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(54) Title: CLAY-BASED MATERIALS FOR ANIMAL FEEDING AND CARE

(57) Abstract: Methods of feeding and/or caring for domesticated animals (e.g., livestock, poultry, equine, and/or animals of aquaculture) comprise improving the growth and/or the health of the domesticated animals. Therapeutic amounts of bioactive clay may be orally administered to the domesticated animals to improve the growth and/or health of the domesticated animals. The bioactive clay generally is a clay that comprises iron sulfide and a source of soluble aluminum ions. The bioactive clay may be formulated by mixing the components of the bioactive clay, may be processed into a feed supplement formulated to be delivered to the domesticated animals, and/or may be processed into a supplemented animal feed formulated to feed the domesticated animals.

CLAY-BASED MATERIALS FOR ANIMAL FEEDING AND CARE

RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/317,318, entitled "CLAY-BASED ANIMAL FEED SUPPLEMENTS AND METHODS OF USE," which was filed on April 1, 2016, the complete disclosure of which is hereby incorporated by reference for all purposes.

TECHNICAL FIELD

The present disclosure relates to clay-based materials for animal feeding and care.

BACKGROUND

To improve access to animal products (*e.g.*, meat, milk, eggs), animals may be raised using a controlled diet to optimize animal nutrition and health. In addition, antibiotics may be administered as growth promoters to improve animal growth rate and feed conversion efficiency. The exact mechanism of action of antibiotic growth promoters is unknown but is believed to relate to reducing clinical and/or sub-clinical infection occurrence. Other potential mechanisms of action include (i) increased nutrient availability due to less transformation by gut flora, (ii) increased nutrient absorption due to a more efficient small intestine, and (iii) decreased exposure to pathogenic bacterial toxins.

Antibiotic growth promoters may increase production and/or reduce the cost of animal products. However, widespread use of antibiotic pharmaceuticals is linked to the spread of antibiotic resistance. The spread of antibiotic resistance may reduce the effectiveness of traditional antibiotic growth promoters in agriculture and may lead to human pathogens with antibiotic resistance (and hence diseases that may be harder to treat).

Hence, there is a need for growth promoters that may improve the growth rate and/or the health of domesticated animals and reduce the spread of deleterious antibiotic resistance.

SUMMARY

Methods of feeding and/or caring for a domesticated animal comprise orally administering a therapeutically effective quantity of a bioactive clay to the domesticated animal. The administration of the bioactive clay may affect the growth and/or health of the domesticated animal, for example increasing the growth rate, increasing the feed intake, increasing the feed conversion ratio, improving in fecal consistency, and/or decreasing morbidity, each in relation to a control domesticated animal that does not receive the bioactive clay. The therapeutically effective dosage of the bioactive clay generally is a daily equivalent dosage of about 0.5 mg (milligrams) to about 50 mg, per kilogram of body mass of the domesticated animal.

The bioactive clay is a clay that comprises iron sulfide and a source of soluble aluminum ions. For example, the bioactive clay may comprise about 1 wt% (weight percent) to about 70 wt% of iron sulfide and may comprise aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM (millimolar). The bioactive clay may further comprise aluminum phyllosilicate clay (*e.g.*, rectorite). The bioactive clay may be Oregon Blue clay or a composition comprising Oregon Blue clay. The bioactive clay may be granulated and/or in a form suitable for oral administration to the domesticated animal. For example, the bioactive clay may be in the form of a supplemented animal feed that includes the bioactive clay (*e.g.*, at an inclusion rate of about 100 ppm (parts per million) to about 5,000 ppm).

DESCRIPTION

Methods of feeding and/or caring for domesticated animals (*e.g.*, livestock, poultry, equine, and/or animals of aquaculture) comprise improving the growth and/or the health of the domesticated animals. Therapeutic amounts of bioactive clay may be orally administered to the domesticated animals to improve the growth and/or health of the domesticated animals. The bioactive clay generally is a clay that comprises iron sulfide and a source of soluble aluminum ions. The bioactive clay may be formulated by mixing the components of the bioactive clay, may be processed into a feed supplement formulated to be delivered to the domesticated animal, and/or may be processed into a supplemented animal feed formulated to feed the domesticated animal.

Clay is a natural rock that is composed principally of one or more phyllosilicate minerals. Phyllosilicate minerals are minerals of silicon and oxygen (with additional metal cations in some minerals). Phyllosilicate minerals are hydrated, including hydroxyl groups and/or complexed water. Clay typically includes an aluminum phyllosilicate mineral. Aluminum phyllosilicate minerals (also referred to as aluminosilicate minerals) are minerals of silicon, aluminum, and oxygen. Examples of phyllosilicate minerals include kaolinite, illite, muscovite, chlorite, rectorite, smectite, montmorillonite, and/or pyrophyllite. Clay may include other silicates, metal oxides, salts, and/or organic matter. For example, clay may include silica, alumina, water, iron, alkali metals, alkaline earth metals, gibbsite, quartz, feldspar, calcium carbonate, salts, and/or organic matter.

Bioactive clay is a composition of an aluminum phyllosilicate mineral that includes iron in a reduced state, *e.g.*, iron(II). Bioactive clay may be referred to as active clay, therapeutic clay, and/or antibacterial clay. Bioactive clay may be an aluminum phyllosilicate clay (*e.g.*, natural or native clay) or may be a composition that has the properties of clay (*e.g.*, a synthetic clay material, optionally including synthetic phyllosilicate minerals). Aluminum phyllosilicate clay that may form a basis of the bioactive clay is a clay principally composed of one or more aluminum phyllosilicate minerals. For example, bioactive clay may comprise one or more of illite, smectite, and rectorite. Additionally or alternatively, the aluminum phyllosilicate minerals of the bioactive clay may consist essentially of one or more of illite, smectite, and rectorite.

The bioactive clay may comprise a mixed layer of aluminum phyllosilicate minerals such as the mixed layers of illite and smectite in rectorite. Any of the aluminum phyllosilicate minerals of the bioactive clay may be enhanced or depleted in particular chemical elements relative to common and/or standard minerals. For example, the rectorite may be K-rectorite (potassium-rich rectorite). The bioactive clay may be a non-swelling clay (*e.g.*, not

comprising significant amounts of bentonite). The bioactive clay may not have any significant toxin binding capability for one or more types of toxins. For example, the bioactive clay may exhibit little to no binding and/or removal of mycotoxin.

Bioactive clay typically includes an iron sulfide, which is a chemical compound comprising principally iron and sulfur. Examples of iron sulfides include pyrite, marcasite, mackinawite, troilite, pyrrhotite, greigite, and iron(II) sulfide. The iron of the iron sulfide in the bioactive clay may principally be in reduced form (*i.e.*, a majority, substantially all, or essentially all of the iron in the iron sulfide of the bioactive clay is in reduced form). Examples of iron sulfide minerals with iron principally in reduced form include pyrite and marcasite. Iron sulfides may comprise other metals (which typically substitute in the structure for iron) such as nickel, copper, and zinc. Some or all of the iron sulfide of the bioactive clay may be another iron-sulfur compound (instead of iron sulfide) such as an iron sulfate compound.

Bioactive clay may be formulated by mixing appropriate amounts (as discussed herein) of iron sulfide and clay (*e.g.*, natural and/or synthetic clay). For example, pyrite may be separated (*e.g.*, by sieving, floating, or other mechanical methods) from soil and/or rock that comprises significant amounts of pyrite and then blended into a base clay material to form the bioactive clay. The base clay material may comprise iron sulfide or may be essentially devoid of iron sulfide. Iron sulfide addition may be used to incorporate a consistent amount of iron sulfide into each batch of bioactive clay. The bioactive clay may be configured and/or selected for sustained release of the iron sulfide. For example, the bioactive clay may have aluminum phyllosilicate minerals that include layers and/or channels that may inhibit diffusion of water, iron ions, and/or sulfur ions into and/or out of the bioactive clay.

As used herein, "Oregon Blue clay" refers to a mixed-layer clay that is a bioactive clay. Oregon Blue clay includes a generally ordered, mixed layer illite/smectite (*e.g.*, rectorite or K-rectorite). For example, Oregon Blue clay has a generally regular alternation of illite and smectite layers substantially parallel to the "c" axis and thus includes the non-swelling properties of illite and the swelling properties of smectite. Additionally, Oregon Blue clay can be described as a high-sulfide K-rectorite or a montmorillonite smectite. Oregon Blue clay may be sourced from volcanic deposits associated with the Oregon Cascade Mountains and/or Mount Mazama (Crater Lake, Oregon, USA). Oregon Blue clay is commercially available from TECTTONIC, LLC, Grants Pass, Oregon, USA. Generally, Oregon Blue clay in native form has a pH that is near neutral (*e.g.*, a pH of 6-8).

As used herein, "weathered Oregon Blue clay" refers to Oregon Blue clay that has been oxidized by weathering, such that it does not contain significant pyrite (an iron sulfide). Weathered Oregon Blue clay is not a bioactive clay. However, weathered Oregon Blue clay may be a component of the bioactive clay. For example, the weathered Oregon Blue clay may be augmented with iron sulfide (*e.g.*, pyrite) to form the bioactive clay. Additionally or alternatively, weathered Oregon Blue clay may be chemically reduced (*e.g.*, iron contained in the weathered Oregon Blue clay may be reduced to the iron(II) state). For example, oxalic acid may be used to chemically reduce iron(III) in the weathered Oregon Blue clay to iron(II) to form the basis of the bioactive clay. Other acids such as phosphoric acid may be used in addition or in alternate to oxalic acid. Generally, weathered Oregon Blue clay in native form has a pH that is acidic (*e.g.*, 2-6). Weathered Oregon Blue clay may be incorporated in the bioactive clay to reduce, establish, and/or stabilize a lower pH for the bioactive clay as described herein.

Some clay compositions and clay minerals, such as Oregon Blue clay, are reported to be antibacterial and therapeutic for skin lesions. *See, e.g.*, U.S. Patent Application Publication No. 2013/0004544 to Metge *et al.* (Metge). Further, Metge reports *in vitro* experiments with *Escherichia coli* and *Staphylococcus epidermidis* that demonstrate the antibacterial nature of Oregon Blue clay and other clay compositions. Based on these findings, Metge suggests use of antibacterial clay compositions for treatment of skin infections and skin diseases. Metge reports that weathered Oregon Blue clay has substantially no antibacterial nature *in vitro*.

Metge provides mineralogical compositions, in weight percent (wt%), of a sample of Oregon Blue clay and a sample of weathered Oregon Blue clay (see Table 1). The clays of Table 1 were analyzed by quantitative X-ray diffraction using the ROCKJOCK computer program (see Eberl, "User guide to RockJock—A program for determining quantitative mineralogy from X-ray diffraction data," USGS Open File Report OF 03-78, 40p (2003)).

Table 1

	Oregon Blue clay	Weathered Oregon Blue clay
NON-CLAYS (wt%)		
Quartz	44.6	46.0
Feldspar	0.9	2.9

	Oregon Blue clay	Weathered Oregon Blue clay
Amphibole	0.5	0.0
Pyrite	9.6	0.0
Barite	0.0	0.0
Gypsum	0.0	0.9
Anatase	0.0	0.2
Jarosite	0.2	0.0
Magnetite/maghemite/goethite	0.0	0.0
Calcite	0.0	0.0
Total non-clays	55.8	50
CLAYS (wt%)		
Kaolinites	0.7	2.9
Illite + muscovite	7.6	30.9
Chlorite	4.4	0.8
Rectorite	48.4	24.9
Smectite	0.0	0.0
Pyrophyllite	0.0	0.0
Gibbsite	0.0	0.0
Total clays	61.1	59.5
Total	**116.9**	**109.5**

Bioactive clay may be characterized by the elemental composition and/or the amount and character of soluble ions. For example, Oregon Blue clay leachate may be formed by mixing 100 mg of Oregon Blue clay in 100 mL (milliliter) of water. The Oregon Blue clay mixture may be shaken for 24 hours and then centrifuged to separate undissolved minerals (the leachate being the supernatant). As shown in Table 2, leachates of samples of Oregon Blue clay were compared against minimum inhibitory concentrations (MIC) and minimum

bactericidal concentrations (MBC) for *E. coli*. Concentrations in Table 2 are expressed in millimolar (mM). At the MIC or above, *E. coli* exhibited no visible growth. At the MBC, the leachate killed 99.9% of *E. coli*. Aluminum, calcium, and iron were the most significant elements observed in the leachates. Amounts for most elements were below the MBC for that element. Aluminum was above the MIC for aluminum. Without being bound by any one particular theory, the reduction-oxidation (redox) state (*e.g.*, the redox state of iron in the bioactive clay) may be more important than concentration. In Table 2, elemental analysis of the leachate is by inductively coupled plasma atomic emission spectroscopy (ICP-OES). MIC and MBC data for *E. coli* are respectively from Nies (Nies, DH, (1999) "Microbial heavy-metal resistance," *Appl. Microbiol. Biotechnol*, 51:730-750) and Harrison (Harrison, JJ, Turner, RJ, Ceri, H, (2005) "High-throughput metal susceptibility testing of microbial biofilms," *BMC Microbiol,* 5:1471-2180).

Table 2

Element	Oregon Blue clay (mM)	MIC (mM)	MBC (mM)
Mg	1.0	---	---
Al	4.8	2.0	19.0
Si	0.1	---	---
P	0.23	---	---
K	0.04	---	---
Ca	6.6	---	---
V	0.01	---	---
Cr	0.02	0.2	---
Mn	0.06	20.0	198.0
Fe	15.8	---	---
Ni	0.01	1.0	17.0
Cu	0.01	1.0	17.0
Zn	0.004	1.0	31.0
As	0.002	4.0	---

Element	Oregon Blue clay (mM)	MIC (mM)	MBC (mM)
Ag	0.01	0.02	0.06
Cd	0.0008	0.5	2.3
Pb	0.002	5.0	---

The bioactive clay may comprise iron sulfide at a level of at least 1 wt%, at least 2 wt%, at least 5 wt%, at least 8 wt%, at least 15 wt%, at least 20 wt%, at least 25 wt%, about 1 wt% to about 80 wt%, about 1 wt% to 20 wt%, about 5 wt% to about 70 wt%, about 20 wt% to about 70 wt%, about 20 wt% to about 50 wt%, about 10 wt% to about 50 wt%, at most 80 wt%, at most 70 wt%, at most 60 wt%, at most 50 wt%, and/or at most 40 wt%.

The bioactive clay may comprise aluminum phyllosilicate minerals (as a group) at a level of at least 5 wt%, at least 10 wt%, at least 20 wt%, at least 30 wt%, at least 50 wt%, at least 80 wt%, about 10 wt% to about 95 wt%, about 10 wt% to about 80 wt%, about 10 wt% to about 50 wt%, about 5 wt% to about 40 wt%, at most 95 wt%, at most 80 wt%, at most 60 wt%, at most 50 wt%, at most 40 wt%, and/or at most 30 wt%.

The bioactive clay may comprise aluminum (*e.g.*, as part of the aluminum phyllosilicate minerals, alumina, gibbsite, aluminum hydroxide minerals, aluminum salt, or other compounds of aluminum) at a level of at least 2 wt%, at least 5 wt%, at least 10 wt%, at least 20 wt%, about 5 wt% to about 50 wt%, about 5 wt% to about 40 wt%, about 10 wt% to about 40 wt%, at most 60 wt%, at most 50 wt%, at most 40 wt%, and/or at most 30 wt%.

The bioactive clay may comprise aluminum a level (*e.g.*, at a sufficient weight percent of aluminum in the bioactive clay) to produce a soluble aluminum ion concentration of about 2 mM, about 5 mM, about 10 mM, at least 0.5 mM, at least 1 mM, at least 2 mM, at least 4 mM, at least 8 mM, about 1 mM to about 20 mM, about 1 mM to about 10 mM, about 2 mM to about 8 mM, about 4 mM to about 6 mM, and/or about 6 mM to about 10 mM. Additionally or alternatively, the bioactive clay may comprise aluminum at a level to produce a soluble aluminum ion concentration that is at or above the minimum inhibitory concentration for aluminum. The soluble aluminum ion concentration may be determined by ICP-OES (*e.g.*, as performed with respect to Table 2) or other method of assaying aluminum ions in solution by using a solution of the bioactive clay formed at a concentration of 100 mg/mL (add the bioactive clay to water, shake 24 hours, centrifuge to separate undissolved minerals, and analyze the supernatant).

The bioactive clay may be formed of a mixture of Oregon Blue clay (or other clay base) and an aluminum admixture. The aluminum admixture is a composition that principally includes an aluminum compound. The aluminum admixture may be a source of aluminum ions in a solution of water. The aluminum admixture may include alumina, gibbsite, aluminum hydroxide minerals, aluminum salt, and/or other compounds of aluminum. The aluminum admixture may be incorporated into the bioactive clay to achieve a desired soluble aluminum ion concentration at a level as described herein. Where the bioactive clay includes the aluminum admixture, the bioactive clay may comprise the aluminum admixture at a level of at least 1 wt%, at least 2 wt%, at least 4 wt%, about 1 wt% to about 50 wt%, about 1 wt% to about 20 wt%, about 1 wt% to about 10 wt%, about 2 wt% to about 10 wt%, about 4 wt% to about 40 wt%, at most 50 wt%, at most 20 wt%, and/or at most 10 wt%. Additionally or alternatively, the aluminum admixture may be incorporated into the bioactive clay to achieve a desired weight percent of aluminum as described herein.

The bioactive clay may comprise silica, which includes silica as a constituent of the aluminum phyllosilicate minerals and/or silicon dioxide minerals such as quartz. The bioactive clay may comprise silica at a level of at least 5 wt%, at least 10 wt%, at least 20 wt%, at least 30 wt%, at least 50 wt%, about 5 wt% to about 60 wt%, about 10 wt% to about 80 wt%, about 10 wt% to about 60 wt%, about 20 wt% to about 95 wt%, about 20% to about 80 wt%, at most 95 wt%, at most 80 wt%, at most 60 wt%, and/or at most 50 wt%.

The bioactive clay may comprise silicon dioxide minerals such as quartz. The bioactive clay may comprise silicon dioxide minerals at a level of at least 1 wt%, at least 2 wt%, at least 5 wt%, at least 10 wt%, at least 20 wt%, about 1 wt% to about 10 wt%, about 1 wt% to about 5 wt%, at most 50 wt%, at most 20 wt%, and/or or at most 10 wt%.

The bioactive clay may have a low pH and/or may buffer the pH in aqueous solutions. The bioactive clay may have higher activity and/or therapeutic effectiveness at lower pH (*e.g.*, at the pH of the stomach). Additionally or alternatively, the bioactive clay may be activated by exposure to an acidic environment and/or a low pH (*e.g.*, a pH of at most 6, 1-6, 2-6, 1-4, and/or 2-4) in aqueous solution and retain higher activity and/or therapeutic effectiveness after sufficient activation at low pH (*e.g.*, an exposure of at least 1 second, at least 1 minute, at least 1 hour, at most 24 hours, at most 6 hours, at most 2 hours, and/or at most 10 minutes). Hence, the bioactive clay may be activated in the stomach of a domesticated animal that ingests the bioactive clay (by the low pH of the stomach contents) and may remain activated as the bioactive clay transitions from the stomach to the higher pH environments of the small and large intestines. In ruminants, the bioactive clay may be inactive in the rumen (which has a relatively high pH and generally beneficial bacteria) and may be activated in the low pH of the true stomach (abomasum).

In some embodiments, the bioactive clay may be dormant or substantially inactive when dry and may become active when hydrated and activated by a low pH aqueous environment. Activation by hydration and/or acidic conditions may transform the bioactive clay into an active state for an extended period of time (*e.g.*, at least 1 hour, at least 4 hours, at least 8 hours or at least 24 hours). Activation also may rely on the presence of reactive oxygen (*e.g.*, molecular oxygen and/or reactive oxygen species).

The intrinsic pH of the bioactive clay is the pH established in an aqueous solution of the bioactive clay (without interference of other acids, bases, or buffers). The bioactive clay may have an intrinsic pH of at most 9, at most 8, at most 7, at most 6, at most 5, at most 4, 1 to 8, 4 to 8, 6 to 8, 2 to 6, and/or 2 to 4. The bioactive clay may be formulated to have a low pH by including acids and/or buffers that have a low pH and/or pKa. Further, the bioactive clay may be pre-activated (also referred to as aged and/or primed) by formulating, selecting, and/or reacting the bioactive clay to have a low pH. For example, a base clay composition with pyrite may be reacted by exposure to water (*e.g.*, humidity) and oxygen to oxidize a portion of the pyrite and create an acidic bioactive clay (having a low pH).

Higher concentrations of iron sulfide may be associated with lower pH bioactive clay. For example, iron sulfide at a level of at least 10 wt%, at least 20 wt%, at least 40 wt%, and/or at least 50 wt% may be (each independently) associated with lower pH such as less than 7, less than 6, less than 5, less than 4, and/or a low pH.

The bioactive clay may be selected and/or formulated to have low calcium and/or low calcium carbonate minerals (*e.g.*, limestone). For example, the bioactive clay may comprise calcium at a level of at most 10 wt%, at most 5 wt%, at most 2 wt%, at most 1 wt%, and/or at most 0.5 wt%. Low calcium and/or low calcium carbonate minerals in the bioactive clay may permit and/or facilitate, relative to a bioactive clay with higher calcium and/or higher calcium carbonate minerals, a low pH bioactive clay, a bioactive clay that activates with less acid, and/or a bioactive clay that remains activated longer.

The bioactive clay may be granulated, *e.g.*, crushed, sieved, ground, milled, and/or formed into granules. For example, the bioactive clay (*e.g.*, rock that sources the clay minerals) may be finely crushed (typically forming granules less than 2,000 μm in diameter), pulverized (typically forming granules less than 100 μm in diameter), and/or jet milled (capable of forming granules less than 1 μm in diameter). The bioactive clay may include granules having a size of at least 25 mesh (less than about 710 μm). Granules may be finer than 50 mesh (about 300 μm), 100 mesh (about 150 μm), 325 mesh (about 44 μm), 500 mesh (about 25 μm), and/or 625 mesh (about 20 μm). Granules may have an average effective diameter of less than 1,000 μm, less than 100 μm, less than 10 μm, less than 1 μm,

less than 0.1 μm, about 500 μm, about 100 μm, about 10 μm, about 1 μm, greater than 0.01 μm, greater than 0.1 μm, greater than 1 μm, greater than 10 μm, and/or greater than 100 μm. The mesh size and/or the granule average effective diameter of the granulated bioactive clay may affect the properties of the bioactive clay. For example, a bioactive clay having a smaller granule size has a greater surface area to volume ratio in comparison to a bioactive clay having a larger granule size. Accordingly, the granule size of the bioactive clay can modify the dosages of the bioactive clay disclosed herein. For example, a bioactive clay having a smaller granule size may be administered at a lower dosage than a bioactive clay having a larger granule size. Additionally or alternatively, smaller granule sizes may be more readily dissolved and/or suspended in solution than larger granule sizes. Larger granule sizes may have more buffering capacity in solution and/or may better act as time release agents for iron and/or aluminum.

As used herein, "domesticated animal" refers to an animal that has been adapted, converted, and/or tamed (*e.g.*, via breeding and/or training) for use by and/or for the benefit of humans. Domesticated animals include agriculturally raised animals, captive-bred animals, and wild-caught animals. Domesticated animals include, but are not limited to, livestock (*e.g.*, ruminants and swine), poultry (*e.g.*, chickens, ducks, geese, and turkeys), equine, and/or animals used in aquaculture (*e.g.*, fish, shellfish, crustaceans, and mollusks).

Methods of improving growth and/or health of domesticated animals may comprise the oral administration of a therapeutically effective dosage and/or amount of the bioactive clay (*e.g.*, a composition that comprises Oregon Blue clay). The bioactive clay may be therapeutically effective based on improved growth (*e.g.*, weight gain) and/or health of the domesticated animal. The therapeutically effective dosage and/or amount may be selected to effect the desired improved growth and/or improved health of the domesticated animal relative to a control animal that does not receive the bioactive clay. The improvement in the health of the domesticated animal may be one or more of an increase in feed intake, an increase in feed conversion ratio, an improvement in fecal consistency, an improvement in coliform count, an improvement in digesta pH, an improvement in one or more colon digesta organic acids, an optimization of organ weight, an optimization of intestinal morphology, and a decrease in morbidity. When administered to the domesticated animal, the bioactive clay may reduce the prevalence and/or burden of bacterial pathogens. Examples of bacterial pathogens that may be affected by the bioactive clay include *E. coli* (*e.g.*, enterotoxigenic *E. coli*), *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Salmonella*, *Clostridium perfringens*, *Pseodomonas aeruginosa*, *Aeromonas salmonicida*, and *Edwardsiella tarda*. When administered to the domesticated animal, the bioactive clay may bind and/or immobilize toxic material (*e.g.*, heavy metals and/or bacteriotoxins) in the

gastrointestinal tract and thereby limit the biological availability and/or toxicity of the toxic materials.

The therapeutically effective dosage of the bioactive clay may be a daily equivalent of about 0.5 milligrams of bioactive clay per kilogram of animal body mass (mg/kg) to about 50 mg/kg, for example, an equivalent daily dosage of about 1 mg/kg to about 30 mg/kg, about 1 mg/kg to about 10 mg/kg, about 3 mg/kg to about 30 mg/kg, about 5 mg/kg to about 45 mg/kg, about 10 mg/kg to about 40 mg/kg, about 15 mg/kg to about 35 mg/kg, about 20 mg/kg to about 30 mg/kg, or about 23 mg/kg to about 27 mg/kg. In absolute amounts, the equivalent daily therapeutically effective amount may be selected according to the animal's body mass. The equivalent daily therapeutically effective amount of bioactive clay may be about 5 mg to about 50 g (grams), about 100 mg to about 10 g, or about 500 mg to about 1 g.

Though dosages and amounts may be expressed as daily dosages and amounts, the bioactive clay may be orally administered once a day, several times a day (*e.g.*, twice daily), and/or on a multiday schedule (*e.g.*, every other day, semiweekly, weekly, biweekly, and/or monthly). Administration of the bioactive clay may be on a regular or irregular schedule. Amounts of bioactive clay administered may be scaled to achieve the desired equivalent daily dosage or amount. For example, an equivalent daily dosage of about 1 mg/kg to about 50 mg/kg of the bioactive clay may be administered as about 7 mg/kg to about 350 mg/kg of the bioactive clay once per week.

The domesticated animal may be a swine, for example, any of a sow, a nursery pig, or a finishing pig. The therapeutically effective amount of the bioactive clay for a sow may be about 0.1 g/day to about 2 g/day, about 0.5 g/day to about 2 g/day, about 0.8 g/day to about 1.7 g/day, or about 1 g/day to about 1.5 g/day. For example, a sow may be administered the equivalent daily amount of 0.5 g/day of 100-mesh bioactive clay. As discussed herein, finer mesh bioactive clays may be administered in smaller amounts. For example, 0.5 g/day of 100-mesh bioactive clay may have the same therapeutic effectiveness as 0.1 g/day of 625-mesh bioactive clay. The therapeutically effective amount of the bioactive clay for a nursery pig may be about 10 mg/day to about 50 mg/day, about 15 mg/day to about 45 mg/day, about 20 mg/day to about 40 mg/day, or about 25 mg/day to about 35 mg/day. The therapeutically effective amount of the bioactive clay for a finishing pig may be about 0.1 g/day to about 1.5 g/day, about 0.3 g/day to about 1.2 g/day, about 0.5 g/day to about 0.9 g/day, or about 0.6 g/day to about 0.8 g/day.

The domesticated animal may be a chicken, for example, a broiler chicken or a layer chicken. The therapeutically effective amount of the bioactive clay for the broiler chicken

and/or the layer chicken may be about 5 mg/day to about 20 mg/day, about 6 mg/day to about 18 mg/day, about 7 mg/day to about 16 mg/day, about 8 mg/day to about 14 mg/day, or about 8.5 mg/day to about 11.5 mg/day.

The domesticated animals may be cattle, for example, a dairy cow or a feeder cow. The therapeutically effective amount for the dairy cow may be about 0.5 g/day to about 20 g/day, about 5 g/day to about 20 g/day, about 0.5 g/day to about 10 g/day, about 0.5 g/day to about 5 g/day, about 8 g/day to about 14 g/day, or about 8.5 g/day to about 11.5 g/day. The therapeutically effective amount per head of feeder cattle may be about 0.1 g/day to about 15 g/day, about 1 g/day to about 15 g/day, about 2 g/day to about 10 g/day, about 3 g/day to about 8 g/day, or about 4 g/day to about 6 g/day.

The domesticated animals may be animals of aquaculture, for example, a fish, a shellfish, a crustacean, or a mollusk. The bioactive clay may be administered orally, for example the bioactive clay may be delivered by feeding (*e.g.*, mixed in aquatic animal feed) or may otherwise be configured for ingestion by the animal of aquaculture (*e.g.*, suspended in water for filter feeding animals such as clams oysters, etc.). The equivalent daily therapeutically effective dosage for oral administration to animals of aquaculture may be at least 0.5 mg/kg, at least 1 mg/kg, about 3 mg/kg to about 30 mg/kg, about 1 mg/kg to about 50 mg/kg, about 5 mg/kg to about 45 mg/kg, about 10 mg/kg to about 40 mg/kg, about 15 mg/kg to about 35 mg/kg, about 20 mg/kg to about 30 mg/kg, at most 50 mg/kg, at most 20 mg/kg, and/or at most 10 mg/kg.

In contrast to livestock and poultry, animals of aquaculture live in water. Water may have varying temperature, pH, salinity, oxygen concentration, mineral content (*e.g.*, softness/hardness), etc. Accordingly, while the bioactive clay may be administered orally to animals of aquaculture, the bioactive clay may also or alternatively be suspended, dissolved, and/or released in the water environment (*e.g.*, forming a bioactive clay solution) in which the animals of aquaculture are immersed. Furthermore, any of the characteristics of the water (*e.g.*, temperature, pH, etc.) may affect the dosage of the bioactive clay and/or the route of administration of the bioactive clay.

Feed conversion ratio is the ratio of feed intake to average daily gain (*i.e.*, feed conversion ratio = feed intake / average daily gain) in an individual domesticated animal. Furthermore, each of the feed intake and the average daily gain are generally measured in grams (g) or kilograms (kg). The feed conversion ratio observed in a domesticated animal that is administered the bioactive clay may be from about 1 to about 3, about 2 to about 4, or about 2.5 to about 4.

Administration of the bioactive clay may improve average daily weight gain in domesticated animals by about 1% to about 20% (*e.g.*, about 1% to about 5%, about 1% to about 10%, about 10% to about 20%). Improvement in average daily weight gain is in comparison to domesticated animals that do not receive the bioactive clay but are otherwise treated the same (these animals may be referred to as control domesticated animals). Administration of the bioactive clay may improve feed efficiency in domesticated animals by about 4% to about 10% in comparison to control domesticated animals. Administration of the bioactive clay may improve feed conversion rate in domesticated animals by about 1% to about 5%. Administration of the bioactive clay may improve average daily feed intake in domesticated animals by about 0.1% to about 2%. Administration of the bioactive clay may decrease the mortality rate in domesticated animals by about 40% to about 60%. Domesticated animals that are administered the bioactive clay may gain from about 1% to about 10% more weight in a lifetime (on average) than domesticated animals that are not administered the bioactive clay.

The improvement in the health of swine during a starting phase that are administered the bioactive clay may be an improvement of from about 10% to about 20% in average daily weight gain in comparison to control swine during the starting phase. The feed conversion ratio of swine during the starting phase that are administered the bioactive clay may also improve by about 4% to about 10% in comparison to control swine during the starting phase. The improvement in the health of swine during a growing phase that are administered the bioactive clay may be an improvement of about 5% to about 15% in average daily weight gain in comparison to control swine during the growing phase. The feed conversion ratio of swine during the growing phase that are administered the bioactive clay may also improve by from about 2% to about 8% in comparison to control swine during the growing phase. The improvement in the health of swine during a growing-finishing phase that are administered the bioactive clay may be an improvement of from about 2% to about 8% in average daily weight gain in comparison to control swine during the growing-finishing phase. The feed conversion ratio of swine during the growing-finishing phase that are administered the bioactive clay may also improve by from about 1% to about 5% in comparison to control swine during the growing phase.

Administration of the bioactive clay may improve average daily weight gain in starter pigs by about 10% to about 20% in comparison to control starter pigs (*i.e.*, starter pigs that do not receive the bioactive clay). Administration of the bioactive clay may improve average daily weight gain in swine by about 1% to about 5% in comparison to control swine. Administration of the bioactive clay may improve feed efficiency in starter pigs by about 4% to about 10% in comparison to control starter pigs. Administration of the bioactive clay may

improve feed conversion rate in swine by about 1% to about 5% in comparison to control swine. Administration of the bioactive clay may improve average daily feed intake in swine by about 0.1% to about 2% in comparison to control swine. Administration of the bioactive clay may decrease the mortality rate in young pigs by about 40% to about 60% in comparison to control pigs.

In a lifetime, an average weight increase of from about 1% to about 10% may be observed in chickens receiving the bioactive clay in comparison to chickens that do not receive the bioactive clay.

Methods of improving growth and/or health of domesticated animals may comprise the oral administration of a feed supplement that comprises the bioactive clay, for example a feed supplement that includes Oregon Blue clay. The feed supplement may be referred to as bioactive feed supplement. The feed supplement may be administered in the dosage, amount, and/or manner described with respect to the bioactive clay. Dosages and amounts may be scaled according to the fraction of the bioactive clay in the feed supplement. For example, a 10 mg/kg daily dosage may be achieved by administration of 20 mg/kg per day of a feed supplement that includes 50% bioactive clay.

The feed supplement may comprise the bioactive clay and one or more adjuncts such as synergistic compounds that enhance the therapeutic effectiveness of the bioactive clay, stabilize the bioactive clay formulation, and/or facilitate handling, mixing, and/or delivery of the bioactive clay. The feed supplement may comprise adjuncts that operate synergistically and/or cooperatively with the soluble iron and/or aluminum of the bioactive clay. For example, adjuncts may comprise acidic compounds to lower the pH of a solution of the feed supplement relative to the bioactive clay. As discussed herein, the bioactive clay may be more effective at lower pH and/or may be activated at low pH. The acidic compounds of the feed supplement may improve the effectiveness of the bioactive clay and/or may facilitate activation of the bioactive clay when ingested by the domesticated animal. Examples of acidic compounds include acids, esters, and/or salts of formic acid (formate), propionic acid (propionate), lactic acid (lactate), butyric acid (butyrate), phosphoric acid (phosphate), phytic acid, and oxalic acid (oxalate).

Adjuncts may comprise enzymes of other compounds that may affect the bioavailability of the bioactive clay (*e.g.*, phytase may increase the availability of inorganic phosphorus and/or may chemically reduce iron). Adjuncts may comprise chelating agents to reduce the effects of metals other than iron, aluminum, and/or other active metals of the bioactive clay (*e.g.*, oxalate may bind calcium). Adjuncts may comprise oligodynamic metals such as zinc, copper, silver, and/or gold. Adjuncts may comprise solubilizing and/or

dispersing agents (*e.g.*, salts, surfactants) that may facilitate effective delivery of the bioactive clay in the feed supplement. Adjuncts may comprise a carrier, such as a tablet, a capsule, microspheres, etc., similar to that used for peroral pharmaceutical delivery.

Another aspect of the disclosure relates to supplemented animal feeds. The supplemented animal feed may include an animal feed (*e.g.*, a concentrate, a roughage, and/or a mixed feed) and the bioactive clay as a supplement (*e.g.*, the feed supplement that includes bioactive clay). The supplemented animal feed may be referred to as the bioactive animal feed and/or the bioactive supplemented animal feed.

The bioactive clay may be administered separately from the animal feed (*e.g.*, the bioactive clay may be administered alone and/or as part of the feed supplement) and/or the bioactive clay may be administered with the animal feed (*e.g.*, the bioactive clay may be mixed with and/or incorporated in the animal feed to form the supplemented animal feed). In the supplemented animal feed, the adjuncts and/or other components of the feed supplement may be incorporated into the animal feed (in addition to or in alternate to incorporating the feed supplement). The bioactive clay may be provided as a suspension in a liquid (*e.g.*, without limitation, milk). Further, the bioactive clay may be provided in a flake, a powder, or a tablet. Other formulations and methods of administering the bioactive clay are also within the scope of this disclosure.

The bioactive clay, the feed supplement, and/or the supplemented animal feed may comprise essential elements for animal health (*e.g.*, copper, zinc, iodine, manganese, and/or selenium). The bioactive clay may comprise one or more essential elements at a level formulated to provide more than the amount necessary and/or recommended for animal nutrition.

An inclusion rate of the bioactive clay in the supplemented animal feed may be about 100 ppm to about 5,000 ppm. The inclusion rate of the bioactive clay is the relative amount of the bioactive clay in the supplemented animal feed and may be expressed as a fraction, *e.g.*, a percentage or ppm. The inclusion rate of the bioactive clay may be referred to as the bioactive clay concentration in the supplemented animal feed. The inclusion rate of the bioactive clay may be about 500 ppm to about 2,000 ppm, about 200 ppm to about 4,000 ppm, about 300 ppm to about 3,000 ppm, or about 1,000 ppm to about 1,500 ppm.

The supplemented animal feed may be formulated for use by a domesticated animal. For example, the supplemented animal feed may be formulated for use by at least one of livestock, poultry, equine, and/or animals of aquaculture. Generally, the animal feed mixed with the bioactive clay and/or that includes the bioactive clay is formulated to satisfy the nutrition requirements of the target domesticated animal. The animal feed may have total

protein at a level of about 1 wt% to about 30 wt%, at least 1 wt%, at least 5 wt%, at most 50 wt%, and/or at most 30 wt%. The animal feed may have total fat at a level of at least 0.1 wt%, at least 0.5 wt%, about 0.1 wt% to about 10 wt%, at most 25 wt%, at most 10 wt%, and/or at most 5 wt%.

The supplemented animal feed may be formulated with low calcium, low calcium carbonate minerals (*e.g.*, limestone), low amounts of high pH buffers (*e.g.*, with a pKa of 6 or greater), and/or low amounts of basic compounds. For example, the supplemented animal feed may comprise limestone at a level of less than 2 wt%, less than 1 wt%, or less than 0.5 wt%.

The supplemented animal feed may be formulated to improve the growth and/or the health of a domesticated animal. The improvement in the growth and/or the health of the domesticated animal may be one or more of an increase in weight gain, an increase in feed intake, an increase in feed conversion ratio, an improvement in fecal consistency, an improvement in coliform count, an improvement in digesta pH, an improvement in one or more colon digesta organic acids, an optimization of organ weight, an optimization of intestinal morphology, and a decrease in morbidity.

Methods of improving growth and/or health of domesticated animals may comprise the oral administration of a therapeutically effective dosage and/or amount of an iron sulfide (*e.g.*, iron pyrite). The iron sulfide may be administered to the domesticated animal at dosages that are a fraction of the dosages disclosed herein with regard to the bioactive clay, for example, from about 0.5% to about 25%, about 5% to about 15%, and/or about 10%. The iron sulfide may be administered in the form of the bioactive clay (*e.g.*, the bioactive clay has an iron sulfide content of about 0.5% to about 25%).

EXAMPLES

Methods of use of bioactive clay and compositions of bioactive clay may be further understood with reference to the following illustrative, non-exclusive examples.

Example 1 - Enterotoxigenic *E. coli* (ETEC) Challenge Test on Weaned Pigs

An ETEC challenge test may be conducted on weaned pigs to evaluate the effects of the oral administration of bioactive clay (*e.g.*, compositions including Oregon Blue clay) on the health of weaned pigs challenged with *E. coli*. The ETEC challenge test may be applied to other types of pigs (*e.g.*, nursery pigs, finishing pigs, and/or sows) and other types of land animals in an analogous manner.

Multiple response criteria (*i.e.*, indicators of weaned pig health) may be measured and assessed in the tested weaned pigs. The response criteria may include: average daily weight gain, average daily feed intake, feed conversion ratio, fecal consistency score, total coliform count (*e.g.*, from ileal mucosa scrapings and colon digesta), *E. coli* K88 count (*e.g.*, from ileal mucosa scrapings and colon digesta), digesta pH (*e.g.*, in the ileum and the colon), colon digesta organic acids (*e.g.*, formic acid, acetic acid, propionic acid, lactic acid, and branched chain volatile fatty acids), organ weight (*e.g.*, of the stomach, small intestine, large intestine, total gastrointestinal tract, spleen, and/or liver), and/or intestinal morphology (*e.g.*, villi height (VH), crypt depth (CD), and VH:CD ratio).

For assessment and comparison of the response criteria, test treatments and control treatments are conducted. For example, the treatments include: a negative control, which receives a basal diet; a positive control, which receives a basal diet and zinc oxide (ZnO), *e.g.*, at 3,000 ppm; a first test treatment, which receives a basal diet and 30 mg/day of the Oregon Blue clay; and a second test treatment, which receives a basal diet and 30 mg/day of an aluminum-enhanced clay mixture of the Oregon Blue clay and an aluminum admixture. The aluminum-enhanced clay mixture has sufficient aluminum admixture to produce a soluble aluminum ion concentration of 10 mM.

The test includes a statistically significant number of pigs. For example, the test may include a total of 60 pigs (4 treatments, 15 replicates per treatment), wherein the pigs weigh about 5 kg. The pigs may be littermate barrows from 15 litters, with 1 pig from each litter in each treatment.

The pigs may be housed individually in pens, *e.g.*, with plastic covered expanded metal flooring. Feed and water is provided *ad libitum* and the room temperature is maintained at a suitable temperature, *e.g.*, at 30.0 °C (± 1 °C). The diets may be in meal form (*e.g.*, from HUBBARD MILLING) and the diets may be sampled at the beginning of the study.

Dietary treatments begin on day 0, wherein the pigs are weighed and the feed added is weighed. On subsequent days (*e.g.*, for some or all of a 14 day course of testing), the pigs and orts (*i.e.*, uneaten scraps or remainders) are weighed. On a selected day (*e.g.*, day 7 of 14) the pigs are orally inoculated with ETEC. Scour scores (*i.e.*, livestock diarrhea scores) may be taken at about the same time of day on days after inoculation (and optionally before inoculation). For example, Scour scores may be collected on days 7, 8, 9, 10, 11, 12, 13, and 14. At the end of the test course (*e.g.*, day 14), the pigs are sacrificed and the gastrointestinal tract and organs are collected.

Data collection during the study may include weighing the pigs at the beginning, at inoculation, and at the end of the test course (*e.g.*, on days 0, 7, and 14), and weighing the feed additions and orts on the inoculation day and the final day. The data may be analyzed using the MIXED PROCEDURE of statistical analysis (SAS Institute, Cary, NC) to evaluate the effects of dietary treatments by Analysis of Variance for this complete randomized design. Individual pigs may serve as the experimental unit. The statistical model may include the fixed effect of dietary treatment. Weaning body weight and gender may be used as covariates in the model if they are significant (*e.g.*, $p < 0.05$). Multiple comparisons among treatments may be performed using the Tukey adjustment option of SAS. All results may be reported as least squares means. The significance level chosen may be $\alpha = 0.05$. For example, treatment effects may be considered significant if $p < 0.05$, whereas values between $0.05 \leq p \leq 0.10$ may be considered statistical trends.

Pigs are initially exposed to dietary treatments for an initial period before inoculation (*e.g.*, 7 days), during which time feed intake and body weight may be monitored to evaluate pre-infection performance. For inoculation, the pigs are orally challenged with *E. coli* K88+ (*i.e.*, ETEC). For some tests, ciprofloxacin-resistant ETEC may be used. The pure ETEC strain may be made resistant to ciprofloxacin by exposing the ETEC strain to increasing doses of ciprofloxacin in Mueller Hinton broth (BECTON, DICKINSON AND COMPANY, Franklin Lakes, NJ) as described by Opapeju, *et al.* (2009 J. Anim. Sci. 87:2635–2643).

Before being used to challenge pigs, the ciprofloxacin-resistant ETEC may be confirmed to be positive for K88 fimbrial antigen, heat labile enterotoxin, and heat stable enterotoxin genes by polymerase chain reaction (PCR) genotyping using published primers (*see* Kotlowski, *et al.* 2007 Gut 56:669–675 and Setia, *et al.* 2009 J. Anim. Sci. 87:2005–2012). Each pig may be orally dosed with 6 mL (2×10^9 colony-forming units (CFU)/mL) of freshly grown ETEC inoculants via a polyethylene tube attached to a syringe placed in the back of the oral cavity. The bacteria-rich solution may then be slowly dribbled into the pig's throat so that the swallowing reflex is triggered and the risk of passage of the inoculant into the lungs is minimized.

To monitor post-challenge growth performance, feeders are emptied and orts and body weight are recorded 2 hours prior to ETEC challenge. Immediately after ETEC challenge, feeders are refilled with each treatment's respective diet. At 7 days post-challenge, all pigs and the uneaten feed may be weighed to evaluate post-challenge performance. Occurrence and severity of post-weaning diarrhea may be monitored and assessed on a pen basis using a fecal consistency scoring system (0 = normal; 1 = soft feces; 2 = mild diarrhea; 3 = severe diarrhea; *see* Marquardt, *et al.* 1999 FEMS Immunol.

Med. Microbiol. 23:283–288) at 24, 48, 72, 96, 120, 144, and 168 hours post-challenge by two trained personnel with no prior knowledge of dietary treatment allotment.

On day 7 post-challenge (*i.e.*, day 14 of the trial), all pigs are euthanized, *e.g.*, as previously described by Kiarie, *et al.* (2007 J. Anim. Sci. 85:2982–2993) and the abdominal cavity may be opened from the sternum to the pubis to expose the total gastrointestinal tract. The spleen and the liver may be removed, washed with cold PBS or saline solution, blotted dry with paper towels, and weighed. The gastrointestinal tract may be separated into 4 segments (*i.e.*, stomach, small intestine, cecum, and colon) using clamps to minimize digesta movement. Intestine samples may be immediately placed on ice to preserve villi integrity. The small intestine may be stripped free of its mesentery and further divided into 2 sections. The ileum may be designated as the intestine from the ileal-cecal junction to 80 cm cranial to this junction, and the remaining portion may be designated as the duodenum and the jejunum. The colon may be further sectioned into the proximal colon (*i.e.*, from the cecal-colonic junction to the apex of the spiral colon) and the distal colon (*i.e.*, from the apex of the spiral colon to the rectum). About 5 cm of ileal tissue may be cleaved, placed in a sterile container, and transferred to a laboratory (*i.e.*, within 30 minutes of collection) for enumeration of bacteria attached to the mucosal scrapings. Another section of ileum may be removed and fixed by immersion in Carnoy's solution (*i.e.*, ethanol, chloroform, and acetic acid; 6:3:1) at 4 °C for histomorphology analysis (*see* Puchtler, *et al.* 1968 Histochemie 16:361–371).

Thereafter, all sections may be emptied of their digesta and weighed. Prior to immersion in Carnoy's solution, intestine samples may be flushed with cold saline solution. Digesta contents of the distal colon may be emptied into 2 separate sterile 50 mL plastic tubes and digesta contents of the ileum may be emptied into 1 sterile 50 mL plastic tube. For the distal colon samples, 1 tube may be placed on ice and immediately transferred to the laboratory for microbial count. The pH may be determined in the ileum digesta and the colon digesta (second tube) using an electronic pH meter (ACCUMET BASIC, FISHER SCIENTIFIC, Fairlawn, NJ). Digesta may be filtered through 4 layers of cheesecloth to remove solid particles. Thereafter, filtered liquid digesta from the distal colon may be stored at -20 °C until used for organic acids analysis.

After 3 hours in Carnoy's solution, samples may be transferred to vials containing 80% ethanol and sent for processing at a commercial laboratory (*e.g.*, Cancer Center Histopathology Core Facility, University of Minnesota). VH from the tip of the villi to the villus-crypt junction and CD from the villus-crypt junction to the base of the crypt may be measured at 10x magnification (AXIOSTAR PLUS microscope; CARL ZEISS MICROSCOPY, Oberkochen, Germany) equipped with a camera (CANON CANADA Inc.,

Mississauga, ON, Canada) and image software (National Institute of Health, Bethesda, MD) in at least 15 well-oriented villus and crypt columns. The VH to CD ratio (VCR) may then be calculated.

Organic acids (*e.g.*, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and lactate) may be assayed using gas chromatography according to Erwin, *et al.* (1961 J. Dairy Sci. 84:1768–1771). Briefly, an aliquot of 2.5 mL of digesta fluid may be mixed with 0.5 mL of 25% meta-phosphoric acid in a centrifuge tube and the mixture may be frozen overnight. Thawed samples may be mixed with 200 µL (microliters) of 25% NaOH and vortexed; followed by addition of 320 µL of 0.3 M (molar) oxalic acid. The samples may then be centrifuged at 3,000 x *g* for 20 minutes at room temperature and the supernatant (2 mL) may be transferred to GLC vials (QORPAK, Chicago, IL). The organic acids may be determined using a glass column packed with 80/120 CARBOPACK B-DA/4% CABOWAX 20M (SUPELCO, Bellefonte, PA) in a gas chromatograph (Model 3400; VARIAN, Inc., Palo Alto, CA).

Ileal segments may be aseptically opened longitudinally and the mucosa scraped from the luminal surface using a sterile glass microscope slide. One gram of ileal mucosal scrapings may be transferred in 15 mL tubes containing beads and 9 mL peptone water (FISHER SCIENTIFIC, Fairlawn, NJ) and vortexed. One gram of digesta from the distal colon may be added to 9 mL of sterile 0.1% peptone water, vortexed for 60 seconds, and then serially diluted 10-fold in sterile peptone water. Total coliform and ETEC K88+ in the serially diluted samples may be quantified using Eosin Methylene Blue agar (BECTON, DICKINSON AND COMPANY) without and with ciprofloxacin (0.5 µg/mL (micrograms per milliliter)), respectively. The tubes may be incubated aerobically at 37 °C for 24 hours to 48 hours.

In comparison to the pigs in the negative control, the weaned pigs in the first test treatment (Oregon Blue clay) may exhibit an improvement in daily weight gain, for example a weight gain improvement of 2-10%. The pigs in the first test treatment may also exhibit an improvement in feed efficiency, for example a feed efficiency improvement of 2-8%. In comparison to the pigs in the negative control, the pigs in the second test treatment (Oregon Blue clay and aluminum admixture) may exhibit an improvement in daily weight gain and/or feed efficiency. And, the pigs in the second test treatment may exhibit an improvement in daily weight gain and/or feed efficiency over the pigs in the first treatment. For example, the pigs in the second treatment may exhibit a weight gain improvement of 5-20% and/or a feed efficiency improvement of 4-10% over the negative control group.

Example 2 - Effects of Clay Administration on Growth of Nursery Pigs

The effects of the oral administration of the bioactive clay on the post-weaning growth performance of nursery pigs may be evaluated following the methods of this example.

A total of about 1,296 nursery pigs (also referred to as weanling pigs) may be utilized. The pigs may be weaned from multiple sow barns but should have the same genetics. 48 pens may be allotted with 27 pigs per pen to one of 4 dietary treatments: a negative control, a low clay test treatment (Oregon Blue clay at a 700 ppm inclusion rate), a high clay test treatment (Oregon Blue clay at a 2,800 ppm inclusion rate), and an aluminum-enhanced test treatment (Oregon Blue clay and aluminum admixture at a combined inclusion rate of 700 ppm). In the aluminum-enhanced test treatment, the aluminum admixture is added at a level to achieve a soluble aluminum ion concentration of 10 mM. This may result in 12 replicates per treatment for evaluation of average daily gain (ADG), average daily feed intake (ADFI), and feed:gain (F:G) data.

A first room may be filled within one day using all available weanling pigs from multiple sow barns. The ratio of pigs from each sow barn may be similar in each pen. Weanling pigs may be sorted to pens (gate cut by weight) with 27 pigs per pen at arrival (day 0). The number of barrows and gilts may be balanced by pen (either 13 barrows or 14 barrows per pen). Non-test pigs (fallbacks) may be removed to hospital pens located in a second room. Pens may be ranked and blocked by weight and by day. Pens within a block may be randomly allotted to one of the 4 treatments resulting in 12 pens per treatment.

Pigs may be fed the experimental diets over a period of 24 days in 2 phases. Specifically, a first ration may be provided at about 4 lb/head for about 10 days immediately after weaning. A second ration may be provided at about 15.5 lb/head for about 14 days. The first ration may be referred to as the 4-15 lb. ration and the second ration may be referred to as the 15-25 lb. ration. Ground feed may be provided through the FEEDLOGIC SYSTEM (FEEDLOGIC Corp., Willmar, MN) allowing collection of feed intake data by pen.

Pigs may be weighed on a pen basis at weaning and when the pigs complete each ration. Feed leftover of each pen may also be measured on the same days when pigs are weighed. Data from the FEEDLOGIC SYSTEM may be saved for every feeding activity. These data may allow the calculation of weekly and overall ADG, ADFI, and F:G of each pen. Feeding may generally be avoided on weigh days to better determine the feed intake for each period. Date and time of measuring feed leftover may be recorded.

The basal diet may be a standard nursery formulation without bentonite, ZnO_2, tribasic copper chloride, or medications (*see* Table 3). The test products (*i.e.*, the Oregon

Blue clay and the Oregon Blue clay/aluminum admixture) may be added on top to replace a corresponding amount of corn.

Table 3

Ingredients	Basal Diet	
	1st Ration (lb.)	2nd Ration (lb.)
Corn	667.00	985.76
Soybean meal, 46%	387.00	539.00
Nursery base	850.00	300.00
Steamed rolled oats	–	50.00
Vitamins and trace minerals	3.00	3.00
Fat	68.40	63.60
Dicalcium/Monocalcium Phosphate	–	20.00
Limestone	–	12.60
Salt	–	6.40
Lysine HCL 78.8%	8.40	8.50
L-Threonine 98.5%	4.40	3.80
DL Methionine	4.40	4.20
L-Tryptophan	1.10	0.70
BETAGRO protein (NUTRIQUEST, Mason City, IA)	6.00	2.50
Total	2000	2000

Blood samples may be taken on day 0 (at weaning) and at the end of Phase 2. One pig per pen, with a total of 12 pigs (6 barrows and 6 gilts) from the 4 dietary treatments, may be selected randomly for blood sampling. These pigs may be ear-tagged so they may be identified for subsequent bleeding. Approximately 5 mL blood samples may be collected using BD serum collection tubes (BECTON, DICKINSON AND COMPANY). After collection,

tubes may be centrifuged at 2000 x *g* for 20 minutes and serum may be separated and transferred to new 1.5 mL tubes. Samples may be stored at -80 °C until future analysis.

Farm attendants may provide daily care of the pigs during the entire experimental period, which may include the following:

1. Daily monitoring of the health and comfort of the pigs by entering into the pens and stirring the pigs in each pen to look for depressed, sick, or injured animals. Pigs may be treated as needed. Any treated pigs should be properly identified and recorded in accordance with standard operating procedure.

2. If sudden death occurs, pig body weight and date may be recorded and the pig may be removed from the pen. Fallback pigs may be identified, weighed, and removed after weighing with their pen.

3. Check feeders and waterers daily in each pen to ensure they are functioning properly.

All feed may be prepared using the base mix from CARGILL (Minnetonka, MN) containing no yeast additives and may be non-medicated.

Multiple response criteria may be measured and compared between the negative control and the test treatments to assess post-weaning growth performance of the nursery pigs. The response criteria may include: average daily weight gain, average daily feed intake, feed conversion ratio, fecal consistency score, total coliform count, digesta pH, colon digesta organic acids, organ weight (*e.g.*, of the stomach, small intestine, large intestine, total gastrointestinal tract, spleen, and/or liver), and/or intestinal morphology (*e.g.*, VH, CD, and VH:CD ratio).

In comparison to the pigs in the negative control, the nursery pigs in the low clay test treatment may exhibit an improvement in daily weight gain, for example a weight gain improvement of 2-10%. The pigs in the low clay test treatment may also exhibit an improvement in feed efficiency, for example a feed efficiency improvement of 2-8%. In comparison to the pigs in the negative control, the pigs in the high clay test treatment may exhibit an improvement in daily weight gain and/or feed efficiency. And, the pigs in the high clay test treatment may exhibit an improvement in daily weight gain and/or feed efficiency over the pigs in the low clay test treatment. For example, the pigs in the high clay test treatment may exhibit a weight gain improvement of 5-20% and/or a feed efficiency improvement of 4-10% over the negative control group. In comparison to the pigs in the negative control, the pigs in the aluminum-enhanced test treatment may exhibit an improvement in daily weight gain and/or feed efficiency. And, the pigs in the aluminum-

enhanced test treatment may exhibit an improvement in daily weight gain and/or feed efficiency over the pigs in the low clay test treatment. The pigs in the aluminum-enhanced test treatment may exhibit similar responses as the pigs in the high clay test treatment. For example, the pigs in the aluminum-enhanced test treatment may exhibit a weight gain improvement of 5-20% and/or a feed efficiency improvement of 4-10% over the negative control group.

Example 3 - Necrotic Enteritis (NE) Test on Broiler Chickens

The effects of the oral administration of the bioactive clay on the health of broiler chickens that have been experimentally induced with NE may be evaluated. For example, one-day-old male broiler chickens (n=800) may be used in this study. The duration of the study may extend from one day of age up to slaughter (*i.e.*, 35 days). The birds may be allotted into 4 equal groups consisting of 200 birds each and assigned into 10 equal replicates of 20 birds. All groups may run contemporaneously. Chickens of groups 1 and 3 may be treated with 10 mg/day of the Oregon Blue clay while groups 2 and 4 may be fed on a plain ration without treatment.

Immunosuppressed chickens may be more likely to develop NE. Methods of inducing immunosuppression may include the use of infectious bursal disease (IBD) vaccine, which has been shown to result in a significant increase in NE lesions (*see* McReynolds, *et al.* 2004 Poultry Sci. 83:1948–1952). Accordingly, vaccination against IBD using 228-E vaccine may be given at the 14th day of age. At the same day of age, birds of groups 1 and 2 may be individually infected by crop gavages with 4×10^8 CFU/mL/bird of *C. perfringens* in phosphate buffered saline (PBS) for 4 successive days (*see* Olkowski, *et al.* 2006 Res Vet Sci. 81:99-108; Gholamiandehkordi, *et al.* 2007 Pathol. 36:375–382; and Timbermont, *et al.* 2009 Comp Immunol Microbiol Infect Dis, 32:503-512). Chickens of groups 3 and 4 may be kept without infection. Strain type A B2 NET B of *C. perfringens* may be isolated from cases of chicken NE. All experimented birds may be vaccinated against different diseases. Chicken groups 1 and 2 (*C. perfringens* challenged) may be kept separately from those of groups 3 and 4 (non-challenged). All chickens may be floor reared in separate pens and kept in environmentally controlled rooms.

Chickens may be fed *ad libitum* a mash commercial starter diet (23% crude protein and 3000 kcal ME/kg diet) during the first 2 weeks of age; a commercial grower diet (22% crude protein and 3150 kcal ME/kg diet) from 2-4 weeks of age; and a commercial finisher diet (19% crude protein and 3200 kcal ME/kg diet) from 4-5 weeks of age. Chickens may have free access to water. No chemotherapy (*e.g.*, antibiotic, anticoccidial, or coccidiostat) may be added during the entire period of the trial.

For measuring intestinal colonization of *C. perfringens*, 10 chickens from the 4 groups may be randomly sacrificed at 14, 21, and 28 days post infection (1 chicken/replicate). Following euthanasia, birds may be necropsied and 0.2 g of intestinal contents from each bird may be serially diluted in sterile PBS to 1:100, 1:1000, and 1:10000, and 0.1 mL of each dilution may be poured on the surface of sheep blood agar plates and tryptose sulfite-cycloserine (TSC) agar (supplemented by D-cycloserine) with egg yolk emulsion. These may be overlaid with the same medium but without egg yolk. After anaerobic incubation at 37 °C for 24 hours, typical *C. perfringens* colonies (black colonies) on TSC agar or large dome-shaped colonies with a double zone of hemolysis on blood agar plates may be counted and reported as CFU per gram. The colonies may be picked and confirmed by the criteria of Harmon (1984 Association of Official Analytical Chemists, Arlington, VA: 1701-1710) and Carrido, *et al.* (2004 Applied and Environmental Microbiology, 70 (9): 5208-5215).

Necropsied chickens at days 21 and 28 may be examined for gross pathological lesion scoring of the small intestine. The scoring system criteria may be the six-point system of Keyburn, *et al.* as modified by Shojadoost, *et al.* (*see* 2006 Infect. Immun. 74:6496–6500 and 2012 Veterinary Research 43:74-86) as follows: 0 = no gross lesions; 1 = thin or friable walls, or diffuse superficial but removable fibrin; 2 = focal necrosis or ulceration, or non-removable fibrin deposit 1 to 5 foci; 3 = focal necrosis or ulceration, or non-removable fibrin deposit 6 to 15 foci; 4 = focal necrosis or ulceration, or non-removable fibrin deposit 16 or more foci; 5 = patches of necrosis 2 to 3 cm long variable; 6 = diffuse necrosis typical of field cases variable, but extensive.

Multiple response criteria may be measured and compared between the treated groups and the non-treated groups to assess the effects of the administration of the Oregon Blue clay on the health of broiler chickens that have been experimentally induced with NE. The response criteria may include: NE lesion scoring, average daily weight gain, average daily feed intake, feed conversion ratio, fecal consistency score, total coliform count, digesta pH, colon digesta organic acids (*e.g.*, formic acid, acetic acid, propionic acid, lactic acid, and branched chain volatile fatty acids), organ weight (*e.g.*, of the stomach, small intestine, large intestine, total gastrointestinal tract, spleen, and/or liver), and/or intestinal morphology (*e.g.*, VH, CD, and VH:CD ratio).

In comparison to the chickens without treatment, the treated chickens may exhibit an improvement in daily weight gain, for example a weight gain improvement of 2-20%. The treated chickens may also exhibit an improvement in feed efficiency, for example a feed efficiency of 2-10%.

Example 4 - Effects of Bioactive Clay on Volatile Fatty Acid (VFA) Production in the Rumen

VFAs are produced through ruminal fermentation and may provide greater than about 70% of a ruminant's energy supply. An *in vitro* test of the impact of the Oregon Blue clay as disclosed herein on VFA production in cattle may be conducted. Four growing Simmental male cattle, with an average live weight of 372 (±6) kg, and fitted with rumen cannulas, may be used as donors of rumen fluid. The daily ration for the cattle may consisted of 6.0 kg rye and 2.0 kg concentrate mixture. In a negative control sample, the concentrate mixture may be composed of 58% corn, 20% soybean meal, 18% wheat bran, 2% calcium hydrogen phosphate, 1% sodium chloride, and 1% trace element mixture. In a test sample, the concentrate mixture may be composed of 1% Oregon Blue clay, 57% corn, 20% soybean meal, 18% wheat bran, 2% calcium hydrogen phosphate, 1% sodium chloride, and 1% trace element mixture.

The negative control sample ration may be divided and fed to two of the cattle. Likewise, the test sample ration may be divided and fed to the other two cattle. Each of the cattle may be fed equally-sized meals at 0700 hours and 1700 hours. Fresh drinking water may be freely available at all times.

The *in vitro* incubation technique of Menke and Steingass (*see* 1988 An. Res. Dev. 28:7-55) may be used for the measurement of acetate, propionate, butyrate, and total VFA production of feed samples. Glass syringes, with a calibrated volume of 100 mL, may be used as the incubation vessels. 200 mL of rumen fluid may be taken from each cattle through the rumen fistulas, 2 hours after feeding in the morning. The rumen fluid from the two control cattle and the two test cattle, respectively, may be mixed and immediately strained through four layers of gauze into a pre-warmed bottle (39 °C). 300 mL of rumen fluid and 600 mL of buffer may be mixed and continuously gassed with carbon dioxide. Each syringe may contain 0.2000 g feed sample and the syringes may be pre-warmed at 39 °C. Four syringes may be used for each feed mixture, as replicates, and three syringes without feed samples may be used as the blanks for each batch of samples.

Each syringe may be filled with 30 mL of rumen fluid-buffer mixture. The air in the syringes may be transpired and the heads of the syringes may be sealed. The syringes may then be kept in a water bath at 39 °C for a 48 hour incubation. At the end of incubation, the pH may be immediately measured. An aliquot of 0.80 mL of incubation liquid may be taken and mixed with 0.20 mL of 25% metaphosphoric acid containing 20 mmol 2-ethyl butyric acid (internal standard). Subsequently, the samples may be centrifuged at 10,000 × *g* for 20 minutes at 4 °C to obtain a clear supernatant for VFA analysis.

In comparison to the negative control cattle, the test cattle (*i.e.*, the cattle receiving the Oregon Blue clay) may exhibit enhanced VFA production in the rumen. Chemical analysis and calculations may be performed according to the methods of Dong and Zhao (*see* 2014 PLoS ONE 9(12):e116290).

Example 5 - Disk Agar Diffusion Test for Antibacterial Susceptibility

The following *in vitro* protocol may be conducted to determine the antibacterial effect of the Oregon Blue clay as disclosed herein on a common aquaculture pathogen, *P. aeruginosa*.

A disk diffusion method may be performed using Mueller-Hinton Agar (MHA). MHA is generally used for routine susceptibility tests because it has good reproducibility; it is low in sulfonamide, trimethoprim, and tetracycline inhibitors; and it gives satisfactory growth of most bacterial pathogens.

The inoculum for the disk diffusion method may be prepared using a suitable broth such as tryptic soy broth. This medium may be prepared according to manufacturer's instructions, dispensed in tubes at 4-5 mL and sterilized. Sterile 0.9% salt solution may also be used. The medium may be supplemented with 1-2% sodium chloride (NaCl) if intended for marine organisms.

The MHA may be prepared from a dehydrated medium according to the manufacturer's instructions. The medium should be prepared using distilled water or deionized water. The hydrated medium mixture may be heated with frequent agitation and boiled to dissolve the medium completely. The prepared medium may be sterilized by autoclaving at 121 °C for 15 minutes. The pH of each preparation after it is sterilized should be between 7.2 and 7.4 at room temperature. The pH may be checked by macerating a small amount of prepared medium in a little distilled water or by allowing a little amount of medium to gel around a pH meter electrode. The agar medium may be allowed to cool to about 40-50 °C. The agar may be poured into sterile glass or plastic petri dishes on a flat surface to a uniform depth of 4 mm and allowed to solidify.

Prior to use, the plates (i.e., the prepared agar medium in the petri dish) may be dried at 30-37 °C in an incubator, with lids partly ajar, for not more than 30 minutes or until excess surface moisture has evaporated. The medium should be moist but free of water droplets on the surface. Presence of water droplets may result in swarming bacterial growth, which could give inaccurate results. Such plates are also easily contaminated.

From a pure *Pseudomonas aeruginosa* bacterial culture (not more than 48 hours old, except for slow growing organisms), four or five colonies may be taken with a wire loop. The

colonies may be transferred to 5 mL of Trypticase soy broth or 0.9% saline to form a bacterial broth. The bacterial broth may be incubated at 30 °C or at an optimum growth temperature until it achieves or exceeds the turbidity of 0.5 MacFarland standard (prepared by adding 0.5 mL of 0.048 M $BaCl_2$ to 99.5 mL of 0.36 NH_2SO_4; commercially available).

The turbidity of the bacterial broth may be compared with that of 0.5 MacFarland (vigorously shaken before use) against a white background with contrasting black line under adequate light. The turbidity may be reduced to 0.5 MacFarland by adding sterile saline or broth to produce a standardized bacterial suspension.

A sterile cotton swab may be dipped into the standardized bacterial suspension. Excess inoculum may be removed by lightly pressing the swab against the tube wall at a level above that of the liquid. The prepared agar plate may be inoculated by streaking with the swab containing the inoculum. The plate may be rotated by 60° and the streaking procedure may be repeated two times. This may ensure an even distribution of the inoculum. The surface of the medium may be dried for 3-5 minutes, but not longer than 15 minutes, to allow for absorption of excess moisture.

One or more disks may be prepared, which comprise the Oregon Blue clay. The disk diameter may be about 6 mm. Using sterile forceps or disk dispenser, each disk comprising Oregon Blue clay may be placed on the surface of the inoculated and dried plate. When a disk is placed, the disk may be immediately pressed down lightly with a suitable instrument to ensure complete contact between the disk and the agar surface. Disks will not be moved after coming into contact with the agar surface since some diffusion of the Oregon Blue clay (or components of the Oregon Blue clay) may occur instantaneously. The disks may be positioned such that the minimum center distance between disks is 24 mm and no disk is closer than 10 to 15 mm from the edge of the petri dish. A maximum of six disks will be placed in a 9-cm petri dish and 12 disks on a 150 mm plate. The number of disks applied per plate may be reduced if overlapping zones of inhibition are encountered.

One plate inoculated with a control strain may be included for every set of plates and the plates may be incubated together. Observation of the zone of inhibition may be conducted after 16-18 hours. Slow growing organisms may require a longer incubation period. The zone of inhibition is the point at which no growth is visible to the unaided eye. The presence of individual colonies within zones of inhibition may be recorded. Occurrence of “fuzzy” zones may also be recorded. In measuring the zone diameter, the fuzzy portion of the zone should be ignored as much as possible. The zone limit is the inner limit of the zone of normal growth.

The diameter of the zones of inhibition may be read using a ruler graduated to 0.5 mm. The zone diameter measurement may be rounded up to the nearest millimeter. The diameter of the zone of inhibition of the test isolates may be compared with those in an available chart of interpretative standard for veterinary pathogens (see Tendencia, E. A. (2004). Chapter 2. Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment (pp. 13–29). Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department).

The results may be reported as resistant (R), intermediate (I), or susceptible (S). The following results will not be read: plates on which growth of test bacteria have isolated colonies or less than semi-confluent growth; zones of inhibition of two adjacent disks that overlap, to the extent that measurement of the zone diameter cannot be made; and zones showing distortion from circular. All data collected in a particular set will be rejected if the zones of inhibition produced on plate inoculated with a control strain are not within the tolerance limits set.

Example 6 - *In vivo* Analysis of Bioactive Clay in Aquaculture

The effect of the bioactive clay on loaches (*Misgurnus anguillcaudatus*) exposed to *E. tarda* may be assessed. A test group of loaches may be fed an animal feed comprising the Oregon Blue clay at an inclusion rate of 500 ppm. A control group may be fed an animal feed lacking the Oregon Blue clay. *E. tarda* may be suspended in tap water at 1 x 10^8 CFU/mL. Loaches in the test group and the control group may be immersed in the *E. tarda* suspension for one hour and subsequently monitored for mortalities (*e.g.*, during the 7 day period following exposure to *E. tarda*). The 7-day mortality of the test loaches may be 0-40% and the 7-day mortality of the control loaches may be 90-100%. Increased survival of the loaches in the test group will indicate the protective effects of the bioactive clay. Accordingly, the Oregon Blue clay may be useful in reducing or preventing *E. tarda* infection and/or promoting growth and/or health in loaches.

Examples of inventive subject matter according to the present disclosure are described in the following enumerated paragraphs.

A1. A bioactive clay comprising:

about 1 wt% to about 70 wt% of iron sulfide; and

aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM.

A2. The bioactive clay of paragraph A1, wherein the iron sulfide comprises iron(II).

A3. The bioactive clay of any of paragraphs A1-A2, wherein the iron sulfide comprises pyrite.

A4. The bioactive clay of any of paragraphs A1-A3, wherein the bioactive clay comprises at least 2 wt%, at least 5 wt%, at least 8 wt%, at least 15 wt%, about 1 wt% to about 20 wt%, about 20 wt% to about 70 wt%, about 10 wt% to about 50 wt%, about 20 wt% to about 50 wt%, at most 60 wt%, at most 50 wt%, and/or at most 40 wt% of iron sulfide.

A5. The bioactive clay of any of paragraphs A1-A4, wherein the bioactive clay further comprises about 10 wt% to about 95 wt% of aluminum phyllosilicate clay.

A5.1. The bioactive clay of paragraph A5, wherein the aluminum phyllosilicate clay comprises one or more of kaolinite, illite, muscovite, chlorite, rectorite, smectite, montmorillonite, and pyrophyllite.

A5.1.1. The bioactive clay of paragraph A5.1, wherein the aluminum phyllosilicate clay comprises one or more of illite, smectite, and rectorite.

A5.1.2. The bioactive clay of any of paragraphs A5.1-A5.1.1, wherein the aluminum phyllosilicate clay is rectorite.

A5.2. The bioactive clay of any of paragraphs A5-A5.1.2, wherein the bioactive clay comprises at least 20 wt%, about 10 wt% to about 60 wt%, about 20 wt% to about 40 wt%, about 20 wt% to about 60 wt%, at most 80 wt%, and/or at most 60 wt% of aluminum phyllosilicate clay.

A6. The bioactive clay of any of paragraphs A1-A5.2, wherein the bioactive clay further comprises about 5 wt% to about 50 wt% of silica.

A6.1. The bioactive clay of paragraph A6, wherein the silica is a constituent of aluminum phyllosilicate clay.

A6.2. The bioactive clay of any of paragraphs A6-A6.1, wherein the bioactive clay comprises at least 10 wt%, at least 20 wt%, about 5 wt% to about 50 wt%, about 10 wt% to about 40 wt%, about 20 wt% to about 40 wt%, at most 60 wt%, and/or at most 50 wt% of silica.

A7. The bioactive clay of any of paragraphs A1-A6.2, wherein the bioactive clay further comprises an aluminum admixture of one or more aluminum compounds selected from the group consisting of alumina, gibbsite, an aluminum hydroxide mineral, and an aluminum salt.

A7.1. The bioactive clay of paragraph A7, wherein the aluminum admixture comprises one or more of gibbsite, an aluminum hydroxide mineral, and an aluminum salt, and optionally wherein the aluminum admixture is gibbsite.

A7.2. The bioactive clay of any of paragraphs A7-A7.1, wherein the bioactive clay comprises at least 1 wt%, at least 2 wt%, about 1 wt% to about 30 wt%, about 1 wt% to

about 20 wt%, about 1 wt% to about 10 wt%, at most 30 wt%, at most 20 wt%, and/or at most 10 wt% of the aluminum admixture.

A8. The bioactive clay of any of paragraphs A1-A7.2, wherein the weight percent of aluminum is formulated to produce a soluble aluminum ion concentration of at least 2 mM, at least 4 mM, about 1 mM to about 20 mM, about 1 mM to about 10 mM, about 2 mM to about 8 mM, about 4 mM to about 6 mM, about 6 mM to about 10 mM, at most 20 mM, and/or at most 10 mM.

A9. The bioactive clay of any of paragraphs A1-A8, wherein the bioactive clay has a pH of at most 8, at most 7, at most 6, about 2 to about 4, about 2 to about 6, and/or about 2 to about 8.

A10. The bioactive clay of any of paragraphs A1-A9, wherein the bioactive clay is granulated.

A10.1. The bioactive clay of paragraph A10, wherein the bioactive clay has granules with a mesh size of 25 to 625, 50 to 500, and/or 100 to 325.

A11. The bioactive clay of any of paragraphs A1-A10.1, wherein the bioactive clay has granules with an average effective diameter less than 1,000 μm, less than 100 μm, less than 10 μm, less than 1 μm, less than 0.1 μm, about 500 μm, about 100 μm, about 10 μm, about 1 μm, greater than 0.01 μm, greater than 0.1 μm, greater than 1 μm, greater than 10 μm, and/or greater than 100 μm.

A12. The use of the bioactive clay of any of paragraphs A1-A11 to at least one of improve growth of a domesticated animal and improve health of the domesticated animal.

B1. A supplemented animal feed, comprising:

an animal feed; and

the bioactive clay of any of paragraphs A1-A11;

wherein an inclusion rate of the bioactive clay in the supplemented animal feed is about 100 ppm (parts per million) to about 5,000 ppm, about 500 ppm to about 2,000 ppm, about 200 ppm to about 4,000 ppm, about 300 ppm to about 3,000 ppm, and/or about 1,000 ppm to about 1,500 ppm.

B2. The supplemented animal feed of paragraph B1, wherein the supplemented animal feed is formulated to be orally administered to a domesticated animal to achieve a daily equivalent dosage of the bioactive clay of about 0.5 mg to about 50 mg, about 1 mg to about 30 mg, about 1 mg to about 10 mg, about 3 mg to about 30 mg, or about 10 mg to about 40 mg, per kilogram of body mass of the domesticated animal.

B3. The supplemented animal feed of any of paragraphs B1-B2, wherein the animal feed is formulated to feed a/the domesticated animal.

B3.1. The supplemented animal feed of paragraph B3, wherein the domesticated animal is selected from the group consisting of a ruminant, a cow, a pig, an equine, poultry,

a bird, a chicken, a duck, a goose, a turkey, an animal of aquaculture, a fish, a shellfish, a crustacean, and a mollusk.

B4. The supplemented animal feed of any of paragraphs B1-B3.1, wherein the animal feed comprises total protein at a level of about 1 wt% to about 50 wt%.

B5. The supplemented animal feed of any of paragraphs B1-B4, wherein the animal feed comprises total fat at a level of about 0.1 wt% to about 10 wt%.

B6. The use of the supplemented animal feed of any of paragraphs B1-B5 to at least one of feed a/the domesticated animal, improve growth of the domesticated animal, and improve health of the domesticated animal.

C1. A method of caring for a domesticated animal, comprising:

administering orally to the domesticated animal a therapeutically effective quantity of a bioactive clay comprising about 1 wt% to about 40 wt% of iron sulfide and aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM.

C2. The method of paragraph C1, wherein the bioactive clay is the bioactive clay of any of paragraphs A1-A11.

C3. The method of any of paragraphs C1-C2, wherein administering includes administering the bioactive clay in the form of the supplemented animal feed of any of paragraphs B1-B5.

C4. The method of any of paragraphs C1-C3, wherein the therapeutically effective quantity is a quantity configured to cause an improvement in health of the domesticated animal and wherein the improvement in health is one or more of an increase in feed intake, an increase in feed conversion ratio, an improvement in fecal consistency, an improvement in coliform count, an improvement in digesta pH, an improvement in one or more colon digesta organic acids, an optimization of organ weight, an optimization of intestinal morphology, and a decrease in morbidity.

C4.1. The method of paragraph C4, wherein the improvement in health includes an increase in feed efficiency of at least 4%

C4.2. The method of any of paragraphs C4-C4.1, wherein the improvement in health includes an increase in feed conversion ratio of at least 1%.

C4.3. The method of any of paragraphs C4-C4.2, wherein the improvement in health includes a decrease in mortality rate of at least 40%.

C5. The method of any of paragraphs C1-C4.3, wherein the therapeutically effective quantity is a quantity configured to cause an improvement in growth of the domesticated animal.

C5.1. The method of paragraph C5, wherein the improvement in growth is an increase in average daily weight gain of at least 5%.

C6. The method of any of paragraphs C1-C5.1, wherein the therapeutically effective quantity of the bioactive clay is a therapeutically effective amount of about 0.5 mg per day to about 50 g per day.

C7. The method of any of paragraphs C1-C6, wherein the therapeutically effective quantity of the bioactive clay is a therapeutically effective dosage of about 0.5 mg per day to about 50 mg per day, per kilogram of body mass of the domesticated animal.

C8. The method of any of paragraphs C1-C7, wherein the bioactive clay is activated in a stomach of the domesticated animal by the domesticated animal ingesting the bioactive clay.

C9. The method of any of paragraphs C1-C8, wherein the domesticated animal is selected from the group consisting of a ruminant, a cow, a pig, an equine, a bird, a chicken, a duck, a goose, a turkey, a fish, a shellfish, a crustacean, and a mollusk.

C10. The method of any of paragraphs C1-C9, wherein the domesticated animal is a sow and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 0.1 g to about 2 g, about 0.5 g to about 2 g, or about 1 g to about 1.5 g.

C11. The method of any of paragraphs C1-C10, wherein the domesticated animal is a nursery pig and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 10 mg to about 50 mg, about 15 mg to about 45 mg, or about 20 mg to about 40 mg.

C12. The method of any of paragraphs C1-C11, wherein the domesticated animal is a finishing pig and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 0.1 g to about 1.5 g, about 0.3 g to about 1.2 g, or about 0.5 g to about 0.9 g.

C13. The method of any of paragraphs C1-C12, wherein the domesticated animal is a chicken and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 5 mg to about 20 mg, about 6 mg to about 18 mg, about 7 mg to about 16 mg, or about 8 mg to about 14 mg.

C14. The method of any of paragraphs C1-C13, wherein the domesticated animal is a dairy cow and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 0.5 g to about 20 g, about 5 g to about 20 g, about 0.5 g to about 10 g, about 0.5 g to about 5 g, or about 8 g to about 14 g.

C15. The method of any of paragraphs C1-C14, wherein the domesticated animal is a feeder cow and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily amount of about 0.1 g to about 15 g, about 1 g to about 15 g, about 2 g to about 10 g, about 3 g to about 8 g, or about 4 g to about 6 g.

C16. The method of any of paragraphs C1-C15, wherein the domesticated animal is an animal of aquaculture and wherein the therapeutically effective quantity of the bioactive clay is an equivalent daily dosage of at least 0.5 mg, at least 1 mg, about 3 mg to about 30 mg, about 1 mg to about 50 mg, about 5 mg to about 45 mg, about 10 mg to about 40 mg, at most 50 mg, and/or at most 20 mg, per kilogram of body mass of the animal of aquaculture.

C16.1. The method of paragraph C16, wherein the animal of aquaculture is selected from the group consisting of a fish, a shellfish, a crustacean, and a mollusk.

C17. The method of any of paragraphs C1-C16.1, wherein the domesticated animal is an/the animal of aquaculture that is a filter feeder and wherein administering includes suspending the bioactive clay in a water environment in which the animal of aquaculture is immersed such that the animal of aquaculture ingests the bioactive clay.

As used herein, the terms "adapted" and "configured" mean that the element, component, or other subject matter is designed and/or intended to perform a given function. Thus, the use of the terms "adapted" and "configured" should not be construed to mean that a given element, component, or other subject matter is simply "capable of" performing a given function but that the element, component, and/or other subject matter is specifically selected, created, implemented, utilized, programmed, and/or designed for the purpose of performing the function. It is also within the scope of the present disclosure that elements, components, and/or other recited subject matter that is recited as being adapted to perform a particular function may additionally or alternatively be described as being configured to perform that function, and vice versa. Similarly, subject matter that is recited as being configured to perform a particular function may additionally or alternatively be described as being operative to perform that function.

As used herein, the phrase, "for example," the phrase, "as an example," and/or simply the term "example," when used with reference to one or more components, features, details, structures, embodiments, and/or methods according to the present disclosure, are intended to convey that the described component, feature, detail, structure, embodiment, and/or method is an illustrative, non-exclusive example of components, features, details, structures, embodiments, and/or methods according to the present disclosure. Thus, the described component, feature, detail, structure, embodiment, and/or method is not intended to be limiting, required, or exclusive/exhaustive; and other components, features, details, structures, embodiments, and/or methods, including structurally and/or functionally similar and/or equivalent components, features, details, structures, embodiments, and/or methods, are also within the scope of the present disclosure.

It will be readily understood that the embodiments, as generally described herein, are exemplary. The following more detailed description of various embodiments is not intended to limit the scope of the present disclosure, but is merely representative of various embodiments. Moreover, the order of the steps or actions of the methods disclosed herein may be changed by those skilled in the art without departing from the scope of the present disclosure. In other words, unless a specific order of steps or actions is required for proper operation of the embodiment, the order or use of specific steps or actions may be modified.

As used herein, the phrases "at least one of" and "one or more of," in reference to a list of more than one entity, means any one or more of the entities in the list of entities, and is not limited to at least one of each and every entity specifically listed within the list of entities. For example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently, "at least one of A and/or B") may refer to A alone, B alone, or the combination of A and B.

As used herein, the singular forms "a", "an" and "the" may be intended to include the plural forms as well, unless the context clearly indicates otherwise.

The various disclosed elements of systems and steps of methods disclosed herein are not required of all systems and methods according to the present disclosure, and the present disclosure includes all novel and non-obvious combinations and subcombinations of the various elements and steps disclosed herein. Moreover, any of the various elements and steps, or any combination of the various elements and/or steps, disclosed herein may define independent inventive subject matter that is separate and apart from the whole of a disclosed system or method. Accordingly, such inventive subject matter is not required to be associated with the specific systems and methods that are expressly disclosed herein, and such inventive subject matter may find utility in systems and/or methods that are not expressly disclosed herein.

INDUSTRIAL APPLICABILITY

The clay-based materials and methods disclosed herein are applicable to agricultural industries.

It is believed that the following claims particularly point out certain combinations and subcombinations that are directed to one of the disclosed inventions and are novel and non-obvious. Inventions embodied in other combinations and subcombinations of features, functions, elements and/or properties may be claimed through amendment of the present claims or presentation of new claims in this or a related application. Such amended or new claims, whether they are directed to a different invention or directed to the same invention,

whether different, broader, narrower, or equal in scope to the original claims, are also regarded as included within the subject matter of the inventions of the present disclosure.

CLAIMS

1. A method of improving growth of a domesticated animal, comprising:

administering orally to the domesticated animal a therapeutically effective dosage of a bioactive clay comprising about 1 wt% (weight percent) to about 50 wt% of iron sulfide and aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM (millimolar);

wherein the therapeutically effective dosage is about 0.5 mg (milligrams) per day to about 50 mg per day, per kilogram of body mass of the domesticated animal.

2. The method of claim 1, wherein the therapeutically effective dosage is selected to effect an increase in average daily weight gain of the domesticated animal of at least 5% relative to a control domesticated animal that does not receive the bioactive clay.

3. The method of any one of claims 1 or 2, wherein administering comprises orally administering the bioactive clay to the domesticated animal selected from the group consisting of a ruminant, a swine, an equine, poultry, and an animal of aquaculture.

4. The method of any one of claims 1-3, wherein the domesticated animal is a nursery pig and wherein administering comprises administering to the nursery pig an equivalent daily amount of the bioactive clay of about 10 mg to about 50 mg.

5. The method of any one of claims 1-3, wherein the domesticated animal is a finishing pig and wherein administering comprises administering to the finishing pig an equivalent daily amount of the bioactive clay of about 0.1 g to about 1.5 g.

6. The method of any one of claims 1-3, wherein the domesticated animal is a chicken and wherein administering comprises administering to the chicken an equivalent daily amount of the bioactive clay of about 5 mg to about 20 mg.

7. The method of any one of claims 1-3, wherein the domesticated animal is a feeder cow and wherein administering comprises administering to the feeder cow an equivalent daily amount of the bioactive clay of about 0.1 g to about 15 g.

8. The method of any one of claims 1-3, wherein the domesticated animal is an animal of aquaculture and wherein administering comprises administering to the animal of

aquaculture an equivalent daily dosage of about 3 mg to about 30 mg, per kilogram of body mass of the animal of aquaculture.

9. The method of any one of claims 1-8, wherein the bioactive clay further comprises about 10 wt% to about 80 wt% of aluminum phyllosilicate clay.

10. The method of any one of claims 1-9, wherein the bioactive clay is granulated and has granules with an average effective diameter less than 100 μm.

11. A method of improving health of a domesticated animal, comprising:

administering to the domesticated animal a therapeutically effective dosage of a bioactive clay comprising about 1 wt% to about 50 wt% of iron sulfide and aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM;

wherein the therapeutically effective dosage is about 0.5 mg per day to about 50 mg per day, per kilogram of body mass of the domesticated animal.

12. The method of claim 11, wherein the domesticated animal is a sow and wherein administering comprises administering to the sow an equivalent daily amount of the bioactive clay of about 0.1 g to about 2 g.

13. The method of claim 11, wherein the domesticated animal is a chicken and wherein administering comprises administering to the chicken an equivalent daily amount of the bioactive clay of about 5 mg to about 20 mg.

14. The method of claim 11, wherein the domesticated animal is a dairy cow and wherein administering comprises administering to the dairy cow an equivalent daily amount of the bioactive clay of about 5 g to about 20 g.

15. A supplemented animal feed, comprising:

an animal feed for a domesticated animal; and

a bioactive clay comprising:

about 1 wt% to about 50 wt% of iron sulfide; and

aluminum at a weight percent to produce a soluble aluminum ion concentration of at least 1 mM;

wherein an inclusion rate of the bioactive clay in the supplemented animal feed is about 100 ppm (parts per million) to about 5,000 ppm.

16. The supplemented animal feed of claim 15, wherein the supplemented animal feed is formulated to be administered to the domesticated animal to achieve a daily equivalent dosage of the bioactive clay of about 0.5 mg to about 50 mg, per kilogram of body mass of the domesticated animal.

17. The supplemented animal feed of any one of claims 15 or 16, wherein the iron sulfide comprises iron(II).

18. The supplemented animal feed of any one of claims 15-17, wherein the bioactive clay further comprises about 10 wt% to about 80 wt% of aluminum phyllosilicate clay.

19. The supplemented animal feed of claim 18, wherein the aluminum phyllosilicate clay is rectorite.

20. The supplemented animal feed of any one of claims 15-19, wherein the bioactive clay further comprises about 20 wt% to about 80 wt% of silica.

21. The supplemented animal feed of any one of claims 15-20, wherein the bioactive clay has a pH of about 6 to about 8.

22. The supplemented animal feed of any one of claims 15-20, wherein the bioactive clay has a pH of about 2 to about 6.

23. The supplemented animal feed of any one of claims 15-22, wherein the bioactive clay comprises about 20 wt% to about 50 wt% of iron sulfide.

24. The supplemented animal feed of any one of claims 15-23, wherein the weight percent of aluminum is formulated to produce a soluble aluminum ion concentration of at least 4 mM and at most 10 mM.

25. The supplemented animal feed of any one of claims 15-24, wherein the animal feed comprises total protein at a level of about 1 wt% to about 30 wt% and comprises total fat at a level of about 0.1 wt% to about 10 wt%.

26. The supplemented animal feed of any one of claims 15-25, wherein the bioactive clay is granulated with a mesh size of at least 50.

27. The supplemented animal feed of claim 26, wherein the bioactive clay has granules with an average effective diameter less than 300 μm and greater than 50 μm.

28. The supplemented animal feed of claim 26, wherein the bioactive clay has granules with an average effective diameter less than 10 μm and greater than 0.05 μm.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/25587

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23K 1/175 (2017.01)
CPC - A23K 20/28, A23K 50/30

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 2014/0134290 A1 (BAMFORD et al.) 15 May 2014 (15.05.2014); para [0011-0013] [0015-0016] [0022-0023] [0029] [0032] [0036] [0050] [0054-0055]	1, 3, 11-17 ---------- 2
Y	WO 2015/107218 A1 (PALM et al.) 23 July 2015 (23.07.2015); Fig. 6, page 1 para 5, page 2 para 3, page 7 para 7	2
A	US 2009/0155380 A1 (HAMON et al.) 18 June 2009 (18.06.2009); entire document	1-3, 11-17
A	US 3,687,680 A (KRCHNAVI et al.) 29 August 1972 (29.08.1972); entire document	1-3, 11-17
A	US 2014/0044834 A1 (ORTIZ NIEMBRO et al.) 13 February 2014 (13.02.2014); entire document	1-3, 11-17
A	US 5,192,547 A (TAYLOR) 9 March 1993 (09.03.1993); entire document	1-3, 11-17
A	US 5,935,623 A (ALONSO-DEBOLT) 10 August 1999 (10.08.1999); entire document	1-3, 11-17

☐ 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	Date of mailing of the international search report
5 June 2017	27 JUN 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/25587

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.: 4-10, 18-28
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.