

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

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
9 June 2016 (09.06.2016)

(10) International Publication Number  
**WO 2016/090366 A1**

(51) International Patent Classification:

A23K 1/16 (2006.01) A23K 1/175 (2006.01)  
A23K 1/18 (2006.01) A23L 1/304 (2006.01)

(21) International Application Number:

PCT/US2015/064293

(22) International Filing Date:

7 December 2015 (07.12.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/088,035 5 December 2014 (05.12.2014) US

(71) Applicant: CAN TECHNOLOGIES, INC. [US/US];  
9380 Excelsior Blvd., Hopkins, Minnesota 55343 (US).

(72) Inventors: VAN DEN BOSCH, Moniek; Duifkruid 41,  
5831 PB Boxmeer (NL). VAN WESEL, Ad; Lignestraat  
19, 4921 ET Made (NL).

(74) Agent: SKAROHLID, Gretchen, P.; Cargill, Incorporated,  
15407 McGinty Road West, MS24, Wayzata, Min-  
nesota 55391 (US).

(81) Designated States (unless otherwise indicated, for every

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

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, 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).

Published:

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



WO 2016/090366 A1

(54) Title: ANIMAL FEED SUPPLEMENT AND METHOD

(57) Abstract: A premix is disclosed. The premix includes a nitrate compound and salts thereof in the amount of greater than about 50% by weight of the premix. The premix also includes vitamins and trace minerals. The premix is formulated for use in at least one of a gestation or lactation phase of an animal and in an amount of less than about five percent by weight (5.0 wt%) inclusion per metric ton of the premix. A supplement for an animal feed for feeding during at least one of an animal's gestation phase and/or lactation phase is also disclosed. A method for feeding an animal is also disclosed.

## ANIMAL FEED SUPPLEMENT AND METHOD

## CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of the United States Provisional Patent Application No. 62/088,035, filed December 5, 2014, entitled ANIMAL FEED SUPPLEMENT AND METHOD, which is hereby incorporated by reference in its entirety.

## BACKGROUND

[0002] Feeding calcium nitrate and other ingredients to ruminants is one way to mitigate methane production. For example, U.S. Patent No. 8,771,723 titled "Compositions For Reducing Gastro-Intestinal Methanogenesis In Ruminants" issued to Hindrik Bene Perdok et al. and assigned to CAN Technologies, Inc. discloses one such method. But such method feeds ruminants the calcium nitrate and other ingredients for the purpose of hydrogen gas removal in the rumen of the ruminant.

[0003] It is also known to feed to gestating sows a product enriched in L-arginine. One such known method is described in PCT Patent Application Publication No. WO 03/009703A1 titled "Dietary Modifications To Improve Fertility" filed by Tette Van der Leande and assigned to Nutreco Netherland B.V. However, such known method is directed to feeding gestating sows the L-arginine for the purpose of improving fertility of pigs by improvement of placentation in the pig (e.g. decreasing embryonic and fetal mortality in the placenta).

[0004] Accordingly, it would be advantageous to provide a feed for an animal for increasing vasodilatation of the animal during gestation and lactation phases.

## SUMMARY

[0005] The present invention is directed to an animal feed containing a nitric oxide producing compound. In one aspect, the nitric oxide producing compound is at least one of calcium nitrite, magnesium nitrite or salts thereof. The nitric oxide producing compound may be introduced into the animal feed through a premix, for example, which can be further incorporated into the animal feed product. A supplement containing a nitric acid producing compound is also contemplated by the present invention. The present invention is further directed to a method of supplying an animal such animal feed or supplement containing the nitric oxide producing compound. In one aspect of the present invention, the animal feed or

supplement containing the nitric oxide producing compound is supplied during at least one of a gestation feed or lactation feed. In a particular aspect of the present invention, the animal feed or supplement containing the nitric oxide producing compound is supplied during at least one of a gestation feed or lactation feed through a liquid application in an amount less than 90 grams of nitric oxide producing compound per animal per day. The present invention is also directed at the use of a nitric oxide producing compound in animal feed to increase vasodilation of the placenta and the mammary glands of the animal. The present invention may also be directed to the use of a nitric oxide producing compound in animal feed to increase systemic vasodilation for the reduction of animal fatigue and farrowing time. In one aspect, the present invention involves a premix. In this aspect, the premix includes a nitrate compound and salts thereof in the amount of greater than about 50% by weight of the premix. The premix may also include vitamins and trace minerals. The premix may be formulated for use in at least one of a number of phases of swine production including the gestation or lactation phase of an animal and in an amount of less than about five percent by weight (5.0 wt%) inclusion per metric ton of the premix.

[0006] In a further aspect, the present invention involves a supplement for an animal feed for feeding during at least one of an animal's gestation phase and/or lactation phase. In this aspect, the supplement may include a nitric oxide producing compound in an amount of less than 10kg/metric ton of the total weight of the feed.

[0007] In yet another aspect, the present invention involves a method for feeding an animal. The method includes providing to the animal an animal feed product containing at least one of gestation feed and a lactation feed having a nitric oxide producing compound in an amount of less than 10kg/metric ton of the total weight of the feed. The method also includes providing the feed to the animal 110 days after gestation of the animal.

#### DETAILED DESCRIPTION

[0008] Specific details of several embodiments of the disclosure are described below with reference to an animal feed supplement. The animal feed supplement is intended for feeding to a non-ruminant animal, such as a monogastric animal, for example, a sow.

#### Swine Production Stages

[0009] Breeding and Gestation. Swine production can be logically separated into a number of phases, beginning with the sow being bred. The sow is bred during her estrous

period. In the phase between weaning the sow and breeding, a special breeding feed can be fed to the sow. After breeding, the sow "gestates" her litter for 113 to 116 days before the piglets are born or "farrowed." As used in this disclosure, the term "gestation" or gestation phase means the 113 to 116 day period when the sow is pregnant from breeding until farrowing.

[00010] Farrowing. The process of giving birth is called farrowing. Sows are normally moved into a farrowing room a few days prior to farrowing and remain in this location through lactation. In certain instances, sows are moved into a farrowing room five to seven days prior to farrowing. Sows typically farrow from eight to fourteen piglets, which as a group are called a litter. It is also possible for sows to farrow from eight to eighteen piglets, which as a group are also called a litter. The piglets are born weighing about three pounds at birth. As used in this disclosure, the term "farrowing" means birth, and the term "farrowing phase" means the period from birth to weaning. The period of time called the transition period is typically seven days pre-farrowing until one to five days post farrowing. The piglets stay with the lactating sow for about 19 – 35 days after farrowing, during which time the piglets drink milk produced by the lactating sow. As used in this disclosure, the term "lactating," lactation, or lactation phase means the period when a sow is producing milk and providing the milk to her piglets and the period of time from farrowing until weaning is called the lactation period. During these periods a farm can provide one feed (typically called a lactation feed) or two feeds (typically a transition feed and a lactation feed).

[00011] Weaning. The piglets are weaned from the sow at anywhere from five days to five weeks, with most operations weaning pigs at two to four weeks after farrowing. As used in this disclosure, the term "weaning" means the process of removing the piglets from the sow and moving them to the nursery. During a weaning or nursery phase, the piglets remain in the nursery (est. until 42 days after farrowing).

[00012] Growing and Finishing. Pigs are normally removed from the nursery after the weaning (or nursery) phase and placed in a grow-finishing building until they reach market weight. Swine Production Diets

[00013] During the gestation phase, the sow is typically fed a gestation diet. A gestation diet is characterized primarily by its time of feeding. The gestation diet is provided in fixed daily amounts to achieve targeted nutrient intakes. See TABLE 1. On about day 110 of the gestation phase, or when the sow is moved into farrowing, the sow is typically switched to a fixed amount of lactation feed. A lactation feed is characterized primarily by the time of feeding and contains the appropriate nutrients and energy to support milk production. See TABLE 1.

During the lactation phase, the sow is fed a lactation feed ad libitum or in fixed daily amounts. After the lactation phase, the sow is separated from its piglets (the weaning phase for the piglets) and the sow enters rebreeding.

[00014] During the gestation phase, the fetus receives nutrients from the placenta of the sow. During the lactation phase, the piglets receive nutrients from the milk produced by the sow. During the nursery phase, the piglets are fed a nursery diet. A weaning diet is characterized by the time of feeding, which begins at the time of weaning and end at approximately 25-35 kg body weight of the piglet.

#### Animal Feed Supplement

[00015] The monogastric animal is fed a supplement that is included in an animal feed during at least one of the gestation and lactation phases according to an exemplary embodiment. For instance, the monogastric animal is fed a supplement that is included in at least one of a breeding feed or transition feed. The animal feed supplement of the present invention comprises a nitrate compound, typically a physiologically acceptable or tolerated nitrate compound. The nitrate compound has sufficient solubility in water according to a preferred embodiment. The nitrate compound is an ionic nitrate compound according to preferred embodiments, most preferably an inorganic nitrate salt. Exemplary salts include sodium nitrate, potassium nitrate, calcium nitrate, or ammonium nitrate, all of which are readily soluble in water at standard temperature and pressure. In another aspect, exemplary salts include sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, ammonium nitrate, or a combination thereof, all of which are readily soluble in water at standard temperature and pressure. In a particular aspect, the salt is a magnesium nitrate. The salts can include different hydrated forms. The salts can also include double salts (e.g. calcium nitrate and ammonium nitrate).

[00016] According to an exemplary embodiment, the nitrate is provided as inorganic calcium nitrate having the formula  $\text{Ca}(\text{NO}_3)_2$ . Calcium nitrate is also referred to as calcium dinitrate, Kalksalpeter, nitrocalcite, Norwegian saltpeter, and lime nitrate. Calcium nitrate may be produced by treating limestone with nitric acid, followed by neutralization with ammonia according to the reaction:  $\text{CaCO}_3 + 2\text{HNO}_3 \rightarrow \text{Ca}(\text{NO}_3)_2 + \text{CO}_2 + \text{H}_2\text{O}$ . In another exemplary embodiment, the nitrate is provided as an inorganic salt of magnesium nitrate hexahydrate having the formula  $(\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O})$ . This product contains 10.8% N from nitrate and 9.5% Magnesium.

[00017] A variety of related complex inorganic salts of calcium nitrate include calcium ammonium nitrate decahydrate and calcium potassium nitrate decahydrate. Calcium ammonium nitrate is a double salt (calcium nitrate and ammonium nitrate) having the formula  $5\text{Ca}(\text{NO}_3)_2 \cdot \text{NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$ . According to an exemplary embodiment, the calcium ammonium nitrate is pentacalcium ammonium nitrate decahydrate commercially available from Bri-Chem Supply Limited with the following specification: Ammonium-N ( $\text{NH}_4\text{-N}$ ) 1.1%; Nitrate-N ( $\text{NO}_3\text{-N}$ ): 14.4%; Total N: 15.5%; Calcium (Ca): 18.8%. According to another exemplary embodiment, the calcium nitrate is BOLIFOR CNF calcium nitrate feed grade having the formula  $5\text{Ca}(\text{NO}_3)_2 \cdot \text{NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$  commercially available from Yara Phosphates Oy of Helsingborg Sweden. According to an exemplary embodiment, the calcium nitrate can have the following specification: Calcium (Ca): 18.9%; Nitrogen (N) 15.5%; pH (10% solution): 6; bulk density  $\text{kg/m}^3$ : 1050; appearance: prilled; size: <1.0mm: 2%; 1.0-2.0mm: 78%; >2mm: 20%. Exemplary formulations of calcium nitrate lacking ammonia include  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ . An exemplary anhydrous air-stable derivative of calcium nitrate includes the urea complex  $\text{Ca}(\text{NO}_3)_2 \cdot 4[\text{OC}(\text{NH}_2)_2]$ .

[00018] The nitrate may be provided by a variety of plant ingredients according to alternative embodiments. Such plant ingredients may include, for example, leafy greens such as spinach, arugula and beetroot. Beetroot has an inorganic nitrate content typically ranging from 110 to 3670 mg nitrate/kg.

[00019] The supplement including the nitrate compound may be fed to the sow such that the amount of nitrate fed per sow per day is from 90 grams to less than 0.5 grams. In a particular aspect, the supplement including the nitrate compound may be fed to the sow such that the amount of nitrate fed per sow per day is less than 10 g nitrate per sow per day, also for example, less than 5g nitrate per sow per day, also for example, less than 4g nitrate per sow per day, also for example, less than 3g nitrate per sow per day, also for example, less than 2g nitrate per sow per day also for example, less than 1g nitrate per sow per day, also for example, less than 0.5g nitrate per sow per day according to suitable embodiments.

[00020] The supplement including the nitrate compound may also be fed to the sow such that the amount of nitrate fed per sow per day is from 90 mg nitrate per kg of body weight of the sow to less than 5 mg nitrate per kg of body weight of the sow. In an exemplary aspect, the supplement including the nitrate compound may also be fed to the sow such that the amount of nitrate fed per sow per day is less than 35 mg nitrate per kg of body weight of the sow, also for example, less than 30 mg nitrate per kg of body weight of the sow, also for example, less than

25 mg nitrate per kg of body weight of the sow, also for example, less than 20 mg nitrate per kg of body weight of the sow, also for example, less than 15 mg nitrate per kg of body weight of the sow, also for example, less than 10 mg nitrate per kg of body weight of the sow, also for example, less than 5 mg nitrate per kg of body weight of the sow according to suitable embodiments. The amount of nitrate in the feed (and the amount of nitrite) may be measured via ion chromatography. According to the ion chromatography method, samples are extracted with water, filtered, diluted and then applied to an anion exchange column. Nitrate is separated and identified using isocratic carbonate/bicarbonate elution coupled with suppressed conductivity detection. Concentration is determined using a standard curve of known nitrate solutions.

[00021] The supplement including the nitrate compound may be included in a complete animal feed. The amount of calcium nitrate and its related salts in the complete feed is less than 1 wt% of the total weight of the feed, for example less than 0.5 wt%, also for example less than 0.4 wt%, also for example less than 0.3 wt%, also for example less than 0.2 wt%, also for example less than 0.1 wt%, also for example less than 0.09 wt%, also for example less than 0.08 wt%, also for example less than 0.07 wt%, also for example less than 0.06 wt%, also for example less than 0.05 wt% according to suitable embodiments.

[00022] The supplement comprising the nitrate compound may be included in a premix feed. In certain aspects, the premix feed is then added to an animal feed and provided to the animal (e.g. sow) such that the amount of nitrate fed per animal per day is from 90 mg nitrate per kg of body weight of the sow to less than 5 mg nitrate per kg of body weight of the animal. The amount of calcium nitrate and its related salts in the premix feed is greater than 50 wt% of the total weight of the feed, for example, greater than 60 wt%, also for example, greater than 70 wt%, also for example, greater than 80 wt%, also for example, greater than 90 wt%, also for example, greater than 95 wt% according to suitable embodiments.

#### Nitrate-Nitrite-NO Pathway

[00023] Without intending to be limited to any particular theory, it is believed the nitrate in the supplement is a source for the biological messenger nitric oxide (NO) according to a non-enzymatic pathway for the generation of NO (nitrate-nitrite-NO pathway). Unlike arginine conversion to nitric oxide, nitrate conversion to nitric oxide via the nitrate-nitrite-NO pathway is not dependent upon oxygen levels. During the farrowing process, sows may experience fatigue and periods of hypoxia or lower oxygen levels.

[00024] It is believed the release of NO in the animal is important for the control of vascular tone, smooth muscle growth, platelet aggregation and inflammation. The release of NO in the animal, for example, in a sow fed the supplement, is believed to induce vasodilation and increased blood flow and exchange of oxygen.

[00025] In one aspect of the present invention is a method of supplying an animal (e.g. a sow) a NO producing compound in at least one of a gestation or lactation feed through a liquid application such as drinking water. An exemplary dosage using this method is less than 90 grams of NO producing compound per animal per day.

#### Vasodilation

[00026] The term “vasodilation” (or vasodilatation) as used in this disclosure refers to the widening of blood vessels in the animal. Vasodilation results from relaxation of smooth muscle cells within the vessel walls, in particular in the large veins, large arteries, and smaller arterioles. When blood vessels dilate in the animal, the flow of blood is increased due to a decrease in vascular resistance. Vasodilation may be localized to a specific organ (depending on the metabolic needs of a particular tissue, as during stress), or it may be systemic (seen throughout the entire systemic circulation). The primary function of vasodilation is to increase blood flow in the body to tissues that need it most. This is often in response to a localized need of oxygen, but can occur when the tissue in question is not receiving enough glucose or lipids or other nutrients.

[00027] Without intending to be limited to any particular theory, it is believed the nitrate in the supplement produces NO when consumed by the animal, which causes vasodilation in specific organs of the animal during certain periods. For example, the animal feed supplement fed during gestation may result in vasodilation in the placenta -- thereby increasing the exchange of oxygen and nutrients between the placenta and the fetus and further thereby decreasing the number of stillbirths. Also for example, the supplement fed during gestation through lactation may result in vasodilation of the placenta of the sow -- thereby reducing stillborn births during farrowing. Also for example, the supplement fed during the gestation phase through the lactation phase may result in improved nutrient and oxygen delivery throughout the entire systemic circulation -- thereby reducing stillborn births due to less sow fatigue and reducing farrowing time. Also for example, the supplement fed during the lactation phase may result in vasodilation of the mammary glands of the sow -- thereby increasing milk production and further thereby reducing piglet mortality and increasing piglet vitality during the weaning phase.

### Benefits of Supplement

[00028] Feeding the supplement during at least one of the gestation and lactation phases of swine production may result in certain physiological benefits to the sow and/or her offspring compared with not feeding the supplement. For example, a sow fed the supplement during the pre-farrowing phase may result in fewer stillborn piglets being born. Also for example, a sow fed the supplement during at least one of the lactation phase or weaning phase may result in piglets showing greater vitality and reduced mortality.

[00029] The number of total born piglets in a litter is determined during ovulation, insemination, and early gestation. The number of piglets born alive is determined by: (i) mortality in utero (mortality in early gestation results in fewer total born piglets while mortality in late gestation can result in an increased number of piglets born mummified); and (ii) mortality during the farrowing process (stillborn). Stillbirth can occur during gestation (Type I stillbirth), which is often due to an infectious cause, or intra-partum (Type II stillbirths), which is often non-infectious.

[00030] Total born piglets. Feeding the supplement including the nitrate compound to the animal (e.g. sow) during the gestation phase may promote embryo survival during implantation. As a result, an increase may be observed in the total number of offspring (e.g. piglets) born due to increased embryo survival during implantation. In sows, attachment of the placental membranes of the conceptus to the endometrium takes place 12 to 13 days after ovulation (initial attachment occurs at day 12 and is well established at day 18-20 after ovulation). After initial placental development, two important processes in creating a functional placenta are angiogenesis and vasculogenesis. These processes are essential for the embryo to take up nutrients such as amino acids, glucose and oxygen from the maternal bloodstream and to lose waste products, such as CO<sub>2</sub>. Angiogenesis is the expansion of blood vessels from pre-existing vessels in the endometrium of the mother, while vasculogenesis is the formation of new blood vessels in the fetal membranes. When vascularization is not developed properly, problems can occur, one of them being placental insufficiency. Placental insufficiency is caused by a poor placentation (a poor vascular development at the utero-placental surface) and leads to a degenerating placental functioning and a decrease in exchange of oxygen and nutrients from placenta to fetus. As a result, the fetus will have a shortage of oxygen, or hypoxemia. Without intending to be limited to any particular theory, it is believed feeding the animal feed supplement to the sow during the gestation phase causes vasodilation to increase blood flow to the placenta during early gestation resulting in improved embryonic survival and implantation,

further resulting in an increased number of total live born piglets and further having improved uniformity, birth weight and vitality. According to an exemplary embodiment, feeding the supplement to the sow during the gestation phase (including the transition phase) may result in vasodilation resulting in: (i) improved development of a functional placenta through increased angiogenesis and/or vasculogenesis; (ii) decreased placental insufficiency; and/or (iii) increased exchange of oxygen and nutrients from the placenta to the fetus.

[00031] Stillbirth. Feeding the animal feed supplement comprising the nitrate compound to the animal (e.g. sow) during the gestation phase may reduce the number of stillborn during farrowing. The major cause of stillbirths is asphyxiation. This is caused by the cumulative effects of successive contractions of the uterus, which result in reduced oxygenation of the unborn piglets. Reduced oxygenation (asphyxiation) results in piglet death in utero or results in reduced piglet vitality which is associated with a higher mortality risk during the first three days after farrowing. Asphyxiation is further complicated by the farrowing time, as increased farrowing time due to sow fatigue results in higher stillbirth piglets. Increased uterine contractions due to long farrowing times also increase the risk of damage, occlusion or rupture of the umbilical cord. Without intending to be limited to any particular theory, it is believed feeding the supplement to the sow during gestation through lactation phases causes vasodilation to increase blood flow to the placenta and therefore oxygen flow to the placenta during the farrowing process to support uterine contractions. Furthermore, systemic effects of feeding the supplement will reduce sow fatigue and reduce farrowing time. According to an exemplary embodiment, feeding supplement to the sow during the gestation through lactation phases may result in vasodilation resulting in: (i) decreased asphyxiation of the fetus; and/or (ii) reduced farrowing duration.

[00032] Reduced Piglet Post-Farrowing Mortality. Feeding the supplement including the nitrate compound to the animal (e.g. sow) during the lactation phase and/or transition phase may decrease or reduce piglet post-farrowing mortality. Without intending to be limited to any particular theory, it is believed the nitrate in the animal feed supplement produces nitric oxide which causes vasodilation of the mammary glands of the sow, thereby increasing milk production. Increased milk production by the sow results in increased uptake of colostrum and milk by the piglets in the litter during the first few days post-farrowing. Increased colostrum and milk uptake by the piglets leads to reduced mortality during the first week post-farrowing. Without intending to be limited to any particular theory, it is believed that the sow vitality can

be improved resulting in less crushing of piglets and better start of feed intake in the early lactation phase.

[00033] Increased Piglet Gain and Vitality Post-Farrowing. Feeding the animal feed supplement including the nitrate compound to the animal (e.g. sow) during the lactation phase and/or transition phase may increase the growth and vitality of the piglet post farrowing. Without intending to be limited to any particular theory, it is believed the nitrate in the supplement produces nitric oxide which causes vasodilation of the mammary glands of the sow, thereby increasing milk production. Increased milk production by the sow results in increased uptake of milk by the piglets in the litter, and a greater average daily gain over the entire suckling period. Also for example, the piglets show increased vitality as demonstrated by reduced time from birth to first time suckling. Vitality is calculated as the amount of time from birth until nursing.

[00034] Other Benefits. In addition to the benefits of vasodilation from the nitrate in the supplement, the animal may receive additional benefits. Additional benefits from the supplement may include, for example, post weaning performance of piglets in gain, feed intake, efficiency, immunity, meat quality, gut health, color/bloom, ability to cope with heat stress, ear necroses, streptococcus. Other additional benefits may include, for example, an increased effect on feed intake, efficiency, udder quality, milk production, milk quality, incidence of lameness, heat stress and hair growth.

#### Animal Feed

[00035] The supplement refers to an additive or premix that includes a nitrate compound. The supplement may be added to an animal feed. The term "animal feed" as used in this disclosure means a feed ration produced for consumption by an animal.

[00036] The animal feed supplement may be included in a compound animal feed according to an exemplary embodiment. The term "compound feed" as used in this disclosure means an animal feed blended to include two or more ingredients which assist in meeting certain daily nutritional requirements of an animal. The animal feed may be a complete animal feed according to an exemplary embodiment. The term "complete feed" as used in this disclosure means an animal feed which is a complete feed, e.g. a nutritionally balanced blend of ingredients designed as the sole ration to provide all the daily nutritional requirements of an animal to maintain life and promote production without any additional substances being consumed except for water. The animal feed may also be a concentrate animal feed according to an exemplary

embodiment. The term “concentrate feed” as used in this disclosure means an animal feed that typically includes a protein source blended with supplements or additives or vitamins, trace minerals, other micro ingredients, macro minerals, etc. to provide a part of the ration for the animal. The concentrate feed may be fed along with other ingredients (e.g., forages in ruminants). The animal feed may include a premix according to an alternative embodiment. As used in this disclosure, the term “premix” means a blend of primarily vitamins and/or minerals along with appropriate carriers in an amount of less than about five percent (5.0%) inclusion per ton of complete feed. The animal feed may also include a base mix according to an exemplary embodiment. The term “base mix” as used in this disclosure means a blend containing vitamins, trace minerals and/or other micro ingredients plus macro minerals such as calcium, phosphorus, sodium, magnesium and potassium, or vitamin or trace mineral in an amount of less than ten percent (10.0%) inclusion per ton of complete feed. The animal feed may be a feed “supplement.” The term “additive” as used in this disclosure means an ingredient such as a protein source, salt, mineral, additive, or buffer that is added to an animal feed. An example of an additive includes calcium, zinc, manganese, copper, iodine, cobalt, selenium and other trace ingredients. The supplement may be top dressed, dissolved in a liquid such as drinking water or any other consumable liquid presented to the animal, etc. according to other alternative embodiments.

[00037] The term “animals” as used in this disclosure includes ruminant and monogastric animals. As used in this disclosure, the term “ruminant” means any mammal that has a multi-compartment stomach and is associated with digestion by regurgitation and repeated chewing of a bolus or cud. Such ruminant mammals include, but are not limited to, bovine animals such as buffalo, bison, and all cattle, including calves, steers, heifers, cows, and bulls. As used in this disclosure, the term “monogastric” means any organism having a simple single-chambered stomach. Such monogastric animals include, but are not limited to, porcine, equine, caprine, ovine, avian animals, seafood (aquaculture) animals. Porcine monogastric animals include, for example, feeder pigs and breeding pigs, including piglets, sows, gilts, barrows, and boars. While the description is primarily with reference to sows, it is not limited as such and should be understood that the disclosure is applicable to other monogastric and ruminant animals.

### Nutrients

[00038] The animal feed is the vehicle to deliver nutrients to the animal. There are six major classes of nutrients: carbohydrates, fats, proteins, vitamins, minerals, and water. These

nutrient classes can be categorized as either macronutrients (needed in relatively large amounts) or micronutrients (needed in smaller quantities). The macronutrients are carbohydrates, fats, fiber, proteins, and water. The micronutrients are minerals and vitamins. The macronutrients (excluding water) provide structural material (amino acids from which proteins are built, and lipids from which cell membranes and some signaling molecules are built) and energy. Vitamins, minerals, fiber, and water do not provide energy, but are required for other reasons. Micronutrients include antioxidants and phytochemicals. Nutrients are delivered by sources of ingredients.

[00039]       Macromineral (also referred to as bulk minerals) nutrients include, for example, calcium, chlorine (as chloride ions), magnesium, phosphorus, potassium, sodium, and sulfur. Micromineral (also referred to as trace minerals) nutrients include, for example, cobalt, copper, chromium, iodine, iron, manganese, molybdenum, nickel, selenium, vanadium, and zinc.

[00040]       Vitamins nutrients include, for example, vitamin A. Ingredient sources of vitamin A include, for example, vitamin A supplement, vitamin A oil, etc.. Vitamins also include, for example, vitamin B1, vitamin B2, vitamin B3, vitamin B4, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12, and vitamin C. Vitamins also include, for example, vitamin D. Ingredient sources of vitamin D include, for example, vitamin D supplement. Vitamins also include, for example, vitamin E. Ingredient sources of vitamin E include, for example, vitamin E supplement. Vitamins also include, for example, vitamin K. Other vitamin product ingredients may include, for example, riboflavin, vitamin D3 supplement, niacin, betaine, choline chloride, tocopherol, inositol, etc.

#### Ingredients

[00041]       The animal feed may include a combination or compound of various ingredients to deliver the nutrients. Examples of ingredients include protein ingredients, grain products, grain by-products, roughage products, fats, minerals, vitamins, additives or other ingredients according to an exemplary embodiment. Protein ingredients may include, for example, animal derived proteins such as: dried blood meal, meat meal, meat and bone meal, poultry by-product meal, hydrolyzed feather meal, etc. Protein ingredients may also include, for example, marine products such as: fish meal, crab meal, shrimp meal, condensed fish solubles, fish protein concentrate, etc. Protein ingredients may further include, for example, plant products such as: algae meal, beans, coconut meal, cottonseed meal, rapeseed meal, canola meal, linseed meal, peanut meal, soybean meal, sunflower meal, peas, soy protein concentrate, dried yeast, active

dried yeast, etc. Protein ingredients may also include, for example, milk products such as: dried skim milk, condensed skim milk, dried whey, condensed whey, dried hydrolyzed whey, casein, dried whole milk, dried milk protein, dried hydrolyzed casein, etc. Grain product ingredients may include, for example, corn, milo, oats, rice, rye, wheat, etc. Grain by-product ingredients may also include, for example, corn bran, peanut skins, rice bran, brewers dried grains, distillers dried grains, distillers dried grains with solubles, corn gluten feed, corn gluten meal, corn germ meal, flour, oat groats, hominy feed, corn flour, soy flour, malt sprouts, rye middlings, wheat middlings, wheat mill run, wheat shorts, wheat red dog, feeding oat meal, etc. Grain product ingredients may also include, for example high-moisture processed grain by-products. Such high-moisture processed grain by-products result from the processing of a number of different grains such as corn, wheat, and milo. Examples of high-moisture processed grain by-products include, without limitation, gluten, non-grain feed ingredients (e.g., molasses, beet pulp and other crop residues), and wet distiller's grain. Roughage product ingredients may include, for example, corn cob fractions, barley hulls, barley mill product, malt hulls, cottonseed hulls, almond hulls, sunflower hulls, oat hulls, peanut hulls, rice mill byproduct, bagasse, soybean hulls, soybean mill feed, dried citrus pulp, dried citrus meal, dried apple pomace, dried tomato pomace, straw, hay, etc. Fat product ingredients may include, for example, beef fat, poultry fat, pork fat, restaurant grease, soy oil, corn oil, tallow, hydrolyzed animal fat, hydrolyzed vegetable fat, calcium salts of long chain fatty acids, hydrogenated glycerides, etc. Mineral product ingredients may include, for example, basic copper chloride, bone ash, bone meal, calcium acetate, calcium carbonate, calcium chloride, calcium gluconate, calcium hydroxide, calcium iodate, calcium iodobenenate, calcium oxide, calcium sulfate (anhydrous or dihydrate), cobalt acetate, cobalt carbonate, cobalt chloride, cobalt oxide, cobalt sulfate, copper carbonate, copper chloride, copper gluconate, copper hydroxide, copper orthophosphate, copper oxide, copper pyrophosphate, copper sulfate, cuprous iodide, dicalcium phosphate, diiodosalicylic acid, disodium phosphate, ethylenediamine dihydroiodide, ferrous fumarate, iron ammonium citrate, iron carbonate iron chloride, iron gluconate, iron oxide, iron phosphate, iron pyrophosphate, iron sulfate, reduced iron, magnesium acetate, magnesium carbonate, magnesium oxide, magnesium sulfate, manganese acetate, manganese carbonate, manganese chloride, manganese citrate (soluble), manganese gluconate, manganese orthophosphate, manganese oxide, manganese phosphate (dibasic), manganese sulfate, monocalcium phosphate, monosodium phosphate, dicalcium phosphate, phosphate deflourinated, rock phosphate, potassium acetate, potassium bicarbonate, potassium carbonate, potassium chloride, potassium iodate, potassium iodide,

potassium, sulfate sodium acetate, sodium chloride, sodium bicarbonate, sodium iodate, sodium iodide, sodium sulfate, sodium, sodium sesquicarbonate, selenium, sulfur, thymol iodide, tricalcium phosphate, tripolyphosphate, zinc acetate, zinc carbonate, zinc chloride, zinc oxide, zinc sulfate , etc.

[00042] Vitamin product ingredients may include, for example, vitamin A supplement, vitamin A oil, vitamin D, vitamin B12 supplement, vitamin E supplement, riboflavin, vitamin D3 supplement, niacin, betaine, choline chloride, tocopherol, inositol, etc. Additive product ingredients can be used, for example, to protect animals from disease and/or stress (e.g. antibiotics, probiotics, etc.) and/or to stimulate or control growth and behavior (e.g. hormones).

[00043] Feed additives can be used, for example, to help provide a balanced diet (e.g., vitamins and/or trace minerals), to protect the animals from disease and/or stress (e.g., antibiotics, probiotics) and/or to stimulate or control growth and behavior (e.g., hormones). Additive product ingredients may include, for example: growth promoters, medicinal substances, buffers, antioxidants, enzymes, preservatives, pellet-binding agents, direct-fed microbials, etc. Additive product ingredients may also include, for example, ionophores (e.g. monesin, lasalocid, laidlomycin, etc.),  $\beta$ -agonist (zilpaterol, ractompamine, etc.), antibiotics (e.g., chlortetracycline (CTC), oxytetracycline, bacitrain, tylosin, aureomycin), probiotics and yeast cultures, coccidiostats (e.g., amprolium, decoquinatate, lasalocid, monensin), and hormones (e.g., growth hormones or hormones that inhibit estrus and/or ovulation such as melengestrol acetate), pheromones, nutraceuticals, pharmaceuticals, flavanoids, nutritive and non-nutritive supplements, detoxicants, etc.

[00044] Examples of a feed used in gestation and lactation to which the supplement may be added is shown in TABLE 1 and TABLE 2.

TABLE 1 – Gestation and Lactation Feed

	Gestation	Lactation
PROTEIN	14.0 (wt%)	18.0 (wt%)
FAT	4.5 (wt%)	6.0 (wt%)
MOISTURE	12.0 (wt%)	12.0 (wt%)
FIBER	7.0 (wt%)	5.0 (wt%)
CALCIUM	0.8 (wt%)	1.0 (wt%)
PHOS	0.5 (wt%)	0.7 (wt%)
NET ENERGY	2000 KCAL	2200 KCAL
LYSINE	0.6 (wt%)	0.9 (wt%)

**TABLE 2 – Gestation and Lactation Feed**

	Gestation	Lactation
PROTEIN	15.0 (wt%)	24.0 (wt%)
FAT	6.0 (wt%)	6.0 (wt%)
MOISTURE	12.0 (wt%)	12.0 (wt%)
FIBER	5.0 (wt%)	3.2 (wt%)
CALCIUM	0.90 (wt%)	0.90 (wt%)
PHOS	0.70 (wt%)	0.60 (wt%)
NET ENERGY	2000 KCAL	2200 KCAL
LYSINE	0.506807 (wt%)	0.90 (wt%)

**EXAMPLE 1**

[00045] Example 1 determines the effect of calcium nitrate in the diet of a sow. The sows entered a farrowing room on average on day 111 of gestation and received a standard lactation diet (see TABLE 1A and TABLE 1B) without (negative control) or with (positive control) the 1.0 kg/metric ton BOLIFOR CNF inorganic feed material of calcium nitrate feed grade (calcium nitrate, containing 63.1% of nitrate) commercially available from Yara Phosphates Oy of Helsingborg, Sweden. During lactation, sows were fed either the positive control or negative control standard lactation diet according to a step-up feeding schedule. Until farrowing the sows were fed a fixed amount of either the positive or negative controls of the standard lactation diet twice a day. After farrowing the amount of feed either the positive or negative controls of the standard lactation diet fed was increased gradually till a maximum of 8 kg/sow per day. The amount of which could be adjusted based on the appetite of the sow. Piglets were fed Ad libitum (no milk was provided other than from the sow a commercial creep feed (meal, dry) from day 4-16 of age and a pelleted commercial prestarter from day 16 till weaning. In total, 150 sows were allocated over the three rooms based on parity. Within 2 days after farrowing, litters were standardized to 13-14 piglets per sow.

**TABLE 1A**

Ingredients	Positive Control (wt%)	Negative Control (wt%)
Bolifor (calcium nitrate)	0.1	0
Natuphos m2346 0.75%	0.75	0.75

Wheat Midds By-Product 27-34%		
NDF	17	17
Canola Meal - 00 RSM	2.5	2.5
Barley	10	10
Maize (bulk)	19.534	19.347
Wheat	17.076	17.177
Sunflower Meal 35% Protein	1.5	1.5
Soybean Hulls	1.5	1.5
Zeug Ad Lib 1%	1	1
Limestone powder	1.423	1.373
Monocalcium phosphate	0.83	0.83
Salt	0.398	0.398
L-Threonine	0.053	0.053
Soybean Meal - 48% Protein	8.405	8.412
Fat - Palm Oil	2.788	2.817
Beet Pulp - Dry	4.5	4.5
Palm Kernel Meal - Expeller	2.5	2.5
Beet - Standard Molasses	3.5	3.5
Soybean-Gr FF heated (PL FD)	4	4
Fat - Fish Oil - Red Salmon	0.5	0.5
L-Lysine HCL	0.243	0.243

TABLE 1B

Nutrients	Units	Positive Control (wt%)	Negative Control (wt%)
Dry matter	%	88.446	88.358
Moisture	%	11.554	11.642
Protein	%	15.846	15.846
Fat	%	6.315	6.338
Ash	%	6.124	6.074
Fiber	%	5.467	5.465
Calcium	%	0.857	0.838
Phosphorus	%	0.625	0.624
Net energy	kcal	2450	2450
Lysine	%	0.906	0.906

[00046] The following measurements were recorded as shown in TABLE 1C: (i) sow weight and backfat thickness when entering the farrowing room (day 111 of gestation) and at weaning (22 days post farrowing); (ii) sow reproductive performance was recorded right after

farrowing (number of piglets born total, alive, stillborn, mummified); (iii) piglets weights were recorded at birth, day 5 after birth and at weaning; (iv) creep feed intake of piglets between day 4 and weaning was recorded; (v) number of piglets alive at day 4 and at weaning were registered; (vi) when piglets died, the reason for dying was also registered; and (vii) interventions during the birth process (e.g. the use of planate, oxytocin and birth assistance).

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TABLE 1C

Lactation (day 111-Weaning)	1 Negative Control	2 NC+0.1% Bolifor CNF <sup>1</sup>	Pooled SEM	P Trt
Number of sows	120	114	-	-
Parity	3.5	3.6	-	-
<i>Sow technical performance</i>				
Average day of gestation when entering farrowing room <sup>2</sup>	111	111	-	-
Average gestation length (days) <sup>2</sup>	116	116	-	-
Weight when entering the farrowing room (kg) <sup>2</sup>	276.5	275.8	-	-
Calculated weight at farrowing (kg)	258.9	257.9	9.6	0.69
Weight at weaning (kg) <sup>3</sup>	243.9	243.1	10.2	0.72
Weight change from farrowing – weaning (%) <sup>3</sup>	7.2	7.1	1.1	0.77
Backfat when entering the farrowing room (mm) <sup>2</sup>	22.5	22.3	-	-
Backfat at weaning (mm) <sup>3</sup>	19.7	19.6	0.7	0.69
Backfat change during lactation (%) <sup>3</sup>	14.0	14.0	2.3	0.95
Total feed intake during lactation (kg) <sup>4</sup>	133.6	133.2	6.3	0.84
ADFI during lactation (kg/sow/day) <sup>4</sup>	6.0	6.0	0.2	0.91
<i>Sow reproductive performance</i>				
Lactation length (days) <sup>2</sup>	21.6	21.7	-	-
Total Born <sup>2</sup>	16.5	16.3	-	-
Total Born Alive <sup>2</sup>	15.5	15.3	-	-
Total Still Born <sup>2</sup>	1.1	1.0	-	-
Probability Born Alive (%) <sup>8</sup>	93.3	93.8	1.4	0.51
Probability Still Born (%) <sup>8</sup>	6.7	6.2	1.4	0.51
Probability Born Mummified (%)	2.3	2.3	0.6	0.99
Total number of piglets at the sow after cross fostering <sup>2</sup>	13.5	13.5	-	-
Total Weaned <sup>2</sup>	13.2	13.1	-	-
<i>Piglet weights</i>				
Litter weight Pre fostering (kg)	20.2	19.8	0.65	0.34
Average birth weight (kg)	1.38	1.36	0.04	0.29
Litter weight post fostering (kg) <sup>5</sup>	30.1	30.8	1.26	0.33
Average weight post fostering (kg)	2.26	2.30	0.09	0.35
Litter weight at weaning (kg) <sup>6</sup>	81.1	82.0	3.29	0.48
Average weight at weaning (kg) <sup>6</sup>	6.62	6.71	0.26	0.38
ADG 0-5 (g/day) <sup>6</sup>	154	157	16.31	0.62
ADG 5 - weaning (g/day) <sup>7</sup>	245.9	245.3	10.75	0.88
ADFI day 5-16 of age (grams / piglet / day)	6.4	6.8	0.58	0.07
ADFI day 16-23 of age (grams / piglet / day)	20.3	20.6	2.49	0.88
ADFI day 5-23 of age (grams / piglet / day)	12.3	12.3	1.29	0.96
<i>Piglet mortality</i>				
Probability of piglets alive at day 4-5 (%) <sup>8</sup>	92.8	92.5	0.9	0.73
Probability of piglet mortality from cross fostering (day 4-5)-weaning (%) <sup>8</sup>	1.9	2.0	0.6	0.86
Probability of piglet Livability <sup>7,8</sup>	89.5	89.8	1.8	0.81

<sup>1</sup>Bolifor CNF is a calciumnitrate source (5Ca(NO<sub>3</sub>)<sub>2</sub>.NH<sub>4</sub>NO<sub>3</sub>.10H<sub>2</sub>O) containing 18.9% of Calcium and 63.1% of Nitrate.

<sup>2</sup>Numbers shown are means

<sup>3</sup>Numbers are corrected for lactation length and number of piglets weaned

<sup>4</sup>Sows were fed restricted. Feed intake reported is from farrowing till weaning.

<sup>5</sup>Is a calculated and not a measured value

<sup>6</sup>Are corrected for lactation length

<sup>7</sup>Piglet livability per sow is calculated by; 1-((number of stillborn + Number of piglets that died pre weaning)/total pigs after cross fostering))

<sup>8</sup>Induction of sows used as a covariable

[00047] As generally shown in TABLE 1C, the addition of 0.1% calcium nitrate to the diet resulted in: (i) a numerically higher probability of born alive; (ii) a numerically lower probability of still born; (iii) a numerically higher probability of piglet livability; and (iv) a numerically higher litter weight at weaning.

EXAMPLE 2

[00048] Example 2 determined the effect of calcium nitrate in the diet of a sow. 600 sows and all sows were fed two diets over the test period. The first diet (phase 1) was fed from approximately 5 days pre-farrowing until 5 days post-farrowing. The second diet (phase 2) was fed from day 5 post-farrowing until weaning. For the first 138 days both phase 1 and 2 diets did not contain any test product (i.e. negative control). For the subsequent 90 days, all sows were fed phase 1 diets that contained 1.0 kg/metric ton BOLIFOR CNF inorganic feed material of calcium nitrate feed grade (calcium nitrate, containing 63.1% of nitrate) commercially available from Yara Phosphates Oy of Helsingborg, Sweden (i.e. positive control). For the final 34 days both phase 1 and 2 diets did not contain any test product (i.e. negative control).

[00049] The following measurements were recorded as shown in TABLE 2A: (i) gestation length; (ii) sow reproductive performance was recorded right after farrowing (number of piglets born total, alive, stillborn); (iii) piglet; (iv) total piglets weaned; (v) weaning weights; and (vi) piglets per sow per year; and (v) .

TABLE 2A

	No calcium nitrate	0.1% Calcium nitrate	No calcium nitrate
	01-01-2014 till 18-05-2014	19-05-2014 till 16-08-2014	17-08-2014 till 20-9-2014
# Days	138	90	34
Pregnancy rate (%)	92.6	94.5	98.1
Gestation days	118.2	118.2	117.7
# Farrowings	539	382	156
Weaning age	28.0	27.2	26.9
Total Born	18.0	17.3	18.2
Born Alive	16.0	15.8	16.3

Still Born	2.0	1.5	2.0
Mortality (%)	13.0	10.5	8.8
Piglets Weaned	13.9	14.3	14.0
Weaning weight (kg)	6.7	6.7	
Piglets/sow/year	31.9	34.1	33.2
Piglet Livability (%)	77.2	82.7	81.9

[00050] As generally shown in TABLE 2A, feeding calcium nitrate resulted in: (i) a decrease in the number of stillborn; (ii) an increase in piglets weaned; and (iii) an increase in piglet livability.

### EXAMPLE 3

[00051] Example 3 is directed to the effect of calcium nitrate in lactation feed on reproduction performance of sows, number of stillborn piglets, placental blood flow and viability of piglets; demonstrating the lower probability on stillbirth of piglets and a higher probability of live born piglets.

### Material and Methods

[00052] A field trial was conducted on a commercial farm in Denmark with 650 highly productive Danbred sows (Landrace x Yorkshire) inseminated with a pure line Duroc boar, between November 17, 2014 and January 16, 2015. A total of 139 sows (total number before analyzes) were included in the trial, divided over 3 subsequent sow groups farrowing over a time span of 6 weeks. For all analyzes SAS software (version 9.3, SAS Institute Inc., Cary, NC) was used. Sows received a dry meal transition feed (from gestation to lactation), fed from day 111 of gestation till day 5 of lactation, and were allocated to one of the two treatment groups based on parity. Sows in the control group received the transition feed without an addition of calcium nitrate ( $5\text{Ca}(\text{NO}_3)_2 \cdot \text{NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$ , Bolifor CNF<sup>®</sup> with 63.1% nitrate, Yara Animal Nutrition, Oslo, Norway) of 0.1% inclusion in final feed. The total amount of calcium was equal between both experimental treatments. Number born alive (NBA), stillborn and mummies, litter birth weight, as well as back fat of the sows before farrowing (day 110 of gestation) and at weaning (day 24 of lactation) were measured. Litter weights at 24 hours and 3 days after birth were measured as well. On day 3 after birth blood samples were taken from 6 piglets (2 biggest, 2

medium and 2 smallest) from 65 litters of their tails when they were cut and analyzed on immunoglobulin G (IgG) concentration. Placentas of the sows were collected after the farrowing process was finished, stored in freezers and afterwards scored on redness for an indication of the vascularization of these placentas.

## Results

[00053] Absolute number of stillborn piglets was significantly lower in litters of sows receiving the 0.1% Bolifor CNF<sup>®</sup> compared to the control sows (1.32 vs. 1.79 respectively,  $p < 0.05$ ) and lower for parity 1 & 2 sows ( $p < 0.05$ ) compared to the control sows. An interaction between diet and parity is seen ( $p = 0.077$ ) for NBD. A trend can be seen for higher parities ( $>4$ ), where addition of Bolifor CNF<sup>®</sup> shows a higher reduction of NBD for the highest parity group compared to the same parity group in the control group (2.51 vs. 1.21 for control and treatment group respectively). NBA was significantly higher in litters of sows receiving the 0.1% Bolifor CNF<sup>®</sup> compared to the control sows (16.84 vs. 16.34 respectively,  $p < 0.05$ ) and higher for parity 1 & 2 sows (17.14 vs. 16.36 and 16.27 for parity 1 & 2, parity 3 & 4 and parity  $>4$  respectively). Birth weight ( $p = 0.291$ ), back fat loss ( $p = 0.748$ ) and TNB ( $p = 0.139$ ) did not differ between both diets. Weights and growth were higher in litters of sows receiving the 0.1% Bolifor CNF<sup>®</sup> compared to the control sows, however, litter start- ( $p = 0.543$ ), litter 24 hour- ( $p = 0.489$ ), and litter 3 days weight ( $p = 0.404$ ) did not differ between diets as did average piglet start- ( $p = 0.803$ ), 24 hour- ( $p = 0.549$ ) and 3 days weight ( $p = 0.478$ ). The same applies to growth where no differences were found between litters of sows receiving the 0.1% Bolifor CNF<sup>®</sup> compared to the control sows for litter growth from start to 24 hour weight ( $p = 0.542$ ), 24 hour to 3 days weight ( $p = 0.342$ ) and start to 3 days weight ( $p = 0.309$ ) as for average piglet growth from start to 24 hour weight ( $p = 0.675$ ), 24 hour to 3 days weight ( $p = 0.859$ ) and start to 3 days weight ( $p = 0.683$ ). No differences were found for calculated colostrum intake (CI) between diets ( $p = 0.409$ ) and parities ( $p = 0.335$ ). Mortality did not differ between sows receiving the 0.1% Bolifor CNF<sup>®</sup> and the control group ( $p = 0.630$ ) and no differences for mortality were found for parity ( $p = 0.212$ ). Standard deviation ( $p = 0.897$ ) and coefficient of variation within litters ( $p = 0.632$ ) of piglet serum IgG concentration did not differ between diets and parity ( $p = 0.919$  and  $p = 0.679$  respectively).

[00054] Supplementation of dietary nitrate decreased NBD and increased NBA. No differences were found on litter weights, growth, CI and IgG concentration. Earlier feeding or .

for a longer period may influence placental vascularity and therefore fetal development and piglet viability.

Table 3a. LS Means  $\pm$  SEM and p-values of diet for different traits

	Control	Control + 0.1% Bolifor CNF <sup>®</sup>	P-value
Number of sows	65	58	
BF* Farrowing	15.05 $\pm$ 0.74	15.44 $\pm$ 0.73	
BF Weaning	12.44 $\pm$ 0.25	12.34 $\pm$ 0.25	
Lactation Days	22.88 $\pm$ 1.08	23.19 $\pm$ 1.08	
Average parity	3.37 $\pm$ 1.49	3.45 $\pm$ 1.45	
Gestation days	117.40 $\pm$ 0.154	117.81 $\pm$ 0.152	0.062
TNB*	18.87 $\pm$ 0.464	17.90 $\pm$ 0.460	0.139
NBA*	16.34 $\pm$ 0.172	16.84 $\pm$ 0.170	<b>0.039</b>
NBD* <sup>1</sup>	1.79 $\pm$ 0.006	1.32 $\pm$ 0.006	<b>0.043</b>
Birth weight (kg)	21.15 $\pm$ 0.617	21.70 $\pm$ 0.614	0.291
BF Loss (mm)	2.71 $\pm$ 0.253	2.80 $\pm$ 0.241	0.748

\* BF, Back Fat; TNB, Total Number Born; NBA, Number Born Alive; NBD, Number Born Death. <sup>1</sup>Log transformed parameter, estimates are transformed back to absolute values.

Table 3b. LS Means  $\pm$  SEM and p-values of diet for different traits

	Control	Control + 0.1% Bolifor CNF <sup>®</sup>	P-value
Number of sows	60	60	
<b>Number of piglets<sup>1</sup></b>			
Start	13.10	12.65	
24 hour	13.10	12.65	
3 days	13.10	12.65	
<b>Litter weight (kg)</b>			
Start	17.05 $\pm$ 0.579	16.70 $\pm$ 0.572	0.543
24 hour	18.04 $\pm$ 0.155	18.14 $\pm$ 0.153	0.489
3 days	19.78 $\pm$ 0.228	19.93 $\pm$ 0.226	0.404
<b>Piglet weight (kg)</b>			
Start	1.31 $\pm$ 0.046	1.32 $\pm$ 0.046	0.803
24 hour	1.40 $\pm$ 0.012	1.41 $\pm$ 0.012	0.549
3 days	1.54 $\pm$ 0.018	1.55 $\pm$ 0.018	0.478

<b>Litter growth (kg/day)</b>			
Start to 24 hour	1.12 ± 0.163	1.21 ± 0.161	0.542
24 hour to 3 days	1.69 ± 0.141	1.80 ± 0.140	0.342
Start to 3 days	2.83 ± 0.236	3.03 ± 0.234	0.309
<b>Piglet growth (kg/day)</b>			
Start to 24 hour	0.087 ± 0.013	0.093 ± 0.013	0.600
24 hour to 3 days	0.132 ± 0.011	0.140 ± 0.011	0.373
Start 3 days	0.220 ± 0.018	0.235 ± 0.018	0.353
<b>Colostrum (g)</b>			
CI*	2688.30 ± 271.59	2858.60 ± 269.68	0.409
CI per piglet	207.60 ± 21.44	222.02 ± 21.29	0.377
Mortality <sup>2</sup>	1.72 ± 0.013	1.87 ± 0.012	0.630

\*CI, Colostrum Intake. <sup>1</sup>Means, numbers and weights are corrected for mortality and cross fostering. <sup>2</sup>Log transformed parameter, estimates are transformed back to absolute values.

Table 3c. LS Means ± SEM and p-values of diet for immunoglobulin G (IgG) concentrations in piglet serum (mg IgG/ml)

	Control	Control + 0.1% Bolifor CNF®	P-value
Number of sows	60	60	
IgG Average	85.57 ± 19.53	80.29 ± 19.66	0.564
IgG SD*	28.90 ± 4.13	28.37 ± 4.26	0.897
IgG CV*	0.39 ± 0.13	0.42 ± 0.13	0.632

\*SD, Standard deviation; CV, Coefficient of variation.

#### EXAMPLE 4

[00055] Example 4 is directed to the effect of nitrate in the diet of a sow.

#### Materials and Methods

[00056] A trial was conducted at the Sterksel Research farm in the Netherlands (part of Wageningen University Research). A total of 207 sows were used in the trial allocated based on parity over 6 treatments (n=34). The sows entered the farrowing room day 107 of gestation and received from that moment onward one of the 6 treatments: being a standard lactation diet

containing respectively 0.0% (negative control), 0.03%, 0.06%, 0.09%, 0.12% and 0.15% nitrate (mixing in the feed as calcium nitrate containing 63.1% nitrate) until 5 days after farrowing.

Calcium levels were the same in all diets.

[00057] Until farrowing, sows received a fixed amount of feed (3.25 kg from day 107-113 and 2.7 kg/sow/day from day 113 till farrowing). During lactation the sows were fed the diets according to a step-up feeding schedule applied (increasing with 0.5 kg/sow/day) the same over the 6 treatments aiming at a peak intake of 7.5 kg/sow/day. Piglets were weaned from the sow at 27 days of age. Performance of sows (e.g. weight, backfat, piglets born alive, stillborn piglets, mortality of piglets) and piglets (e.g. weight at birth, after 48 hours and weaning) was measured on all sows and litters. A subset of sows (19-20 per treatment) was examined more in detail to get insight in the possible mode of action of nitrate. On this subset, blood samples were collected within 4 minutes after birth from 2 piglets out of every 4 piglets born. Blood gasses of umbilical cord blood were analyzed using the i-STAT handheld (Abbott). Vitality of piglets was scored within 30 seconds after birth according to Baxter et al. (2008) with 1 being the lowest and 4 being the highest score for vitality.

Results:

Table 4(a): Sow body condition performance

Level of Nitrate (%)	0	0.03	0.06	0.09	0.12	0.15	
Variable	Treat1	Treat2	Treat3	Treat4	Treat5	Treat6	P value Linear effect
<i>Nr of replicates</i>	35	32	35	36	34	35	-
<i>Parity</i>	3.8	4.0	4.2	3.9	3.9	4.1	-
<i>Days on feed before farrowing</i>	7.7	7.4	7.2	7.3	7.9	7.5	-
Backfat at day 107 of gestation (mm)	15.8	15.1	16.1	16.3	15.7	17.5	-
body weight after farrowing (kg)	241.3	241.6	240.7	240.8	240.0	240.0	0.0670
Suckling days	26.5	26.8	26.9	26.9	26.3	26.5	
Body weight at weaning (kg)	232.0	228.5	228.1	226.6	224.8	227.5	0.0636
Backfat at weaning (mm)	12.8	12.8	12.6	12.2	12.7	12.5	0.2952

Body weight change during lactation	-10.0	-13.4	-12.8	-15.0	-15.4	-13.3	0.1874
%weight change farrow – wean	-3.5	-5.4	-5.0	-5.9	-6.3	-4.8	0.2472
Backfat change during lactation	-2.8	-2.8	-3.0	-3.4	-2.9	-3.1	0.2952
%backfat change	-17.7	-17.0	-19.4	-21.6	-18.2	-19.3	0.3351

[00058] No clear statistical differences were found in these parameters. While there was a significant linear effect of nitrate on body weight at farrowing the low net change (1.8 kg difference across the nitrate range) is considered biologically insignificant. The trend in body weight at weaning could possibly be explained by higher total litter gain (see table 3) which would be associated with more milk production.

Table 4b: sow reproductive performance

Level of Nitrate (%)	0	0.03	0.06	0.09	0.12	0.15	
Variable	Treat1	Treat2	Treat3	Treat4	Treat5	Treat6	P value Linear effect
Total born	16.3	16.8	16.8	16.1	17.1	16.6	-
Total born alive	15.1	15.2	15.7	14.9	15.8	15.3	-
Total still born	1.2	1.6	1.1	1.2	1.3	1.3	-
Total born mummified	0.3	0.6	0.7	0.5	0.5	0.3	-
Total nr. of piglets after cross fostering	14.7	15.0	14.8	14.4	14.7	14.8	-
Total weaned	12.3	12.3	12.4	12.7	13.0	12.6	-
Average mortality %	17.0	16.5	16.2	12.7	14.0	14.8	-
Average livability %	77.2	76.1	78.3	81.3	80.0	79.0	-
Probability born alive	0.96	0.95	0.97	0.96	0.96	0.96	0.8310
Probability weaned	0.91	0.91	0.91	0.94	0.93	0.92	0.0642
Probability mortality	0.10	0.10	0.10	0.07	0.08	0.09	0.1169
Probability livability	0.86	0.85	0.86	0.89	0.88	0.87	0.1322

[00059] Because of the non-normal distribution these parameters were statistically analyzed using probabilities.

[00060] Higher nitrate levels tended to lower mortality and increase livability, resulting in more weaned piglets per litter. Livability is defined as piglets weaned from total born corrected for cross fostering. Probability of weaned piglets tended ( $P < 0.10$ ) is improved with increasing nitrate inclusion.

Table 4c: Piglet technical performance

Level of Nitrate (%)	0	0.03	0.06	0.09	0.12	0.15	
Variable	Treat1	Treat2	Treat3	Treat4	Treat5	Treat6	P value Linear effect
Aver. birth weight born alive pigs (grams)	1262	1308	1320	1329	1326	1355	0.0228
total birth weight litter life born piglets (kg)	19.6	19.5	20.3	20.2	20.8	21.0	0.0341
Average piglet weight after 48 hours (kg)	1.582	1.611	1.598	1.621	1.640	1.692	0.0126
Probability of a higher Vitality score	0.75	0.70	0.74	0.80	0.78	0.79	0.0042
Average weight at weaning (kg)	7.431	7.439	7.321	7.472	7.550	7.608	0.1971
total litter weight weaning (kg)	92.2	91.8	91.8	96.9	98.6	96.8	0.0203

[00061] Increasing nitrate levels resulted in higher average piglet birth weight, total litter birth weight and average piglet weight at 48 h ( $P < 0.05$ ). Vitality score of the piglets is significantly ( $P < 0.05$ ) improved on increasing nitrate addition in the feed. Total litter weight at weaning is also significantly ( $P < 0.05$ ) improved. Increased weight at weaning suggests higher milk production

Table 4d: blood parameters umbilical cord

Variable	Treat1	Treat2	Treat3	Treat4	Treat5	Treat6	Linear effect
Level of Nitrate (%)	0	0.03	0.06	0.09	0.12	0.15	
<i>Nr of replicates</i>	20	19	20	20	19	19	-
<i>Parity</i>	4.1	4.1	4.0	4.0	3.8	4.1	-

<i>Days on feed before farrowing</i>	7.3	7.8	7.3	6.9	7.9	7.5	-
<b>Blood values umbilical cord piglets</b>							
pH	7.5	7.5	7.5	7.5	7.5	7.5	0.8473
pCO <sub>2</sub> (mmHg) - Measured value	33.3	34.1	34.5	34.9	34.5	34.8	0.4116
pO <sub>2</sub> (mmHg) - Measured value	31.1	33.8	35.4	34.5	35.6	35.3	0.0305

[00062] From these parameters, pO<sub>2</sub> values were linearly improved by increasing nitrate addition. This higher amount of oxygen in the blood of piglets direct after birth is indicating less asphyxia during the birth process which could explain better vitality score shown in table 3 and a better vitality score could be the explanation that piglets grow faster during the rest of the suckling period because they suckle more vigorously.

[00063] The inclusion of nitrate in lactation feed for sows improves piglet's livability and enhances their body weight development. The hypotheses that nitrate in feed (via nitrite pathway) can lead to better oxygen supply is confirmed by the blood pO<sub>2</sub> as measured in the umbilical cord of the piglets. This can explain the better vitality score of the fresh born piglets. Statistics show several linear effects suggesting that the higher the dose the better. Looking at the figures numerically, some characteristics reach their optimum already at 0.09% nitrate inclusion. An optimal dose is not clear cut but seems to be somewhere between 0.09 and 0.15% added nitrate.

## CLAIMS

I/We claim:

1. A premix comprising:
  - a nitrate compound and salts thereof in the amount of greater than about 50% by weight of the premix;
  - vitamins and trace minerals;
  - wherein the premix is formulated for use in at least one of a gestation or lactation phase of an animal and in an amount of less than about five percent by weight (5.0 wt%) inclusion per metric ton of the premix.
2. The premix of claim 1, wherein the nitrate compound and salts thereof comprises calcium nitrate and its related salts.
3. The premix of claim 2, wherein the nitrate compound and salts thereof comprises ten hydrated calcium ammonium nitrate  $5\text{Ca}(\text{NO}_3)_2 \cdot \text{NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$ .
4. The premix of claim 2, wherein the calcium nitrate and its related salts comprises at least about 15% calcium and at least about 10% nitrogen.
5. The premix of claim 4, wherein the calcium nitrate is prilled.
6. A supplement for an animal feed for feeding during at least one of an animal's gestation phase and/or lactation phase, comprising:
  - a nitric oxide producing compound in an amount of less than 10 kg/metric ton of the total weight of the feed;
  - wherein the nitric oxide producing compound increases vasodilation of the placenta and the mammary glands of the animal.
7. The supplement of claim 6 wherein the nitric oxide producing compound comprises a plant based ingredient.

8. The supplement of claim 0, wherein the plant based ingredient comprises at least one of leafy greens, spinach, arugula, and beet root.
9. The supplement of claim 6, wherein the nitric oxide producing compound comprises a nitrate compound and its related salts.
10. The supplement of claim 9, wherein the nitrate compound comprises a calcium nitrate compound and its related salts.
11. The supplement of claim 6 wherein the nitric oxide producing compound comprises at least 101 kg/metric ton of the total weight of the lactation feed.
12. The supplement of claim 9, wherein the nitrate is present in a concentration of less than about 1%.
13. A method of feeding an animal, comprising:
  - providing to the animal a complete animal feed comprising at least one of a gestation feed and a lactation feed, the feed comprising a nitric oxide producing compound in an amount of less than 10 kg/metric ton of the total weight of the feed;
  - feeding the feed to an animal 110 days after gestation of the animal;
  - whereby the nitric oxide producing compound increases systemic vasodilation to reduce animal fatigue and reduce farrowing time;
  - whereby the nitric oxide producing compound increases vasodilation of at least one of the placenta and the mammary glands of the animal.
14. The method of claim 13, wherein the nitric oxide producing compound comprises calcium nitrate and its related salts.
15. The method of claim 13 further comprising feeding the feed to the animal at least through farrowing of the animal and wherein the incidence of stillborn offspring of the animal is reduced compared to an animal not fed the nitric oxide producing compound.

16. The method of claim 13, wherein the vasodilation of the placenta increases nutrients and oxygen to the placenta, thereby increasing nutrients and oxygen to a fetus of the animal and supporting uterine contractions to help reduce animal fatigue and farrowing time.
17. The method of claim 13 further comprising feeding the animal feed after farrowing and before weaning.
18. The method of claim 13, wherein the vasodilation of the mammary glands of the animal increases milk production of the animal for feeding to at least one offspring of the animal.
19. The method of claim 18, wherein the offspring of the animal exhibit a reduced incidence of mortality four days after weaning compared to offspring of the animal not receiving the nitric oxide producing compound.
20. The method of claim 18, wherein the offspring of the animal exhibits an increased average daily gain when housed with the animal compared to offspring of the animal not receiving the nitric oxide producing compound.
21. The method of claim 18, wherein the offspring of the animal exhibit an increased weight at weaning compared to offspring of the animal not receiving the nitric oxide producing compound.
22. The method of claim 18, wherein the calcium nitrate is fed to the animal in an amount of up to 10.0 g per animal per day.
23. The method of any one of claims 13-22 wherein the animal is a monogastric animal.
24. The method of any one of claims 13-23, wherein the monogastric animal is a sow.
25. A method of feeding an animal during gestation and/or lactation phases the premix of claim 1.
26. The premix of claim 1, wherein the premix can be included in a complete animal feed.

27. The method of claim 13 wherein the gestation and lactation feed is shown in Table 1.
28. The premix of claim 1, wherein the nitrate compound and salts thereof comprises magnesium nitrate and its related salts.
29. The premix of claim 28, wherein the nitrate compound and salts thereof comprises magnesium nitrate hexahydrate ( $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ).
30. The supplement of claim 9, wherein the nitrate compound comprises a magnesium nitrate compound and its related salts.
31. The method of claim 13, wherein the nitric oxide producing compound comprises magnesium nitrate and its related salts.
32. A method of feeding an animal, comprising:
  - providing to the animal a nitric oxide producing compound in an least one of a gestation or lactation feed through a liquid application, wherein the liquid application is water and the dosage is less than 90 grams of nitric oxide producing compound per animal per day.
33. A method of reducing animal fatigue and farrowing time in an animal comprising:
  - providing to the animal a complete animal feed during at least one of the animal's gestation phase or lactation phase comprising at least one of a gestation feed or a lactation feed, the feed comprising a nitric oxide producing compound in an amount of less than 10 kg/metric ton of the total weight of the feed.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/64293

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(8) - A23K 1/16, 1/18, 1/175, A23L 1/304 (2016.01) CPC - A23K 1/16, 1/175, 1/184, 1/1753 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC (8) - A23K 1/16, 1/18, 1/175, A23L 1/304 (2015.01); CPC - A23K 1/16, 1/175, 1/184, 1/1753; USPC - 426/53, 74 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) PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH), EBSCO, Google/Google Scholar, IP.com; premix, animal feed, gestation, lactation, nitrate compounds, calcium nitrate, salts, vitamins, trace minerals, prilled, magnesium nitrate, vasodilation, leafy greens, spinach, pentacalcium ammonium nitrate decahydrate, calcium ammonium nitrate, sows, monogastric		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	US 8,303,979 B2 (VAN DER LENDE, T) 06 November 2012; column 2, lines 15-18, 58-63; column 3, lines 1 12, 19-31; column 4, lines 42-49; column 5, lines 31-32	6 ---
Y	WO 2012/159186 A1 (GRASP INDUSTRIA E COMERCIO LTDA) 29 November 2012; page 16, lines 3-4, 9-22; page 18, lines 20-22; page 21, lines 9 14	1-5, 7-18, 20-22, 23/13-18, 23/20-22, 25-26, 28-31, 33
Y	(LIDDER, S et al.) Vascular Effects of Dietary Nitrate (As Found in Green Leafy Vegetables and Beetroot) Via The Nitrate-Nitrite-Nitric Oxide Pathway. British Journal of Clinical Pharmacology. 2012. vol. 75, no. 3; page 677, first-second columns; page 678 second column; table 1	1-5, 10, 14, 23/14, 25-26, 28-31
Y	US 7,105,191 B2 (MISHRA, SK) 12 September 2006; column 4, lines 14-34	7-10, 12, 30
Y	(SIAHL, C) Research Spotlight. Swine News NC State University. September 2008. vol. 31, no. 9, [Retrieved on 15 January 2016]. Retrieved from the internet; URL: <https://www.ncsu.edu/project/swine_extension/swine_news/2008/September/september_08.pdf>; page 1, second column, first-third paragraphs; page 2, first column, first paragraph; table 2	11, 13-18, 20-22, 23/13-18, 23/20-22, 31, 33
A	US 6,514,521 B1 (JULIEN, WE) 04 February 2003; column 2, lines 27-33, 38-41; column 4, lines 25-30, 64-66	19, 23/19, 27, 32
A	(WU, G et al.) Important Roles for the Arginine Family of Amino Acids in Swine Nutrition and Production. Livestock Science. 2007. vol. 112; abstract	19, 23/19, 27, 32
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents. "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 18 January 2016 (18.06.2016)		Date of mailing of the international search report <b>19 FEB 2016</b>
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/64293

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

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

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 24  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

**Remark on Protest**

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