



(51) International Patent Classification:

A23K 1/16 (2006.01) A61K 31/716 (2006.01)
A23K 1/175 (2006.01) A61K 31/736 (2006.01)
A61K 38/47 (2006.01) A61K 35/02 (2015.01)
A61K 45/06 (2006.01)

(21) International Application Number:

PCT/US2015/015692

(22) International Filing Date:

12 February 2015 (12.02.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/939,206 12 February 2014 (12.02.2014) US
62/000,986 20 May 2014 (20.05.2014) US

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
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KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
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SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: COMPOSITION AND METHOD FOR PROMOTING REDUCTION OF HEAT STRESS IN ANIMALS

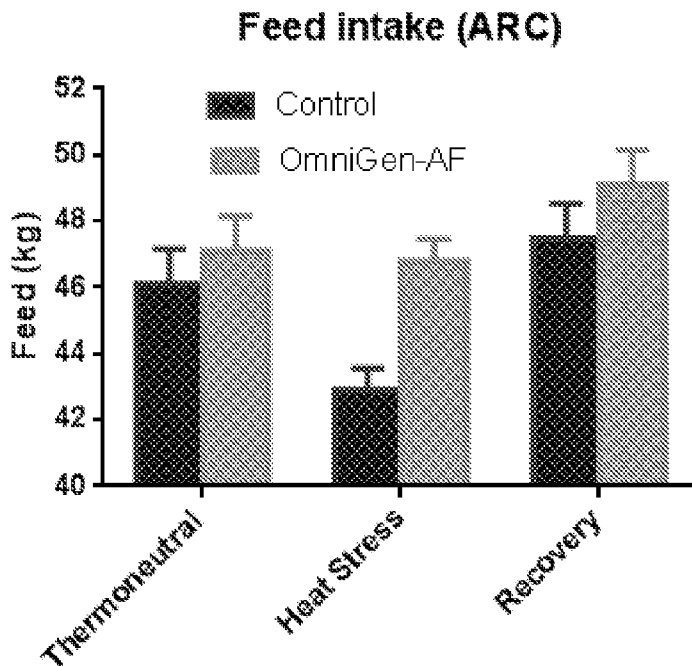


FIG. 5

(57) Abstract: Disclosed herein is a method for promoting reduction of heat stress in animals, as well as a composition for use in the disclosed method. The composition comprises a glucan, silica, mineral clay, mannan, and optionally an endoglucanohydrolase, and may be administered to an animal that is susceptible to or suffers from heat stress.





Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

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

COMPOSITION AND METHOD FOR PROMOTING REDUCTION OF HEAT STRESS IN ANIMALS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of the earlier filing date of U.S. Provisional Application No. 62/000,986, filed May 20, 2014, and the benefit of the earlier filing date of U.S. Provisional Application No. 61/939,206, filed February 12, 2014, each of which is incorporated by reference herein in its entirety.

FIELD

10 This disclosure relates to methods and compositions for promoting reduction of heat stress in animals.

BACKGROUND

Heat stress causes significant economic losses in animal industries, such as dairy
15 industries. Production, reproduction and animal health are impaired by hyperthermia. Physiological and production responses are well documented, but not fully understood. During heat stress, respiration rates and body temperatures increase, and feed intake, milk yield, and reproduction decrease.

Milk synthesis decreases from reduced feed intake. There are additional losses in milk
20 yield that are associated metabolic changes (Rhoads et al., 2009). Countering the production effects of heat stress in lactating dairy cows involves reduced maintain feed intake and controlling metabolic flux.

Immune function and health are also reduced with heat stress. The severity and occasion
of disease are increased when immune and inflammatory responses are impaired. The rumen
25 environment can be altered during thermal stress. Increased respiration rates can cause respiratory alkalosis, rumen acidosis and eventually metabolic acidosis. Oxidative stress can be increased with heat stress and can impair the heat shock response and increase cell damage and death.

30 SUMMARY

The object of the present disclosure is to provide a novel and previously unknown method for promoting reduction of heat stress in animals. Also disclosed herein is a composition suitable for administering to animals in order to substantially prevent and/or

promote reduction of heat stress in animals. The disclosed composition and method for preventing and/or promoting reduction of heat stress may be applied to any animal species susceptible to heat stress, such as mammals, avians, or aquatic animal species. In some embodiments, the animals treated herein can be an animal raised for human consumption, such as livestock (*e.g.*, feed or dairy cattle) or pigs, or avians, such as domestic fowl (*e.g.*, chicken, turkey, goose, duck, cornish game hen, quail, pheasant, guinea-fowl, ostrich, emu, swan, or pigeon), or fish (*e.g.*, salmon, trout and the like). In other embodiments, the animal can be a domestic animal, such as a dog, cat, fish, or rabbit. In some other embodiments, the animal can be a ruminant species, such as a sheep, goat, cow, deer, bison, buffalo, or llama. In yet other
5
10
embodiments, the animal can be an ungulate, such as a horse, donkey, or pig.

Disclosed herein are embodiments of a method for promoting heat stress reduction in an animal, such as a mammal or avian species by administering to the mammal or avian species a composition comprising glucan, silica, mineral clay and mannan. In some embodiments the composition comprises between 15% and 40% silica, between 50% and 81% mineral clay,
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between 1.0% and 5.0% β -glucans, and between 1% and 8.0% mannan. In a particular embodiment the composition consists essentially of β -glucans, β -1,3 (4)-endoglucanohydrolase, diatomaceous earth, a mineral clay, and glucomannan.

In any or all of the above embodiments, the composition can be administered prophylactically, or when the animal has or is at risk of developing heat stress. Heat stress may
20
occur when the animal is exposed to a temperature humidity index of 68 or greater, 75 or greater, or ≥ 79 .

In any or all of the above embodiments, heat stress may be evidenced by a heat stress indicator. Heat stress indicators include, but are not limited to, feed intake, water consumption, respiration rate, rectal temperature, milk yield, milk fat, milk protein, or any combination
25
thereof. In some embodiments the composition is administered prior to the animal experiencing heat stress while the animal is experiencing heat stress, and/or after the animal experiences heat stress. In any or all of the above embodiments, the animal may be immunosuppressed prior to administration of the composition, for example as a result of heat stress.

In any or all of the above embodiments, the composition may be administered at set
30
intervals to the animal for an effective period of time to promote reduction of heat stress. The set interval can be daily, or it can be more or less frequently than that. In some embodiments the effective period of time is from 0 days to 200 days, from 1 day to 200 days, from 1 day to 90 days, from 1 day to 60 days, from 1 day to 45 days, from 1 day to 30 days, from 3 days to

21 days, at least 1 day, at least 3 days, at least 7 days, at least 30 days, at least 60 days, or at least 90 days.

In any or all of the above embodiments, the amount administered to the animal can range from 1 mg/kg body weight per day to 20 g/kg body weight per day. In any or all of the above
5 embodiments, the composition may be administered by mixing the composition with the animal's feed in an amount ranging from 0.1 to 20 kg per ton of feed and providing the composition mixed with the feed to the animal. In some embodiments, the animal is a bovine and the amount administered to that animal can range from 0.5 grams to 100 grams daily.

In any or all of the above embodiments, an amount of the composition administered to
10 the animal may be increased if the temperature humidity index is expected to increase or decreased if the temperature humidity index is expected to decrease.

In any or all of the above embodiments, the animal may be administered an amount of the composition effective to alter a heat stress indicator compared to an animal that has not been administered the composition. In some embodiments, the method further includes measuring
15 the heat stress indicator, and selecting an amount of the composition administered to the animal based at least in part on the measurement of the heat stress indicator.

In particular embodiments the animal is a cow, such as, for example, a dairy cow. Dairy cows may be administered the composition before lactation. This can be from 90 days prior to lactation onset to 1 day prior to lactation onset, preferably from 45 days prior to lactation onset
20 to 10 days prior to lactation onset. The composition may be administered daily prior to lactation onset. In any or all of the above embodiments, the dairy cow may be fed the composition for a period of time to increase milk production and/or reduce milk fat relative to dairy cattle not fed the composition.

The animal that is administered the composition may have reduced water intake relative
25 to animals not fed the composition. The animal also may have a reduced respiration rate relative to animals not fed the composition. In other embodiments, the animal may have a reduced rectal temperature relative to animal not fed the composition. In particular disclosed embodiments, the animal may exhibit increased food intake relative to animal not fed the composition. Also, the animal may exhibit decreased respiratory alkalosis, rumen acidosis, metabolic acidosis, and any
30 and all combinations thereof, relative to animals not fed the composition. In other embodiments, the animal may have decreased serum cortisol levels relative to animals not fed the composition.

In any or all of the above embodiments, the method may further comprise administering to the mammal or avian species a therapeutic process and/or a therapeutic agent suitable for

treating heat stress. Exemplary therapeutic processes and agents include, but are not limited to, provision of shade to the animal, use of a water sprinkler to externally administer water to the animal, use of a fan to provide air movement, addition of bypass fats to feed for the animal, meloxicam, a corticosteroid, a composition comprising one or more electrolytes, an alkalinizing agent, a direct-fed microbial, or a combination thereof.

In any or all of the above embodiments, the method may include administering a first amount of the composition to a group of animals that have or are at risk of developing heat-induced stress for a first period of time, measuring a heat stress indicator of at least one animal of the group of animals, and administering an adjusted amount of the composition to the group of animals for a subsequent period of time, wherein the adjusted amount is based at least in part on the measurement of the heat stress indicator.

In any or all of the above embodiments, the method may include evaluating at least one heat stress indicator of individual animals in a group of animals, determining whether one or more of the individual animals are experiencing heat stress, and administering the composition to the one or more individual animals that are experiencing heat stress.

An exemplary embodiment comprises selecting a dairy cow that has or is at risk of developing heat induced stress and administering to the dairy cow before, during, and/or after lactation a composition comprising glucan, silica, mineral clay, mannan, and optionally an endoglucanohydrolase, wherein the composition is administered at set intervals for an effective period of time sufficient to (1) increase milk production relative to dairy cattle not fed the composition, (2) lower milk fat relative to dairy cattle not fed the composition, (3) reduce water intake relative to dairy cattle not fed the composition, (4) reduce respiration rate relative to dairy cattle not fed the composition, (5) reduce rectal temperature relative to dairy cattle not fed the composition, (6) increase food intake relative to dairy cattle not fed the composition, (7) decrease respiratory alkalosis, rumen acidosis, and/or metabolic acidosis relative to dairy cattle not fed the composition, (8) decrease serum cortisol levels relative to dairy cattle not fed the composition, or (9) any and all combinations thereof. The method may further comprise administering to the dairy cow a therapeutic agent suitable for treating heat stress.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar chart illustrating on-dairy milk yield (kg) by cows that are administered the disclosed composition and those that are not, including both initial and on-dairy conditions.

FIG. 2 is a bar chart illustrating milk yield for control cows and cows that have been administered the disclosed composition during thermoneutral conditions (TN), heat stress conditions (HS), and recovery conditions (Recovery).

FIG. 3 is a graph illustrating mean milk production by day for control cows and those
5 that have been administered the disclosed composition.

FIG. 4 is a graph illustrating feed intake by day for control cows and those that have been administered the disclosed composition.

FIG. 5 is a bar chart illustrating feed intake for control cows and cows that have been administered the disclosed composition during thermoneutral conditions, heat stress conditions,
10 and recovery conditions.

FIG. 6 is a graph illustrating somatic cell count by day for control cows and those that have been administered the disclosed composition.

FIG. 7 is a bar chart illustrating mean serum cortisol for control cows and cows that have been administered the disclosed composition during thermoneutral conditions (TN) and heat
15 stress conditions (HS), measured after a certain number of days.

FIG. 8 is a bar chart illustrating mean serum insulin levels for control cows and cows that have been administered the disclosed composition during thermoneutral conditions (TN) and heat stress conditions (HS), measured after a certain number of days.

FIG. 9 is a bar chart illustrating mean serum glucose levels for control cows and cows
20 that have been administered the disclosed composition during thermoneutral conditions (TN) and heat stress conditions (HS), measured after a certain number of days.

FIG. 10 is a bar chart illustrating IL8R receptor gene expression leukocytes in lactating control cows and cows that have been administered the disclosed composition during thermoneutral conditions and heat stress conditions, measured after a certain number of days.

FIG. 11 is a bar chart illustrating RANTES protein levels in control cows and cows that
25 have been administered the disclosed composition prior to heat stress, during acute heat stress, chronic heat stress, heat stress recovery and long-term recovery.

FIG. 12 is a bar chart illustrating serum ACTH levels in control cows and lactating dairy cows that have been administered the disclosed composition during thermoneutral conditions
30 (TN) and heat stress conditions (HS), measured after a certain number of days.

FIG. 13 is a graph showing rectal temperature of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a subcutaneous lipopolysaccharide challenge.

FIG. 14 is a graph showing change in rectal temperature of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a subcutaneous lipopolysaccharide challenge.

5 FIG. 15 is a graph showing rectal temperature of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

10 FIG. 16 is a graph showing change in rectal temperature of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

FIG. 17 is a bar graph showing overall cortisol concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt during the receiving period (OMN-28) or no OmniGen (CON) during a lipopolysaccharide challenge.

15 FIG. 18 is a graph showing cortisol concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

20 FIG. 19 is a series of bar graphs showing treatment, prior, and post lipopolysaccharide challenge concentrations of blood urea nitrogen (BUN) for newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control).

FIG. 20 is a bar graph showing treatment concentrations of glucose concentrations for newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

25 FIG. 21 is a graph showing glucose concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

30 FIG. 22 is a series of bar graphs showing treatment, prior, and post lipopolysaccharide challenge concentrations of non-esterified fatty acids of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control).

FIG. 23 is a series of bar graphs showing treatment, prior, and post lipopolysaccharide challenge concentrations of interferon - γ concentrations of newly received steers supplemented

with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control).

FIG. 24 is two bar graphs showing treatment and post lipopolysaccharide challenge concentrations of tumor necrosis - α (TNF- α) overall and post LPS challenge concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control).

FIG. 25 is a graph showing tumor necrosis - α (TNF- α) concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

FIG. 26 is a graph showing interleukin-6 (IL-6) concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

FIG. 27 is a graph showing lymphocyte concentrations of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

FIG. 28 is a graph showing the neutrophil to lymphocyte ratio of newly received steers supplemented with the disclosed composition at a rate of 4 g/cwt (OmniGen) during the receiving period (28 d) or no OmniGen (Control) during a lipopolysaccharide challenge.

DETAILED DESCRIPTION

I. Definitions

The following explanations of terms and abbreviations are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. As used herein, “comprising” means “including” and the singular forms “a” or “an” or “the” include plural references unless the context clearly dictates otherwise. The term “or” refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise.

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

be limiting. Other features of the disclosure are apparent from the following detailed description and the claims.

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

Animal: This term includes species that are produced for human consumption or that are domesticated animals. Exemplary species of such animals are provided herein.

Administering: Administration by any route to the subject. As used herein, administration typically refers to oral administration.

Mannans: A class of polysaccharides including the sugar mannose. The mannan family includes pure mannans (*i.e.*, the polymer backbone consists of mannose monomers), glucomannans (the polymer backbone comprises mannose and glucose), and galactomannans (mannans or glucomannans in which single galactose residues are linked to the polymer backbone). Mannans are found in cell walls of some plant species and yeasts.

Mineral Clay: The term “mineral clay” refers to hydrous aluminum silicates. Mineral clays usually include minor amounts of impurities, such as potassium, sodium, calcium, magnesium, and/or iron. Mineral clays typically have a two-layer sheet structure including tetrahedral silicate sheets and octahedral hydroxide sheets or a three-layer structure including a hydroxide sheet between two silicate sheets.

Therapeutic agent: An agent that is capable of providing a therapeutic effect, *e.g.*, preventing a disorder, inhibiting a disorder, such as by arresting the development of the disorder or its clinical symptoms, or relieving a disorder by causing regression of the disorder or its clinical symptoms.

Therapeutically effective amount: A quantity or concentration of a specified compound or composition sufficient to achieve a desired effect in a subject being treated for a disorder. The therapeutically effective amount may depend at least in part on the species of animal being treated, the size of the animal, and/or the severity of the disorder.

Additional disclosure is found in U.S. Patent Application No. 13/566,433, U.S. Patent Application No. 13/872,935, U.S. Patent Publication No. 2013/0017211, U.S. Patent Publication

No. 2012/0156248, U.S. Patent Publication No. 2007/0253983, U.S. Patent Publication No. 2007/0202092, U.S. Patent Publication No. 20070238120, U.S. Patent Publication No. 2006/0239992, U.S. Patent Publication No. 2005/0220846, U.S. Patent Publication No. 2005/0180964, and Australian Patent Application No. 2011201420, all of which are
5 incorporated herein by reference.

II. Composition

The present disclosure is based on the novel discovery that a combination of glucan, silica, mineral clay, mannans can effectively prevent or reduce heat stress in animals.

10 Embodiments of the disclosed composition comprise one or more components as disclosed herein. The composition typically comprises glucan (*e.g.*, β -1,3 (4)glucan), silica, mineral clay and mannans. In some embodiments, the composition further comprises an endoglucanohydrolase, such as β -1,3 (4)-endoglucanohydrolase. Suitable sources of silica include, but are not limited to, sand, quartz, diatomaceous earth, and synthetic silica. In certain
15 embodiments, the mannans comprise glucomannan.

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

In one embodiment, the composition includes 1-40 wt% silica, 1-25 wt% glucan and mannans, and 40-92 wt% mineral clay. In another embodiment, the composition comprises 5-40 wt% silica, 2-15 wt% glucan and mannans, and 40-80 wt% mineral clay. In another
30 embodiment, the composition comprises 20-40 wt% silica, 4-10 wt% glucan and mannans, and 50-70 wt% mineral clay. In another embodiment, the composition comprises 15-40 wt% silica, 1-15 wt% glucans, 0-10 wt% mannans, and 50-81 wt% mineral clay. In another embodiment, the composition comprises 15-40 wt% silica, 1.0-5.0 wt% glucans, 1.0-8.0 wt% mannans, and

50-81 wt% mineral clay. In another embodiment, the composition comprises 20-30 wt% silica, 1.0-3.5 wt% glucans, 1.0-6.0 wt% mannans, and 60-75 wt% mineral clay.

In some embodiments, β -glucans and mannans are obtained from yeast cell wall extract, and Composition I comprises 1-40 wt% silica, 1-30 wt% yeast cell wall extract, 40-92 wt% mineral clay. In one embodiment, Composition I comprises 10-40 wt% silica, 5-20 wt% yeast cell wall extract, and 40-80 wt% mineral clay. In another embodiment, Composition I comprises 15-30 wt% silica, 5-15 wt% yeast cell wall extract, and 55-70 wt% mineral clay.

In any of the above embodiments, the composition may further comprise an endoglucanohydrolase, such as β -1,3 (4)-endoglucanohydrolase. The composition may include from at least 0.05 wt% endoglucanohydrolase to 5 wt% endoglucanohydrolase, such as from 0.05-3 wt% β -1,3 (4)-endoglucanohydrolase. In one embodiment, Composition I consists essentially of 0.1-3 wt% β -1,3 (4)-endoglucanohydrolase, 20-40 wt% silica, 2-20 wt% glucan and mannans, and 50-70 wt% mineral clay. In another embodiment, the composition consists essentially of 0.2-3 wt%, β -1,3 (4)-endoglucanohydrolase, 20-40 wt% silica, 4-10 wt% glucan and mannans, and 50-70 wt% mineral clay. In any of the above embodiments, the silica may be provided by diatomaceous earth. In any of the above embodiments, the glucans may be β -glucans. In some embodiments, the β -glucans can be obtained from yeast, or other materials, such as fungi, algae, or the like. In any of the above embodiments, the mannans may comprise glucomannan.

The glucan and mannans (or yeast cell wall extract) can be prepared by a method commonly known in the art. In an independent embodiment, it can be a commercial source of β -1,3 (4) glucan and glucomannan derived from primary inactivated yeast (*Saccharomyces cerevisiae*) with the following chemical composition:

Moisture 3.5-6.5%

Proteins 1-6%

Fats 0-0.5%

Phosphorus 0-0.2%

Mannans 9-20%

β -1, 3-(4) glucan 9-18%

Ash 75-85%; and in some embodiments, the composition can further comprise Dry matter 97-98%.

In another independent embodiment, the composition can comprise:

Moisture 2-3%

Dry matter 97-98%

Proteins 14-17%

Fats 20-22%

Phosphorus 1-2%

5 Mannans 22-24%

β -1, 3-(4) glucan 24-26%

Ash 3-5%.

The mineral clays (aluminosilicates) used in this composition may be fulfilled by any of a variety of commercially-available clays including, but not limited to, montmorillonite clay,
10 bentonite and zeolite.

In an independent embodiment of the composition, silica, glucan and mannans, and mineral clay are combined at 1-40%, 1-25% and 40-92%, respectively. In an independent embodiment of the composition, β -1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall extract and mineral clay are combined at 0.05-3%, 1-40%, 1-20% and 40-92%,
15 respectively. In an independent composition, β -1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall extract and mineral clay are combined at 0.1-3%, 5-40%, 2-10% and 40-80%, respectively. In another independent embodiment of the composition, β -1,3 (4)-endoglucanohydrolase, diatomaceous earth, yeast cell wall extract and mineral clay are combined at 0.2-3%, 30-40%, 4-6% and 50-65%, respectively.

20 In some embodiments, the composition includes additional components. Additional components may be used for any desired purpose, such as a substantially biologically inert material added, for example, as a filler, or to provide a desired beneficial effect. For example, the composition may include a carbonate (including a metal carbonate such as calcium carbonate), kelp, a vitamin (such as a niacin supplement or vitamin B-12 supplement), biotin, d-
25 calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, menadione dimethylpyrimidinol bisulfite, riboflavin-5-phosphate, folic acid, soybean oil, calcium aluminosilicate, rice hulls, mineral oil, or any combination thereof.

The composition may be formulated in any suitable form, including a powder, a granule, a pellet, a solution, or a suspension. In one embodiment, the composition can be a dry, free-
30 flowing powder suitable for direct inclusion into a commercially-available feed, food product or as a supplement to a total mixed ration or diet. The powder may be mixed with either solid or liquid feed or with water. In another embodiment, the composition can be formed into pellets.

In one embodiment, when incorporated directly into feeds, the composition may be

added in amounts ranging from 0.1 to 100 kg per ton, such as from 0.1 to 20 kg per ton (2000 pounds) of feed. In some embodiments, the composition can be added to animal feedstuffs or to food in amounts from 0.1 kg to 50 kg per ton, from 0.1 to 20 kg per ton, or from 0.5 kg to 10 kg per ton of feed. In certain embodiments, the composition may be added to feeds in amounts
5 ranging from 1 to 5 kg per ton of feed.

When expressed as a percentage of dry matter of feed, the composition may be added to animal feedstuffs or to foods in amounts ranging from 0.01 to 2.5% by weight, such as from 0.0125% to 2% by weight. In one embodiment, the composition can be added to animal feedstuffs or to food in amounts from 0.05 to 1.5% by weight, such as from 0.06% to 1% by
10 weight. In another embodiment, the composition can be added in amounts from 0.1 to 0.7% by weight, such as from 0.125% to 0.5% by weight of feed.

Alternatively, the composition may be fed directly to the animal as a supplement in amounts of from 0.01 gram to 20 gram per kilogram of live body weight per day, such as from 0.01 gram to 10 gram per kilogram, 0.01 gram to 5 gram, 0.01 gram to 1 gram, 0.015 gram to
15 1 gram, or 0.02 gram to 0.4 gram per kilogram of live body weight per day. In some embodiments, the composition may be provided for use with many species in amounts of from 0.05 grams to 0.20 grams per kilogram of live body weight per day.

Additionally, the composition may be fed to mammalian animals or avian species as a supplement (in combination with other feed, or alone) in amounts ranging from 10 grams per
20 head per day to 70 grams per head per day, such as from 40 grams per head per day to 70 grams per head per day, from 45 grams per head per day to 70 grams per head per day, or from 50 grams per head per day to 70 grams per head per day. In exemplary embodiments, the composition can be provided to bovines in an amount ranging from 50 grams per head per day to 60 grams per head per day, such as 56 grams per head per day.

The physical form of the composition can be a dry, free-flowing powder which is suitable for direct inclusion into a feed, food product or as a supplement to a total mixed ration or diet.
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Alternatively, the composition contained in the present composition may be fed directly to mammalian or avian species as a supplement in amounts 0.016 grams/kg to 0.37 grams/kg of
30 live body weight per day. In an independent embodiment, the composition may be provided to mammalian and avian species in amounts of 0.10 grams/kg to 0.20 grams/kg of body weight per day.

In particular disclosed embodiments, the composition may be administered to the mammal or avian species using a carrier. The carrier may be any carrier known to a person of ordinary skill in the art as being suitable for combining with a feed composition, such as molasses. In some embodiments, the composition includes additional ingredients. For example, the composition includes calcium carbonate, dried kelp, niacin supplement, biotin, d-calcium pantothenate, vitamin B-12 supplement, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, silicon dioxide, riboflavin-5-phosphate, folic acid, soybean oil, or any combination thereof.

One of skill in the art can appreciate that the amount of the claimed composition fed can vary depending upon the animal species, size of the animal and type of the feedstuff to which the claimed composition is added.

III. Reducing Heat Stress

In particular disclosed embodiments, the composition disclosed herein may be used to prevent and/or promote reduction of heat stress in animals. Heat stress can impair production, reproduction, and animal health. For example, heat stress can lead to increased respiratory rate, increased body temperature, increased fluid intake, decreased feed intake, decreased weight gain, decreased milk yield, decreased reproduction, respiratory alkalosis, ruminal alkalosis, metabolic acidosis, increased oxidative stress, decreased immune function, and/or increased cell damage and death.. Accordingly, any of the foregoing factors may be assessed as heat stress indicators. In some embodiments, the heat stress indicator is feed intake, water consumption, respiration rate, rectal temperature, milk yield, milk fat, milk protein, an immune biomarker, or any combination thereof. In certain embodiments, the heat stress indicator is feed intake, water consumption, respiration rate, rectal temperature, milk yield, milk fat, milk protein, or any combination thereof.

In some embodiments, heat stress conditions occur when a temperature humidity index (THI) is greater than 68, such as greater than 75, ≥ 79 (regarded as a dangerous level), or ≥ 84 (regarding as an emergency level). In another embodiment, heat stress conditions occur when the heat index, commonly reported by the media for humans, is above 100, such as above 110, above 115, or above 120.

The composition may be administered to an animal that is susceptible to heat stress or that suffers from heat stress. In one embodiment, the composition is administered to the animal when the temperature humidity index is, or is expected to be, greater than 68. The composition

may be administered to the animal at set intervals. For example, the composition may be administered to the animal on a daily basis. In some embodiments, a daily amount of the composition is divided and administered to the animal at two or more feedings.

In some embodiments, the animal can be an animal raised for human consumption including, but are not limited to mammals, such as livestock (*e.g.*, feed or dairy cattle) or pigs; avians, such as domestic fowl (*e.g.*, chicken, turkey, goose, duck, cornish game hen, quail, pheasant, guinea-fowl, ostrich, emu, swan, or pigeon); or fish (*e.g.*, salmon, trout and the like). In other embodiments, the animal can be a domestic animal, such as a dog, cat, fish, or rabbit. In some other embodiments, the animal can be a ruminant species, such as a sheep, goat, cow, deer, bison, buffalo, or llama. In yet other embodiments, the animal can be an ungulate, such as a horse, donkey, or pig.

The disclosed method and composition can be used to promote reduction of heat stress in any mammalian (including human), avian, or aquatic species. In a particular embodiment, the compositions are administered to livestock animals, including both ruminants (*e.g.*, cattle, sheep, goats, cows, deer, bison, buffalo) and non-ruminants (*e.g.*, pigs, horses, sows).

The composition disclosed herein may be administered in an amount effective to promote reduction of heat stress in animals, such as dairy cows, particularly lactating dairy cows. The composition may be administered pre- or post-calving for a suitable number of days. The composition also may be administered prior to lactation or after lactation. For example, the composition may be administered to the animal for a period of from 1 day prior to lactation onset to 100 days prior to lactation onset. In other disclosed embodiments, the composition may be administered to the animal for 40 days to 100 days post calving, or for 45 days to 95 days post calving, or for 50 days to 90 days post calving.

In some embodiments, a dairy cow is fed the composition for a period of time to increase milk production relative to dairy cattle not fed the composition. In particular embodiments, the dairy cow has lower milk fat relative to dairy cattle not fed the composition. In one embodiment, the dairy cow had reduced water intake relative to dairy cattle not fed the composition. In another embodiment, the dairy cow had a reduced respiration rate relative to dairy cattle not fed the composition. In yet another embodiment, the dairy cow had a reduced rectal temperature relative to dairy cattle not fed the composition. The dairy cow may have increased food intake relative to dairy cattle not fed the composition. In particular disclosed embodiments, the dairy cow may have decreased respiratory alkalosis, rumen acidosis, metabolic acidosis, and any and all combinations thereof, relative to dairy cattle not fed the

composition. In another embodiment, the dairy cow has decreased serum cortisol levels relative to dairy cattle not fed the composition.

In additional disclosed embodiments, the composition may be administered prophylactically. In certain embodiments, the composition may be administered prophylactically and continuously. For example, the animal may be administered the composition every day for a certain period of time prior to and during lactation.

The disclosed composition may be formulated for administration to animals in order to prevent and/or promote reduction of heat stress. In particular disclosed embodiments, the effective amount of the composition administered can be affirmatively chosen based on the ability of that effective amount to promote heat stress reduction in an animal based on particular factors disclosed herein. In particular disclosed embodiments, the effective amount may be determined by administering a particular first dose to the animal, monitoring the animal during heat stress, and then adjusting the dose in order to determine an effective amount of a second dose that will ameliorate, or further ameliorate the heat stress of the animal.

In one embodiment, the composition is administered to a group of animals that have or are at risk of developing heat stress. The composition may be administered to the group of animals in a first amount for a first period of time. Subsequently a heat stress indicator of at least one animal in the group of animals is measured. Based at least in part on the heat stress indicator measurement, the amount of composition administered to the group of animals for a subsequent period of time may be adjusted. For example, the amount may be increased if the heat stress indicator measurement indicates that the animals are stressed. Alternatively, the amount may be decreased if the heat stress indicator measurement indicates that the animals are not stressed or have relatively little heat stress.

In another embodiment, individual animals in a group of animals are evaluated for heat stress. Heat stress can be evaluated, for example, by measuring a level of one or more heat stress indicators. Based at least on the measurement, a determination is made whether one or more of the individual animals is experiencing heat stress. Individual animals in the group that are experiencing heat stress may be selected to receive the composition for a period of time effective to promote reduction of heat stress.

In particular disclosed embodiments, the composition may be administered with a molasses carrier once or multiple times a day (*e.g.*, from two to five times per day). The composition may be mixed into the total mixed ration of feed that can be provided to the mammal or avian species, such as in the top one-fourth, top one-third, or top one-half of the total

mixed ration.

In some embodiments, the animal is further administered a therapeutic process and/or a therapeutic agent suitable for treating stress. Exemplary therapeutic processes and agents include, but are not limited to, provision of shade to the animal, use of water sprinklers to externally administer water to the animal, use of a fan to provide air movement, addition of high-fat feeds or bypass fats, meloxicam, corticosteroids (isoflupredone, fludrocortisone, triamcinolone, dexamethasone, betamethasone, flumethasone, methylprednisolone acetate, methylprednisolone sodium succinate), oral electrolytes (*e.g.*, sodium, glucose, glycine, potassium, chloride) alkalizing agents (*e.g.*, bicarbonate, acetate, and/or citrate salts), direct-fed microbials, and combinations thereof.

The ability of the composition to reduce and/or prevent heat stress may be determined by comparing heat stress indicators with animals that are not administered the composition. In particular disclosed embodiments, heat stress indicators may be used to determine the effect of the composition on heat stress. Suitable heat stress indicators/factors include, but are not limited to, feed intake, milk yield, milk fat, milk protein, water consumption, respiration rates, rectal temperatures, and combinations thereof.

In particular disclosed embodiments, animals that are administered the composition will have a higher feed intake during heat stress compared to animals that are not administered the composition. The feed intake during heat stress may increase from 2 kg to 10 kg (or from 2 kg to 8 kg, or from 2 kg to 6 kg, or 2 kg to 4 kg) compared to the feed intake of an animal of the same species has not been administered the composition. In exemplary embodiments, a bovine that is administered the composition will have feed intake of 3 kg higher than a bovine who has not been administered the composition.

The milk yield also may be maintained or increased (as compared to yields from animals that are not administered the composition) during heat stress by administering the composition. For example, the milk yield may increase by 1 kg, 2 kg, 3 kg, 4 kg, up to 10 kg using the disclosed composition. In particular disclosed embodiments, animals that are provided the composition will produce milk having lower milk fat and/or milk protein (in terms of percentage). For example, milk fat may be reduced from 0.2% to 1%, or from 0.2% to 0.8%, or from 0.2% to 0.6%, with exemplary embodiments including a 0.4% reduction. In one embodiment, a dairy cow fed the composition produces a milk fat percentage of 3.8%, whereas dairy cattle not fed the composition have a milk fat percentage of 4.2%.

Water consumption, respiration rates, and serum cortisol levels also are lowered by administering the disclosed composition, with some embodiments exhibiting from 4 to 10 fewer respirations per minute. Another factor indicating that the disclosed composition is capable of reducing heat stress is rectal temperature, which typically may be lowered by 0.10 °C to 0.3 °C, in comparison to the rectal temperatures taken from animals that are not administered the composition. Additionally, animals that have been administered the disclosed composition may have decreased respiratory alkalosis, rumen acidosis, metabolic acidosis, and combinations thereof in comparison to animals that were not administered the composition.

IV. Examples

Example 1

A total of 60 cows on a commercial dairy in Arizona were balanced for DIM, parity and milk production and assigned to 1 of 2 treatment groups fed the disclosed composition (OmniGen-AF® [OG] comprising between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan, 30 cows) or control (CON, 30 cows) diets for 52 d post calving. At 52 d of lactation cows were randomly selected (n = 12) from both groups (6 OG and 6 CON) and housed in environmentally controlled modules for 21 d. The OG was top-dressed 2×/d with molasses as the carrier and the CON cows received the molasses carrier 2×/d. Both were mixed into the top one-third of the TMR (total mixed ration). During the environmental room phase of the study cows fed OG had higher feed intake than CON during heat stress (HS) (46.8 kg vs. 42.9 kg, $P < 0.0001$) and no difference during thermoneutral (TN). A temperature-humidity index (THI) threshold of 68 or greater was used to achieve HS. Feeding OG maintained a numerical 1 kg milk yield advantage compared with CON (30.3 kg vs. 31.4 kg, $P = 0.26$) during HS but not during TN. Cows fed OG had lower milk fat (%) (4.2% vs. 3.8%, $P = 0.02$) and milk protein (%) ($P = 0.04$). There was no difference in 3.5% FCM between treatments. Water consumption was lower (12.4 l/d in OG treated cows, $P < 0.01$) than control cows. Respiration rates were lower in treated cows at 1400 h and 1700 h (4.7 and 8.4 less respirations/min, $P = 0.05$, < 0.001) and rectal temperatures were also lower (0.15°C and 0.25°C lower than CON, $P = 0.05$, < 0.001) in treated cows. Feeding OG reduced physiological responses to heat stress in lactating dairy cows.

Example 2

A total of 30 cows on a commercial dairy in Arizona were balanced for DIM, parity and milk production and assigned to 1 of 2 treatment groups fed the disclosed composition (OmniGen-AF[®] [OG] comprising between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan, 15 cows) or control (CON, 15 cows) diets for 90 d post calving. At 90 d of lactation, cows were randomly selected (n = 12) from both groups (6 OG and 6 CON) and housed in environmentally controlled modules for 21 d at the University of Arizona. The OG was top-dressed 2x/d with molasses as the carrier. The CON cows received the molasses carrier 2x/d. Both were mixed into the top one-third of the TMR. During the environmental room phase of the study, cows fed OG had higher feed intake than CON during heat stress (HS) (46.8 kg vs. 42.9 kg, $P < 0.0001$) and no difference during thermoneutral (TN). A temperature-humidity index (THI) threshold of 68 or greater was used to achieve HS. Feeding OG maintained a numerical 1 kg milk yield advantage compared with CON (30.3 kg vs. 31.4 kg, $P = 0.26$) during HS but not during TN. Cows fed OG had lower milk fat (%) (4.2% vs. 3.8%, $P = 0.02$) and milk protein (%) ($P = 0.04$). There was no difference in 3.5% FCM between treatments. Water consumption was lower (12.4 l/d in OG treated cows, $P < 0.01$) than control cows. Respiration rates were lower in treated cows at 1400 h and 1700 h (4.7 and 8.4 less respirations/min, $P = 0.05$, < 0.001) and rectal temperatures were also lower (0.15°C and 0.25°C lower than CON, $P = 0.05$, < 0.001) in treated cows. Feeding OG reduced physiological responses to heat stress in lactating dairy cows.

Experimental Design: The study consisted of two phases; 1) the commercial dairy, and 2) the controlled environmental chambers. During the commercial dairy phase, multiparous lactating Holstein cows (n=30) were balanced by DIM, milk production and parity (91 ± 5.9 DIM, 36.2 ± 2.5 kg/d, and 3.1 ± 1.4). Cows were separated into one of two groups. The control group received the base TMR with no supplement. The treatment group was fed the base diet plus 56 g/ head/ day of OmniGen-AF composition mixed into the TMR. Daily milk production was measured. The dairy phase lasted for 45 days. The dairy portion was used to meet the manufacture's recommended 45 d feeding for OmniGen-AF composition to function.

After the on-dairy portion was complete, 12 cows (6 control and 6 treatment) were housed in the environmentally controlled rooms at the Agricultural Research Center (ARC). Cows continued the ARC portion in the same treatment groups from the on-dairy portion.

The ARC portion lasted for 21 days. Cows were subjected to 7 days of TN conditions, 10 days of HS, and 4 days of recovery (TN). The diurnal cycle during thermoneutral (TN) and recovery maintained a temperature humidity index (THI) < 68. During HS, the THI was greater than 68 for 16 hours/day. Temperatures mimicked ambient temperatures at a southwest United States dairy during summer heat and TN conditions. Fresh feed was provided twice daily and cows were individually fed. Control animals received base TMR, and OmniGen-AF cows received 56 g/head per day, split between two meals. Feed intake, milk production, and milk composition were measured daily. Rectal temperatures and respiration rates were recorded 3x /d (600, 1400, and 1800 h). Blood samples were taken by venipuncture from the tail (coccygeal) vein on days 7 (TN), 8 (HS), 10 (HS), 17 (HS) and 18 (TN) during the ARC segment. Samples were collected 6 times per day (0400, 0800, 1200, 1600, 2000, and 2400 h) on days 7, 8, 17, and 18, and once per day on day 14 (0800 h). Blood was collected in Vacutainer (BD Vacutainer, Franklin Lakes, NJ) tubes containing sodium heparin for plasma and in sterile blank tubes for serum.

Statistical analyses were performed using the PROC MIXED procedure (version 9.3, SAS Institute, Cary, NC). Cow was the experimental unit (ARC portion). Data is presented in least square means with significance declared with a P-value ≤ 0.05 . (See Table 1, below).

There were no initial differences in milk yield (control = 38.6 kg/day and treatment = 38.6 kg/day) at the start of the on-dairy phase of the study. There was a numerical advantage to feeding OmniGen-AF (FIG. 1) of 1.5 kg of milk/day, but this was not significant (control = 36.8 kg/day and treatment = 38.3 kg/day).

There was a period effect on milk yield ($P < 0.01$) during the environmental room (ARC) phase associated with a decline in milk yield in both groups during HS. Milk yield at the ARC ($P < 0.23$) did not differ between control and OmniGen-AF fed groups (FIGS. 2, 3), however, there was a numerical advantage (1.1 kg/day) for cows fed OmniGen-AF during HS ($P < 0.26$) which was similar to the pattern in milk yield noted during the on-farm phase

Feeding the disclosed composition to heat stressed dairy cows maintained feed intake during heat stress. Feed intakes in the two groups did not differ during TN but was higher during HS in OmniGen-AF-fed cows (46.8 kg/d and 42.9 kg/d, $P < 0.01$, FIGS. 4, 5; Table 1).

Milk protein (%) and fat (%) were lower in OmniGen-AF-fed cows (Table 1) during HS but not during TN. There was no difference in FCM or protein yield between treatments. Cows fed OmniGen-AF displayed decreased somatic cell count (SCC) compared to control cows (59.4 and 26.3×1000 , $P < 0.03$; Table 1) with the greatest difference during the recovery period

(FIG. 6). There was a spike in SCC around day 5 (TN) and during recovery around day 17 (FIG. 6).

Table 1. Effects of OmniGen-AF supplementation in heat-stressed lactating dairy cows								
Item	Control			OmniGen-AF			SEM	P-value
	TN	HS	Recovery	TN	HS	Recovery		
Feed intake (kg)	46.1	42.9	47.5	47.1	46.8*	49.1	1.04	0.01
Milk yield (kg)	33.1	30.3	30.4	33.9	31.4	31.3	1.02	0.23
Fat (%)	4.03	4.22	4.16	3.94	3.82	3.83	0.22	0.04
FCM (kg/d)	35.0	33.7	33.7	34.7	32.8	32.6	1.45	0.39
Protein (%)	2.95	2.98*	2.86	2.95	2.86	2.79	0.07	0.15
Protein (kg)	0.98	0.89	0.90	1.00	0.93	0.92	0.30	0.13
Lactose (%)	4.87	4.85	4.99	4.89	4.78	4.96	0.08	0.61
SCC	20.3	23.9	59.4*	19.6	22.9	26.3	9.12	0.03
* = P -value ≤ 0.05 and indicates the higher value								

Respiration rate and rectal temperatures did not differ between treatments during TN; however, during HS, OmniGen-AF reduced respiration rate, (Table 2, $P < 0.01$) in both environments at 1400 h and in HS animals at 1800 h when environmental heat load was greatest. Rectal temperatures were lower in cows fed OmniGen-AF at 1400 and 1800 h compared to controls when environmental heat loads were maximal.

Table 2. Effects of OmniGen-AF supplementation and environment on respiration rate and rectal temperature in lactating dairy cows

Item	Control			OmniGen-AF			SEM	P-value
	TN	HS	Recovery	TN	HS	Recovery		
Resp/ min								
600	26.9	31.9	28.3	26.6	30.4	27.9	1.40	0.40
1400	34.3	63.1*	35.3	30.1	58.3	35.5	2.99	0.20
1800	34.9*	60.8*	32.1	29.5	52.4	29.7	2.62	0.01
Rectal Temp (°C)								
600	38.2	38.0	37.9	38.2	38.1	38.1	0.05	0.26
1400	38.0	38.7*	38.0	38.1	38.5	38.1	0.09	0.77
1800	38.2	39.1*	38.2	38.2	38.8	38.3	0.08	0.25

* $P \leq 0.05$ and indicates the higher value.

Hormones in plasma are important as potential indicators of the physiological status of a cow and reflect the physiological compensations a cow undergoes at various stages of lactation and exposure to HS. Serum cortisol levels were highest on day 8 (first day of HS, FIG. 7). This is in agreement with prior reports that acute but not chronic HS is associated with increases in circulating cortisol concentrations (Christian and Johnson, 1972, Wise et al., 1988). OmniGen-AF treated cows had significantly lower serum cortisol on day 8 (0.8372 vs. 0.4838 $\mu\text{g/dL}$ for control and OmniGen-AF respectively, $P < 0.006$) and did not differ on other days. This

suggests that OmniGen may reduce impact of acute stress on the cortisol response in lactating dairy cows.

Serum insulin and plasma glucose levels (FIGS. 8 and 9) were not different between groups ($P = 0.8248$ and 0.945). Serum insulin concentrations in both groups rose during the latter part of the HS period and during the recovery period. The reason for this pattern is unknown.

The immune function of cattle on this study was evaluated by looking at the expression of the interleukin-8 receptor (FIG. 10) and expression of Regulated on Activation, Normal T Expressed and Secreted (RANTES) protein (FIG. 11) which is a member of the interleukin-8 family of cytokines.

Heat stress exposure was mild to moderate in this study. The threshold for heat stress in lactating dairy cows is a THI > 68 , respiration rates > 60 bpm, and rectal temperatures $> 38.5^{\circ}\text{C}$ (Zimbleman et al., 2009). OmniGen-AF reduced impact of thermal stress on stress of lactating dairy cows. Cows fed OmniGen-AF had reduced rectal temperatures and respiration rates during periods of peak thermal load. Respiration rates in treated cows did not exceed 60 bpm and mean rectal temperatures were 0.2 to 0.3°C cooler. OmniGen-AF fed cows displayed higher feed intakes during HS as well. Cows fed OmniGen-AF also displayed a lower cortisol spike on the first day of heat stress.

Milk yield decreased with heat stress in both control animals and the OmniGen-AF fed animals. However, feed intake was unchanged in cows fed OmniGen-AF and milk yields were numerically higher. Changes in SCC were consistent between groups. Cows fed OmniGen-AF displayed decreased SCC compared to control cows with the greatest difference during the recovery period.

Serum cortisol levels were similar to previous findings (Christison and Johnson, 1972) and increased within the first day of heat exposure. The animals in the ARC had higher cortisol levels compared to published levels, but the confinement and changes in surrounding from the dairy to the ARC may account for some of the changes.

Cytokine (RANTES) gene expression was higher in cows fed OmniGen-AF during the HS portion of the study but not during recovery. The elevated cytokine gene expression may be associated with improved immune function in cows fed OmniGen-AF.

Example 3

Embodiments of the disclosed composition, such as OmniGen-AF[®] composition comprising between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan (OG), have been demonstrated to reduce physiological measures of heat stress (e.g., body temperature, respiration rate, and water intake) in cattle subjected to temperature and humidity conditions above their thermoneutral zone. Concomitantly, in OG-fed animals, feed intake was significantly increased and milk yield was numerically increased during periods of thermal stress. Furthermore, measures of immune function were improved along with reduced cortisol concentrations during acute heat stress. Paradoxically, adrenocorticotrophic hormone (ACTH) which regulates cortisol secretion from the adrenal cortex was significantly increased in animals fed OG (FIG. 12). This suggests that either the adrenal is less responsive to ACTH or that corticoid binding globulin is increased in animals fed OG. Both conditions would result in increased ACTH secretion from the pituitary since negative feedback in both cases would be reduced. The objective of this study is to determine if feeding OG to lactating dairy cows decreases adrenal responsiveness to ACTH or increases corticosteroid binding globulin in either thermoneutral or heat stress conditions.

Heat stress in dairy cows may result in reductions in dry matter intakes and milk yields and elevated somatic cell counts, respiration rates, body temperature and plasma heat shock protein and serum cortisol. Feeding OG may reduce the impact of heat stress on these measures of stress response. Feeding OG to non-heat stressed lactating dairy cows for 52 days prior to a heat stress bout may increase the expression of immune markers of neutrophil function (L-selectin, IL8-R and IL-1B) associated with altered secretion of cortisol from the adrenal gland. This may be associated with reduced cortisol secretion in response to ACTH infusion in cows fed OG. Heat stress may reduce the expression of immune markers of neutrophils even in cows supplemented with OG. However, the reduced cortisol secretion response may also be present in heat stressed dairy cows fed OG.

Experimental Design:

Animal Selection and Treatment Assignment: Thirty lactating dairy cows will be assigned to one of two treatments (15 head/treatment). Treatment 1 cows will be fed a control diet without OG, and Treatment 2 cows will be fed the control diet plus OG. OG will be pre-blended in a grain mix to provide 56 g/h/d. Each cow will be housed in an individual tie-stall where individual feed and water intake can be controlled and recorded prior to moving to the

Agricultural Research Center (ARC). After 45 days on study, all cows will be given a low dose ACTH challenge (20 mg) via tail vein infusion, and blood samples will be taken at time zero, 1 hour, 4 hours and 8 hours after ACTH challenge. Subsequently, a subgroup of 12 cows, which have been on their respective diets on a commercial dairy for 55 days prior to the moving to the ARC environmental controlled rooms, will be added. From the 30 original cows assigned to treatments, 6 cows from each treatment will be selected for the trial and will continue on their respective treatment diets while in the ARC. Cow will be the experimental unit.

Blood Sample Schedule: Blood samples will be collected on all treatment cows while at the commercial dairy at days 1, 21 and 42 after initial treatment assignment. On days 45- 50 ten cows each day will be subjected to low dose ACTH challenge. On each sampling day, approximately 2 hours after the morning feeding, cows will be restrained in headlocks, taking care to avoid stressing the animals; tail vein blood samples will be obtained from each animal and then infused via tail vein or tail artery with 20 µg (=2IU) of a synthetic analogue of ACTH (ACTH1-24, Synacthen® – Novartis Pharma AG - Stein, CH). Blood samples will also be taken 30 and 60 minutes after ACTH injection, leaving cows restrained in the head locks. All the blood samples will be collected in vacuum Li-heparin tubes, and immediately stored in iced water. In the blood samples, packed cell volume (PCV) will be determined and, after centrifugation (3500g for 16min. at 6°C), plasma cortisol will be measured by the RIA method (Coat-A-Count; DPC, Los Angeles, CA, USA). The integrated response of cortisol over 60 min will be evaluated as area under the curve. For the statistical evaluation, data will be subjected to ANOVA using GLM procedure (SAS Inst. Inc., Cary, NC, release 9.1) including in the model cow, dietary treatment (control or OmniGen-AF) stage of lactation as main factors. A repeated measures analysis will also be conducted on the challenge data.

At arrival to the ARC, cows will be weighed, fitted with halters and blood samples collected. Cows will remain in the ARC chambers for 21 days, the first 7 days at thermal neutral (TN) conditions followed by 10 days of heat stress (HS) and then 4 days in TN conditions. On d 7 of TN, d 8 (HS), d 17 (HS), d 18 (TN) and d 21 (TN) cows will be bled in 4 h intervals at 0400, 0800, 1200, 1600, 2000, and 2400 h following a low dose (20mg) of ACTH infused via the tail vein.

Physiological/Behavior Metrics: To assess the effectiveness of the imposed heat stress model, known physiological and behavioral responses will be measured and recorded daily. These will include feed intake, water consumption, milk yield, milk somatic cell concentrations (cells/ml), respiration rate, rectal temperature and skin temperature measurements (3x/d at 0600,

1400 and 1800 h). Milk samples will be collected and stored in vials containing bronopol tablets for preservation and stored at 4 °C until analysis. Analysis will be done by infrared analysis. All treatment and health events will be recorded daily.

Blood collected for immune biomarkers will be collected and preserved according to protocol (Wang, et al., 2003). The immune biomarkers neutrophil L-selectin, IL8-R and IL-1B will be evaluated on each sample. In addition, on days 17 and 18 from the 0800 and 1600 h samples, neutrophils will be purified and assessed for RANTES (regulated on activation, normal T cell expressed and secreted), phagocytosis ability or ROS (reactive oxygen species) generation. Ionized serum calcium will also be determined on samples collected on days 1, 7, 10 17, 18 and 21. Blood samples collected on day 1 of arrival to the ARC and days 7, 8, 12, 17, 18 and 21 will be assayed for Heat Shock Protein (HSP) and cortisol. These samples will be stored on ice until centrifugation (15,000 x g) for 15 minutes at 4 °C. Plasma will be removed and stored at -20 °C and the buffy coat fraction will be removed and stored in Trizol at -80 °C. On days of blood sampling, additional milk samples will be collected at both the AM and PM 15 milking and neutrophils isolated.

An additional possibility is the sampling of fecal cortisol as a noninvasive measure of cortisol output. Another consideration is to measure corticoid binding globuli (CBG). An increase in CBG would reduce effective concentrations of cortisol and cause increases in ACTH.

20 *Example 4*

Procedures: Calf-fed steers (306 head; BW 263.5 ± 18.6 kg; 36 pens) were utilized in a randomized block design experiment. Steers were received over a two-day span at the feedlot. Upon arrival, steers were allowed access to water and were processed, weighed, and allocated to treatment within 12 h. During processing, steers were identified with an individual ear tag, 25 individually weighed, vaccinated with Bovi-Shield Gold One ShotTM (IBR, BVD), Dectomax injectable[®] (internal and external parasiticide), and Somubac[®] (*Haemophilus somnus disease complex*). Shrunk BW was a single weight collected at time of processing following arrival. Steers were blocked based on arrival date resulting in two blocks. Within blocks, steers were assigned randomly to one of three treatments by gate sorting every two steers to a pen; pen was 30 then randomly assigned to treatment. Treatments included: a control group (Control): no supplementation with the disclosed composition (OmniGen-AF[®] [OG] comprising between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan)

OG supplemented at 4 g/cwt of BW for 28 d (receiving period); and OG supplemented at 4 g/cwt of BW for 215 d (the entire feeding period). Supplementation of OG for both the 28 d and 215 d treatment groups was formulated and added to the daily delivery of the diet prior to feeding. A ninth steer was randomly added to nine pens of the control group and the treatment group that only received OG for 28 d during processing of the 2nd block. These 18 steers (9 hd/treatment group) would allow for the selection of one steer from each of these 18 pens to be utilized for the intravenous lipopolysaccharide (LPS) challenge portion of the trial.

At the conclusion of the 28 d receiving period, steers were limit fed for a period of 4 d at 2% BW to obtain an end of receiving period BW. At the conclusion of the 28 d receiving period, OG was no longer supplemented to the group of steers within the treatment group that only received OG for the 28 d of the receiving period; steers within the other OG treatment group (fed for 215 d) continued to receive OG. OG supplementation for the remaining treatment group was recalculated every 30 d to supply 4 g/cwt of BW on average. All steers were implanted with Revalor[®] XS at the conclusion of the receiving period. During the last 28 d of the finishing period, all cattle were supplemented Optaflexx[®] ractopamine composition at a rate of 300 mg/hd/d. At the end of the trial, steers were transported 51.5 km to a commercial abattoir and held over-night. The following morning steers were harvested; at which time hot carcass weights (HCW) were recorded. Following a 48-h chill, fat thickness, LM area, and USDA marbling score were determined. Final BW, ADG, and F:G were calculated using HCW adjusted to a standard (63%) dressing percentage.

To evaluate the immune response, two LPS challenges were conducted; a subcutaneous LPS challenge (steers from the Control group and the OG fed for 215 d group) and an intravenous LPS challenge (steers from the Control group and the OG fed for 28 d group). For the subcutaneous LPS, three pens from the Control and the OG fed 215 d treatment group were randomly selected. From the selected pens, 6 steers/pen were randomly selected and challenged with LPS via subcutaneous injection on d 21. On d 21, steers from selected pens were removed from the pen and moved to the processing barn. As each steer was processed, BW was determined and an indwelling rectal probe was inserted. After determining BW, each steer was challenged with a subcutaneous injection of LPS at a rate of 0.5µg/kg of BW and then returned to the feedlot pen. For the intravenous LPS challenge, on d 25 of the receiving period, 18 steers (nine steers from Control and OG fed for 28 d treatment groups) were randomly selected from the 9 Control and 9 OG fed for 28 d pens that contained 9 steers/pen. Steers were identified and moved into a tie stall barn. After a 3 d adjustment period, steers were fitted with indwelling

jugular vein catheters for serial blood collection and indwelling rectal temperature (RT) recording devices, set to record RT at 1-min intervals continuously throughout the immune challenge study. After insertion of the jugular catheter and RT probe, steers were returned to the individual tie stalls and allowed to rest for the remainder of the d.

5 On the following day, from 0800 to 1600 h, blood samples were collected at 30-min interval; two h prior to the challenge (0800 – 1000 h) and six h after the challenge (1000 – 1600 h). At 1000 (0 h), following the collection of the blood sample, steers were administered an i.v. dose of lipopolysaccharide (LPS, 0.5 µg/kg BW; purified from *E. coli* O111:B4; Sigma-Aldrich, St. Louis, MO). A final blood sample was collected 24 h post LPS challenge. At each
10 collection point, 9 mL of blood was collected via monovette tubes containing no additive for serum. After collection, blood samples were allowed to clot for 30 min at room temperature and then centrifuged at 2,000 x g for 30 min (39.2°F). Serum was collected and transferred into 1.5 mL microcentrifuge tubes and stored at -112°F until analyzed. Serum was analyzed for cortisol and pro-inflammatory cytokines (tumor necrosis factor - α , TNF- α ; interferon - γ ,
15 IFN- γ ; and interleukin - 6, IL-6).

Feedlot performance data were analyzed as a randomized block design using MIXED procedures of SAS (SAS Institute, Inc., Cary, NC). Steers were blocked by arrival date and pen was the experimental unit; model included the fixed effect of treatment and block was a random effect. Immune response data were analyzed as a completely randomized design with repeated
20 measures using the MIXED procedures of SAS; model included fixed effects of treatment and time, treatment x time was used as the error term to test whole plot effect. For both feedlot and immune data, when results of F-test were significant ($P < 0.05$), group means were compared by use of least significant difference. Pair wise differences among least squares means at various sample times were evaluated with the PDIFF option of SAS. Distribution of USDA Quality
25 Grade data were analyzed as a randomized block design using the Glimmix procedure of SAS.

Results: For the receiving portion of the trial, there was no difference in initial BW ($P = 0.82$), ending BW ($P = 0.43$), ADG ($P = 0.32$), DMI ($P = 0.76$), and F:G ($P = 0.35$) between the three treatments (Table 2). In terms of morbidity related to respiratory diseases, there was no difference ($P = 0.21$) in the % of steers treated (Table 3). There was also no difference in overall
30 feedlot performance; DMI ($P = 0.89$), ADG ($P = 0.66$) and F:G ($P = 0.90$) were similar across the three treatments (Table 2). There was no difference in final BW ($P = 0.59$), HCW ($P = 0.60$), LM area ($P = 0.31$), marbling score ($P = 0.96$), 12th rib fat thickness ($P = 0.86$), or calculated yield grade ($P = 0.52$) between the three treatment groups. The distribution of USDA

Quality Grade was analyzed across all three treatments. There was no difference in the amount of USDA Prime, USDA Choice, or USDA Select Quality Grades for all three treatments (Table 3). While there was no statistical difference in terms of the percentage of carcasses grading USDA Choice or greater, the economic significance is still of major importance. Steers supplemented with OG for 215 d had a rate of USDA Choice that was 81.91%, compared to 76.40 for Control steers. With a \$13.58 Choice / Select spread (11 Dec 14), this difference in Choice between the two treatment groups is the equivalent of ~\$850 difference in carcass value.

Table 3. Receiving period and overall feedlot performance and carcass merit for steers fed no OG (CON), OG during the receiving period (OG-28), or OG for 215 d (OG-215)

	Treatment groups ¹				
Item	Con	OG-28	OG-215	SEM	<i>P-value</i>
<i>Receiving Performance</i>					
Initial BW (kg)	259	256	262	3.2	0.82
Ending BW (kg) ²	300	304	306	3.6	0.43
DMI (kg/d)	7.5	7.7	7.6	0.4	0.76
ADG (kg/d) ³	1.44	1.54	1.58	0.15	0.32
G:F ⁴	0.9	0.9	0.09	-	0.35
Morbidity (%) ⁵	6.9	2.0	3.0	3	0.12
<i>Feedlot Performance</i>					
Initial BW (kg)	261	572	572	6	0.87
Final BW (kg) ⁶	649	642	643	11	0.59
DMI (kg/d)	9.80	9.80	9.88	0.3	0.89
ADG (kg) ⁷	1.81	1.79	1.79	0.04	0.66
G:F	0.18	0.18	0.18	-	0.90
<i>Carcass Merit</i>					
HCW (lbs)	409	405	405	3.2	0.96
LM area (in ²)	94.78	91.36	94.06	1.61	0.31
Calculated YG	3.09	3.28	3.09	0.14	0.52
12 th rib fat (cm)	1.37	1.42	1.40	0.08	0.86
Marbling ⁸	503	498	508	24	0.96
Prime (%)	4.49	2.13	5.32	2.32	0.53

Choice (%)	76.40	76.60	81.91	4.50	0.59
Select (%)	19.10	21.28	13.83	4.22	0.41

¹ CON: No OG; OG-28: OG during receiving, OG: OG for 215 d.

²Limit fed at 2% of BW for 4 d prior to single BW to determine ending BW of receiving period

³Calculated from ending BW of receiving period

⁴Analyzed as G:F, the reciprocal of F:G.

⁵Overall percentage of steers treated for bovine respiratory disease

⁶Calculated from carcass weight, adjusted to 63% common dressing percent.

⁷ADG for the entire feeding period (including receiving period)

⁸Marbling Score: 400 = Small, 500 = Modest, etc.

For the subcutaneous LPS challenge, there was a treatment ($P = <0.001$) and time ($P = <0.001$) effect for RT (FIG. 13). Both treatment groups responded to the subcutaneous LPS challenge, with both treatment groups achieving a maximum RT approximately 5 h post challenge (Control = 40.10°C vs. OG = 39.92°C; FIG. 13). Furthermore, both maintained similar patterns ($P = 1.00$) throughout the subcutaneous LPS challenge. Steers in the Control treatment group had an average RT that was greater than the OG treatment group (39.22°C and 39.07°C, respectively). There was no treatment effect ($P = 0.75$) or treatment x time interaction ($P = 0.99$) for change in RT from baseline (FIG. 14). Again, there was a treatment effect for RT ($P = <0.001$), but this treatment difference was not observed when comparing the change in RT from baseline between the treatment groups (average change from baseline was 0.03°C for Control and 0.03°C for OG).

For the intravenous LPS challenge portion of the trial, there was a treatment ($P = 0.002$) and time effect ($P = <0.001$), however there was no treatment x time interaction ($P = 0.99$) for RT (FIG. 15). Steers supplemented with OG had a greater RT when compared to Control (39.26°C vs. 39.17°C, respectively) during the intravenous challenge. This average RT is very similar to the RT that was observed for the steers that were utilized in the subcutaneous LPS challenge. In terms of response to the intravenous LPS challenge, both treatment groups responded to the LPS challenge similarly ($P = 0.99$). For both groups of steers, maximum RT was observed 2.5 h post intravenous LPS administration, and within 6 h, RT had returned to baseline temperatures (-21 to 0 h prior to the challenge; FIG. 15). There was also no treatment effect ($P = 0.49$) or treatment x time interaction ($P = 0.99$) for change in RT from baseline (-21 to 0 h; FIG. 16). Baseline line temperatures were different ($P < 0.001$) between the two

treatment groups, but the change from baseline was similar ($P = 0.49$) between the Control and OG supplemented steers (FIG. 16).

For serum concentrations of cortisol, there was a treatment ($P = 0.005$) and time effect ($P = < 0.001$); though there was no treatment x time interaction ($P = 0.99$). Steers supplemented OG had decreased concentrations of cortisol, when compared to the CON steers (25.5 vs 29.2 ng/ml, respectively; FIG. 17). In terms of response to the intravenous LPS challenge, both treatment groups responded similarly ($P = 0.99$). Prior to the LPS challenge, both treatment groups had cortisol concentrations below 10 $\mu\text{g/mL}$. Thirty min after the LPS challenge, cortisol concentrations had increased ($P = < 0.001$) to above 35 $\mu\text{g/mL}$; cortisol concentrations for both treatment groups did not return to baseline concentrations until 24 h post LPS challenge (FIG. 18).

Blood urea nitrogen (BUN), non-esterified fatty acids, and glucose were analyzed to evaluate metabolic alterations during the intravenous LPS challenge. For BUN's, there was a treatment ($P = < 0.001$) and time ($P = < 0.001$) effect, but there was no treatment x time interaction ($P = 0.99$). Control steers had a greater ($P = < 0.001$) concentration of BUNs when compared to the OG supplemented steers (FIG. 19). This difference in BUNs was observed prior ($P = 0.01$) and post LPS challenge ($P = < 0.001$). Regardless of the LPS challenge, Control steers' BUN concentrations were on average 12.4 mg/mL while OG-supplemented steers' BUN concentrations were on average 11.5 mg/mL (FIG. 19.) There was a treatment ($P = 0.009$) and time ($P = < 0.001$) effect for glucose concentrations; no treatment x time interaction ($P = 0.19$). Serum glucose concentrations were greater ($P = < 0.001$) for OG-supplemented steers, when compared to Control steers (76.4 mg/mL vs. 72.4 mg/mL, respectively; FIG. 20). This difference in glucose was not apparent prior to the LPS challenge, as baseline concentrations (-2 to 0 h) were similar (0.62; FIG. 21). However, following the LPS challenge, while both treatment groups had a decrease in serum glucose, this reduction in glucose was greater ($P = 0.009$) in the Control steers, when compared to the OG-supplemented steers (FIG. 21). Prior to the LPS challenge, both treatment groups maintained serum glucose concentrations greater than 75 mg/mL. However, following the LPS challenge (1 h post LPS challenge) serum glucose concentrations started to decrease, and continued to decrease until 3.5 h after administrations of the LPS challenge (FIG. 21). During this decrease in glucose concentrations, concentrations in the Control steers decreased to a greater extent than the OG-supplemented steers. While this decrease of glucose concentrations did not result in a treatment x time interaction, this decrease did result in an overall treatment difference between the two treatment groups (FIG. 21). There

was a treatment ($P = < 0.001$) and time ($P = < 0.001$) effect, and a tendency for a treatment x time interaction ($P = 0.007$) for serum NEFA concentrations. Steers within the Control treatment group had greater ($P = < 0.001$) serum NEFA concentrations, when compared to OG-supplemented steers (FIG. 22). This difference in NEFA concentrations was present prior ($P =$
 5 0.006) to the intravenous LPS challenge. However, following the LPS challenge these differences were even greater ($P = < 0.001$; FIG. 22). Prior to the LPS challenge, NEFA concentrations for Control steers were 0.10 mmol/ml while OG-supplemented steers were 0.07. However, following the LPS challenge, NEFA concentrations in the Control steers increased to 0.23 mmol/mL while the OG-supplemented steers only increased to 0.11 mmol/mL.

10 There was a treatment ($P = < 0.001$) and a time effect ($P = < 0.001$) for the pro-inflammatory cytokine IFN – γ ; there was no treatment x time interaction ($P = 0.77$). OG-supplemented steers had a greater production of IFN – γ , when compared to the Control steers (FIG. 23). Prior ($P = 0.05$) and post ($P = < 0.001$) LPS challenge, the concentrations of IFN – γ were greater in the OG-supplemented steers, when compared to the Control steers (FIG. 23).

15 There was also a treatment ($P = 0.03$) and time ($P = < 0.001$) effect for TNF – α ; there was no treatment x time interaction ($P = 0.42$). Overall serum concentrations for TNF – α were greater ($P = 0.03$) in the OG-supplemented steers, when compared to the Control steers (FIG. 24). For both treatment groups, prior to the LPS challenge, no detectable concentrations of TNF – α were present, and this no detectable concentrations continued until 0.5 h post challenge (FIG. 25). At
 20 0.5 h, concentrations of TNF – α dramatically increased ($P = < 0.001$) for both treatment groups. Concentrations of TNF – α returned to near baseline (-2 to 0 h) around 4 h after the LPS challenge (FIG. 25). There was only a time effect ($P = < 0.001$) for the pro-inflammatory cytokine IL- 6 (FIG. 26). As with TNF – α , prior to the LPS challenge, concentrations of IL – 6 were not detectable. However, 1 h after the LPS challenge, concentrations of IL – 6
 25 dramatically increased ($P = < 0.001$); reaching maximum concentrations at 2 h post LPS challenge. From 1 to 8 h post LPS challenge, IL – 6 concentrations remained elevated above baseline concentrations, and did not return to baseline concentrations until 24 h post LPS challenge (FIG. 26).

The mean complete blood cell count (CBC) analysis is reported in Table 4.

30 Supplementation of OG did not impact red blood cell counts ($P = 0.45$) or the monocyte percentage ($P = 0.26$). Hemoglobin concentrations ($P = 0.008$), Hematocrit % ($P = 0.03$), and white blood cell concentration ($P = 0.001$) were greater ($P = 0.008$) in Control steers, when compared to OG-supplemented steers. Neutrophil % ($P = 0.04$) and eosinophils concentration

($P = 0.02$) were greater in OG-supplemented steers, when compared to Control steers. For the % of lymphocytes, there was a treatment x time interaction ($P = 0.006$). Prior to the LPS challenge (- 2 to 0 h), Control steers had a greater ($P = < 0.001$) concentration of lymphocytes compared to the OG-supplemented steers (FIG. 27). One h after the challenge, there was no difference ($P =$

5 0.35) between the treatment groups, and from 1 h to 8 h, there was no difference ($P = 0.66$). However, 24 h after the LPS challenge, Control steers had a greater ($P = 0.005$) concentration of lymphocytes (FIG. 27). There was also a treatment x time interaction ($P = 0.006$) for the neutrophil:lymphocyte ratio. Prior to the LPS challenge, the neutrophil:lymphocyte ration was greater ($P = < 0.001$) for OmniGen steers, when compared to Control steers (FIG. 28).

10 **Table 4.** Mean Complete Blood Cell Count values newly received steers supplemented OmniGen-AF at a rate of 4 mg/cwt during the receiving period (OMN-28) or no OmniGen – AF (CON) during a lipopolysaccharide challenge

Variables	OmniGen	Control	SEM	P - value		
				TRT	TIME	TxT
Red Blood Cells (mil/ μ L)	8.46	8.47	0.30	0.45	0.41	0.99
Hemoglobin (g/dL)	10.61	10.97	0.09	0.008	0.18	0.99
Hematocrit (%)	31.06	32.29	0.38	0.03	0.46	0.99
White Blood Cells (K/ μ L)	4.47	5.42	0.12	0.001	<0.001	0.88
Neutrophils (%)	0.43	0.26	0.05	0.04	<0.001	0.34
Lymphocytes (%)	3.02	3.63	0.09	<0.001	<0.001	0.006
Monocytes (%)	1.06	1.14	0.20	0.26	<0.001	0.23
Eosinophils (K/ μ L)	0.07	0.04	0.005	0.02	0.76	0.76
Neutrophils:Lymphocytes	0.14	0.09	0.016	0.01	<0.001	0.006

Conclusions: In this study, newly received calf-fed steers supplemented with OG for a period of 28 d (receiving period) or for 215 d (entire feeding period) did not impact feedlot

performance or carcass merit. While there was no statistical difference in terms of the percentage of carcasses grading USDA Choice or greater, the economic significance should outweigh the lack of a statistical difference. With a \$13.58 Choice / Select spread (11 Dec 2014), this difference in Choice between the two treatment groups is the equivalent of ~\$850 in carcass value. Furthermore, supplementation of OG alters the immune response. When challenged with a lipopolysaccharide, OG supplementation appears to prime the pro-inflammatory response (as evident by increased concentrations of IFN – γ and TNF – α). In addition, there was the difference in metabolism that was observed. The increased concentrations of BUN's and NEFA's within the Control steers indicate a possible greater energy need to initiate and sustain an immune response, when compared to the OG-supplemented steers. This increase in greater energy demand is further indicated as the OG-supplemented steers had greater concentrations of serum glucose, when compared to the Control steers. Overall, these results suggest that supplementation of OG may enhance the immune response of calf-fed steers upon feedlot entry.

EXAMPLE 5

A study was conducted to identify genes expressed by circulating immune cells that are regulated by a commercial embodiment of Composition I. Rats (n=6 per group) were randomly assigned to Composition I and control groups. Composition I was supplemented in the diet at 0.5% in the Composition I group. Total RNA was purified from whole blood and gene expression was analyzed with the use of the Rat Innate and Adaptive Immune Responses RT2 Profiler Polymerase Chain Reaction (PCR) Array (SABiosciences, Qiagen). A total of 84 target genes were present on the array. Gene expression of circulating immune cells was analyzed at seven, fourteen, twenty-one and twenty-eight days of Composition I supplementation. The expression of 67 genes changed following Composition I supplementation across the time points. Table 5 lists the genes with altered gene expression following Composition I supplementation and includes information indicating stimulation (+) or repression (-) of gene expression.

Table 5

Gene	Repressed	Gene	Induced	Reference Genes
Crp	-	Ifnb1	+	Actb
Mbl2	-	Cd80	+	Ldha
Apcs	-	Tlr1	+	Rplp1

Il5	-	Tlr6	+		
Ifna1	-	Mapk8	+		
Ccl12	-	Nod2	+		
Csf2	-	Ccr8	+		
Il13	-	Irak1	+		
Il10	-	Cd1d1	+		
Gata3	-	Stat4	+		
Stat3	-	Il1r1	+		
C3	-	Faslg	+		
Tlr3	-	Irf3	+		
Ccl5	-	Ifnar1	+		
Mx2	-	Slc11a1	+		
Nfkb1	-	Tlr4	+		
Nfkb1a	-	Cd86	+		
Tlr9	-	Casp1	+		
Cxcl10	-	Ccr5	+		
Cd4	-	Icam1	+		
Il6	-	Camp	+		
Ccl3	-	Tlr7	+		
Ccr6	-	Irf7	+		
Cd40	-	Rorc	+		
Ddx58	-	Cd40lg	+		
Il18	-	Tbx21	+		
Jun	-	Casp8	+		
Tnf	-	Il23a	+		
Traf6	-	Cd14	+		
Stat1	-	Cd8a	+		
		Cxcr3	+		
		Foxp3	+		
		Lbp	+		
		Mapk1	+		
		Myd88	+		

		Stat6	+		
		Agrin	+		
		IL33	+		

EXAMPLE 6

Feeding OmniGen-AF® (Composition I or OG; Prince Agri Products, Inc., Quincy, IL), a branded proprietary product, at 0.5% of the diet supports immune function in ruminant livestock. Targeted profiling of immune-associated genes in whole blood is an established methodology to evaluate the efficacy of feed additives with immune-altering properties. We hypothesized that higher daily inclusion rate of OG than 0.5% may be required to optimize immune function. The objective of this study was to evaluate the effect of dietary OG inclusion rate (1% vs. 0.5%) on the expression profile of immune-associated genes. Male CD rats (5/treatment) weighing 180-200 grams had ad libitum access to a diet with 0 (control), 0.5 (1×), or 1% (2×) of OmniGen-AF® for 28 days. At the end of the feeding period, whole blood was collected. RNA was purified from whole blood samples and used to generate cDNA that acted as template in the *Rat Innate and Adaptive Immune Responses* RT² Profiler PCR array (SABiosciences). Using PROC GLM, we compared cDNA abundance of immune-associated genes between control and supplemented groups (0.5 or 1%) with a $P < 0.05$ cut-off value for significance. Of the 79 immune-associated genes that were expressed above the detection limit in all samples, 16 (7 up-regulated) and 13 genes (8 up-regulated) were altered by 0.5% and 1% OG supplementation, most of which (11 with 6 up-regulated) were altered at both OG inclusion rates. Genes that were up-regulated at both rates include IL13 (0.5%: +3.16, 1%: +3.70 fold-change), IL5 (0.5%: +2.64, 1%: +2.62), Irak1 (0.5%: +2.50, 1%: +1.98), Nod2 (0.5%: +1.83, 1%: +2.02), IFNa1 (0.5%: +1.81, 1%: +2.10), and Cd80 (0.5%: +1.77, 1%: +2.47). Genes that were down-regulated at both inclusion rates include TLR3 (0.5%: -2.22, 1%: -2.39), CxCL10 (0.5%: -2.19, 1%: -2.26), STAT1 (0.5%: -2.07, 1%: -1.99), STAT3 (0.5%: -2.05, 1%: -1.92), and NFκb1 (0.5%: -1.84, 1%: -1.75). In conclusion, our results suggest that OG supplementation inclusion rate independently promotes immune function through various pathways including pathogen recognition, adaptive immune cell activation, and various transcription factors.

EXAMPLE 7

OmniGen-AF® (Composition I or OG; Prince Agri Products, Inc., Quincy, IL) is a branded proprietary product shown to augment immune function in ruminants and other species. Targeted profiling of immune-associated genes in whole blood is an effective platform for
5 identification of multiple immune response markers to feed additives with immune-altering properties. The objective of this study was to identify multiple immune response markers that are increased by dietary OG throughout a 28-d supplementation period. We hypothesized that several immune-associated genes in whole blood are consistently up-regulated during a 28-d supplementation period. Fourteen male CD rats weighing 180-200 grams had ad libitum access
10 to a diet containing 0 (control; n=5, only 28 days) or 0.5% OG for 7 (n=4) or 28 days (n=5). Whole blood was collected at the end of the feeding period. RNA was purified from whole blood samples and used to generate cDNA that acted as template in the *Rat Innate and Adaptive Immune Responses* RT² Profiler PCR array (SABiosciences). Using PROC GLM, we compared cDNA abundance of immune-associated genes between control and supplemented groups (7 or
15 28 d) with a $P < 0.05$ cut-off value for significance. Of the 77 immune-associated genes that were expressed above the detection limit in all samples, 6 genes were up-regulated after 7 d of OG supplementation and 4 genes were up-regulated after 28 d of OG supplementation. Three genes were up-regulated after 7 d (Cd80: +2.40; Irak1: +2.25; Nod2: +2.08 fold-change) as well as after 28 d of OG supplementation (Cd80: +1.77; Irak1: +2.50; Nod2: +1.83 fold-change). In
20 conclusion, our results suggest Cd80, Irak1, and Nod2 as immune response markers that are increased by dietary OG throughout a 28-d supplementation period.

EXAMPLE 8

This study was designed to determine the effect of supplementing feedlot steers with
25 OmniGen-AF (Composition I) on the acute phase response to a lipopolysaccharide (LPS) challenge. Steers (n=18; 270±5 kg BW) were separated into two treatment groups (n=9/treatment): one group was fed a standard receiving diet (Control, Cont) and the other group was fed the same receiving diet supplemented with OmniGen-AF at 4 g/45.4 kg BW for 29d (OmniGen-AF). On d27 steers were fitted with indwelling jugular cannulas and rectal
30 temperature (RT) monitoring devices and placed in individual stalls. On d28, steers were challenged i.v. with LPS (0.5 µg/kg BW at 0 h). Sickness behavior scores (SBS) and two whole blood samples were collected at 30-min intervals from -2 to 8h relative to the challenge at 0h. One vacutainer containing EDTA was collected for complete blood cell count (CBC) analysis,

and the second was collected in 9-mL monovette serum tube; after collection serum was isolated and stored at -80°C until analyzed for cortisol and cytokine concentrations. Rectal temperature, SBS, and cortisol were affected by time ($P<0.001$). Prior to the challenge, RT was greater ($P<0.001$) in Cont steers ($39.31\pm0.03^{\circ}\text{C}$) than OmniGen-AF steers ($39.14\pm0.03^{\circ}\text{C}$). Therefore, post-challenge RT was analyzed as the change in response from baseline values. The change in RT relative to baseline values increased ($P<0.001$) in both groups in response to LPS challenge, but was not affected by treatment ($P=0.49$). Sickness behavior scores increased ($P<0.001$) after LPS challenge and tended ($P=0.09$) to be greater in Control (1.57 ± 0.02) than OmniGen-AF steers (1.51 ± 0.02). Cortisol concentrations were affected by treatment ($P=0.005$) and time ($P<0.001$). For both groups, cortisol increased ($P<0.001$) in response to LPS challenge. Cortisol was greater in Cont (25.2 ± 0.9 ng/mL) than OmniGen-AF steers (25.5 ± 0.9 ng/mL). White blood cell and lymphocyte concentrations were greater ($P\leq0.004$) in Cont than OmniGen-AF steers throughout the study. Neutrophils were decreased ($P = 0.04$) in Cont steers (0.7 ± 0.2 K/uL) compared to OmniGen-AF steers (1.3 ± 0.2 K/uL) prior to the LPS challenge. There was a treatment ($P\leq0.02$) and time ($P<0.001$) effect for tumor necrosis factor- α (TNF α) and interferon- γ (IFN γ). Specifically, TNF α and IFN γ concentrations increased ($P<0.001$) in response to LPS challenge. Furthermore, concentrations of TNF α and IFN γ were decreased in ($P\leq0.02$) in Cont steers compared to OmniGen-AF steers. These data suggest that OmniGen-AF supplementation served to prime the immune system prior to the LPS challenge, allowing for an enhanced response to LPS challenge.

EXAMPLE 9

The use of probiotic feed supplements to enhance animal health and growth are of great interest to the beef industry. Studies have demonstrated that some probiotic supplements may affect metabolism, and therefore influence an animal's response to an immune challenge. This study was designed to determine the effect of supplementing feedlot steers with OmniGen-AF (Composition I) during the receiving period on the metabolic response to a lipopolysaccharide (LPS) challenge. Steers ($n = 18$; 270 ± 5 kg BW) were obtained and transported to the feedlot. Upon arrival steers were processed and separated into 2 treatment groups ($n = 9/\text{treatment}$): one group was fed a standard receiving diet (Control; Cont) and the other group was fed the same receiving diet supplemented with OmniGen-AF at 4 g/45.4 kg BW/d for 29 d (OmniGen-AF). On d 27 steers were fitted with indwelling jugular cannulas and placed in individual stalls. On d 28, steers were challenged i.v. with LPS (0.5 $\mu\text{g/kg}$ BW at 0 h) and blood samples were

collected at 30-min intervals from -2 to 8 h and at 24 h post-challenge. Serum was isolated and stored at -80°C until analyzed for glucose, non-esterified fatty acids (NEFA) and blood urea nitrogen (BUN) concentrations. Glucose concentrations were affected by treatment ($P = 0.009$) and time ($P < 0.001$). Glucose was greater in OmniGen-AF steers compared to Cont steers (76.4 ± 1.1 mg/dL vs. 72.4 ± 1.0 mg/dL). For NEFA concentrations, there was a treatment ($P < 0.001$) and time ($P < 0.001$) effect. Specifically, Cont (0.210 ± 0.007 mmol/L) steers had greater NEFA concentrations than OmniGen-AF steers (0.101 ± 0.010 mmol/L). There was a tendency ($P = 0.07$) for a treatment x time interaction such that NEFA concentrations were greater ($P \leq 0.03$) in Cont steers than OmniGen-AF steers from 3 to 8 hr after LPS challenge. For BUN, there was a treatment ($P < 0.001$) effect such that concentrations were greater in Cont steers (12.4 ± 0.1 mg/dL) than OmniGen-AF supplemented steers (11.5 ± 0.1 mg/dL) throughout the study, and were not affected by time ($P = 0.28$). These data suggest that OmniGen-AF supplementation modulates the metabolic response to a LPS challenge and provides an indication that supplementation of feedlot steers with OmniGen-AF may prevent the breakdown of other substrates (e.g., protein and fat) for energy during an immune challenge.

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

We claim:

1. A composition comprising glucan, silica, mineral clay, and mannan for use in promoting reduction of heat-induced stress in an animal that has or is at risk of developing heat-induced stress, wherein administering the composition to the animal for an effective period of time promotes reduction of heat-induced stress.
2. The composition of claim 1 wherein the composition comprises between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, and between 1% and 8.0% mannan.
3. The composition of claim 1 or 2 wherein the composition further comprises an endoglucanohydrolase.
4. The composition of claim 3 wherein the composition consists essentially of between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan..
5. The composition of any one of claims 1-5 wherein the animal is a mammalian species or an avian species.
6. The composition of claim 5 wherein the animal is a dog, cat, rabbit, sheep, goat, cow, deer, bison, buffalo, llama, horse, donkey, pig, chicken, turkey, goose, duck, cornish game hen, quail, pheasant, guinea-fowl, ostrich, emu, swan, or pigeon.
7. The composition of any one of claims 1-6 wherein the animal is immunosuppressed prior to administration of the composition.
8. The composition of any one of claims 1-7 wherein the composition is administered to the animal at set intervals for the effective period of time.

9. The composition of claim 8 wherein the composition is administered to the animal in an amount from 1 mg/kg body weight per day to 20 g/kg body weight per day.

10. The composition of any one of claims 1-9 wherein the effective period of time is at least 1 day, at least 3 days, or from one day to 200 days.

11. The composition of any one of claims 1-10 wherein the composition is administered to the animal when a temperature humidity index is, or is expected to be, greater than 68.

12. The composition of claim 11 wherein the temperature humidity index is, or is expected to be, greater than 75.

13. The composition of claim 11 wherein the temperature humidity index is, or is expected to be, ≥ 79 .

14. The composition of any one of claims 11-13 wherein:
an amount of the composition administered to the animal is increased if the temperature humidity index is expected to increase; or
an amount of the composition administered to the animal is decreased if the temperature humidity index is expected to decrease.

15. The composition of any one of claims 1-14 wherein the animal is administered an amount of the composition effective to alter a heat stress indicator compared to an animal that has not been administered the composition.

16. The composition of claim 15 wherein the heat stress indicator is feed intake, water consumption, respiration rate, rectal temperature, milk yield, milk fat, milk protein, an immune biomarker, or any combination thereof.

17. The composition of claim 15 or claim 16 wherein:
the heat stress indicator is measured; and

an amount of the composition administered to the animal is selected based at least in part on the measurement of the heat stress indicator.

18. The composition of claim 15 or claim 16 wherein:

5 a first amount of the composition is administered daily to the animal for a first period of time;

the heat stress indicator is measured; and

an adjusted amount of the composition is administered daily to the animal for a subsequent period of time, wherein the adjusted amount is based at least in part on the
10 measurement of the heat stress indicator.

19. The composition of any one of claims 1-18 wherein the composition is incorporated into the animal's feed in an amount ranging from 0.1 to 100 kg per ton of feed, and the composition incorporated into the feed is provided to the animal.

15 20. The composition of any one of claims 1-19 wherein the mammal is a cow.

21. The composition of claim 20 wherein the cow is a dairy cow and the composition is administered to the dairy cow before lactation onset.

20 22. The composition of claim 21 wherein the composition is administered to the dairy cow for a period from 100 days prior to lactation onset to 1 day prior to lactation onset.

23. The composition of claim 21 or claim 22 wherein the composition is
25 administered to the dairy cow during lactation.

24. The composition of any one of claims 20-23 wherein the cow is a dairy cow and the composition is administered to the dairy cow after lactation onset.

30 25. The composition of any one of claims 20-24, wherein the composition is administered to the cow for 40 days to 100 days post calving.

26. The composition of any one of claims 1-25 wherein the animal is further administered a therapeutic process for alleviating heat stress, a therapeutic agent for treating heat stress, or a combination thereof.

5 27. The composition of claim 26 wherein the therapeutic process or therapeutic agent is provision of shade to the animal, use of a water sprinkler to externally administer water to the animal, use of a fan to provide air movement, addition of bypass fats to feed for the animal, meloxicam, a corticosteroid, a composition comprising one or more electrolytes, an alkalinizing agent, a direct-fed microbial, or a combination thereof.

10 28. The composition of any one of claims 1-27 wherein the composition is administered to the animal prior to the animal experiencing heat stress, while the animal is experiencing heat stress, and after the animal experiences heat stress.

15 29. The composition of any one of claims 1-28 wherein:
the composition is administered to a group of animals that have or are at risk of developing heat-induced stress for a first period of time;
a heat stress indicator of at least one animal of the group of animals is subsequently measured; and
20 an adjusted amount of the composition is administered to the group of animals for a subsequent period of time, wherein the adjusted amount is based at least in part on the measurement of the heat stress indicator.

25 30. The composition of any one of claims 1-28 wherein:
at least one heat stress indicator of individual animals in a group of animals is evaluated;
a determination of whether one or more of the individual animals are experiencing heat stress is made; and
the composition is administered to the one or more individual animals that are
30 experiencing heat stress.

31. A method for promoting reduction of heat-induced stress in an animal, comprising:

administering to an animal that has or is at risk of developing heat-induced stress a composition comprising glucan, silica, mineral clay, and mannan, wherein the composition is administered for an effective period of time to promote reduction of heat induced stress.

32. The method of claim 31 wherein the composition comprises between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, and between 1% and 8.0% mannan.

33. The method of claim 31 or 32 wherein the composition further comprises an endoglucanohydrolase.

34. The method of claim 33 wherein the composition consists essentially of between 15% and 40% silica, between 50% and 81% mineral clay, between 1.0% and 5.0% β -glucans, between 0.05% and 3.0% β -1,3 (4)-endoglucanohydrolase and between 1% and 8.0% mannan..

35. The method of any one of claims 31-34 wherein the animal is a mammalian species or an avian species.

36. The method of claim 35 wherein the animal is a dog, cat, rabbit, sheep, goat, cow, deer, bison, buffalo, llama, horse, donkey, pig, chicken, turkey, goose, duck, cornish game hen, quail, pheasant, guinea-fowl, ostrich, emu, swan, or pigeon.

37. The method of any one of claims 31-36 wherein the animal is immunosuppressed prior to administration of the composition.

38. The method of any one of claims 31-37, further comprising administering the composition to the animal at set intervals for the effective period of time.

39. The method of claim 38, further comprising administering the composition in an amount from 1 mg/kg body weight per day to 20 g/kg body weight per day.

40. The composition of any one of claims 31-39 wherein the effective period of time is at least 1 day.

41. The method of any one of claims 31-40, further comprising administering the composition to the animal when a temperature humidity index is, or is expected to be, greater than 68.

42. The method of claim 41 wherein the temperature humidity index is, or is expected to be, greater than 75.

43. The method of claim 41 wherein the temperature humidity index is, or is expected to be, ≥ 79 .

44. The method of any one of claims 41-43, further comprising:
increasing an amount of the composition administered to the animal if the temperature humidity index is expected to increase; or
decreasing an amount of the composition administered to the animal if the temperature humidity index is expected to decrease.

45. The method of any one of claims 31-44, further comprising administering to the animal an amount of the composition effective to alter a heat stress indicator compared to an animal that has not been administered the composition.

46. The method of claim 45 wherein the heat stress indicator is feed intake, water consumption, respiration rate, rectal temperature, milk yield, milk fat, milk protein, an immune biomarker, or any combination thereof.

47. The method of claim 45 or claim 46, further comprising:
measuring the heat stress indicator; and
selecting an amount of the composition administered to the animal based at least in part on the measurement of the heat stress indicator.

48. The method of claim 45 or claim 46, further comprising:
administering a first amount of the composition daily to the animal for a first period of
time;

measuring the heat stress indicator; and

5 administer an adjusted amount of the composition daily to the animal for a
subsequent period of time, wherein the adjusted amount is based at least in part on the
measurement of the heat stress indicator.

49. The method of any one of claims 31-48 wherein administering comprises mixing
10 the composition with the animal's feed in an amount ranging from 0.1 to 100 kg per ton of feed,
and providing the composition mixed with the feed to the animal.

50. The method of any one of claims 31-49 wherein the mammal is a cow.

15 51. The method of claim 50 wherein the cow is a dairy cow, the method further
comprising administering the composition to the dairy cow before lactation onset.

52. The method of claim 51, further comprising administering the composition to the
dairy cow for a period from 100 days prior to lactation onset to 1 day prior to lactation onset.

20

53. The method of claim 51 or claim 52, further comprising administering the
composition to the dairy cow during lactation.

54. The method of any one of claims 51-53 wherein the cow is a dairy cow, the
25 method further comprising administering the composition to the dairy cow after lactation onset.

55. The method of any one of claims 51-54, further comprising administering the
composition to the cow for 40 days to 100 days post calving.

30 56. The method of any one of claims 31-55, further comprising administering to the
animal a therapeutic process for alleviating heat stress, a therapeutic agent for treating heat
stress, or a combination thereof.

57. The method of claim 56 wherein the therapeutic process or therapeutic agent is provision of shade to the animal, use of a water sprinkler to externally administer water to the animal, use of a fan to provide air movement, addition of bypass fats to feed for the animal, meloxicam, a corticosteroid, a composition comprising one or more electrolytes, an alkalinizing agent, a direct-fed microbial, or a combination thereof.

58. The method of any one of claims 1-25 wherein the composition is administered to the animal prior to the animal experiencing heat stress, while the animal is experiencing heat stress, and after the animal experiences heat stress.

59. The method of any one of claims 31-58, further comprising:
administering the composition to a group of animals that have or are at risk of developing heat-induced stress for a first period of time;
subsequently measuring a heat stress indicator of at least one animal of the group of animals; and
administering an adjusted amount of the composition to the group of animals for a subsequent period of time, wherein the adjusted amount is based at least in part on the measurement of the heat stress indicator.

60. The method of any one of claims 31-58, further comprising:
evaluating at least one heat stress indicator of individual animals in a group of animals;
determining whether one or more of the individual animals are experiencing heat stress;
and
administering the composition to the one or more individual animals that are experiencing heat stress.

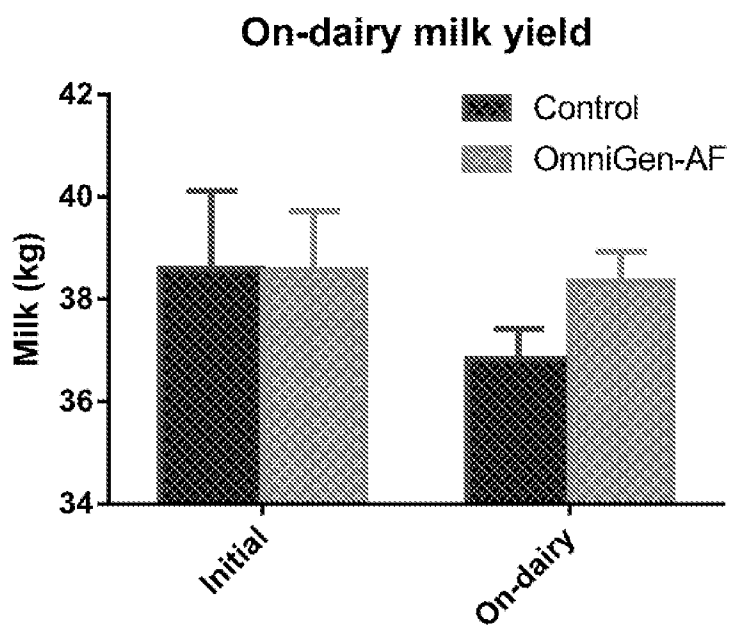


FIG. 1

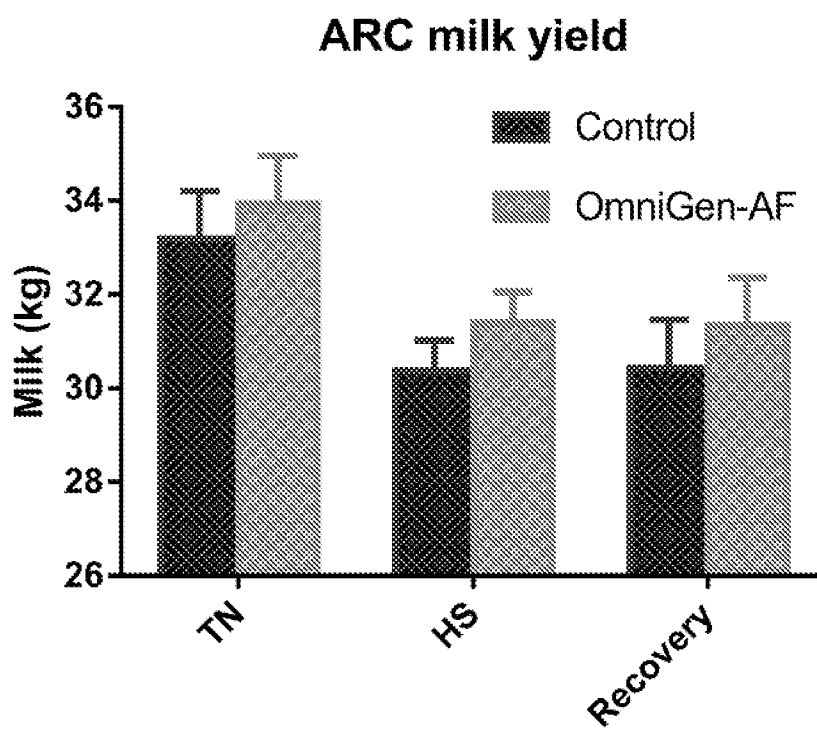


FIG. 2

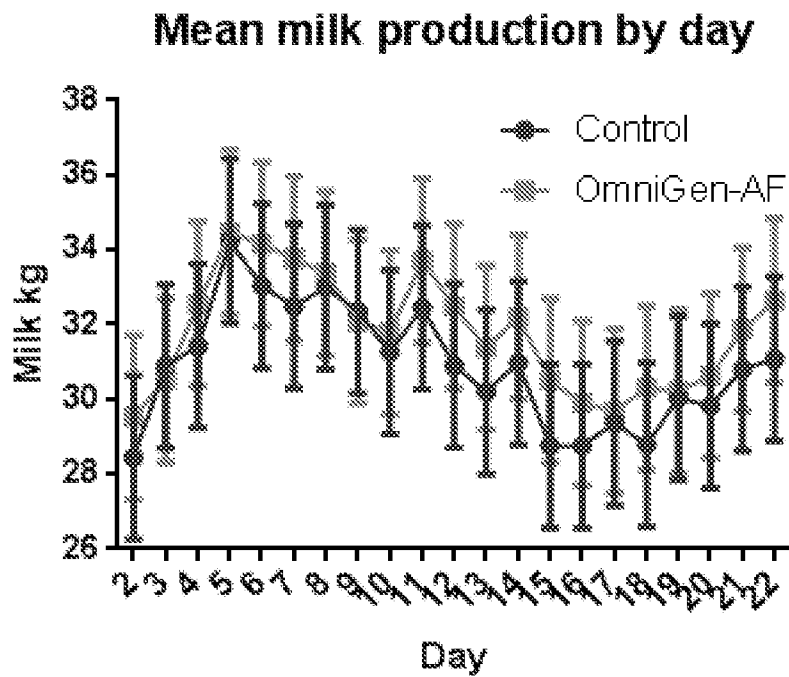


FIG. 3

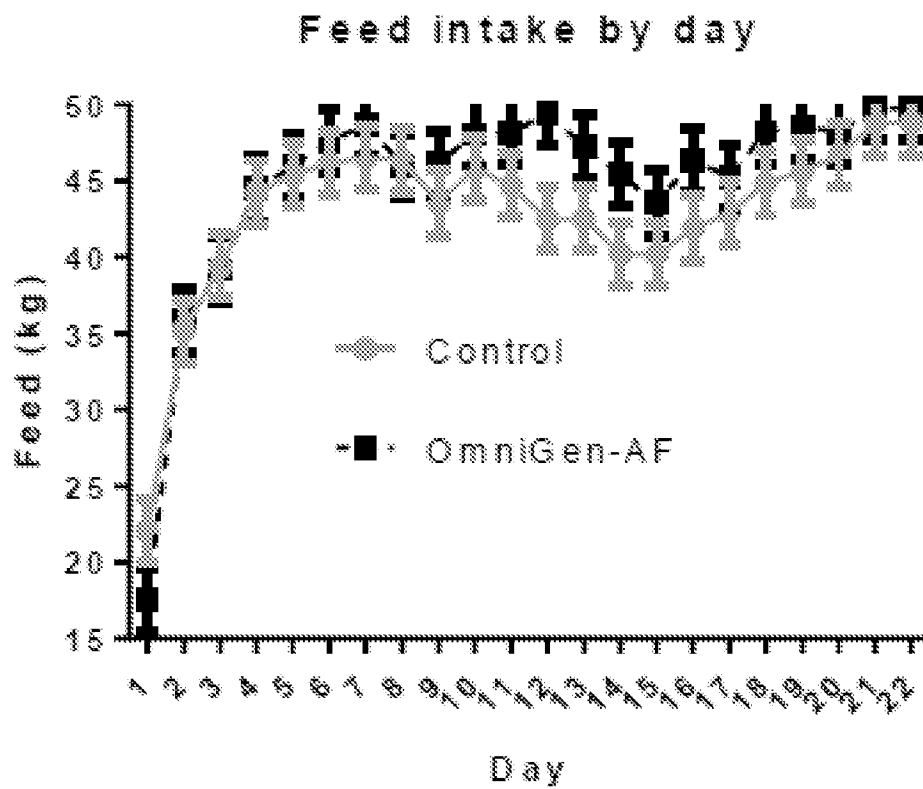


FIG. 4

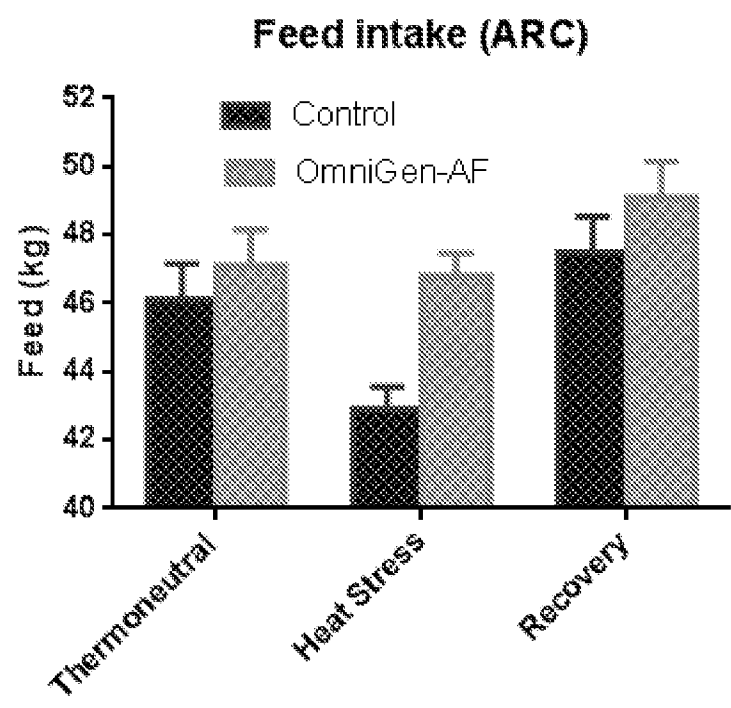


FIG. 5

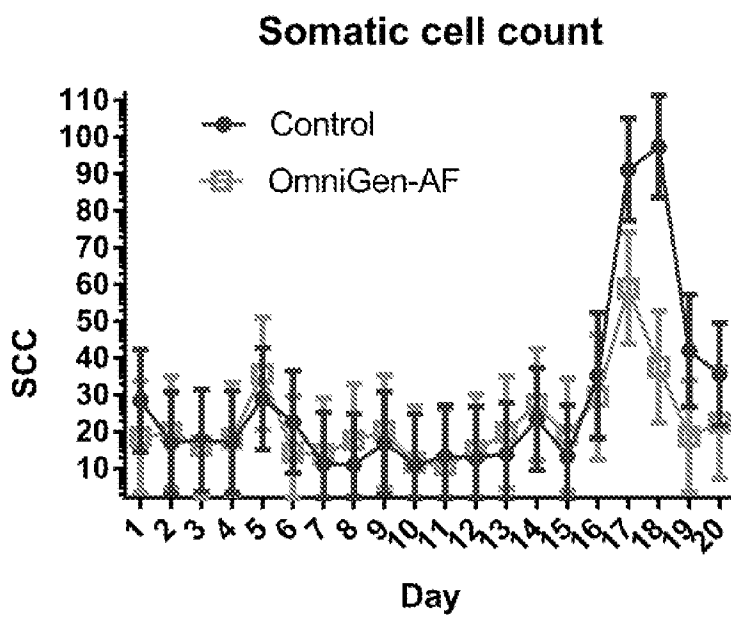


FIG. 6

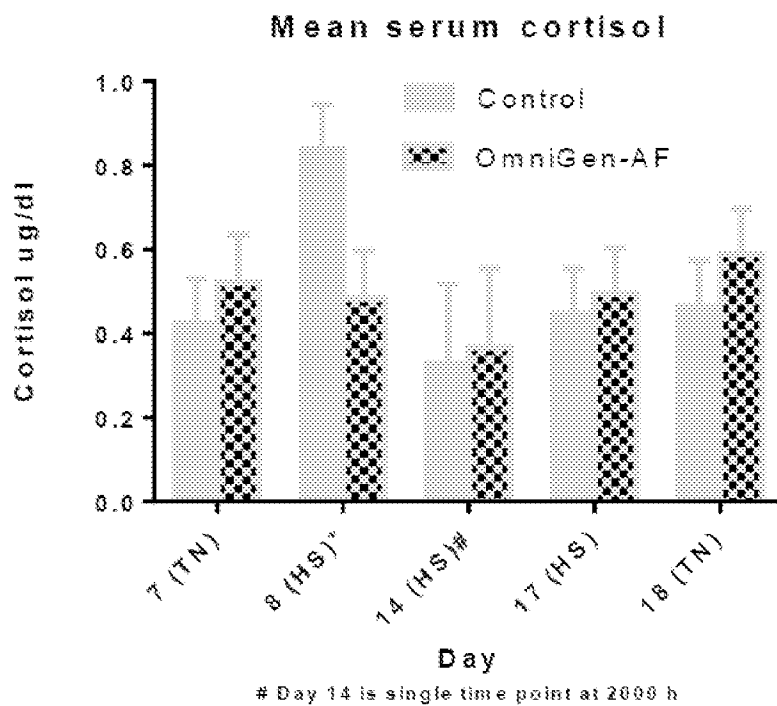


FIG. 7

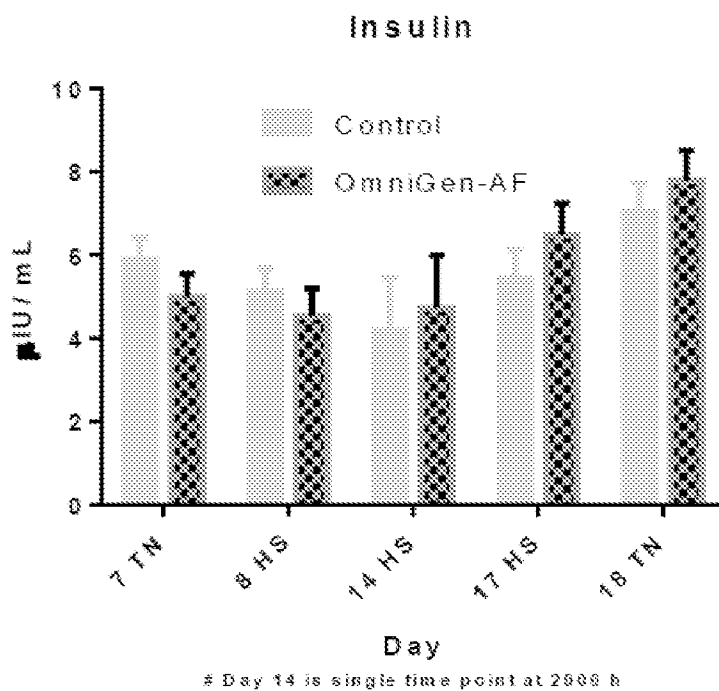


FIG. 8

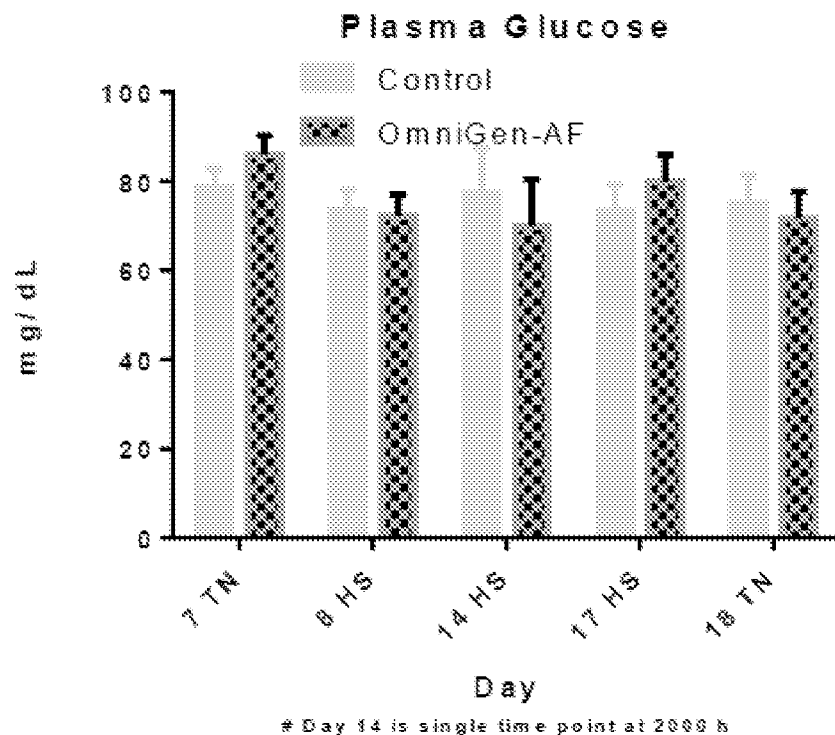


FIG. 9

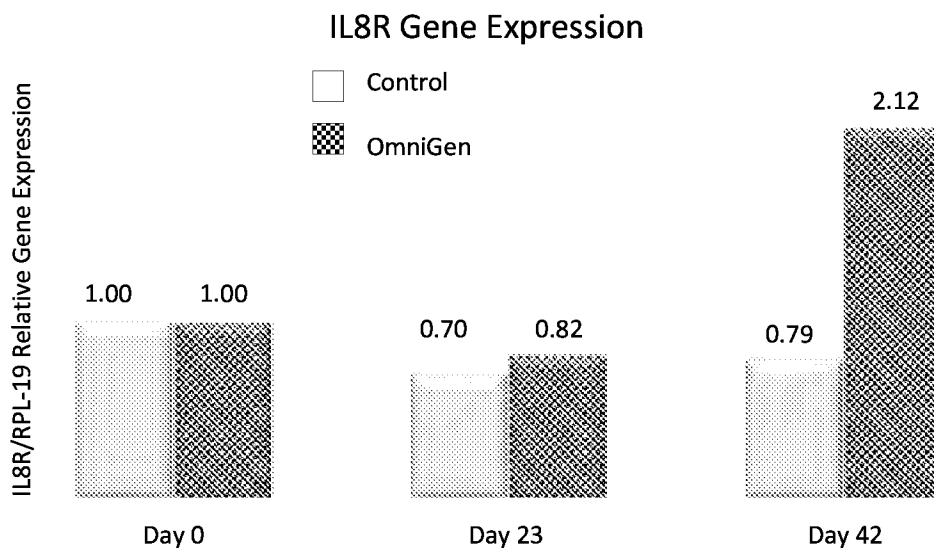


FIG. 10

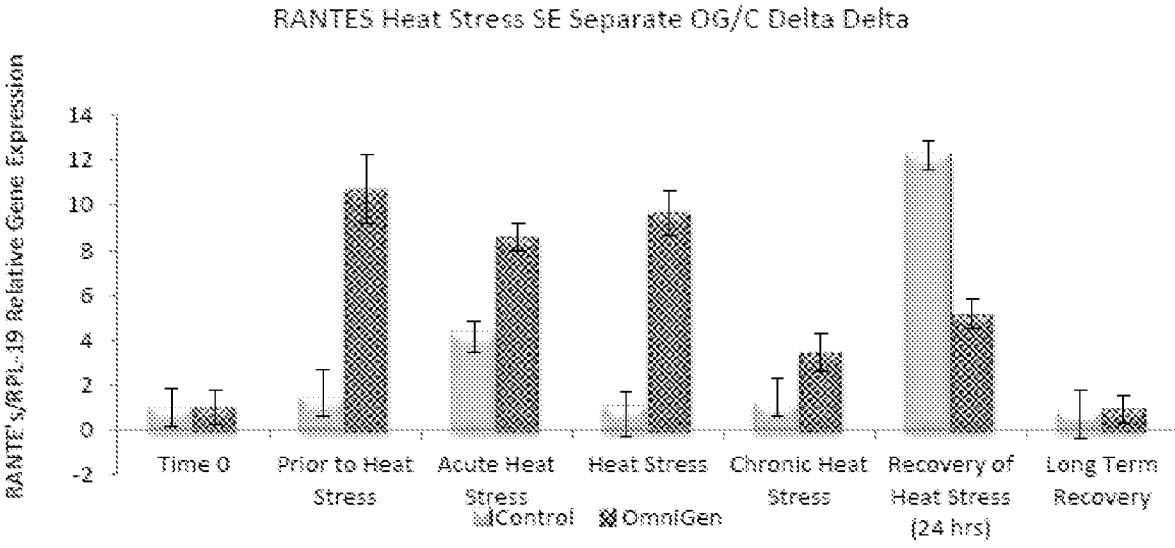


FIG. 11

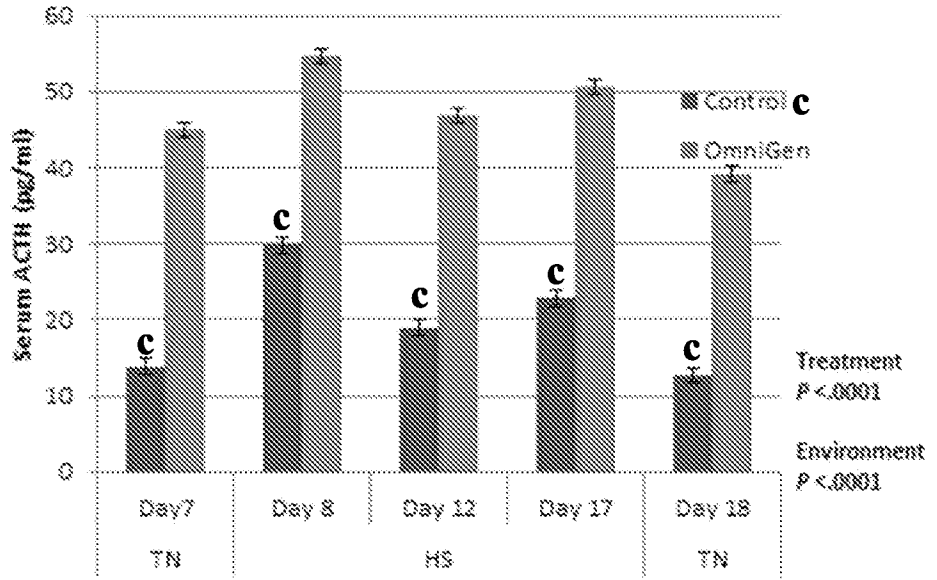


FIG. 12

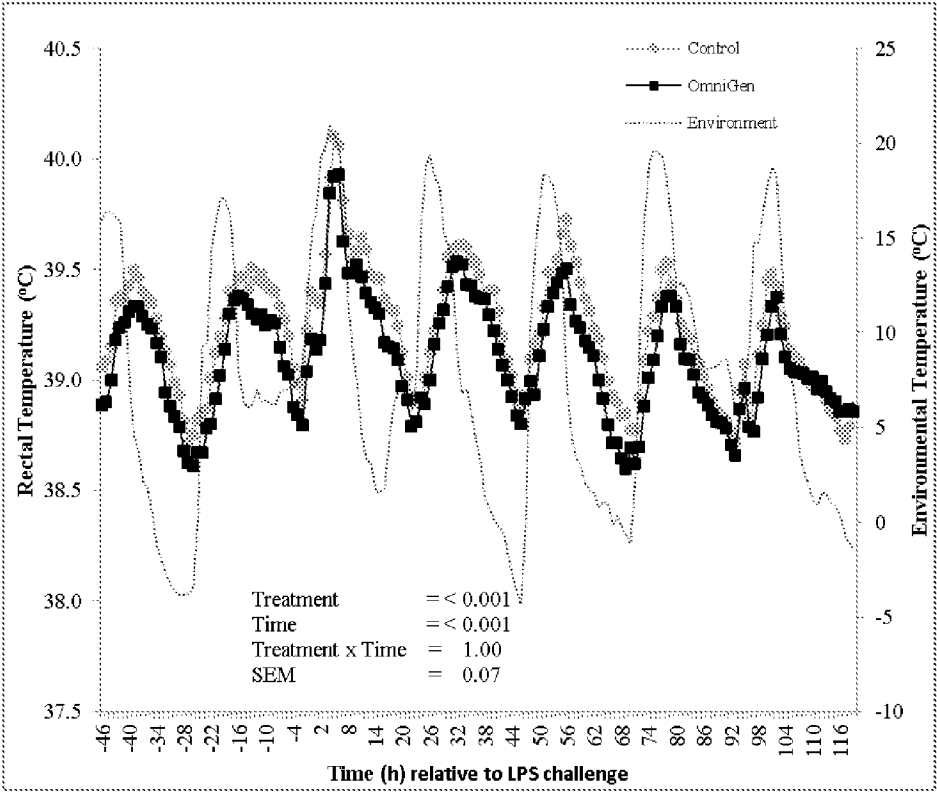


FIG. 13

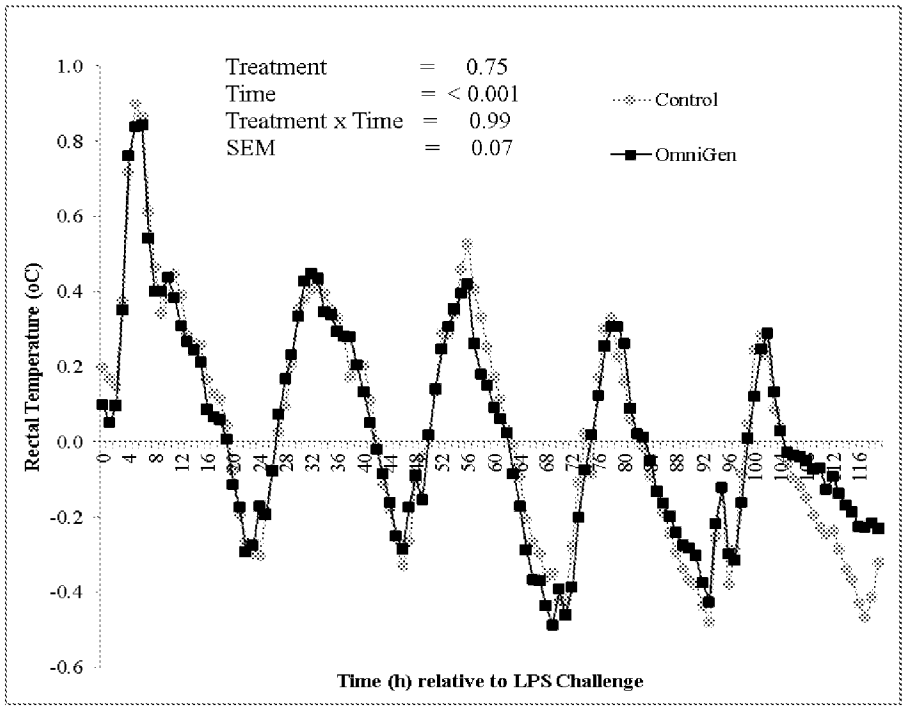


FIG. 14

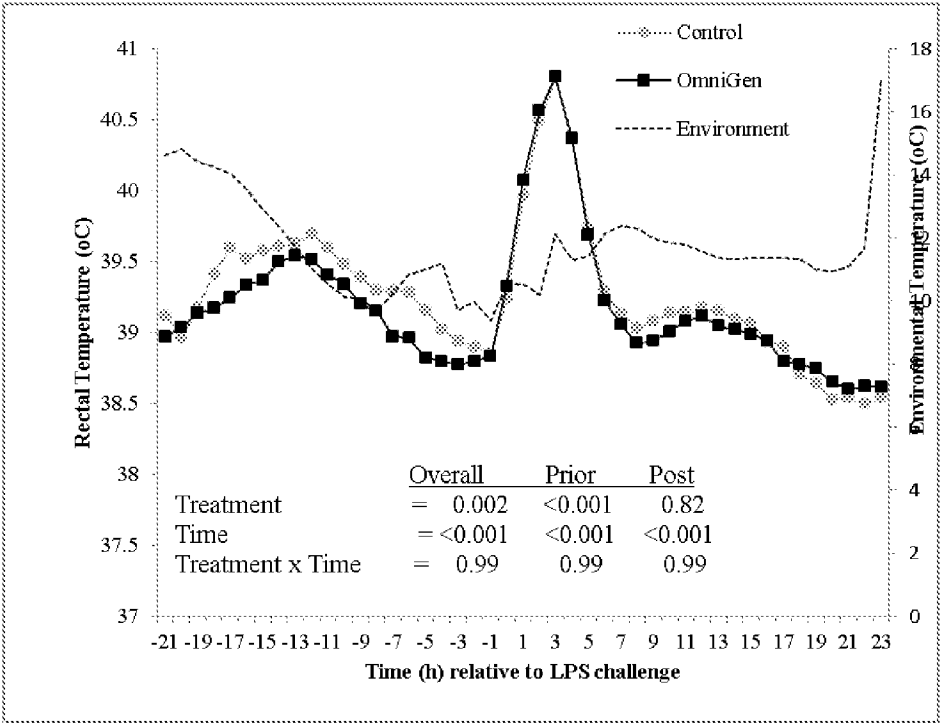


FIG. 15

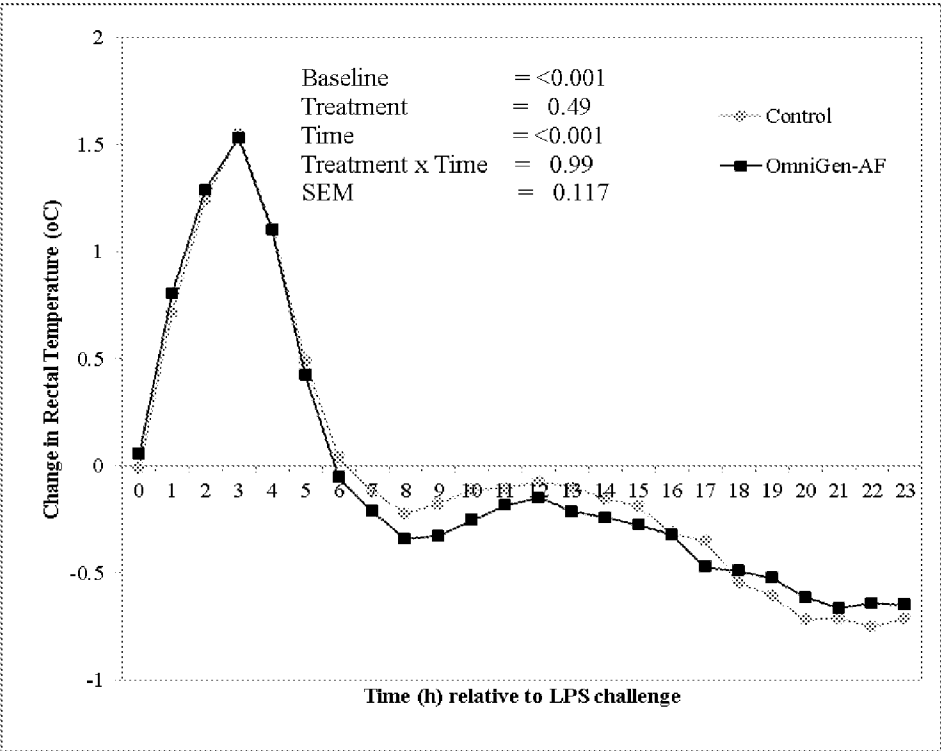
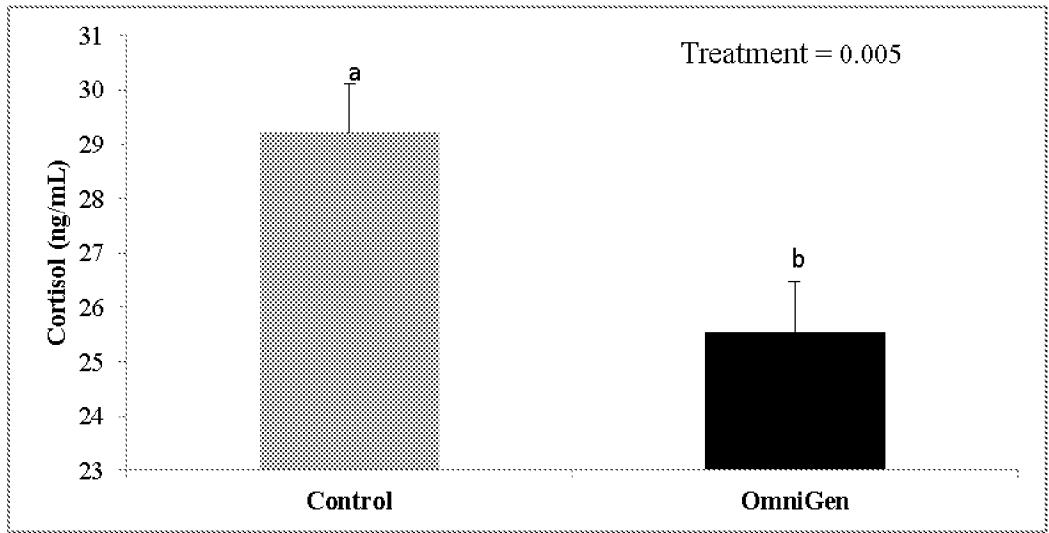


FIG. 16



a, b, Denotes differences ($P < 0.05$) between treatment groups

FIG. 17

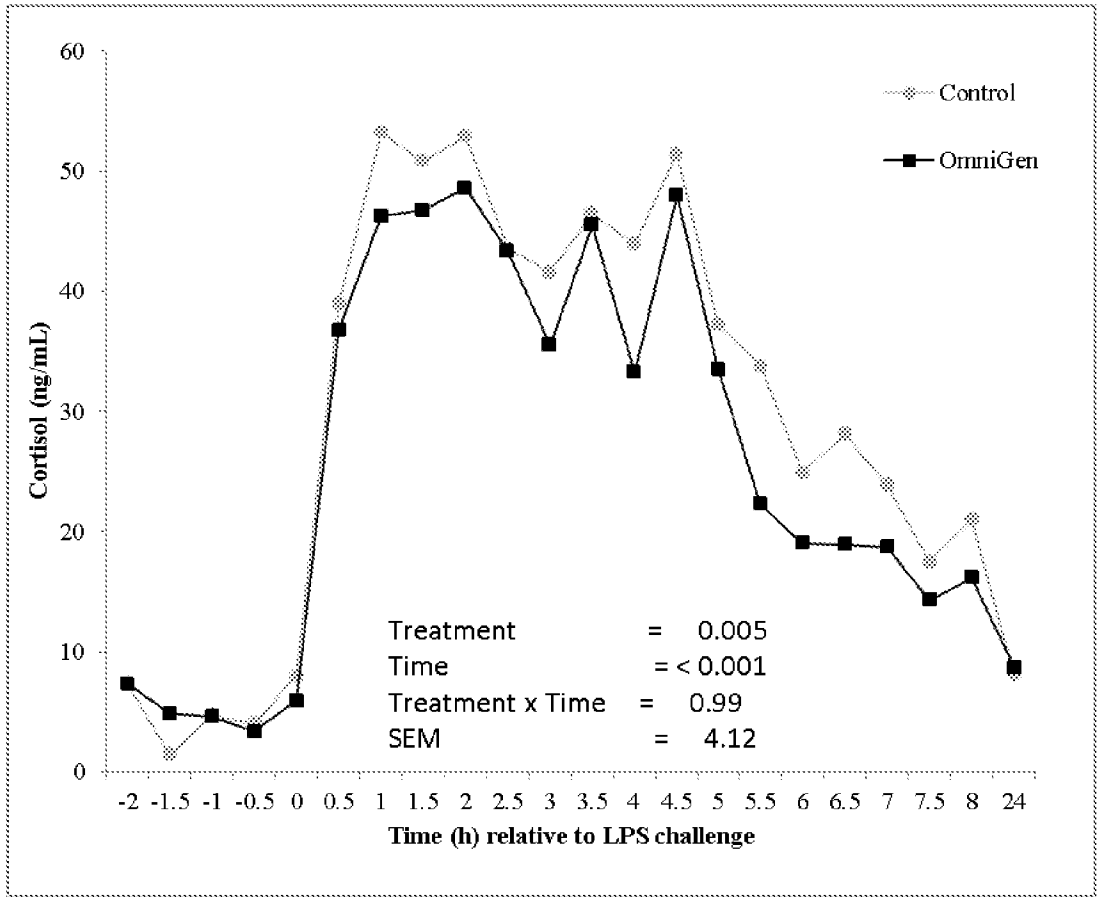


FIG. 18

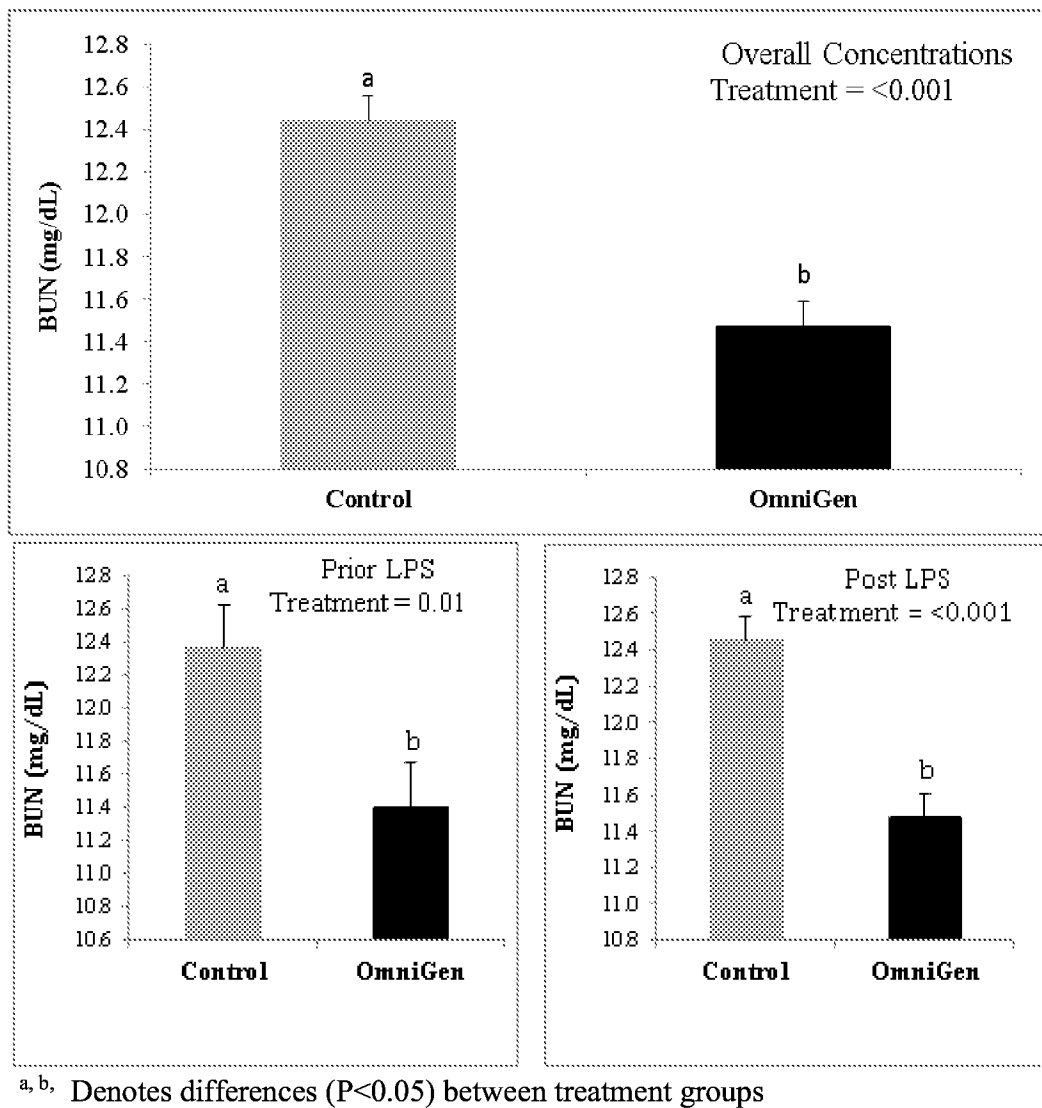


FIG. 19

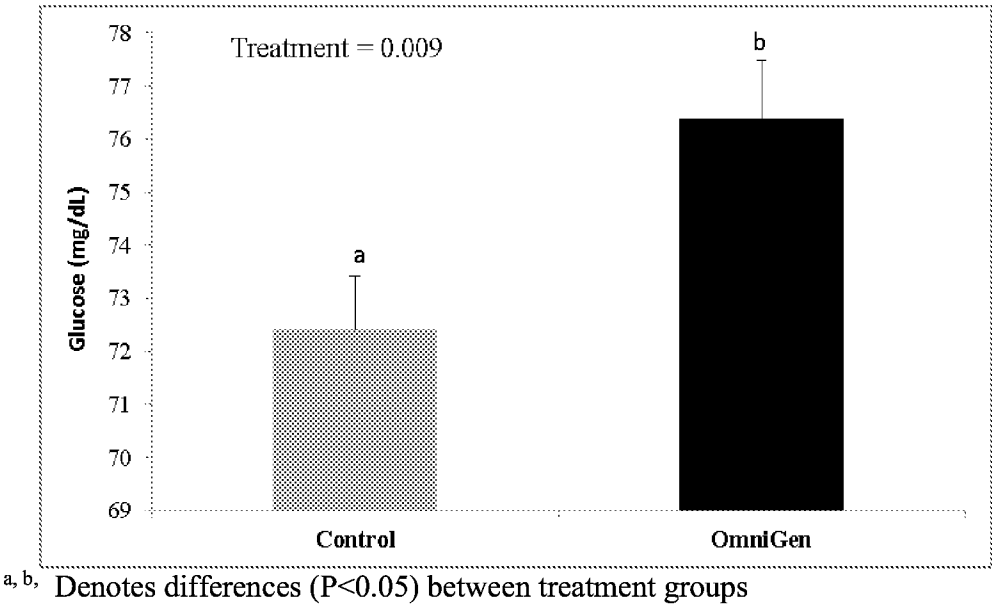


FIG. 20

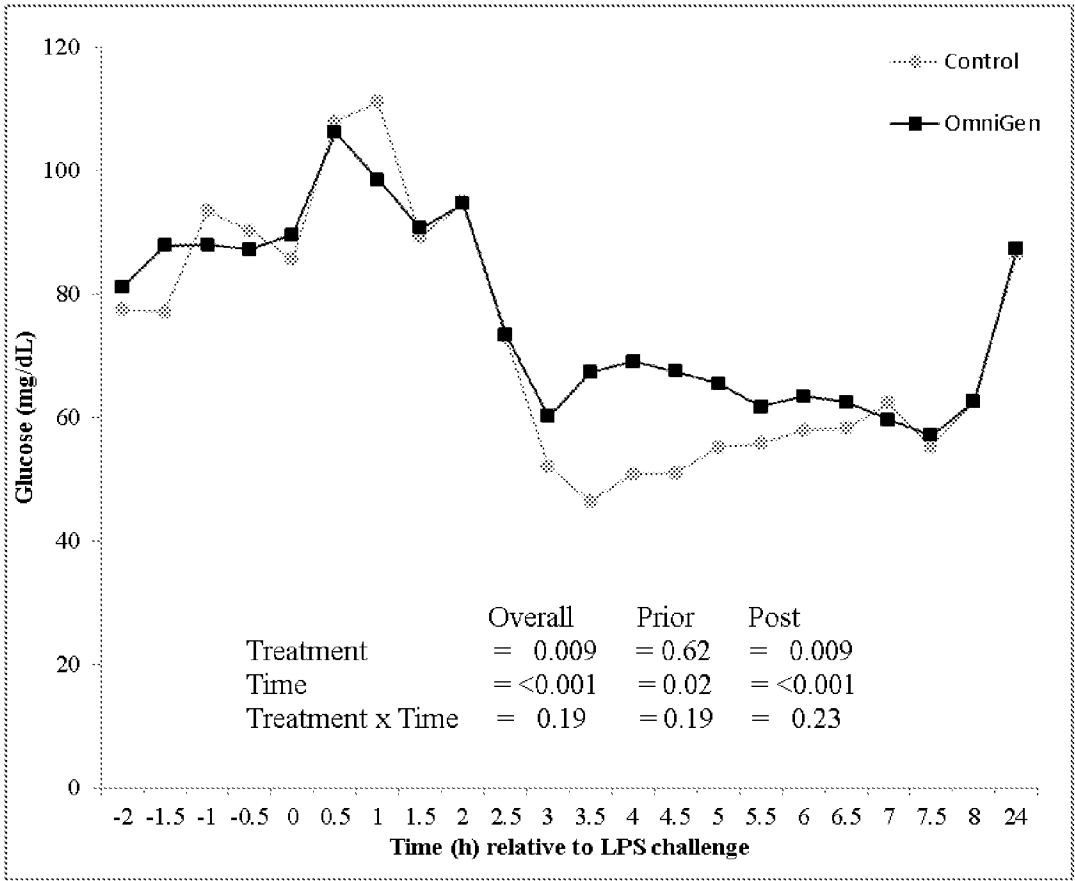
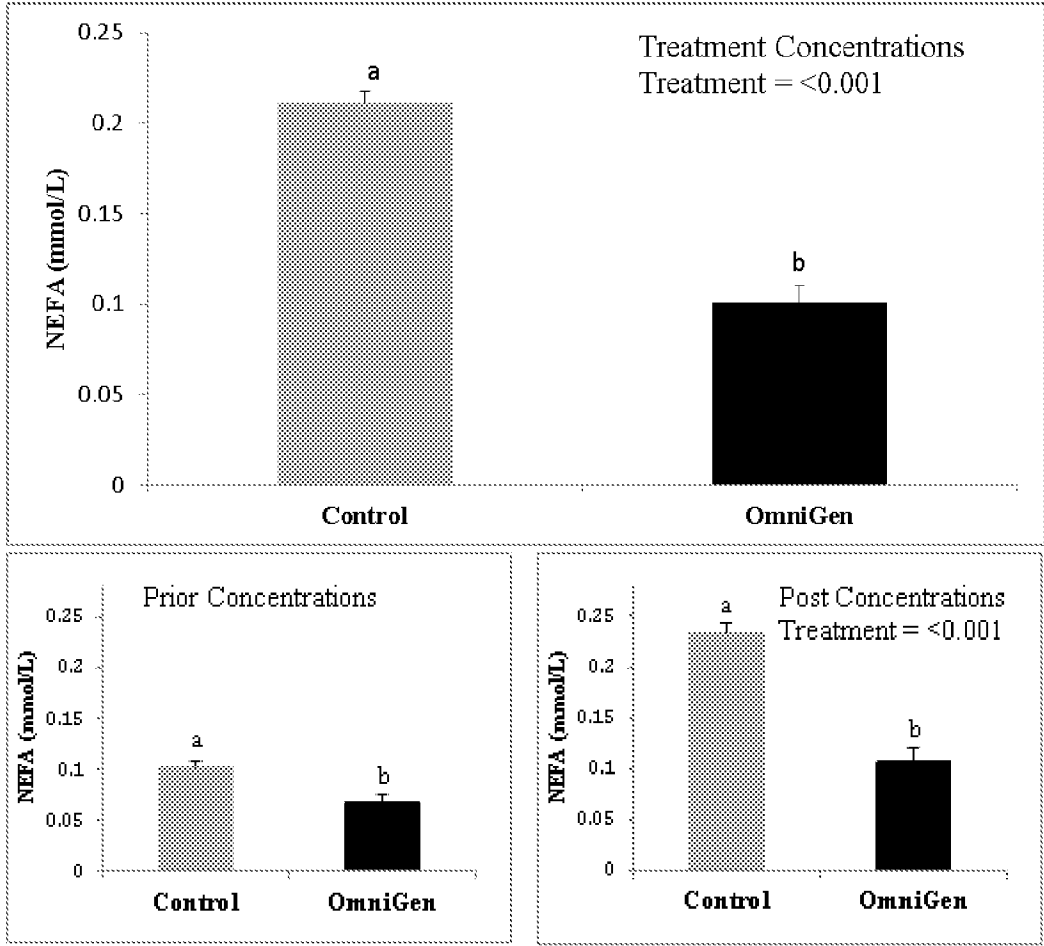


FIG. 21



a, b, Denotes differences (P<0.05) between treatment group

FIG. 22

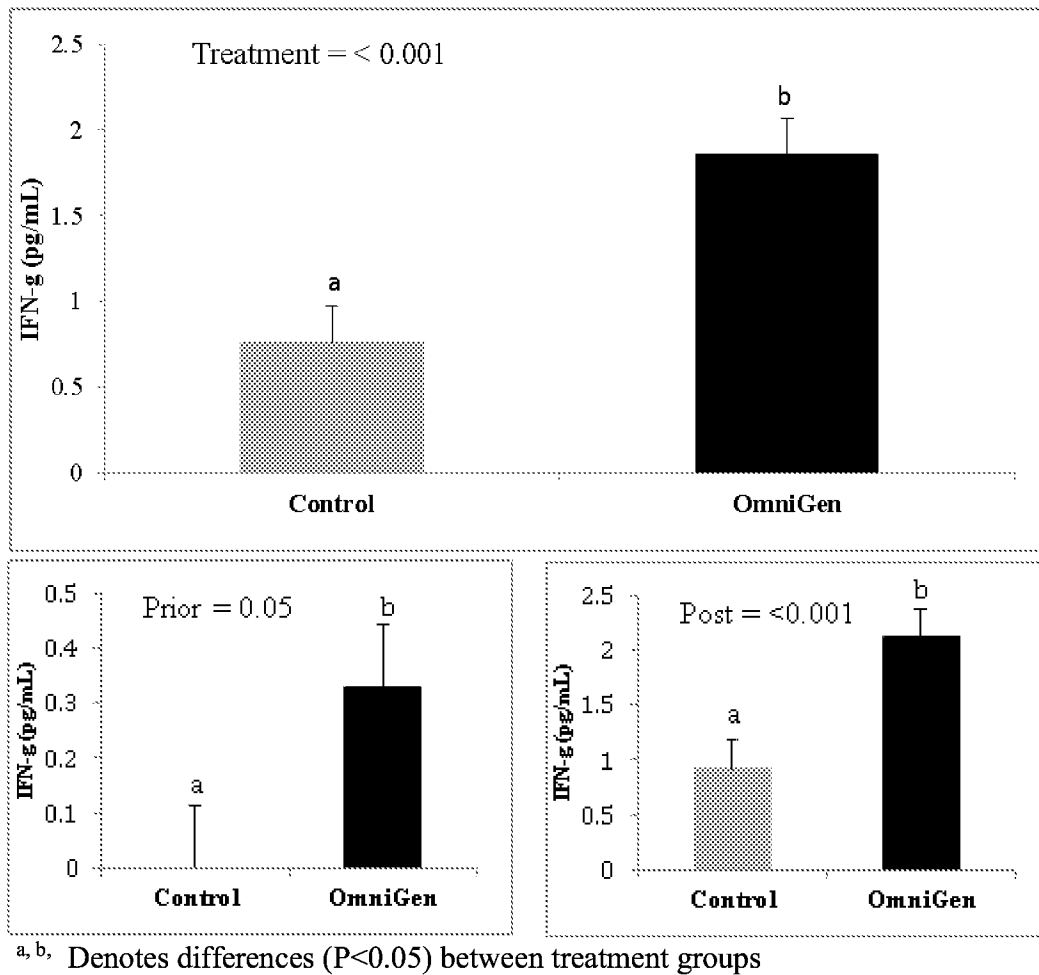
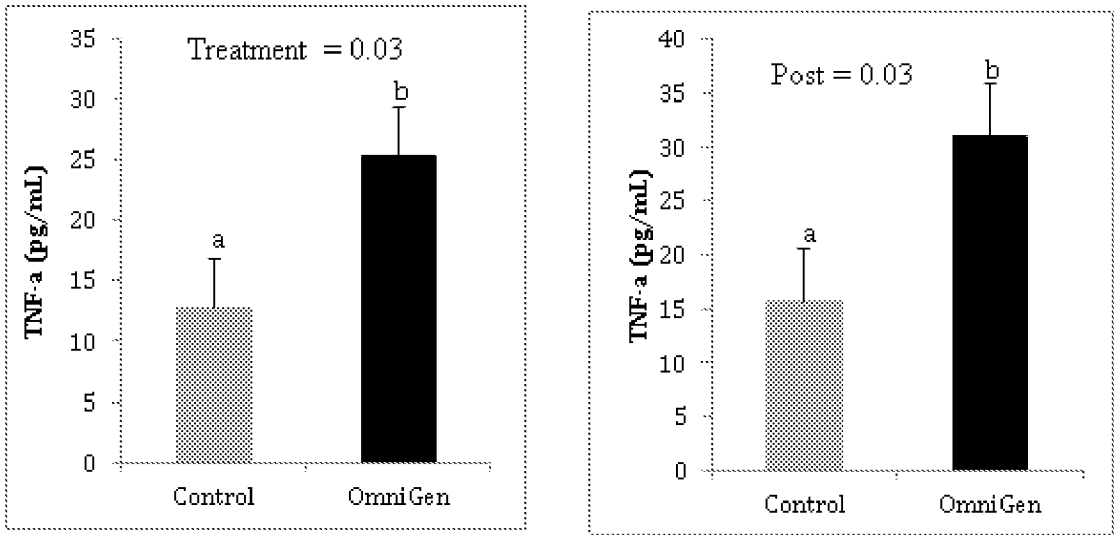


FIG. 23



a, b, Denotes differences ($P < 0.05$) between treatment groups at specific time points

FIG. 24

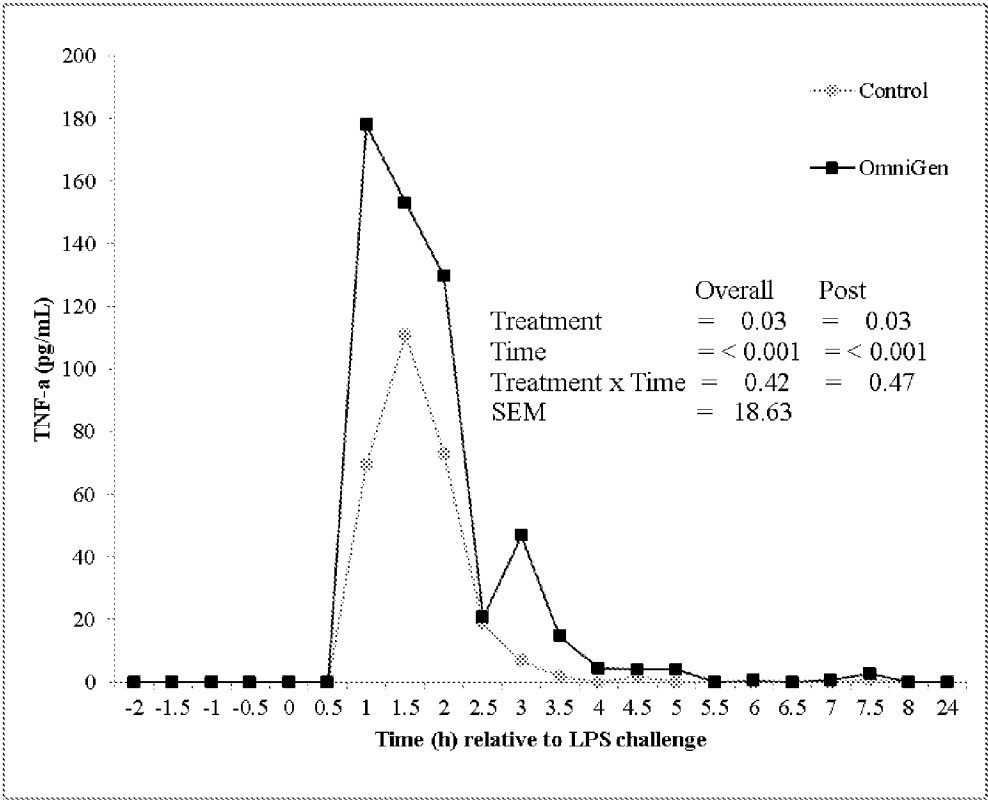


FIG. 25

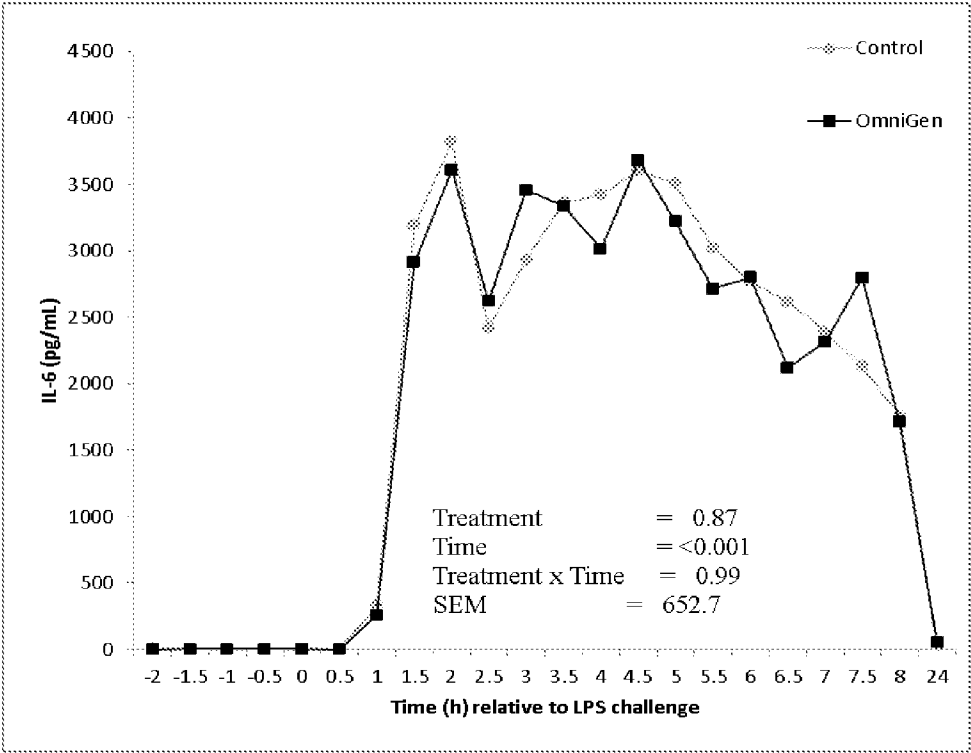
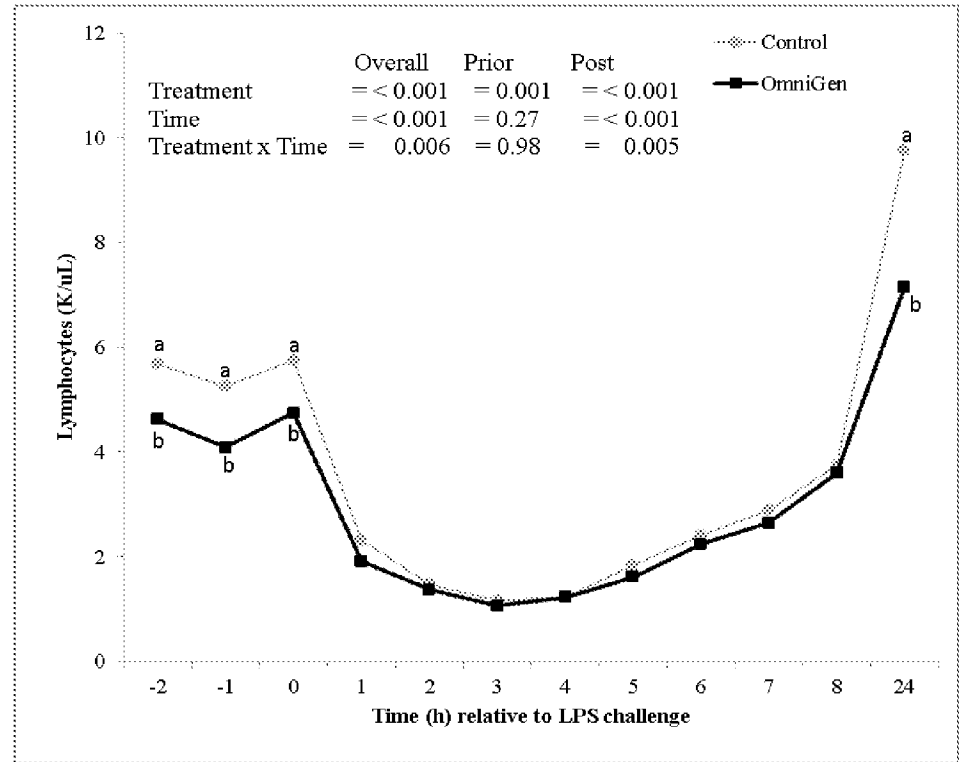


FIG. 26



a, b, Denotes differences ($P < 0.05$) between treatment groups at specific time points

FIG. 27

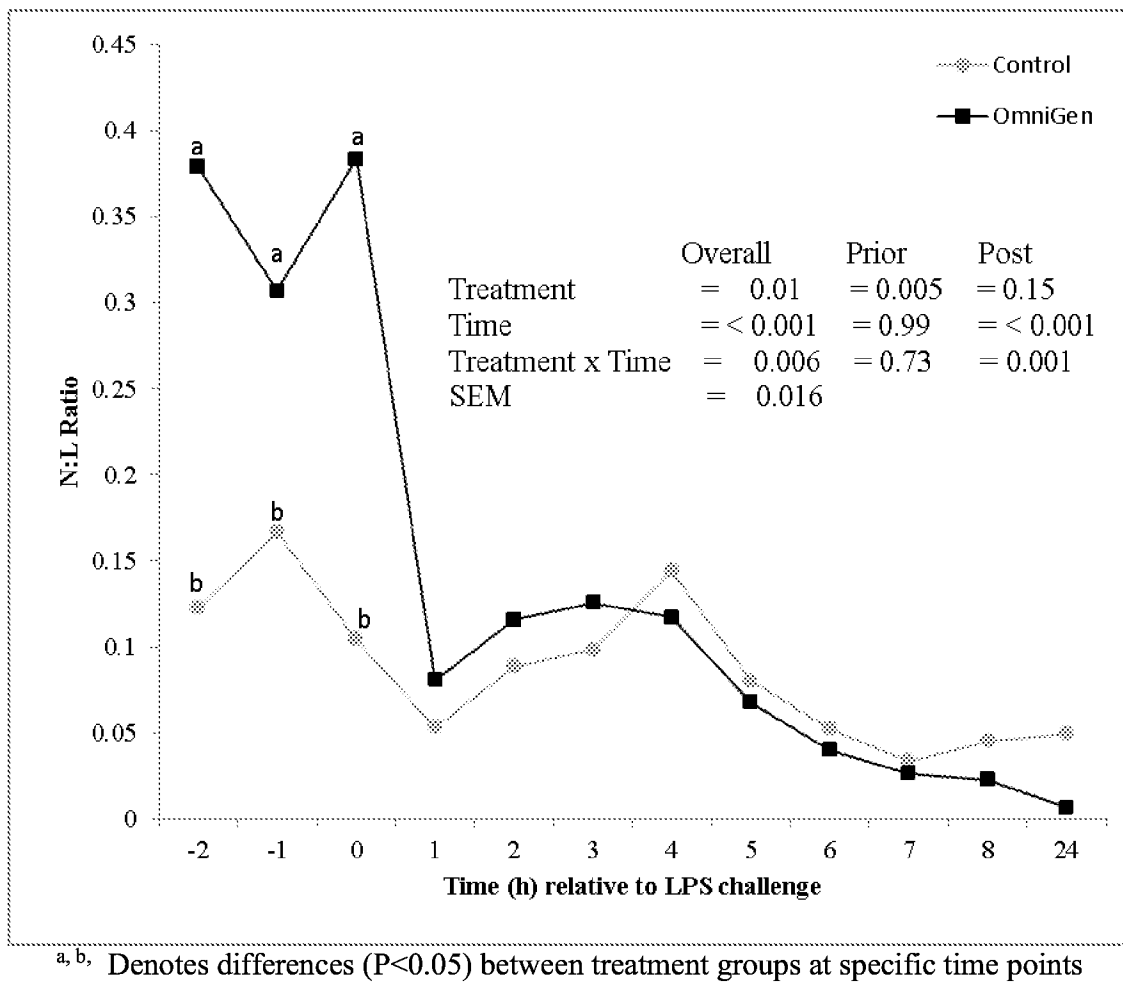


FIG. 28

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/015692

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23K1/16 A23K1/175 A61K38/47 A61K45/06 A61K31/716
A61K31/736 A61K35/02

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23K A61K

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

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

EPO-Internal, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 759 932 A (LAURENT SEBASTIAN M [US] ET AL) 26 July 1988 (1988-07-26) claims; examples -----	1-60
A	US 2011/195146 A1 (RUSSI JUAN PABLO [AR]) 11 August 2011 (2011-08-11) claims 1-3, 7-9, 12 -----	1-60
A	US 2013/196021 A1 (MILLER BILL L [US] ET AL) 1 August 2013 (2013-08-01) claims; examples -----	1-60
A	WO 2007/021262 A1 (PUNTENNEY STEVEN BRUCE [US]; FORSBERG NEIL ELLIOTT [US]) 22 February 2007 (2007-02-22) claims ----- -/--	1-60



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

14 April 2015

Date of mailing of the international search report

04/05/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Smeets, Dieter

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/015692

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2007/253983 A1 (FORSBERG NEIL E [US] ET AL) 1 November 2007 (2007-11-01) cited in the application claims; examples -----	1-60

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2015/015692

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