain UMNPBX17 (deposited in NCBI databases under GENBANK accession number PCYT000000000).

10. The composition of any one of the previous claims, wherein the bacterial species or strains comprise *Lactobacillus helveticus* strain UMNPBX6 (deposited in the NCBI database under GENBANK accession number PCZD000000000).

11. The composition of any one of the previous claims, wherein the bacterial species or strains comprise *Lactobacillus ingluviei* strain UMNPBX19 (deposited in the NCBI database under GENBANK accession number PCYR000000000).

12. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus johnsonii strain UMNLJ21 (deposited in the NCBI database under GENBANK accession numbers CP021701, CP021702, and CP021703);

Lactobacillus johnsonii strain UMNLJ94 (deposited in the NCBI database under GENBANK accession number PCZK000000000); and

Lactobacillus johnsonii strain UMNLJ113 (deposited in the NCBI database under GENBANK accession number PCZJ000000000).

13. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus reuteri strain UMNPBX3 (deposited in the NCBI database under GENBANK accession number PCZG000000000);

Lactobacillus reuteri strain UMNPBX7 (deposited in the NCBI database under GENBANK accession number PCZC000000000);

Lactobacillus reuteri strain UMNPBX10 (deposited in the NCBI database under GENBANK accession number PCZL000000000); and

Lactobacillus reuteri strain UMNPBX18 (deposited in the NCBI database under GENBANK accession number PCYS000000000).

14. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus salivarius strain UMNPBX2 (deposited in the NCBI database under GENBANK accession number PCZH000000000); and

Lactobacillus salivarius strain UMNPBX9 (deposited in the NCBI database under GENBANK accession number PCZA000000000).

15. The composition of any one of the previous claims, wherein the bacterial species or strains comprise at least one of:

Lactobacillus vaginalis strain UMNPBX5 (deposited in the NCBI database GENBANK accession number PCZE000000000); and

Lactobacillus vaginalis strain UMNPBX13 (deposited in the NCBI database under GENBANK accession number PCYX000000000).

16. The composition of any one of the previous claims, wherein the bacterial species or strains comprise *Pediococcus acidolactici* UMNPBX20 (deposited in the NCBI database under GENBANK accession number PCYQ000000000).

17. The composition of any one of claims 1 to 3, the composition comprising at least 4 bacterial species or strains selected from the following:

Clostridium bartlettii;

Lactobacillus aviaries; and

Lactobacillus johnsonii.

18. The composition of any one of claims 1 to 3 or claim 17, wherein the bacterial species or strains comprise:

Clostridium bartlettii DSM 16795 (deposited in the NCBI database under accession number FUXV000000000.1);

Lactobacillus aviarius strain UMNLav12 (deposited in the NCBI database under accession number NZ_LWUE000000000.1);

Lactobacillus aviarius strain UMNLav13 (deposited in the NCBI database under accession number NZ_LWUF000000000.1); and

Lactobacillus johnsonii strain UMN LJ21 (deposited in the NCBI database under accession numbers CP021701, CP021702, and CP021703);

19. The composition of any one of claims 1 to 3, the composition comprising at least 4 bacterial species or strains selected from the following:

Lactobacillus aviarius;

Lactobacillus crispatus;

Lactobacillus gallinarum;

Lactobacillus helveticus;

Lactobacillus johnsonii;

Lactobacillus reuteri;

Lactobacillus salivarius; and

Lactobacillus vaginalis.

20. The composition of any one of claims 1 to 3 or claim 19, the composition comprising at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 bacterial species or strains selected from the following:

Lactobacillus aviarius strain UMN Lav76 (deposited in the NCBI database under GENBANK accession number PCZQ000000000);

Lactobacillus crispatus strain UMN PBX1 (deposited in the NCBI database under GENBANK accession number PCZI000000000);

Lactobacillus crispatus strain UMN PBX8 (deposited in the NCBI database under GENBANK accession number PCZB000000000);

Lactobacillus gallinarum strain UMNPBX4 (deposited in the NCBI database under GENBANK accession number PCZF000000000);

Lactobacillus helveticus strain UMNPBX6 (deposited in the NCBI database under GENBANK accession number PCZD000000000);

Lactobacillus johnsonii strain (deposited in the NCBI database under GENBANK accession number PCZJ000000000);

Lactobacillus reuteri strain UMNPBX3 (deposited in the NCBI database under GENBANK accession number PCZG000000000);

Lactobacillus reuteri strain UMNPBX7 (deposited in the NCBI database under GENBANK accession number PCZC000000000);

Lactobacillus salivarius strain UMNPBX2 (deposited in the NCBI database under GENBANK accession number PCZH000000000); and

Lactobacillus vaginalis strain UMNPBX5 (deposited in the NCBI database GENBANK accession number PCZE000000000).

21. The composition of any one of claims 1 to 3, the composition comprising at least 4 bacterial species or strains selected from the following:

Lactobacillus acidophilus;

Lactobacillus aviarius;

Lactobacillus crispatus;

Lactobacillus gallinarum;

Lactobacillus johnsonii;

Lactobacillus reuteri;

Lactobacillus salivarius; and

Lactobacillus vaginalis.

22. The composition of any one of claims 1 to 3 or claim 21, the composition comprising at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 bacterial species or strains selected from the following:

Lactobacillus acidophilus strain UMNPBX11 (deposited in the NCBI database under GENBANK accession number PCYZ000000000);

Lactobacillus aviarius strain UMNlav97 (deposited in the NCBI database under GENBANK accession number PCZP000000000);

Lactobacillus aviarius strain UMNlav98 (deposited in the NCBI database under GENBANK accession number PCZO000000000);

Lactobacillus crispatus strain UMNPBX12 (deposited in the NCBI database under GENBANK accession number PCYY000000000);

Lactobacillus crispatus strain UMNPBX15 (deposited in the NCBI database under GENBANK accession number PCYV000000000);

Lactobacillus gallinarum strain UMNPBX14 (deposited in the NCBI database under GENBANK accession number PCYW000000000);

Lactobacillus johnsonii strain UMN LJ21 (deposited in the NCBI database under accession numbers CP021701, CP021702, and CP021703);

Lactobacillus reuteri strain UMN PBX10 (deposited in the NCBI database under GENBANK accession number PCZL000000000);

Lactobacillus salivarius strain UMN PBX9 (deposited in the NCBI database under GENBANK accession number PCZA000000000); and

Lactobacillus vaginalis strain UMN PBX13 (deposited in the NCBI database under GENBANK accession number PCYX000000000).

23. The composition of any one of claims 1 to 3, the composition comprising at least 4 bacterial species or strains selected from the following:

Clostridium bartlettii;

Lactobacillus aviarius;

Lactobacillus crispatus;

Lactobacillus gallinarum;

Lactobacillus ingluviei;

Lactobacillus johnsonii;

Lactobacillus reuteri; and

Pediococcus acidolactici.

24. The composition of any one of claims 1 to 3 or claim 23, the composition comprising at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 11 bacterial species or strains selected from the following:

Clostridium bartlettii strain DSM 16795 (deposited in the NCBI database under GENBANK accession number FUXV000000000.1);

Lactobacillus aviarius strain UMNLAv97 (deposited the NCBI database under GENBANK accession number PCZP000000000);

Lactobacillus aviarius strain UMNLAv98 (deposited in the NCBI database under GENBANK accession number PCZO000000000);

Lactobacillus aviarius strain DSM 20653 (deposited in the NCBI database under GENBANK accession number NZ_AYYZ000000000.1);

Lactobacillus aviarius strain DSM 20655 (deposited in the NCBI database under GENBANK accession number NZ_AYZA000000000.1);

Lactobacillus crispatus strain UMNPBX16 (deposited in the NCBI database under GENBANK accession number PCYU000000000);

Lactobacillus gallinarum strain UMNPBX17 (deposited in the NCBI database under GENBANK accession number PCYT000000000);

Lactobacillus ingluviei strain UMNPBX19 (deposited in the NCBI database under GENBANK accession number PCYR000000000);

Lactobacillus johnsonii strain UMNLJ94 (deposited in the NCBI database under GENBANK accession number PCZK000000000);

Lactobacillus johnsonii strain UMNLJ21 (deposited in the NCBI database under GENBANK accession numbers CP021701, CP021702, and CP021703);

Lactobacillus reuteri strain UMNPBX18 (deposited in the NCBI database under GENBANK accession number PCYS000000000); and

Pediococcus acidolactici strain UMNPBX20 (deposited in the NCBI database under GENBANK accession number PCYQ000000000).

25. The composition of any one of the previous claims, wherein the composition is antibiotic free.

26. The composition of any one of the previous claims, wherein the composition comprises at least 1×10^5 colony-forming units (CFU) of each of the bacterial species or strains.

27. A method of using the composition of any one of the previous claims, wherein the method comprises administering the composition to an animal.

28. The method of claim 27, wherein the composition is administered by oral gavage.

29. The method of claim 27 or 28, wherein the animal is a turkey.

30. The method of claim 29, wherein the method comprises administering the composition to a turkey when the turkey is less than a week old.

31. The method of claim 29 or claim 30, wherein the method comprises administering the composition to a turkey when the turkey is greater than a week old.

32. The method of any one of claims 27 to 31, wherein the method further comprises administering a prebiotic to the animal.

33. The method of claim 32, the method comprising administering the prebiotic in the animal's feed.

34. The method of claim 32 or 33, wherein the prebiotic comprises one or more of lactose, a mannan-oligosaccharide, and a β -glucan.

FIG. 1

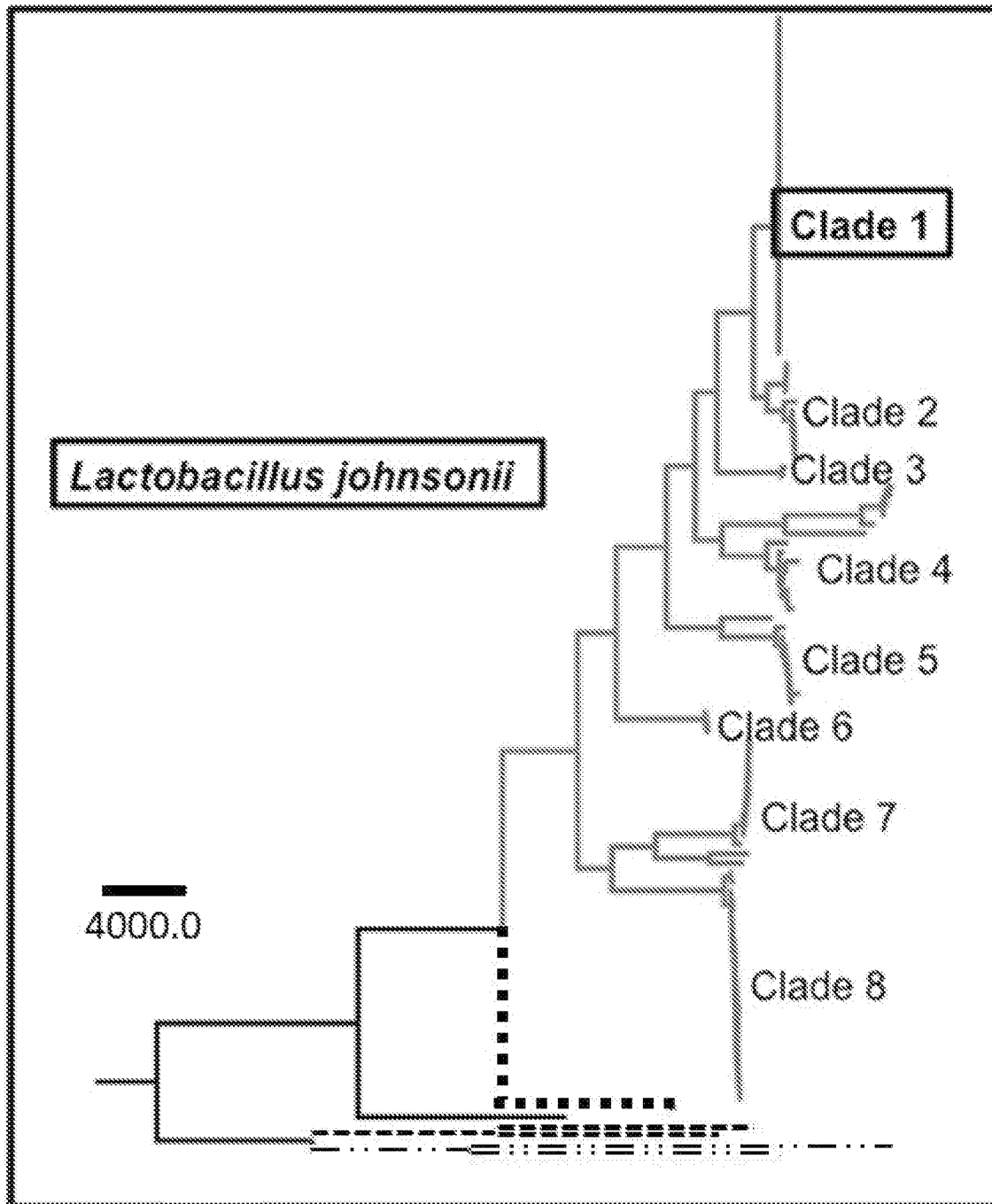


FIG. 2

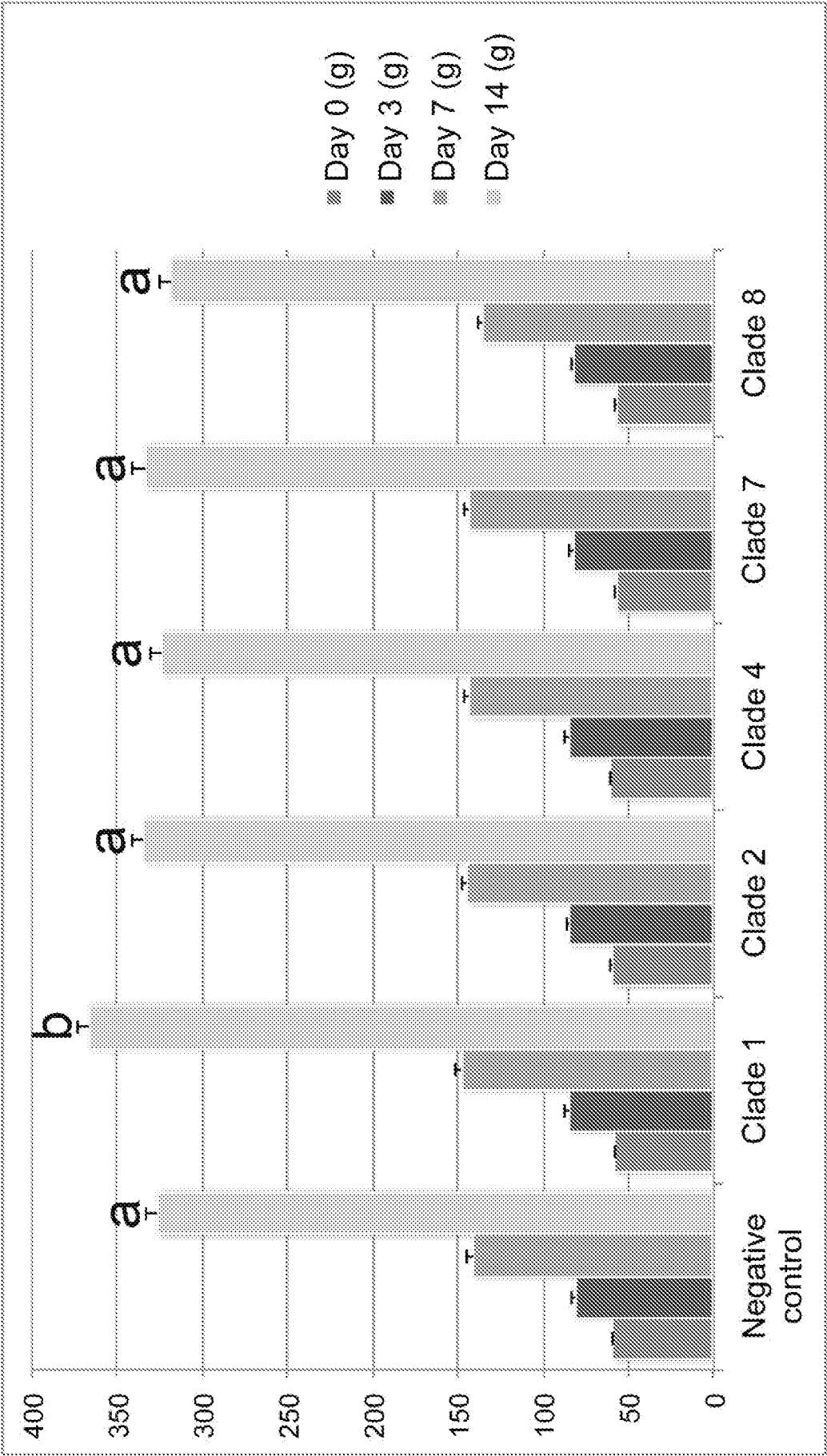


FIG. 3

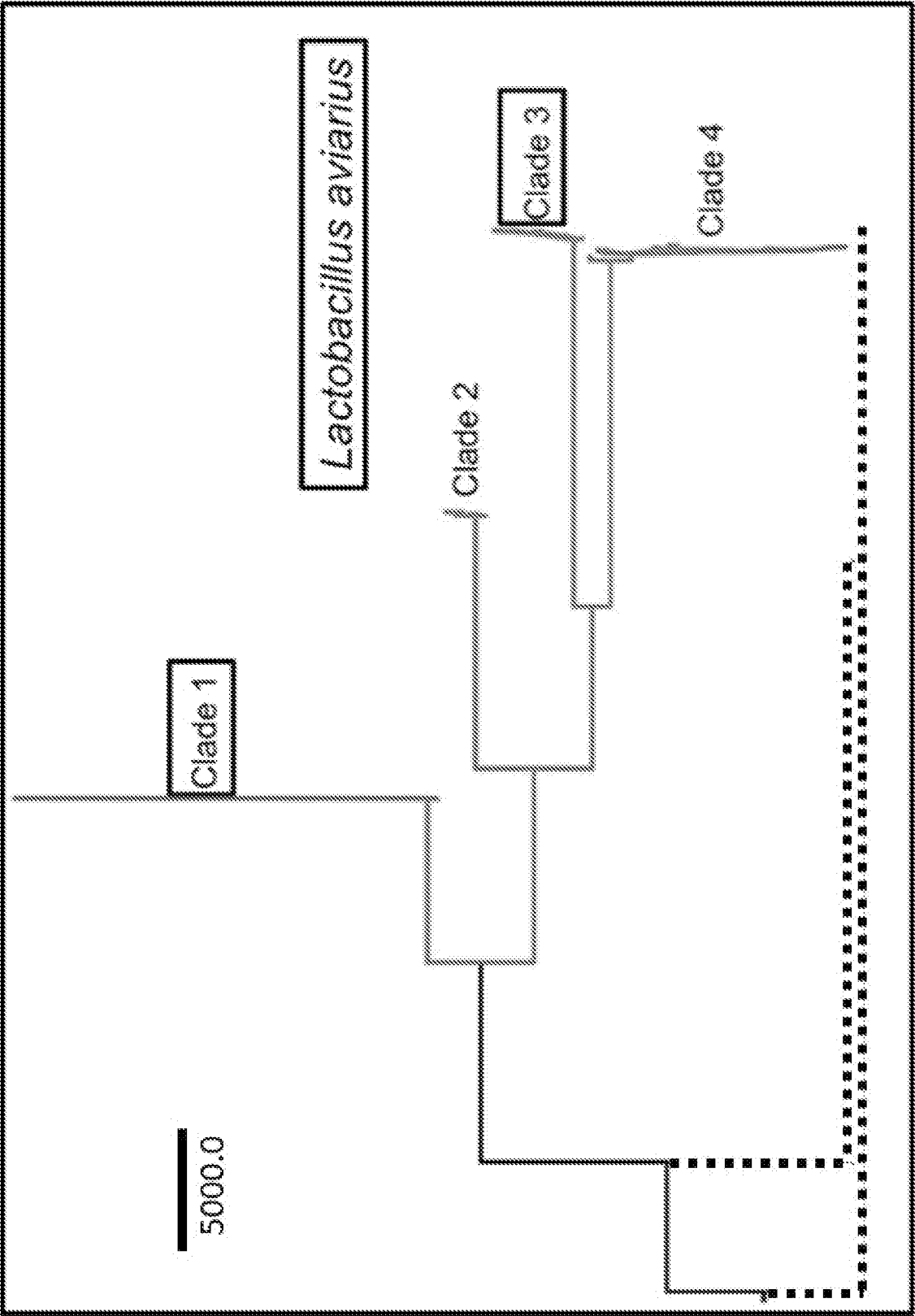


FIG. 4A

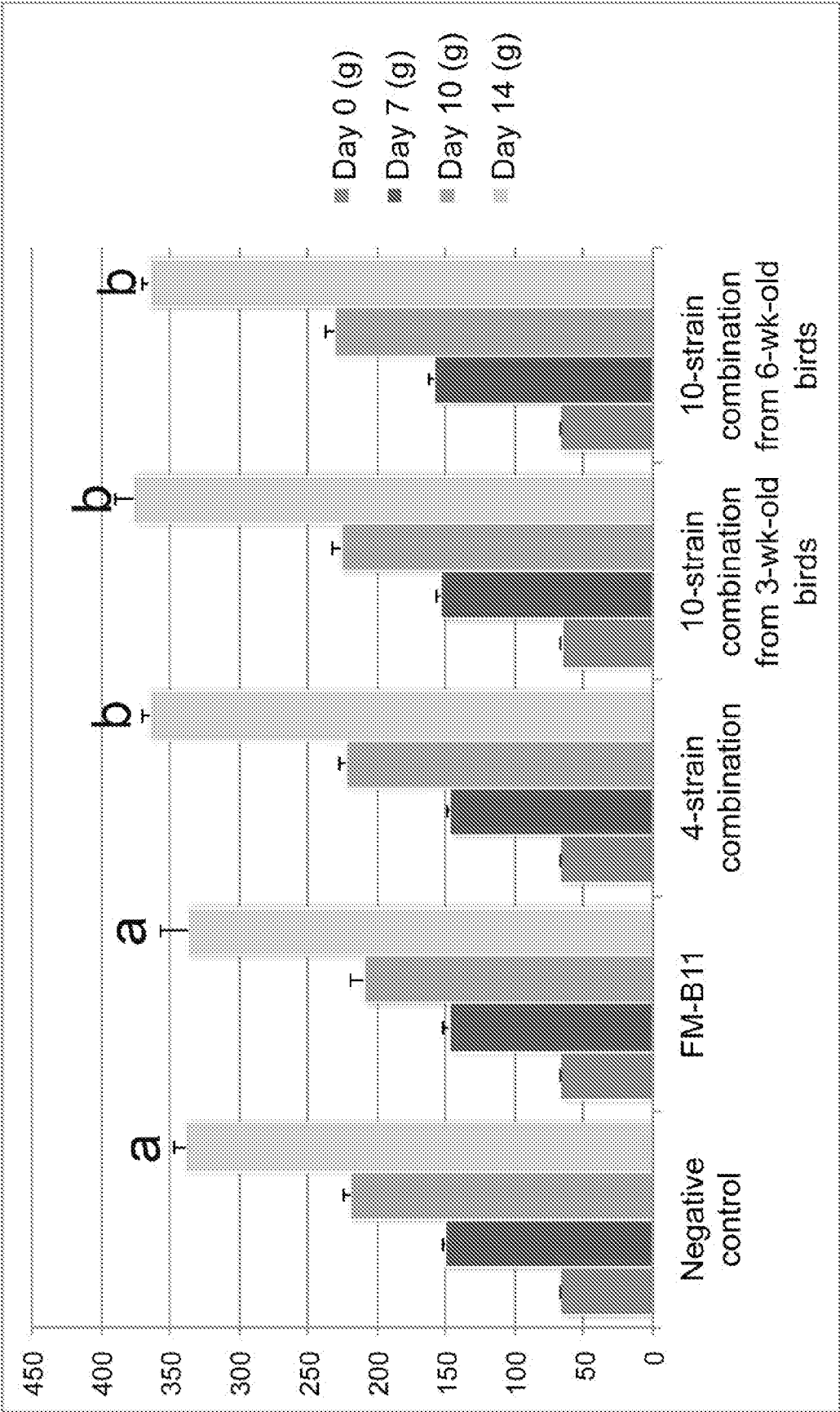


FIG. 4B

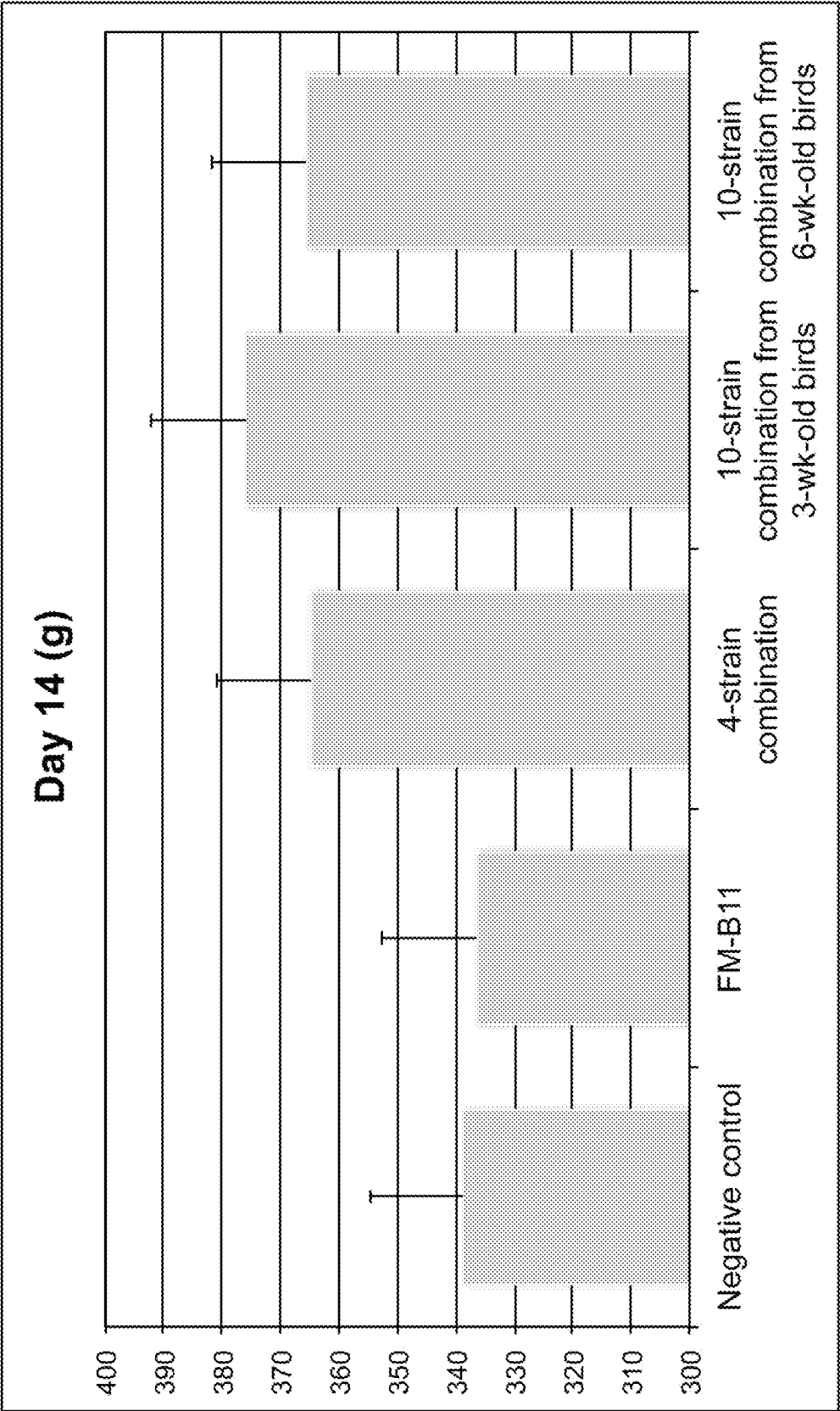


FIG. 5A

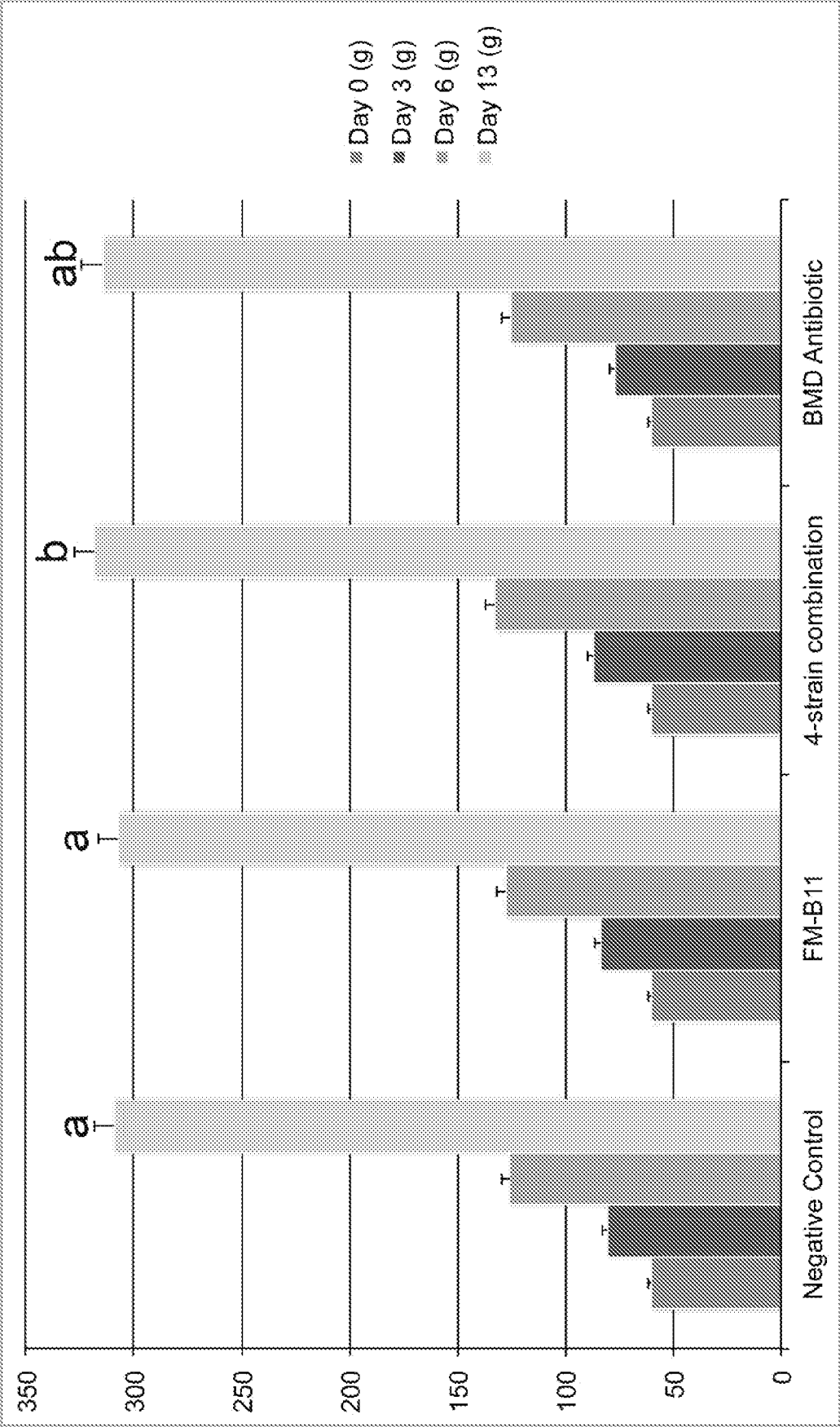
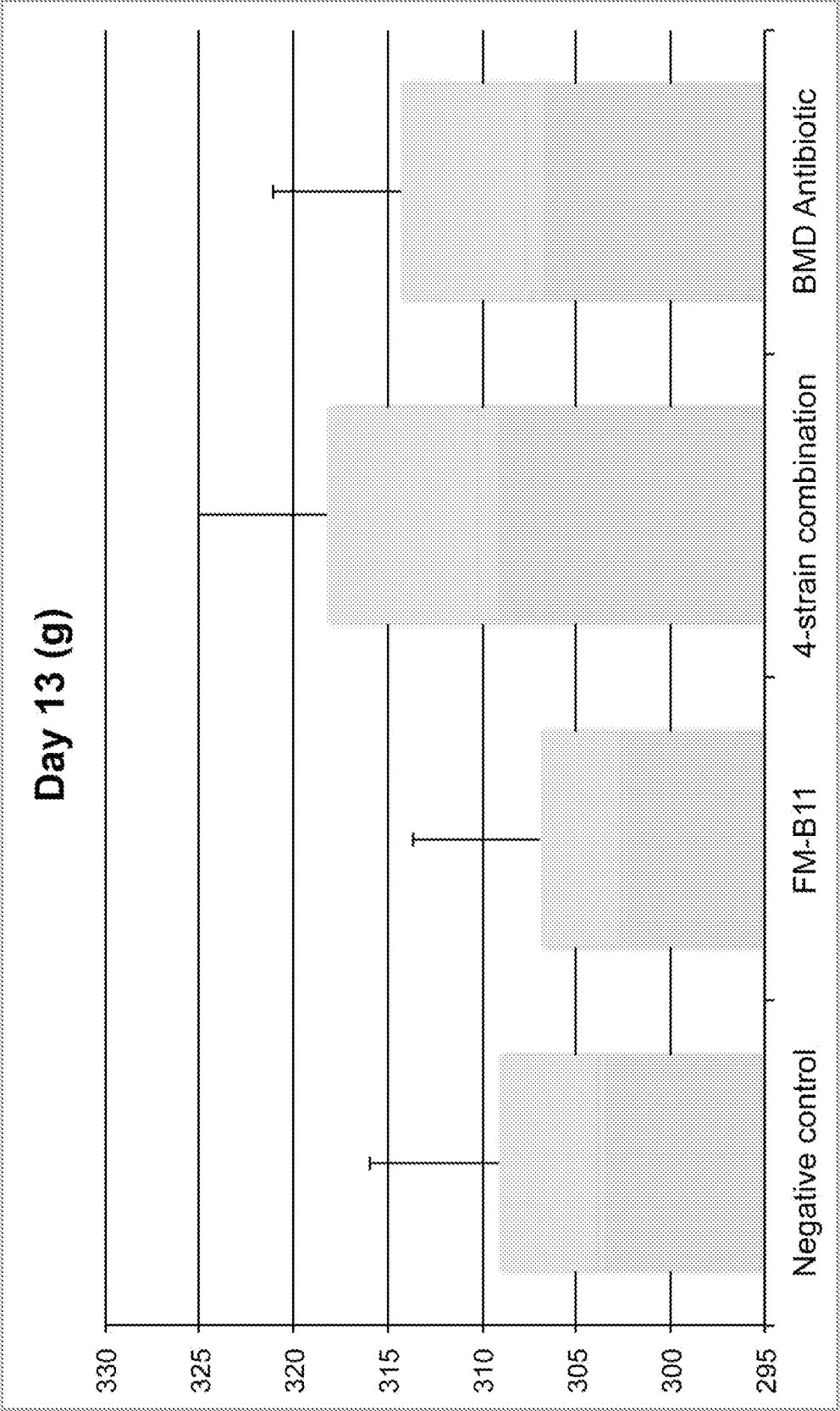


FIG. 5B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/56033

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/56033

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/56033

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/02 A23K 10/10, A23K 10/16 (2018.01)
CPC - A61K 35/747, A23K 50/75, A23K 50/70, A23K 10/18

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)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0328815 A1 (NUTRITION PHYSIOLOGY COMPANY, LLC) 6 November 2014 (06.11.2014) para [0011], [0034]	1 --- 2-3
Y	US 4,980,164 A (MANFREDI et al.) 25 December 1990 (12.25.1990) abstract, col 3, ln 8-20, col 8, ln 64-67	2-3

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

☐ 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

9 December 2018

Date of mailing of the international search report

07 JAN 2019

Name and mailing address of the ISA/US

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