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- (71) Applicants: ANATARA LIFESCIENCES LIMITED [AU/AU]; 433 Logan Road, Stones Corner, Brisbane, QLD 4120 (AU). PHARMACEUTICAL PATENT ATTORNEYS, LLC [US/US]; 55 Madison Avenue, 4th Floor, Morristown, NJ 07960 (US).
- (72) Inventors: MYNOTT, Tracey, L.; 433 Logan Road, Stones Corner, Brisbane, QLD 4120 (AU). WALSH, John; Sidney (AU).
- (74) Agent: POHL, J. Mark; Pharmaceutical Patent Attorneys, LLC, 55 Madison Ave, 4th Floor, Morristown, NJ 07960 (US).

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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
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[Continued on next page]

(54) Title: ANTI-DIARRHEA FORMULATION WHICH AVOIDS ANTIMICROBIAL RESISTANCE

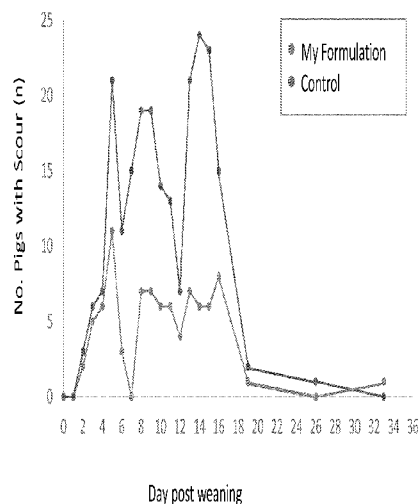


Figure 3 - One group of piglets was administered a single oral dose (4 mL) of My formulation at weaning (day 0), or were untreated (Control). My formulation significantly reduced the incidence of scour (diarrhoea) ($P < 0.05$).

(57) Abstract: An oral formulation of bromelain effective to treat and prevent diarrhea caused by pathogenic microbes. This formulation does not kill pathogenic microbes, and thus does not facilitate the proliferation of anti-microbial resistant organisms. The invention entails formulating an aqueous oral suspension of bromelain, Blanose sodium carboxymethyl cellulose, citric acid anhydrous, Epikuron 135F lecithin oil and ethylenedinitrietetraacetic acid, disodium salt dihydrate ("EDTA").

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ANTI-DIARRHEA FORMULATION WHICH AVOIDS ANTIMICROBIAL RESISTANCE

1

2 Government Interest:

3 None

4 Related Applications:

5 This application claims priority from United States provisional patent filing Serial No.
6 62/041175, filed 25 August 2014, the contents of which are hereby incorporated by reference.

7 Background:

8 Diarrhea is a problem in pigs and people. Scour (diarrhea) in piglets is one of the most
9 common problems in pig farming affecting hundreds of millions of the 1,600 million piglets born
10 globally each year. It can result in reduced weight gain, high cost of treatment, and frequent
11 mortality. Piglets set back in health at an early age, tend to remain at a weight and performance
12 disadvantage in later life. Therefore, scours not only has a detrimental impact on piglet health,
13 but on farm profitability.

14 Diarrhea is also a significant problem in humans. It is the second cause of death in the
15 developing world, killing more than 1.5 million children annually and is a leading cause of
16 malnutrition. About 4 billion cases of diarrhea are estimated to occur every year among children
17 under five years. Diarrhea is also a significant problem for Traveller's to developing areas,
18 affecting 40 million people annually, and is a major problem for military personnel during
19 operations.

20 There are many different causes of diarrhea. Enterotoxigenic Escherichia coli (ETEC) is
21 one of the most common causes of scour in un-weaned and just-weaned piglets. Other important
22 pathogens include rotavirus and coccidiosis caused by the protozoan Isospora suis and affects
23 mainly piglets in the first three weeks of life. High mortality due to coccidiosis is observed in
24 co-infections with ETEC.

25 ETEC is also one of the most common causes of diarrhea in young children in developing
26 countries, and is associated with a higher risk of death than other diarrhea-causing pathogens.
27 ETEC are also the most prevalent cause of diarrhea in Traveler's to developing areas with attack
28 rates as high as 70% in some instances. Other important pathogens affecting children under five
29 in developing countries include rotavirus, Cryptosporidium, Shigella, Aeromonas, Vibrio
30 cholerae O1 and Campylobacter. Enteroaggregative E. coli (EAEC), Shigella, Campylobacter,
31 Salmonella, Aeromonas, Plesiomonas, and noncholera Vibrios are other important causes of
32 Traveler's Diarrhea.

33 These same above pathogens also cause diarrhea in the USA, as well as
34 enterohaemorrhagic E. coli (EHEC, or Shiga toxin-producing E. coli, STEC), Listeria, Yersinia,
35 Cyclospora, Giardia, calciviruses, and other enteric viruses. Clostridium difficile is the major
36 cause of hospital acquired infections. Collectively, these agents are responsible for greater than
37 200 million cases of diarrhea in the USA each year. In many cases, an infectious agent is not
38 always responsible. For example, in HIV affected patients and cancer patients, diarrhea may

1 result as a chronic side effect of chemotherapy. Diarrhea may also be a result of hormonal
2 imbalances induced by endocrine tumours, diabetes or chronic inflammation, as in patients with
3 inflammatory bowel disease.

4 All diarrhea-causing pathogens differ markedly in the clinical syndromes they induce,
5 mechanisms of pathogenesis, virulence and epidemiology. Some pathogens, such as ETEC, are
6 non-invasive and induce diarrhea by attaching via specific adherence factors to enterocyte
7 receptors on the small intestine and by the production of enterotoxins. Other pathogens, such as
8 *Shigella*, are invasive and alter intestinal barrier function and induce diarrhea by inducing
9 inflammation or loss of absorptive surface and malabsorption. This diversity poses an enormous
10 task for researchers who are attempting to design simple and effective prophylaxis or treatment
11 against all causes of diarrhea.

12 Despite the diversity of pathogens and multitude of factors that induce diarrhea, bacterial
13 toxins or inflammatory mediators induce the most common cause of diarrhea. Toxins typically
14 trigger signaling molecules such as cyclic AMP, cyclic GMP or intracellular Ca^{2+} , which, in turn
15 activate intestinal chloride (Cl^-) channels leading to an increase in secretion of Cl^- and
16 consequently fluid secretion. When the level of fluid secretion increases beyond the ability of
17 the colon to reabsorb water and electrolytes lost from the small intestine, diarrhea results that can
18 lead to severe dehydration and eventual death. Chemotherapeutic agents and inflammatory
19 agents also induce diarrhea by activating these signaling molecules. See Figure 1. Agents that
20 target these cyclic nucleotide or calcium signaling pathways would be expected to be anti-
21 secretory agents, and hence effective broad spectrum anti-diarrhea drugs.

22 Ingestion of pathogenic bacteria such as *Escherichia coli* can cause diarrhea in humans
23 and scour in swine. Scour is of particular concern in modern swine farming, which often entails
24 dense or crowded growing conditions.

25 Antibiotics are an effective means to treat diarrhea in humans and scour in swine. In
26 farming, antibiotics may also be routinely added to animal feed to prophylactically prevent scour.
27 Regular and widespread antibiotic use, however, has allowed antibiotic-resistant strains of
28 pathogenic bacteria to proliferate.

29 Thus, there is a need in the art for a way to treat and prevent diarrhea in humans and
30 scour in swine without encouraging the further proliferation of antibiotic-resistant strains of
31 pathogenic bacteria.

32 I found a way. My solution does not kill pathogenic bacteria; it is thus not an “anti-biotic”
33 in the conventional sense of the word, and does not selectively favor the proliferation of
34 antibiotic-resistant bacteria. Rather, my solution merely prevents pathogenic bacteria from
35 adhering to the lining of the gastrointestinal tract. Unable to adhere to the lining of the
36 gastrointestinal tract, the pathogenic bacteria pass through the host animal’s gastrointestinal tract
37 unharmed and are eliminated in host animal’s stool. My solution also works against other
38 diarrhea causing microbes, such as viruses and parasites. It does this by blocking the invasion
39 and secretory pathways within intestinal cells, and not by trying to kill or target the diarrhea
40 causing microbe. My solution is not poisonous and produces no poisonous metabolite or residue;
41 it thus is suitable for use in humans and in food animals such as swine, cattle and poultry, and in

1 aquaculture (for example, farmed fish and shellfish). It is also useful for companion animals
2 such as dogs and cats, ornamental fish etc. I use the term “veterinary” in the appended claims to
3 encompass food animals, companion animals and fish. Further, it may be synthesized using
4 standard industrial polypeptide synthetic techniques such as Fmoc resin synthesis, but may
5 alternatively also be manufactured from certain botanical extracts.

6 Brief Description of the Figures:

7 Figure 1 diagrams several possible causes of diarrhea.

8 Figure 2 diagrams three possible mechanisms of action of my formula.

9 Figure 3 charts the number of post-weaning piglets with scour over time after a single
10 administration of my formula, compared to non-treated piglets.

11 Figure 4 charts Total Clinical Score of post-weaning piglets over time after a single
12 administration of my formula, compared to non-treated piglets.

13 Detailed Description:

14 My invention entails formulating an aqueous oral suspension of bromelain, BLANOSE®
15 sodium carboxymethyl cellulose, citric acid anhydrous, EPIKURON® 135F lecithin oil and
16 ethylenedinitrilotetraacetic acid, disodium salt dihydrate (“EDTA”). For example, a suitable
17 formulation is shown in Table 1.

Table 1

Material	Quantity per Unit Dose	Percent (w/w)
Bromelain q.s	62.5 - 88.4 mg	53.1 - 74.7
BLANOSE® sodium carboxymethyl cellulose	25.9 - 42.4 mg	22 - 36
citric acid anhydrous	4.7 - 14.96 mg	4 - 12.7
EPIKURON® 135F lecithin oil	3.5 - 9.3 mg	3 - 7.9
ethylenedinitrilotetraacetic acid, disodium salt dihydrate	0.8 - 1.9 mg	0.7 - 1.6
Total	97.4 - 156.96 mg	100.0

18

19 This formulation produces a free-flowing powder. It may be suspended in 2 mL of water
20 for oral administration to suckling piglets to prevent E. coli, or other microbe induced diarrhea
21 (“scours”). The amount used may be increased when administering to larger animals or to
22 humans. I now provide further detail on each of these components.

1 Bromelain

2 Bromelain is the collective name for a crude proteolytic extract obtained from the
3 pineapple plant (*Ananas comosus*). Two forms of bromelain are known; fruit bromelain obtained
4 from fresh pineapple fruit, and stem bromelain obtained from the stem of the plant. The main
5 commercial source of bromelain is stem bromelain, and the terms “bromelain” and “stem
6 bromelain” are used interchangeably.

7 Bromelain is prepared from the stems after the fruit is harvested. The stem is peeled,
8 crushed and pressed to obtain a juice containing the soluble bromelain components. Further
9 processing includes concentration, filtration and drying of the pressed juice to get a final white-
10 yellow or tan dry powder. The resultant bromelain extract is a mixture of protein-digesting
11 enzymes—called proteolytic enzymes or proteases—and several other substances in smaller
12 quantities, such as peroxidases, acid phosphatases, certain protease inhibitors, and calcium.

13 The major proteolytic enzyme within bromelain is a protease called stem bromelain, CAS
14 37189-34-7 (EC 3.4.22.32), while the major protease within the fruit bromelain extract is called
15 fruit bromelain (EC 3.4.22.33). These proteases enzymes are referred to as sulfhydryl proteases,
16 since a free sulfhydryl group of a cysteine side-chain is required for function. Stem bromelain
17 has a broad specificity for cleavage of proteins, and has a strong preference for Z-Arg-Arg-|-
18 NHMec among small molecule substrates. Fruit bromelain, has a strong preference for Bz-Phe-
19 Val-Arg-|-NHMec.

20 Unless otherwise qualified (e.g. “fruit bromelain”), I use the term “bromelain” here to
21 refer to the crude extract from pineapple stems, and “stem bromelain protease” to describe the
22 main protease.

23 Pineapples have a long tradition as a medicinal plant among the natives of South and
24 Central America. The first isolation of bromelain was recorded by the Venezuelan chemist
25 Vicente Marcano in 1891 from the fruit of pineapple. In 1892, Russell Henry Chittenden,
26 assisted by Elliott P. Joslin and Frank Sherman Meara, investigated the matter fully, and called it
27 'bromelin'. Later, the term “bromelain” was introduced, and originally the term was applied to
28 any protease from any member of the plant family Bromeliaceae.

29 Bromelain has a long history of folk and modern medicinal use and continues to be
30 explored as a potential healing agent in alternative medicine. It is also widely accepted as a
31 phytotherapeutical drug. Bromelain was first introduced as a therapeutic supplement in 1957.
32 First, research on bromelain was conducted in Hawaii, but more recently has been conducted in
33 countries in Asia, Europe, and Latin America. Recently, researchers in Germany have taken a
34 great interest in bromelain research. Currently, bromelain is the thirteenth most widely used
35 herbal medicine in Germany.

36 Some of the therapeutic benefits of bromelain are reversible inhibition of platelet
37 aggregation, reversible inhibition of angina pectoris, reversible inhibition of bronchitis and
38 sinusitis, treating surgical traumas, thrombophlebitis, and pyelonephritis. It can also be used after
39 surgery or injury to reduce swelling (inflammation), especially of the nose and sinuses. It is also
40 used for preventing muscle soreness after intense exercise. Bromelain also has been reported to

1 interfere with the growth of tumor cells and slow blood clotting. *See*
2 www.nlm.nih.gov/medlineplus/druginfo/natural/895.html. Bromelain is also used for hay fever,
3 treating a bowel condition that includes swelling and ulcers (ulcerative colitis), removing dead
4 and damaged tissue after burns (debridement), preventing the collection of water in the lung
5 (pulmonary edema), relaxing muscles, stimulating muscle contractions, improving the
6 absorption of antibiotics, preventing cancer, shortening labor, and helping the body get rid of fat.
7 In food preparation, bromelain is used as a meat tenderizer, and to clarify beer.

8 Systemic enzyme therapy (consisting of combinations of proteolytic enzymes such as
9 bromelain, trypsin, chymotrypsin, and papain) has been investigated in Europe for the treatment
10 of breast, colorectal, and plasmacytoma cancer patients. In mice with experimental colitis, six
11 months of dietary bromelain from pineapple stem or from fresh juice decreased the severity of
12 colonic inflammation and reduced the number of cancerous lesions in the colon.

13 Bromelain supplements, when taken with other medications (Amoxicillin, antibiotics,
14 anticoagulant/antiplatelet drugs), may increase the risk associated with heart rate, blood clotting
15 and bleeding post-surgery.

16 Bromelain's anti-metastatic and anti-inflammatory activities are apparently independent
17 of its proteolytic activity. Although poorly understood, the diverse biological effects of
18 bromelain seem to depend on its ability to traverse the membrane barrier, a very unusual
19 property of this compound.

20 As a potential anti-inflammatory agent, bromelain may be useful for treating arthritis, but
21 has neither been confirmed in human studies for this use, nor is it approved with a health claim
22 for such an effect by the Food and Drug Administration or European Food Safety Authority. The
23 *Natural Medicines Comprehensive Database* suggests that bromelain, when used in conjunction
24 with trypsin (a different protease) and rutin (a substance found in buckwheat) is as effective as
25 some prescription analgesics in the management of osteoarthritis. A product (WOBENZYME™)
26 that combines bromelain with trypsin and rutin is available commercially and seems to reduce
27 pain and improve knee function in people with osteoarthritis. However, the National Institutes of
28 Health notes, "There isn't enough scientific evidence to determine whether or not bromelain is
29 effective for any of its other uses." *See id.* Bromelain is also available in some countries as a
30 product under the name ANANASE™.

31 Bromelain has not been scientifically proven to be effective in any other diseases and it
32 has not been licensed by the Food and Drug Administration for the treatment of any other
33 disorder.

34 Bromelain is produced in Thailand, Taiwan, and other tropical parts of the world where
35 pineapples are grown.

36 Bromelain has shown promise of an effective, broad spectrum anti-diarrhea drug, as it
37 targets the underlying cause of diarrhea, the inflammatory and secretory pathways. Bromelain
38 has a triple mechanism of action. *See Figure 2.* First, it prevents the attachment of bacteria to
39 the small intestine thereby preventing their colonization. Secondly, it prevents and reverses the
40 action of bacterial toxins, and inflammatory mediators, the underlying cause of excessive fluid

1 secretion and diarrhea. It does this by blocking enterotoxin and inflammatory mediator-induced
2 cyclic AMP, cyclic GMP and Ca^{2+} intracellular signaling pathways that induce intestinal fluid
3 secretion and secretory diarrhea. Thirdly, bromelain also inhibits inflammation by reducing the
4 production of, and action of pro-inflammatory cytokines, including $\text{TNF}\alpha$, $\text{IFN}\gamma$, and IL-6 by
5 preventing activation of the ERK-2, JNK and p38 mitogen activated protein (MAP) kinase
6 pathways. These pathways and pro-inflammatory cytokines play a key role in the intestinal
7 barrier dysfunction induced by Shigella, Salmonella, and Clostridium difficile, and in chronic
8 inflammation, such as in patients with inflammatory bowel disease (IBD).

9 Because bromelain acts on the underlying mechanisms of diarrhea, unlike antibiotics and
10 vaccines that only target specific types of pathogen, bromelain will be effective against a range
11 of different causes of diarrhea. Also because bromelain is not an antibiotic and does not target
12 the pathogens, it should not contribute to the growing problem of antibiotic-resistance, a serious
13 global health problem.

14 The use of my formulation prevents inflammation at weaning, improving gut health, and
15 increasing feed intake. Weaning is a critical period in a piglet's life. It must cope with
16 separation from the sow, the transition from highly digestible milk to a less digestible and more
17 complex solid feed, a new environment, movement and separation from littermates, and
18 exposure to unfamiliar pigs. These stressors can lead to reduced feed intake and reduced piglet
19 growth.

20 Additionally, the newly-weaned pigs' immune and digestive systems are still maturing,
21 making the piglet more susceptible to antigenic challenges (nutritional or microbial), which can
22 lead to inflammatory responses. Inflammation induces a negative impact on the digestive and
23 absorptive capabilities of the gut, and overall gut health creating an opportunity for an animal to
24 become more susceptible to pathogens. An animal whose reduced feed intake is poor at the time
25 of pathogen exposure will become sick.

26 The absence of feed in a piglet's stomach results in a microbial imbalance, leading to
27 higher occurrences of diseases. Thus, this short weaning phase in a pig's life can have far-
28 reaching consequences, negatively affecting the pig's entire rearing period.

29 The anti-inflammatory activity of bromelain reduces gut inflammation, and protects
30 piglets from disease, as well as increases piglet food intake during the post-weaning period.

31 BLANOSE® sodium carboxymethyl cellulose

32 Cellulose for industrial use is mainly obtained from wood pulp and cotton. The kraft
33 process is used to separate cellulose from lignin, another major component of plant matter.
34 Cellulose has no taste, is odorless, is hydrophilic with the contact angle of 20–30, is insoluble in
35 water and most organic solvents, is chiral and is biodegradable. It can be broken down
36 chemically into its glucose units by treating it with concentrated acids at high temperature.

37 Cellulose is derived from D-glucose units, which condense through $\beta(1\rightarrow4)$ -glycosidic
38 bonds. This linkage motif contrasts with that for $\alpha(1\rightarrow4)$ -glycosidic bonds present in starch,
39 glycogen, and other carbohydrates. Cellulose is a straight chain polymer: unlike starch, no

1 coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like
2 conformation, aided by the equatorial conformation of the glucose residues. The multiple
3 hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the
4 same or on a neighbor chain, holding the chains firmly together side-by-side and forming
5 *microfibrils* with high tensile strength. This confers tensile strength in cell walls, where cellulose
6 microfibrils are meshed into a polysaccharide *matrix*.

7 Compared to starch, cellulose is also much more crystalline. Whereas starch undergoes a
8 crystalline to amorphous transition when heated beyond 60–70 °C in water (as in cooking),
9 cellulose requires a temperature of 320 °C and pressure of 25 MPa to become amorphous in
10 water.

11 Several different crystalline structures of cellulose are known, corresponding to the
12 location of hydrogen bonds between and within strands. Natural cellulose is cellulose I, with
13 structures I_α and I_β. Cellulose produced by bacteria and algae is enriched in I_α while cellulose of
14 higher plants consists mainly of I_β. Cellulose in regenerated cellulose fibers is cellulose II. The
15 conversion of cellulose I to cellulose II is irreversible, suggesting that cellulose I is metastable
16 and cellulose II is stable. With various chemical treatments it is possible to produce the structures
17 cellulose III and cellulose IV. Many properties of cellulose depend on its chain length or degree
18 of polymerization, the number of glucose units that make up one polymer molecule. Cellulose
19 from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant
20 fibers as well as bacterial cellulose have chain lengths ranging from 800 to 10,000 units.
21 Molecules with very small chain length resulting from the breakdown of cellulose are known as
22 cellodextrins; in contrast to long-chain cellulose, cellodextrins are typically soluble in water and
23 organic solvents.

24 Methyl cellulose (or methylcellulose) is a chemical compound derived from cellulose. It
25 is a hydrophilic white powder in pure form and dissolves in cold (but not in hot) water, forming a
26 clear viscous solution or gel. It is sold under a variety of trade names and is used as a thickener
27 and emulsifier in various food and cosmetic products, and also as a treatment of constipation.
28 Like cellulose, it is not digestible, not toxic, and not an allergen.

29 Methyl cellulose does not occur naturally and is synthetically produced by heating
30 cellulose with caustic solution (e.g. a solution of sodium hydroxide) and treating it with methyl
31 chloride. In the substitution reaction that follows, the hydroxyl residues (-OH functional groups)
32 are replaced by methoxide (-OCH₃ groups).

33 Different kinds of methyl cellulose can be prepared depending on the number of hydroxyl
34 groups substituted. Cellulose is a polymer consisting of numerous linked glucose molecules,
35 each of which exposes three hydroxyl groups. The *Degree of Substitution* (DS) of a given form
36 of methyl cellulose is defined as the average number of substituted hydroxyl groups per glucose.
37 The theoretical maximum is thus a DS of 3.0, however more typical values are 1.3–2.6. Different
38 methyl cellulose preparations can also differ in the average length of their polymer backbones.

39 Methyl cellulose has a lower critical solution temperature (LCST) between 40 °C and
40 50 °C. At temperatures below the LCST, it is readily soluble in water; above the LCST, it is not
41 soluble, which has a paradoxical effect that heating a saturated solution of methyl cellulose will

1 turn it solid, because methyl cellulose will precipitate out. The temperature at which this occurs
2 depends on DS-value, with higher DS-values giving lower solubility and lower precipitation
3 temperatures because the polar hydroxyl groups are masked. Preparing a solution of methyl
4 cellulose with cold water is difficult: as the powder comes into contact with water, a gel layer
5 forms around it, dramatically slowing the diffusion of water into the powder, hence the inside
6 remains dry.

7 Carboxymethyl cellulose (CMC) or cellulose gum is a cellulose derivative with
8 carboxymethyl groups (-CH₂-COOH) bound to some of the hydroxyl groups of the
9 glucopyranose monomers that make up the cellulose backbone. It is often used as its sodium salt,
10 sodium carboxymethyl cellulose.

11 CMC is used in food science as a viscosity modifier or thickener, and to stabilize
12 emulsions in various products including ice cream. It is also a constituent of many non-food
13 products, such as personal lubricants, toothpaste, laxatives, diet pills, water-based paints,
14 detergents, textile sizing, and various paper products. It is used primarily because it has high
15 viscosity, is nontoxic, and is generally considered to be hypoallergenic as the major source fiber
16 is either softwood pulp or cotton linter. CMC is used extensively in gluten free and reduced fat
17 food products. CMC is also used in pharmaceuticals as a thickening agent.

18 Sodium carboxymethyl cellulose, also known as croscarmellose sodium, is an internally
19 cross-linked sodium carboxymethylcellulose. It is used as a superdisintegrant in pharmaceutical
20 formulations.

21 The exemplary formula in Table 1 uses BLANOSE® brand food-grade sodium
22 carboxymethyl cellulose, commercially available from Ashland Chemical Co., Covington KY
23 USA. Food-grade and pharmaceutical-grade sodium carboxymethyl cellulose is available from
24 other vendors, e.g., Sigma-Aldrich Inc. and Spectrum Chemical, Inc. Other gelling agents may
25 be used in addition to or in lieu of sodium carboxymethyl cellulose.

26 Citric acid anhydrous

27 Citric acid is a commodity chemical, commercially available from a wide variety of
28 suppliers. It is used mainly as an acidifier, as a flavoring, and as a chelating agent.

29 Citric acid is a weak organic acid with the formula C₆H₈O₇. It is a natural
30 preservative/conservative and is also used to add an acidic or sour taste to foods and drinks. In
31 biochemistry, the conjugate base of citric acid, citrate, is important as an intermediate in the
32 citric acid cycle, which occurs in the metabolism of all aerobic organisms. It consists of 3
33 carboxyl (R-COOH) groups.

34 At room temperature, citric acid is a white crystalline powder. It can exist either in an
35 anhydrous (water-free) form or as a monohydrate. The anhydrous form crystallizes from hot
36 water, while the monohydrate forms when citric acid is crystallized from cold water. The
37 monohydrate can be converted to the anhydrous form by heating above 78 °C.

38 The dominant use of citric acid is as a flavoring and preservative in food and beverages,
39 especially soft drinks. The buffering properties of citrates are used to control pH in

1 pharmaceuticals. I prefer that the citric acid used conform to the purity requirements for citric
2 acid as a food additive are defined by the Food Chemicals Codex published by the United States
3 Pharmacopoeia.

4 Without intending to be bound to any theoretical mechanism, I believe that the citric acid
5 in my formulation functions as an emulsifying agent to keep the lipophilic lecithin oil from
6 separating from the hydrophilic bromelain. Further, citric acid is an excellent chelating agent,
7 binding metals. For example, it is used to remove limescale from boilers and evaporators. It can
8 be used to soften water, which makes it useful in soaps and laundry detergents. By chelating the
9 metals in hard water, it lets these cleaners produce foam and work better without need for water
10 softening. Chelation activity is important in my formula because metal ion may interfere with
11 the biological activity of bromelain.

12 EPIKURON® 135F lecithin oil

13 Lecithin has emulsification and lubricant properties, and is a surfactant. Commercial
14 lecithin, as used by food manufacturers, is a mixture of phospholipids in oil. The lecithin can be
15 obtained by water degumming the extracted oil of seeds. It is a mixture of various phospholipids,
16 and the composition depends on the origin of the lecithin. A major source of lecithin is soybean
17 oil. Other sources of lecithin (e.g., sunflower oil) may be used to avoid soy allergy concerns.
18 The main phospholipids in lecithin from soya and sunflower are phosphatidyl choline,
19 phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidic acid. They often are
20 abbreviated to PC, PI, PE, and PA, respectively. Purified phospholipids are produced by
21 companies commercially.

22 To modify the performance of lecithin to make it suitable for the product to which it is
23 added, it may be hydrolyzed enzymatically. In hydrolysed lecithins, a portion of the
24 phospholipids have one fatty acid removed by phospholipase. Such phospholipids are called
25 lysophospholipids. The most commonly used phospholipase is phospholipase A2, which
26 removes the fatty acid at the C2 position of glycerol. Lecithins may also be modified by a
27 process called fractionation. During this process, lecithin is mixed with an alcohol, usually
28 ethanol. Some phospholipids, such as phosphatidylcholine, have good solubility in ethanol,
29 whereas most other phospholipids do not dissolve well in ethanol. The ethanol is separated from
30 the lecithin sludge, after which the ethanol is removed by evaporation to obtain a
31 phosphatidylcholine-enriched lecithin fraction.

32 Soybean-derived Lecithin dietary supplements are composed of 19-21%
33 phosphatidylcholine, 8-20% Phosphatidylethanolamine, 20-21% Inositol phosphatides, 33-35%
34 Soybean oil, 2-5% Sterols, 5% Carbohydrates/free, 1% Moisture, and 5-11% Other phosphatides.
35 Lecithin is used for applications in human food, animal feed, pharmaceuticals, paints, and other
36 industrial applications. In the pharmaceutical industry, lecithin acts as a wetting, stabilizing
37 agent and a choline enrichment carrier, helps in emulsifications and encapsulation, and is a good
38 dispersing agent. Lecithin is approved by the United States Food and Drug Administration for
39 human consumption with the status "generally recognized as safe." Lecithin is also permitted by
40 the European Union as a food additive (designated E322). The exemplary formula of Table 1
41 uses lecithin as an emulsifier. As an emulsifier, lecithin imparts several advantages. In animal
42 feed, it enriches fat and protein content and improves pelletization. Research studies show soy-

1 derived lecithin has significant effects on lowering serum cholesterol and triglycerides, while
2 increasing HDL ("good cholesterol") levels in the blood of rats. It can be totally metabolized by
3 humans, so is well tolerated by humans and non-toxic when ingested. (In contrast, certain other
4 emulsifiers can only be excreted via the kidneys). Other emulsifiers, however, may be used in
5 addition to or in lieu of lecithin.

6 Ethylenedinitrilotetraacetic acid, disodium salt dihydrate ("EDTA")

7 Ethylenediaminetetraacetic acid, widely abbreviated as EDTA, is a chelating agent. It is
8 colorless, water-soluble solid. Its conjugate base is ethylenediaminetetraacetate.

9 Its usefulness arises because of its role as a chelating agent, i.e. its ability to sequester
10 metal ions such as Ca^{2+} and Fe^{3+} . Chelating activity is advantageous for my formulation
11 because metal ion (e.g., metal ion commonly present in tap water used in livestock farming) may
12 interfere with or inhibit the activity of bromelain.

13 After being bound by EDTA, metal ions remain in solution but exhibit diminished
14 reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium
15 EDTA.

16 In industry, EDTA is mainly used to sequester metal ions in aqueous solution. In the
17 textile industry, it prevents metal ion impurities from modifying colors of dyed products. EDTA
18 inhibits the ability of metal ions, especially Mn^{2+} , from catalyzing the disproportionation of
19 hydrogen peroxide. In a similar manner, EDTA is added to some food as a preservative or
20 stabilizer to prevent catalytic oxidative decoloration, which is catalyzed by metal ions. In soft
21 drinks containing ascorbic acid and sodium benzoate, EDTA mitigates formation of benzene (a
22 carcinogen).

23 EDTA is used to bind metal ions in the practice of chelation therapy, e.g., for treating
24 mercury and lead poisoning. It is used in a similar manner to remove excess iron from the body.
25 This therapy is used to treat the complication of repeated blood transfusions, as would be applied
26 to treat thalassemia. The U.S. FDA approved the use of EDTA for lead poisoning on July 16,
27 1953, under the brand name of Versenate, which was licensed to the pharmaceutical company
28 Riker. Some alternative medical practitioners believe EDTA acts as a powerful antioxidant to
29 prevent free radicals from injuring blood vessel walls, therefore reducing atherosclerosis. The
30 U.S. FDA has not approved it for the treatment of atherosclerosis.

31 Without intending to be bound by any hypothetical mode of action, EDTA may also
32 serve in my formulation as a preservative, perhaps to enhance the preservative action of citric
33 acid.

34 In addition to or in lieu of EDTA, one may use another chelating agent(s). For example,
35 one could use a chelating ligand which binds metal ion but also has a higher biodegradability and
36 a lower content of nitrogen than does EDTA.

37 For example, one may use Iminodisuccinic acid (IDS). Commercially used since 1998,
38 iminodisuccinic (IDS) acid biodegrades about 80% after only 7 days. IDS binds to calcium
39 exceptionally well and forms stable compounds with other heavy metal ions. In addition to

1 having a lower toxicity after chelation than does EDTA, the production of IDS is environment-
2 friendly. Additionally, IDS is degraded through the use of IDS-epimerase and C-N lyase found
3 in *Agrobacterium tumefaciens* (BY6) which can be harvested on a large scale. Additionally, the
4 reactions catalyzed by both enzymes does not require any cofactors and can thus be applied
5 directly.

6 Similarly, one may use Polyaspartic acid. Polyaspartic acid, commercially available as
7 BAYPURE™ DS 100, is produced in an environmentally friendly manner. Polyaspartic acid,
8 like Iminodisuccinic acid binds to calcium and other heavy metal ions. It has a higher value of
9 7.2 meq/g than does EDTA, which only has 6.0 meq/g. While it has a higher theoretical capacity,
10 in practical applications it exhibits low efficiency in lower ion concentration solutions. DS has
11 many practical applications including corrosion inhibitors, waste water additives, and
12 agricultural polymers. A BAYPURE™ DS 100 based laundry detergent was the first laundry
13 detergent in the world to achieve the EU flower ecolable.

14 Similarly, one may use Ethylenediamine-N,N'-disuccinic acid (EDDS). As a structural
15 isomer of EDTA, ethylenediamine-N,N'-disuccinic acid can exist three isomers: (S,S),
16 (R,S)/(S,R) and (R,R), but only the S,S-isomer is readily biodegradable. EDDS exhibits a
17 surprisingly high rate biodegradation at 83% in 20 days. Biodegradation rates also varies the
18 different metal ions chelated. For example, the complexes of lead and zinc with EDDS have
19 relatively the same stability but the lead complex is biodegrades more efficiently than the zinc
20 complex. As of 2002, EDDS has been commercially prominent in Europe on a large scale with
21 an estimated demand rate increase of about 15% each year.

22 Similarly, one may use Methylglycinediacetic acid (MGDA). Commercially available
23 from BASF GmbH, methylglycinediacetic acid (MGDA) is produced from glycine. MGDA has
24 a high rate of biodegradation >68%, but unlike many other chelating agents can degrade without
25 the assistance of adapted bacteria. Additionally, unlike EDDS or IDS, MGDA can withstand
26 higher temperatures while maintaining a high stability as well as the entire pH range. As a result,
27 the chelating strength of MGDA is stronger than many commercial chelating agents.

28 Similarly, one may use L-glutamic acid N,N-diacetic acid, tetra sodium salt (GLDA).
29 Such aminopolycarboxylate-based chelates are used to control metal ions in water-based systems.

30 EXAMPLES

31 I tested my formulation on swine grown in three different types of environments and
32 three different climates, under actual commercial farming conditions, assessing two different age
33 groups of pigs - suckers (unweaned) and weaners.

34 • Trial 1 (Spain) – My formulation reduced the incidence, severity and duration of
35 post-weaning scour in piglets. My formulation also reduced the requirement for antibiotics and
36 improved feed conversion ratios (FCR).

37 • Trial 2 (France) – My formulation improved average daily weight gain, and
38 improved food conversion ratios when compared to antibiotics in feed.

1 • Trial 3 (Philippines) - My formulation reduced piglet deaths in sucker piglets
2 compared with antibiotics.

3 • Trial 4 (Australia) - My formulation reduced piglet deaths in sucker piglets
4 compared with antibiotics.

5 EXAMPLE 1 - Spain (just weaned piglets).

6 Aim: The objective of this study was to compare whether a single oral dose of my
7 formulation (4 ml) at weaning could reduce scour in a commercial piggery with a history of E.
8 coli.

9 Study Outline: This study was a blinded, randomised field trial comparing two parallel
10 groups of piglets. I used two Test Groups (n = 72 per group): 1. My formulation, and 2. No
11 treatment. On the day of weaning (day 0), piglets in each litter were randomly assigned to two
12 different treatment groups, weighed and then given a unique identification (ID) number. A
13 single dose of my formulation was then administered to one group of piglets which were then
14 transported to the weaning pens. The other group of piglets were left untreated, but handled in
15 an identical manner.

16 Clinical Parameters: Piglets were monitored daily for scour and signs of any other
17 disease. Once piglets showed signs of scour, their ID numbers, fecal consistency and the general
18 condition of piglets were recorded using a scoring system (Table 2).

19

20 Table 2. Scoring fecal consistency and piglet general condition.

Fecal consistency	General condition
0: normal	0: normal
1: pasty or partially formed (mild)	1: mildly depressed
2: loose, semi liquid (moderate)	2: severely depressed.
3: profuse and watery (severe)	

21

22 Classification of the animals as healthy, unwell or moribund were based on the total
23 clinical score (Table 3). The total clinical score is the sum of the fecal consistency score plus the
24 general condition score for each pig. This score gives an overall indication of piglet health.
25 Some piglets may have scour, but still appear healthy, whereas other piglets may have mild scour,
26 but be moribund. My formulation significantly reduced the clinical score of piglets, and
27 therefore improved their overall health compared to untreated pigs.

28 Table 3. Classification of the piglets by their total clinical score.

Total clinical score	Classification
0	Healthy
1	Healthy
2	Unwell
3	Moribund and requires individual animal treatment.
4+	Moribund and requires removal and/or euthanasia

1

2 Antibiotic treatments: All antibiotic treatments administered during the study were
3 recorded (animal ID, date, product, dose and route of administration).

4 Piglet Weight: Piglets were individually weighed on day 0 (at weaning), day 7 and day 14.
5 The average daily weight gain (ADG) was calculated.

6 Feed Intake: The feed intake per pen was also assessed to determine feed conversion
7 ratios (FCR). The FCR is equal to the feed intake divided by the weight of the pig.

8 Data Analysis: To evaluate the incidence of scour, clinical score or morbidity (general
9 condition + fecal consistency), the treatment rate and mortality rate, the statistical procedures
10 used was a Linear Mixed Model with poisson and binomial errors. Room/Sex were as random
11 effects and Treatment as a fixed effect. Analyses were conducted with GenStat for Windows.
12 (2007). 10th Edn. VSN International Ltd., Hemel Hempstead, UK. For body weight and average
13 daily weight gain the statistical procedures used were Linear Mixed Models with normal errors.
14 The Type 1 error was ≤ 0.05 .

15 Results:

16 Clinical Parameters:

17 • A single dose of my formulation administered at weaning significantly reduced
18 the incidence of scour (from day 0 to day 19 post weaning) by 40% when compared to untreated
19 pigs (Figure 3) ($p < 0.05$). This single dose protected piglets for 19 days, indicating a long
20 duration of effect. The cause of scour on this farm was E. coli.

21 • Over the duration of the study, the total diarrhea score (sum of all diarrhea scores)
22 in the group of piglets treated with my formulation was 98, compared with 253 in the untreated
23 group.

24 • My formulation significantly improved the overall health in piglets or they had a
25 reduced clinical score, therefore less severe disease, when compared with untreated piglets
26 (Figure 4) ($p < 0.05$).

1 • My formulation significantly reduced the number of sick pigs by 58% (n = 16)
2 when compared to untreated pigs (n = 38) (p<0.05).

3 • My formulation reduced the requirement for the number of antibiotic treatments
4 by 55% (15 treatments) versus controls (33 treatments).

5 • There were equal numbers of deaths (all causes) in the group treated with my
6 formulation (4) and in the untreated group (3).

7 Pig Performance:

8 • The average daily weight gain in piglets treated with my formulation in the two
9 weeks post weaning was 22% higher than in untreated piglets (50 ± 7.1 g vs 39 ± 7.0 g,
10 respectively). At 42 days post-weaning, my formulation treated piglets were 0.2 kg (1.6%)
11 heavier than control piglets, but this increase was not significant.

12 • In the first 2 weeks after weaning, piglets treated with my formulation had a
13 significantly better feed conversion ratio of 8.4% than untreated pigs (2.84 ± 1.22 vs 3.1 ± 1.20 ,
14 respectively). Overall (day 0 to 42) my formulation had a 2.7% improvement in feed conversion
15 ratios (1.46 ± 0.06 versus 1.50 ± 0.06).

16 Although the overall improved FCR of my formulation compared to the untreated group
17 is modest (0.04 or 2.7%), it should be noted that every 0.01 improvement in FCR will reduce
18 feed cost by \$0.28 to \$0.30 per pig (based on 2008 figures - cost of feed has gone up
19 significantly, so current benefits will be greater). It has also been calculated that a 0.1 %
20 improvement in grower FCR can improve the profitability of a 200-sow unit by approximately
21 \$6,000 per annum. A 5% improvement in FCR has a potential value of \$28 million to the
22 Australian pork Industry (<http://www.australianpork.com.au>).

23 The improved performance in FCR is important, as the cost of feed is the major cost to
24 pork production, usually accounting for over 60 to 80 percent of all production expenses. Every
25 improvement in FCR will reduce feed costs and improve profitability.

26 Conclusion:

27 A single dose of my formulation administered at weaning reduces the incidence, duration
28 and severity of scour. My formulation also reduces the requirement for antibiotic treatments, and
29 improves growth in piglets. Therefore, my formulation improves piglet health and performance,
30 and therefore farm productivity.

31 EXAMPLE 2 - France (weaned piglets).

32 Background: Antibiotics as a feed additive to promote growth is now banned in Europe,
33 however, addition of prescribed antibiotics to feed under veterinary supervision is allowed for
34 the prophylaxis and treatment of acute conditions, such as scour.

35 Aim: The objective of this study was to compare the feed conversion ratios of piglets
36 administered a single dose of my formulation with in feed colistin, an antibiotic.

1 Study Outline: This study was a blinded, controlled, randomised field trial comparing
 2 three parallel groups of piglets, (n=89 per group): 1. Colistin; 2. My formulation; and 3. No
 3 treatment. In this study, whole litters are randomised to receive different treatments, so litters are
 4 treated on a whole litter basis.

5 Weaning on this farm occurs in 2 stages. In stage 1, piglets are weaned by removing the
 6 sow (at day -5). Then on day 0, piglets are moved to their weaning pen.

7 At day -5, Group 1 piglets were administered colistin (9 kg antibiotic premix per Ton of
 8 feed) in pre-starter feed for 14 days (day -5 to day 9). The other two groups were administered
 9 pre-starter feed alone. On day 0 (5 days post weaning), when piglets are moved to their weaner
 10 pens, Group 2 piglets received a single dose of my formulation. Group 3 piglets were untreated.

11 Analysis: As per Study 1.

12 Results:

13 Pig Performance

14 • My formulation treated piglets had a significantly higher average daily weight
 15 gain during all phases of the study compared to piglets receiving colistin (P<0.05, Table 4).
 16 Piglets treated with colistin in their feed had the lowest weight gain of all groups, including less
 17 weight gain than untreated pigs (P<0.05).

18 Table 4 - Average Daily Weight gain (g) of all groups.

	My formulation	Colistin	Untreated
Pre-starter (d-5 to d9)	252 ± 74	167 ± 67	236 ± 63
Starter	537 ± 105	505 ± 110	542 ± 110
Post weaning	429 ± 81	377 ± 82	426 ± 75
Post weaning and fattening (overall)	692 ± 65	664 ± 67	678 ± 56

19

20 • Overall (at 150 days of age) the average total weight gain of piglets administered
 21 my formulation was 2% (or 1.71 kg) higher than control pigs, and 3.9% (or 3.25 kg) higher than
 22 piglets administered colistin in feed (P<0.05).

1 • The feed intake was determined for the pre-starter phase only (d-5 to d9). My
 2 formulation treated piglets had a higher feed intake over the weaning period compared to piglets
 3 receiving colistin in feed (Table 5).

4 Table 5. Feed intake (g) during the pre-starter phase

	My formulation	Colistin	Control
Number of pens	6	6	6
Min – Max	408 – 499	358 – 479	420 – 529
Arithmetic Mean	450	417	460

5

6 • The feed conversion ratio (FCR) was determined for the pre-starter phase only (d-
 7 5 to d9). My formulation alone treated piglets had the best FCR (1.82) (P<0.009) than all the
 8 other groups (Table 5). Piglets treated with my formulation had a 33% improvement over piglets
 9 treated with colistin (2.71). Despite piglets treated with my formulation having a lower feed
 10 intake than untreated pigs (Table 5), they still had a 7% improvement in FCR over untreated pigs
 11 (1.96) (Table 6). These performance results show that my formulation has a significant
 12 advantage over colistin in feed.

13 Table 6. Feed Conversion Ratio during the pre-starter phase per Group

	My formulation	Colistin	Control
Number of pens	6	6	6
Median	1.77	2.59	2.01
Min – Max	1.59 – 2.10	1.75 – 3.42	1.63 – 2.11
Arithmetic Mean	1.82	2.71	1.96

14

15 The negative effect of colistin on FCR may be because of its adverse effect on the gut
 16 flora, and thus a negative effect on pig gut health and nutrition.

17 Conclusion: My formulation improved weight gains of piglets and improved food
 18 conversion ratios.

19 EXAMPLE 3 - Philippines (unweaned piglets or sucker piglets).

1 Background: This farm in the Philippines has a high death rate due to scour.

2 Aim: The objective of this study was to compare the efficacy of my formulation with
3 antibiotics in unweaned piglets.

4 Study Outline: This study was a randomised field trial comparing two groups of piglets.
5 Since the mortality rate on this farm is very high, there is no negative control (non-medicated
6 group). Test Groups (n = 38 per group) were 1. My formulation (2 doses), and 2. Antibiotics
7 (orally administered every 3 days). Piglets received my formulation at 3 days of age. A follow
8 up dose was given at 6 days of age.

9 Results:

10 Clinical Parameters

11 • There were few deaths due to scour in this study. But there were significantly
12 lower deaths of 5% (from all causes) in the group treated with my formulation. The death rate
13 was 21% in piglets receiving antibiotics (P<0.05).

14 Performance Parameters

15 • At weaning, piglets receiving my formulation weighed 0.1 kg more than piglets
16 treated with antibiotics. Since there were no negative controls in this study, it is unknown
17 whether my formulation had increased weight over untreated pigs, as observed in other field
18 trials.

19 EXAMPLE 4 - Australia (unweaned piglets or sucker piglets).

20 Aim: The objective of this study was to investigate the efficacy of my formulation (2
21 mL) in reducing piglet mortality and morbidity on an Australian farm with a history of pre-
22 weaning (sucker) scour.

23 Study Outline: The study was conducted on a commercial piggery located in Northern
24 Victoria, Australia. This farm has a history of problems with pre-weaning scour usually
25 occurring at 3-4 days following birth. Faecal samples obtained from the farm in the month prior
26 to the study indicated that the scour was due to a combined infection of *E. coli* (K99, STa toxin
27 genes) and Rotavirus. Current approaches such as vaccines as well as antibiotics had failed to
28 adequately control the problem.

29 This study was a blinded, placebo controlled, randomised field trial comparing two
30 parallel groups of piglets. The farmer and farm workers were blinded to the treatments.

31 There were 21 litters (233 piglets) administered my formulation (2 mL) at 2 days of age
32 (Group 1), while 23 litters (229 piglets) received a placebo (Group 2).

33 Each group contained equal gilt litters (or first time mothers).

1 The usual management routines of the farm were allowed to continue, including usual
2 medications such as sow vaccinations, antibiotics, and coccidiostats, as well as cross fostering of
3 piglets, where small piglets or those of ill health may be moved to another sow.

4 The trial was conducted by independent veterinarians and investigators from the Pig
5 Specialist Centre, Victorian Department of Economic Development, Jobs, Transport and
6 Resources. The appropriate statistical analysis was determined and applied by an independent
7 biometrician.

8 The trial investigated the incidence of death and scour, morbidity (or piglet clinical
9 condition), as well as weight gains, and average daily weight gain (ADG) from 2 to 21 days of
10 age. Piglets were monitored daily for scour and signs of any other disease. Once piglets showed
11 signs of scour, their ID numbers, faecal consistency and the general condition of piglets were
12 recorded using the scoring system described in Table 2.

13 Any piglet found to be severely depressed was euthanized on humane grounds. Piglets
14 euthanized or found dead during the trial were necropsied within 12 hours of death.

15 **Data Analysis:**

16 There were 21 – 23 replications of the two treatments randomly positioned in a shed.

17 A litter of piglets in a pen is the experimental unit.

18 The appropriate statistical analysis was determined and applied by an independent
19 biometrician.

20 Average maximum scour scores and morbidity scores per pen were analysed in a one way
21 Analysis of Variance (ANOVA) after $\log_e + 0.05$ transformed to stabilize variances. ADG for all
22 piglets was unsuitable for ANOVA because residuals were neither normally distributed or
23 homogeneous over the range of fitted values; so we used the non-parametric (distribution free)
24 Kruskal-Wallis test.

25 Mortalities and the incidences of scouring and morbidity were analysed using Exact
26 Binary Regression, suitable for small cell sizes. Two tailed tests of significance were used.

27 ANOVAs and the Kruskal Wallis Test were performed using R version 2.7.2 (2008). The
28 R Foundation for Statistical Computing. Exact Binary Regression was performed with StatExact
29 (Cytel Statistical Software, Cytel Software Corporation, MA, USA).

30 **Results:**

31 **Clinical Parameters**

32 The study was designed as a prophylactic study, where my formulation was to be
33 administered to piglets prior to the expected onset of scour. However, prior to product
34 administration, scour was evident in 59 of 462 piglets (12.8%). Despite the earlier than expected
35 onset of scour, all piglets were included in the study, and there were no exclusions.

1 Table 7 shows the number of pre-weaning mortalities in both groups due to all causes.
 2 My formulation significantly reduced piglet mortality by 47.8% (p < 0.02). Of the piglets treated
 3 with my formulation, 19 of 233 (8.2%) piglets died compared with 36 of 229 (15.7%) piglets
 4 which died in the control group.

5 **Table 7 – Pre-weaning mortality (all causes)**

Treatment	No. Pigs per group	No. pigs	% mortality
My formulation	233	19	8.2%
Untreated	229	36	15.7%
% reduction			47.8%

6
 7 The primary cause of death of piglets in this study (81.8%) was diagnosed as scour and ill thrift
 8 based on post-mortem findings.

9 **Etiology**

10 Samples were collected from 24 piglets that were less than 7 days of age. No
 11 predominant pathogen was identified in this study. Twenty three faecal samples were subject to
 12 culture (aerobic and anaerobic) to isolate possible bacterial causes of diarrhoea (*E. coli* and
 13 Clostridia). One piglet treated with my formulation tested positive for non-haemolytic *E. coli*
 14 (STa positive), and two haemolytic *E. coli* isolates (K88) were obtained from two piglets from the
 15 Control group. One of these Control piglets had a co-infection with non-haemolytic *E. coli*
 16 (K88). No Clostridia spp. were isolated. Nine of 21 (42.8%) faecal samples tested as positive or
 17 weakly positive by Rotavirus ELISA (IDEXX Rota-Corona-K99, IDEXX Montpellier SAS,
 18 France). However, none of 7 samples were positive on the rotavirus RTPCR, nor had intestinal
 19 lesions indicative of rotavirus infections.

20 A high proportion 26/53 (49%) of piglets autopsied had empty stomachs suggesting that
 21 the diarrhoea/ill-thrift present on this farm could have been attributed to inadequate colostrum
 22 and milk intake in piglets, leading to sub-optimal nutritional intake in piglets and poor lactogenic
 23 protection.

24 **Morbidity**

25 My formulation also reduced severe morbidity, or life threatening disease (Score 4). Of
 26 the piglets that had life threatening disease, or were considered moribund, 36 of 38 control
 27 piglets died, compared with 19 of 28 piglets that were moribund in group treated with my
 28 formulation.

29 **Performance Parameters**

1 Table 8 shows the mean and range of weight gains (from day of treatment, Day 2 to Day
2 21) and the average daily weight gain (ADG) for both groups.

3 Table 8 – Weight gains and ADG from Day 2 to weaning at 21 days.

Treatment	No. Pens	Litter size (weaned)	Average Weight Gain (g) (min – max)	Average ADG (g/day) (min – max)
My Formulation	21	10.19	4,188 (2,512 to 5,279)	199 (120 to 251)
Control	23	8.48	3,964 (939 to 5,205)	189 (45 to 248)
% increase			5.7%	5.6%

4

5 My formulation increased the weight gain and average daily weight gain by 5.7% (or 224 g per
6 piglet) and 5.6%. But these gains were not statistically significant ($p=0.49$) at the litter level (but
7 was significant at the individual piglet level, $p<0.04$).

8 Discussion and Conclusion:

9 The results of this study suggest that my formulation can be used reduce pre-weaning
10 mortality on farms that have a problem with diarrhea/ill-thrift of non-specific etiology.

11 Ill-thrift and failure-to-thrive is a major cause of death among piglets in the first week of
12 life. Piglets that fail to ingest colostrum in the first 24 hours after birth are at risk of early death
13 as a result of crushing by the sow or exposure due to inadequate energy intake. Unlike human
14 babies, no antibodies are transferred to piglets via the placenta from the sow. So without
15 maternal antibodies, the piglet is highly susceptible to infection. If they survive the first few days,
16 but continue to have inadequate milk intake, piglets are more likely to succumb to infectious
17 disease due to low lactogenic immunity (from milk antibodies and immune factors in the sow's
18 milk) compared with their more robust littermates. Those that fail to sustain adequate milk
19 intake, either through poor milk production by the sow or low consumption by the piglet are
20 again more likely to succumb to an early death due to an inability to compete with littermates or
21 to infectious disease later in life.

22 In this study, my formulation halved the pre-weaning mortality rate among sucker piglets
23 on a commercial farm. It would appear that its mode of action was through improving the vigour
24 and therefore the survivability of moribund piglets in the first week of life. This is demonstrated
25 by the lower proportion (45.2%) of piglets treated with my formulation that were classified as
26 clinically moribund that died or had to be euthanized, compared with 76.6% in the Control group
27 in the same clinical category.

28 Piglets treated with my formulation grew 5.7% faster than piglets in the control group.
29 Although this weight gain difference was not significant, this equates to approximately a 225g

1 difference in live-weight at weaning. Weaning weight is positively associated with subsequent
2 growth and survival of piglets.

3 Summary

4 Given my disclosure here, one may readily make certain variants and alternatives. For
5 example, the formulation of Table 1 provides a dosage suitable for prophylactic treatment of a
6 suckling piglet to prevent scour. Use to treat (rather than prevent) scour may require a different
7 dose; the artisan would readily be able to derive the appropriate dose. Similarly, use to treat a
8 mature adult pig, or a human may require a larger dose; the artisan would readily be able to
9 derive the appropriate dose.

10 One may provide my formulation as an oral drench, for example as a granulated powder
11 requiring reconstitution with water. To prevent post-weaning scour, my formulation may be
12 given as a once only oral dose 4 ml (0.24 g) on the day of weaning (1-2 days before the expected
13 on set of scour). To prevent pre-weaning scour, a 2 ml (0.12 g) single oral dose can be
14 administered at 2-5 days of age, depending on a particular farm's problem period. A repeat dose
15 may be required 3-7 days later. As a treatment, my formulation may be administered (either 2
16 ml or 4 ml) immediately when symptoms of disease occur.

17 One may provide my formulation as a feed additive, for example prepared as a granulated
18 powder that can added to pig feed. To ensure thorough dispersion of the product it should first be
19 mixed with a suitable quantity of feed ingredients before incorporation in the final mix. My
20 formulation may be fed as a pre-mix only, or the pre-mix incorporated in the final mix. The
21 recommended dose level is 40 mg my formulation /kg bodyweight fed daily for 14 consecutive
22 days.

23 One may also deliver my formulation in water via drinking systems. Alternatively, one
24 may use bromelain to make an equivalent oral drench, formulated with excipients and requiring
25 reconstitution in liquid. To prevent post-weaning scour, this may be given as a once only oral
26 dose (125 mg) on the day of weaning (1-2 days before the expected on set of scour). To prevent
27 pre-weaning scour, a 62.5 mg single oral dose may be administered at 2-5 days of age,
28 depending on a particular farm's problem period. A repeat dose may be required 3-7 days later.
29 As a treatment, it may be administered (either 62.5 mg or 125 mg) immediately when symptoms
30 of disease occur.

31 Alternatively, one may use bromelain to make an equivalent feed additive, for example as
32 a powder that can added to pig feed. To ensure thorough dispersion of the product it should first
33 be mixed with a suitable quantity of feed ingredients before incorporation in the final mix. It may
34 be fed as a pre-mix only, or the pre-mix incorporated in the final mix. The recommended dose
35 level is 20 mg bromelain/kg bodyweight fed daily for 14 consecutive days.

36 Alternatively, my formulation may be provided as tablet and capsules, and other
37 appropriate dose forms for humans.

38 The skilled artisan may adjust my formulation for different indications. For example, it
39 may be used for the prevention and treatment of scour in production animals (cattle, swine etc.)
40 and diarrhea in humans. It may also be used for improved gut health by reducing inflammation.

1 Alternatively, it may be formulated to promote increased feed intake in production animals, thus
2 promoting weight gains and feed conversion efficiency. It may be used to reduce the
3 requirement for antibiotics in animal feed, and for acute administration to humans. It may also
4 be used to ameliorate Inflammatory Bowel Disease in humans.

5 I thus intend the legal coverage of this patent to be defined not by the specific example
6 recited here, but by the legal claims and permissible equivalents thereof.

7

8

9

CLAIMS

- 1
2 1. An oral veterinary formulation comprising an anti-diarrhea effective amount of bromelain,
3 substantially free of enteric polymer.
- 4 2. An oral veterinary formulation comprising an anti-diarrhea effective amount of bromelain,
5 substantially free of phthalate.
- 6 3. The oral veterinary formulation of claim 2, wherein said phthalate comprises cellulose
7 acetate phthalate.
- 8 4. An oral veterinary formulation comprising an anti- diarrhea effective amount of
9 bromelain, and chelating agent.
- 10 5. The oral veterinary formulation of claim 4, wherein the chelating agent comprises EDTA.
- 11 6. An oral veterinary formulation comprising an anti- diarrhea effective amount of
12 bromelain and an emulsifier in an amount effective to improve the solubility of said bromelain in
13 water.
- 14 7. The oral veterinary formulation of claim 6, said emulsifier comprising lecithin.
- 15 8. The oral veterinary formulation of claim 7, further comprising citric acid.
- 16 9. An anti-diarrheal oral dosage form comprising carboxymethylcellulose.
- 17 10. The dosage form of claim 9, further comprising bromelain.
- 18 11. The dosage form of claim 10, where the oral dosage form is a drink or oral drench.
- 19 12. The dosage form of claim 10, where the dosage form is a veterinary dosage form.
- 20 13. A method for preventing the proliferation of anti-microbial resistant pathogenic gut
21 microbes in humans, animals or fish, comprising: (a) identifying a human, animal or fish
22 vulnerable to infection by pathogenic gut microbes, and then (b) orally administering to said
23 human, animal or fish an amount of bromelain effective to prevent said human, animal or fish
24 from becoming diarrheal from said pathogenic gut microbes, whereby said human, animal or fish
25 is no longer vulnerable to infection by said pathogenic gut microbes, whereby said human,
26 animal or fish is not administered an anti- microbial effective amount of an anti-microbial to
27 treat or prevent diarrhea by said pathogenic gut bacteria.
- 28 14. A method for preventing the proliferation of pathogenic gut microbes in humans, animals or
29 fish, comprising: (a) identifying a human, animal or fish vulnerable to infection by pathogenic gut
30 microbes, and then (b) orally administering to said human, animal or fish an amount of bromelain
31 effective to prevent said human, animal or fish from becoming sick from said pathogenic gut
32 microbes, whereby said human, animal or fish is no longer vulnerable to infection by said pathogenic
33 gut microbes, whereby said human, animal or fish is not administered an anti-microbial effective
34 amount of an anti-microbial to treat or prevent said infection by said pathogenic gut microbes.

- 1 15. The dosage form of Claim 2, where the dosage form is animal feed.

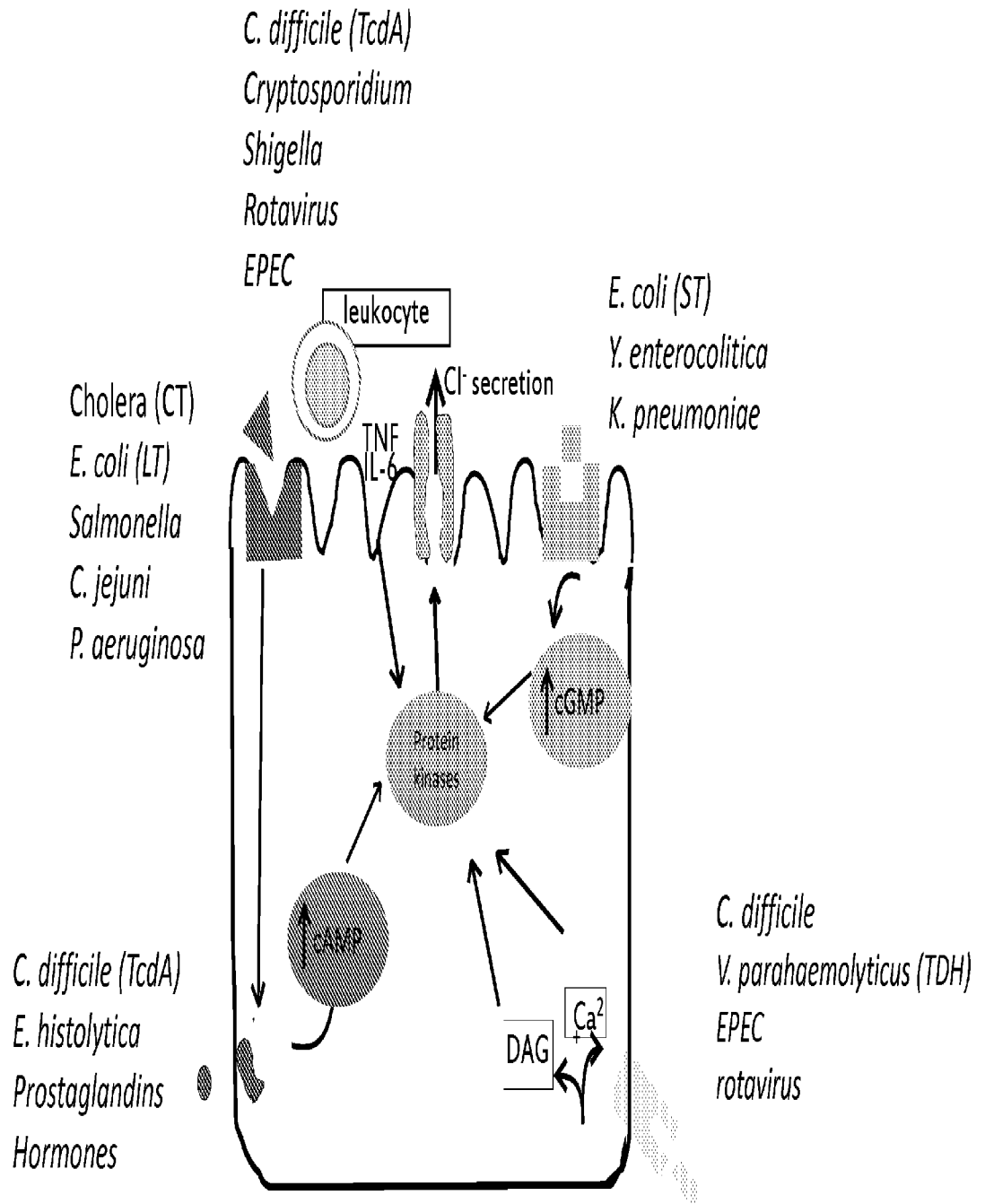


Figure 1 - There are many different causes of diarrhea that act by activating one or more cell secretory pathways. Viswanathan et al., *Nature Review Microbiol*, 2009; Hodges & Gill. *Gut Microbes*. 2010.

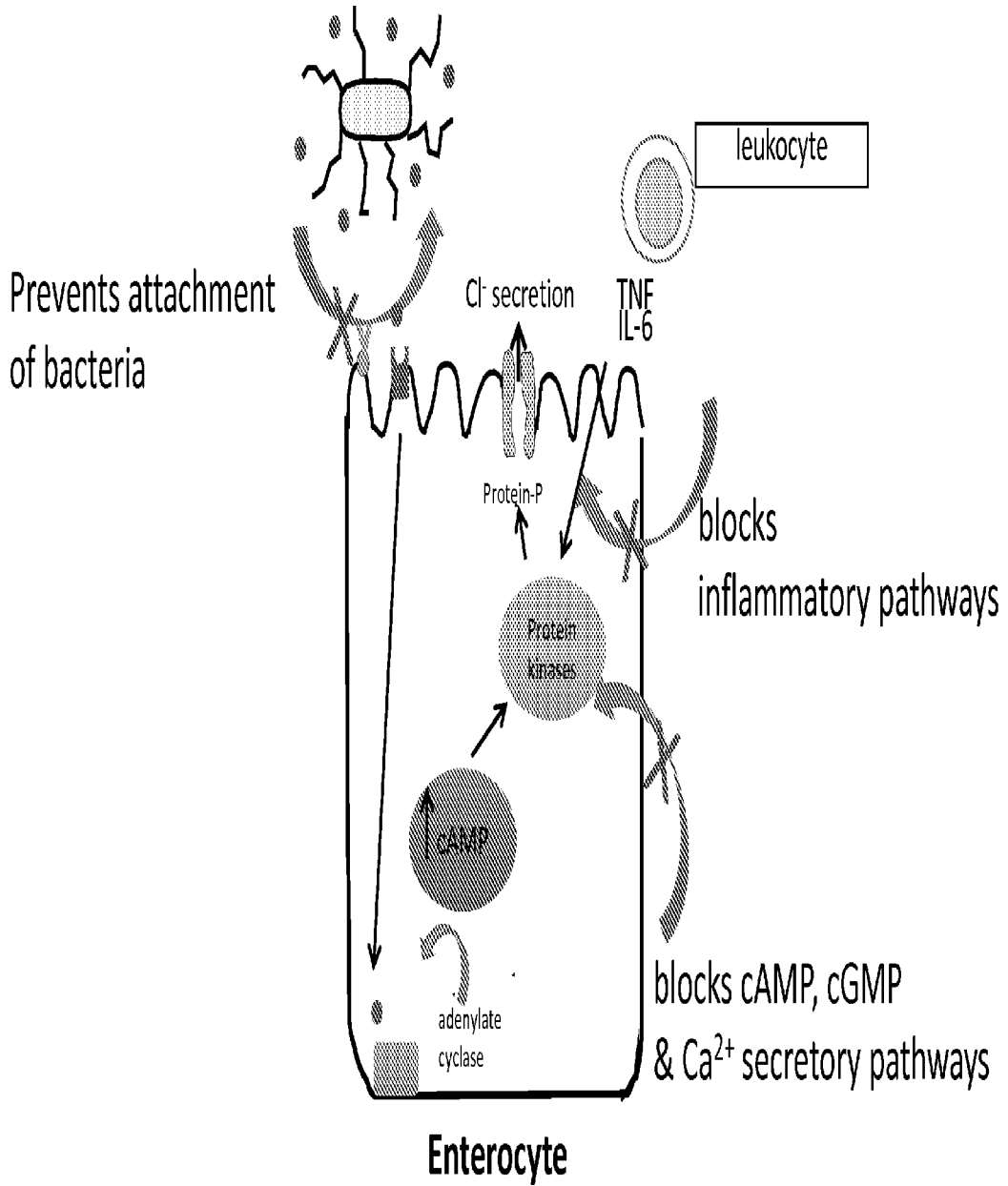


Figure 2 – Mechanism of action of My formulation. Triple mechanism of action – provides broad spectrum protection against bacteria, parasites, viruses and inflammation.

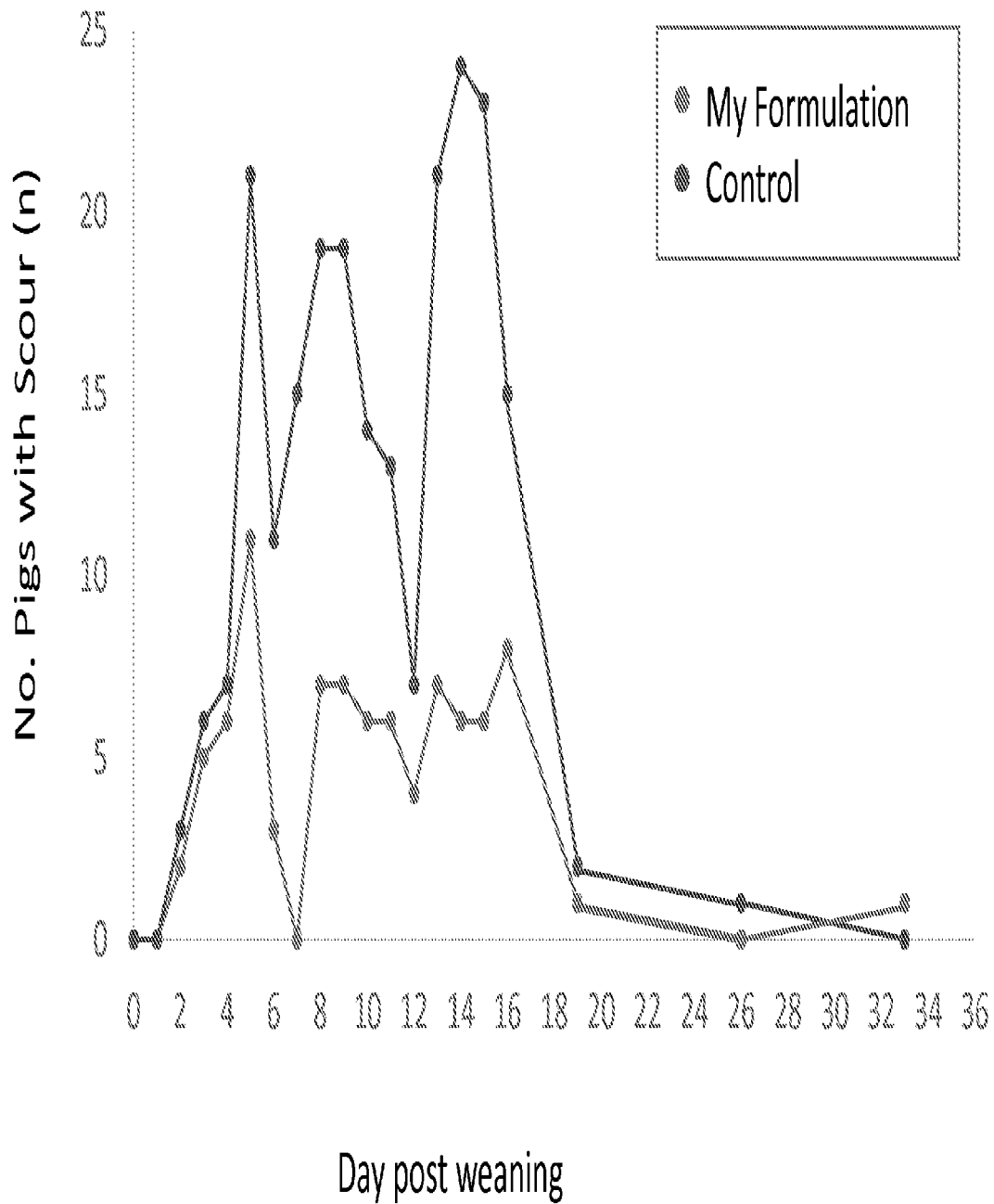


Figure 3 – One group of piglets was administered a single oral dose (4 mL) of My formulation at weaning (day 0), or were untreated (Control). My formulation significantly reduced the incidence of scour (diarrhoea) ($P < 0.05$).

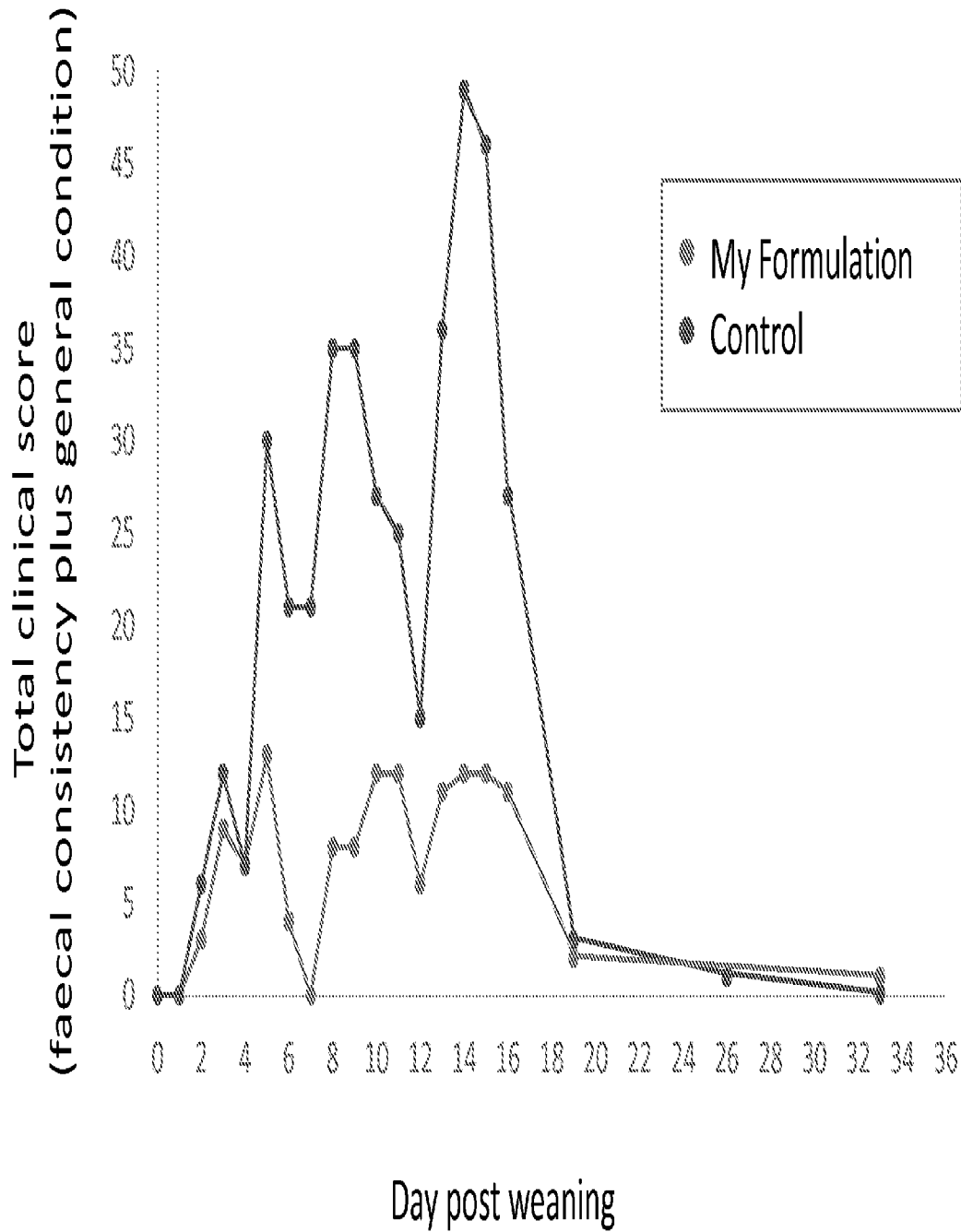


Figure 4 – One group of piglets was administered a single oral dose of My formulation at weaning (day 0). A second group of piglets were untreated (Control). My formulation significantly reduced the clinical score, and therefore improved the health of piglets ($P < 0.01$).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/046509

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23K 1/165 (2015.01)

CPC - A23K 1/1653 (2015.10)

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)

IPC(8) - A23K 1/00, 1/16, 1/165, 36/00, 36/31, 36/886; A61P 1/12 (2015.01)

CPC - A23K 1/1631, 1/1634, 1/1643, 1/1646, 1/1653, 1/1866, 1/1893; A61K 36/00, 36/31, 36/886 (2015.10)

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

CPC - A23K 1/1631, 1/1634, 1/1643, 1/1646, 1/1653, 1/1866, 1/1893; A61K 36/00, 36/31, 36/886 (2015.10) (keyword delimited)

USPC - 424/50, 94.65, 442

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

Orbit, Google Patents, Google Scholar.

Search terms used: (anti-diarrheal OR antidiarrheal) bromelain EDTA (lecithin OR phosphatidylcholine) carboxymethylcellulose (citrate OR citric) inassignee:anataara ininventor:mynott, walsh (animal OR veterinary) (beverage OR drink OR liquid)

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 2006/0052438 A1 (HO et al) 09 March 2006 (09.03.2006) entire document	9-12 ----- 4-8, 15
X - Y	CHANDLER et al. "Bromelain protects piglets from diarrhea caused by oral challenge with K88 positive enterotoxigenic Escherichia coli," Gut, 01 August 1998 (01.08.1998), Vol. 43, Pgs. 196-202. entire document	13, 14 ----- 1-8, 15
Y	GASPANI et al. "In vivo and in vitro Effects of Bromelain on PGE2 and SP Concentrations in the Inflammatory Exudate in Rats," Pharmacology, 01 May 2002 (01.05.2002), Vol. 65, Pgs. 83-86. entire document	1-8, 15
A	US 6,080,403 A (SHIELDS, JR et al) 27 June 2000 (27.06.2000) entire document	1-15
A	MYNOTT et al. "Bromelain Prevents Secretion Caused by Vibrio cholerae and Escherichia coli Enterotoxins in Rabbit Ileum In Vitro," Gastroenterology, 01 July 1997 (01.07.1997), Vol.113, Pgs. 175-184. entire document	1-15
A	MYNOTT et al. "Oral administration of protease inhibits enterotoxigenic Escherichia coli receptor activity in piglet small intestine," Gut, 01 January 1996 (01.01.1996), Vol. 38, Pgs. 28-32. entire document	1-15
A	FAIBROTHER et al. "Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies," Animal Health Research Reviews, 01 June 2005 (01.06.2005), Vol. 6, Pgs. 17-39. entire document	1-15
A	WO 2009/009879 A1 (AQUIENCE, INC) 22 January 2009 (22.01.2009) entire document	1-15

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

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"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

23 October 2015

Date of mailing of the international search report

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Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774