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(73) Patenthaver: **Agro Innovation International, 18 avenue Franklin Roosevelt, 35400 Saint-Malo, Frankrig**

(72) Opfinder: **LAZA-KNOERR, Anca L., 5 rue Gabriel Degrés, 35400 Saint-Malo, Frankrig**
**ALIS, Yves, 4 impasse des Serres, 35400 Saint-Malo, Frankrig**
**ANGLADE, Nicolas M., 69 rue des Mottes Castrales, 35430 Chateauneuf d'Ille et Villaine, Frankrig**
**DESPREZ, Benjamin, 15 rue du Perret, 22980 Plelan le Petit, Frankrig**

(74) Fuldmægtig i Danmark: **Budde Schou A/S, Dronningens Tværgade 30, 1302 København K, Danmark**

(54) Benævnelse: **Produkt til forbedring af udnyttelsen af fibre i foder**

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The present invention relates to the field of the nutrition of non-human mammals, in particular the nutrition of ruminants, and to improving microbial metabolism, the digestion of fibers, and thus the utilization of rations.

Among animals, herbivores, and in particular ruminants, are mammals that receive a diet based on plant fibers that, among soluble fibers, contain insoluble fibers of the cellulose and hemicellulose type; these fibers are very difficult to digest.

A distinctive feature of a ruminant is that it digests via a "fermentation vat", namely the rumen (130 liters (L) to 180 L) inserted into the anterior portion of the digestive tract. The rumen contains a wide variety of bacterial microflora and protozoa, i.e. it carries out obligate, priority, and highly effective fermentative digestion. Thus, microbial flora conditions the digestibility of carbohydrates, and of proteins, and the self-production of vitamins or other nutrients. Although the microbial metabolism assists them in digesting these fibers, it is not always sufficient, which means that the ration is not being used efficiently, and thus the quantity of feed that is necessary increases, along with production costs. Thus, there continues to be a need for improving the digestibility of dietary fibers for this type of animal (ruminants). There is thus a keen interest in making maximum use of the digestive distinctiveness of ruminants by stimulating microbial activity and by directing it in a manner such that it benefits productivity, health and the quality of the produce as much as possible.

The inventors have now surprisingly discerned that the use of a mixture of co-products from cereal distillation or from beer production, with or without solubles (DDG and DDGS), concentrated distiller's solubles (CDS), prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes, has a positive impact on the digestion of non-human mammals, in particular herbivores (such as ruminants, horses, lagomorphs). As an example, with ruminants, the digestibility of the matter and fibers, in particular in fodder and concentrates, is increased by improving the consumption index and/or the feed efficiency, by promoting weight gain, by increasing rumination activity by reducing the emission of gas, in particular methane, by inhibiting the protozoa in the flora, by reducing the degradation of proteins and/or by directing the fermentation towards producing volatile fatty acids (VFA), and more specifically towards propionic acid. Propionic acid is a precursor of glucose and thus is a source of energy for the ruminant. A reduction in the supply of propionate is equivalent to a loss of energy, having a negative effect on the energy balance for the animal.

It is known to use a mixture of co-products from cereal distillation or from beer production with or without solubles (DDG and DDGS) and from concentrated distiller's solubles (CDS) in animal feeds. Patent US 5 260 089 thus describes an animal nutrition supplement without molasses for animal consumption, comprising a mixture of this type. However, that patent indicates that only minerals and vitamins need to be

added to that supplement and recommends the use of more DDG and DGS than CDS, or at best an equivalent quantity. Furthermore, it does not state that those compounds could have an impact on the use of the ration in a manner such as to improve the digestibility. It has also been demonstrated, by means of comparative tests (comparative test 1), that a composition containing only a mixture of co-products from cereal distillation or from beer production with or without solubles (DDG and DDGS) and concentrated distiller's solubles (CDS) had no significant effect on the concentration of $N\text{-}NH_3$ and on the pH.

In Application WO 2015/11663, the use of an enzyme composition having cellulase, xyalanase, and β-glucanase enzyme activities obtained by fermentation of cereals and/or cereal co-products and/or oleaginous co-products in a solid medium with a filamentary fungus of the genus Aspergillus section Nigri, more particularly of the genus Aspergillus tubingensis, is known in order to improve the zootechnical performances of a ruminant. However, the tests presented do not demonstrate a significant or large effect on corn (i.e. maize) silage or hay, which are highly fibrous foods, in particular as regards the release of gas, for example methane, during ruminal fermentation, and the digestibility of the feeds. The composition of the invention has a much more interesting and important effect as regards directing the fermentations in the rumen, even for rations based on corn silage and/or hay.

Thus, the present invention concerns a composition for the nutrition of a non-human mammal, comprising a mixture of co-products from cereal distillation or from beer production, with or without solubles (DDG and DDGS), concentrated distiller's solubles (CDS) and a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal and the nutritional yeast *Saccharomyces cerevisiae*, said cereals being fermented with the aid of said nutritional yeast *Saccharomyces cerevisiae*.

The co-products from cereal distillation or from beer production with or without solubles (DDG and DDGS) and concentrated distiller's solubles (CDS) are by-products from the alcoholic fermentation of grains, as in beer, and are in general obtained by separating wet grains (from which the alcohol has been removed) from the grain alcohol still in the form of a liquid fraction (containing concentrated distiller's solubles) and from a solid fraction (containing the co-products from cereal distillation). Products of this type are commercially available.

Advantageously, the inventors have discerned that the ratio by weight between the concentrated distiller's solubles and the co-products from cereal distillation or from beer production with or without solubles in the composition of the invention has a particularly interesting effect when it lies in the range 1/1 to 5/1, advantageously in the range 2/1 to 4/1, and yet more advantageously in the range 2.5/1 to 3.5/1. In fact, it is

more important to have a higher concentrated distiller's solubles content than a content of the co-products from cereal distillate or from beer production with or without solubles.

Advantageously, the concentrated distiller's solubles represent 41% to 80%, more advantageously 60% to 79%, in particular 65% to 75%, yet more advantageously 68% to 75% by weight of the total weight of the composition. Advantageously, the co-products from cereal distillation or from beer production with or without solubles represent 15% to 40%, advantageously 17% to 35%, in particular 20% to 30%, yet more advantageously 21% to 28% by weight of the total weight of the composition. Thus, the composition of the invention may have the composition as indicated in Table 1 below:

· Table 1: Examples of compositions of the invention

| **Components** | **% by weight** | **Preferred % by weight** |
|---|---|---|
| DDG or DDGS | 41-80 | 50-75 |
| CDS | 15-50 | 21-46 |
| Mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes | Balance to 100 | Balance to 100 |

Advantageously, the prebiotic fibers of the composition of the invention are oligosaccharides or polysaccharides, in particular selected from larch arabinogalactans, trans-galactooligosaccharides, fructans such as inulin, oligofructoses such as fructo-oligosaccharides (FOS) (in particular from cereals, such as barley or wheat), mannan-oligosaccharides (MOS) (in particular from *Saccharomyces cerevisiae)*, beta-glucans (in particular from cereals such as oats, wheat and barley, more particularly wheat and/or barley, and/or yeast, in particular from *Saccharomyces cerevisiae,* more particularly beta-glucans from oats) arabino-xylo-oligosaccharides(AXOS) (in particular from cereals such as wheat), xylo-oligosaccharides (XOS) (in particular from cereals such as wheat), and mixtures thereof, more advantageously selected from fructo-oligosaccharides, inulin, mannan-oligosaccharides, beta-glucans, arabino-xylo-oligosaccharides, xylo-oligosaccharides, and mixtures thereof; yet more advantageously it is a mixture of fructo-oligosaccharides, mannan-oligosaccharides, beta-glucans, arabino-xylo-oligosaccharides and xylo-oligosaccharides.

In an advantageous embodiment, the digestive enzymes of the composition of the invention are selected from amylolytic enzymes (in particular for starch and sugar such as alpha-amylases, beta-amylases, pullulanases and/or amyloglucosidases), fibrolytic enzymes (in particular for fibers such as cellulases and/or hemicellulases or xylanases), proteolytic enzymes (in particular for proteins such as exopeptidases (or exoproteases), aminopeptidases, carboxypeptidases and/or endopeptidases (or endoproteases)) and phospholytic enzymes (in particular for minerals such as phosphorus, but also phytic acid, in particular it is a phytase), and mixtures thereof; advantageously, it is a mixture of amylolytic, fibrolytic, proteolytic and phospholytic enzymes. These enzymes are obtained from barley malt in particular.

In another advantageous embodiment, the probiotics of the composition of the invention are selected from *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Saccharomyces uvarum*, *Saccharomyces ludwigi*, *Kluyveromyces lactis*, *Torulaspora delbrueckii*, *Candida utilis/Pichia jadini*, *Brettanomyces spp*; advantageously, it is a yeast, in particular a nutritional yeast such as *Saccharomyces cerevisiae*.

In yet another advantageous embodiment, the proteins of the composition of the invention are selected from wheat proteins, oat proteins, flax proteins, barley malt proteins, and mixtures thereof; advantageously, it is a mixture of wheat proteins, oat proteins, flax proteins and barley malt proteins. Advantageously, the protein content of the mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes of the composition of the invention lies in the range 20% to 30% by weight relative to the total weight of this mixture.

Particular amino acids of the composition of the invention that may be mentioned are methionine and cystine.

In an advantageous embodiment, the lignans of the composition of the invention are flax lignans, such as secoisolariciresinol and matairesinol or their glycosylated forms (diglucosides).

According to the invention, the mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes that may be used in the composition of the invention is obtained from wheat, oats, barley malt, flax, flax meal, and from nutritional yeast. The cereals are fermented with the aid of nutritional yeast (*Saccharomyces cerevisiae*) to eliminate starch, concentrate the proteins and produce the prebiotic fibers, and the enzymes used in the process are stabilized in order to be able once again to act in the digestive system of animals with the metabolites from growth of the yeast.

The composition of the invention may also comprise one or more ingredients selected from:

· essential oils, in particular the essential oil from *Thymus Vulgaris*, advantageously obtained from flowering shoot, more particularly by steam distillation, yet more particularly comprising thymol and para-cymene;

· plant extracts, such as chestnut extract (*Castanea Sativa Mill.*), in particular obtained by water extraction, advantageously with no other solvent, more particularly chestnut tannin extract;

· humates such as leonardite;

· peat;

· aluminosilicates (such as zeolites or clays);

· tannin, such as chestnut tannins (*Castanea Sativa Mill*), in particular esterified and glycosidic, more particularly obtained by aqueous extraction, in particular with no other solvent;

· linseed;

· polyphenols;

· saponins, advantageously steroidal and/or triterpenoid saponins, in particular obtained from the plants *Quillaja saponaria*, *Trigonella foenum graecum*, *Yucca schidigera*, *Chenopodium quinoa* and/or *Camelia SP* such as FIX'N marketed by NOR-FEED;

· algae such as microalgae, for example spirulina or macroalgae for example green, red or brown algae, and mixtures thereof;

· algae extracts;

· the product of the fermentation of *Aspergillus oryza,* in particular *Aspergillus oryzae* NRRL 458, more particularly containing endo-1,4-beta-glucanase EC 3.2.1.4 and alpha-amylase EC 3.2.1.1, such as AMAFERM marketed by ZOOTECH; and

· a mixture of these ingredients.

The composition of the invention may thus comprise essential oils, plant extracts, leonardite, peat, and/or aluminosilicates (such as zeolites or clays).

It may also comprise tannin, linseed, polyphenols, and/or saponins.

Furthermore, it may comprise algae and/or algae extracts such as microalgae, for example spirulina or macroalgae, for example green, red or brown algae.

Advantageously, it comprises saponins, in particular in a quantity lying in the range 5% to 15% by weight relative to the total weight of the composition. These saponins are advantageously steroidal and/or triterpenoid saponins, in particular obtained from the plants *Quillaja saponaria*, *Trigonella foenum graecum*, *Yucca schidigera*, *Chenopodium quinoa* and/or *Camelia SP*.

In fact, because saponins have the intrinsic property of carrying out the targeted lysis of protozoa in the rumen, this results in modulation of ruminal fermentation, leading in the end to: a reduction in the concentration of $NH_3$ in the rumen, and thus to

better exploitation of nutritional nitrogen of the nitrogenous metabolism towards more proteosynthesis and to a reduction in the acetate (C2)/propionate (C3) ratio, leading to an optimization of energy metabolism.

Advantageously, it comprises chestnut extract (Castanea Sativa Mill.), in particular obtained by water extraction, advantageously with no other solvent, more particularly chestnut tannin extract.

Advantageously, it comprises the essential oil of *Thymus Vulgaris*, advantageously obtained from flowering shoot, more particularly by steam distillation, yet more particularly comprising thymol and para-cymene.

Advantageously, it comprises humates, in particular leonardite, advantageously in a quantity lying in the range 1 gram per day (g/d) to 100 g/d.

Advantageously it comprises the product of the fermentation of *Aspergillus oryzae,* in particular of *Aspergillus oryzae* NRRL 458, more particularly containing endo-1,4-beta-glucanase EC 3.2.1.4 and alpha-amylase EC 3.2.1.1, such as AMAFERM marketed by ZOOTECH. AMAFERM therefore not only contains the product of the fermentation of *Aspergillus oryzae* in a quantity by weight of 4-5% (endo-1,4-beta-glucanase EC 3.2.1.4: 3 IU(1)/g; alpha-amylase EC 3.2.1.1: 40 IU (2)/g), but also 94-95% by weight of wheat bran and 1% by weight of steel grit containing 5% by weight of cobalt carbonate.

Advantageously, the quantity of the product of the fermentation of *Aspergillus oryzae* in the composition of the invention lies in the range 0.5 g/d to 10 g/d, in particular in the range 0.5 g/d to 3 g/d, more particularly in the range 1 g/d to 2 g/d.

The composition of the present invention is intended for the nutrition of non-human mammals, in particular herbivores (such as ruminants, horses, lagomorphs) and more particularly ruminants.

"Ruminants" includes, for example, bovines, ovines, caprines, cervids, and camelids.

The term "bovines" as used in the context of the present invention should be understood to mean a sub-family of Bovidae comprising several major species of farm animals. In particular, "bovines" includes the cow, in particular the dairy cow, the suckler cow, the heifer, the calf, the weanling, the bullock, the steer, the fattening steer, the bull, the buffalo, the yak, the gaur, and the banteng.

The term "ovines" as used in the context of the present invention should be understood to mean the ruminant herbivores of the genus Ovis. In particular, "ovines" includes the mouflon, the sheep, the ewe, the theave, and the lamb.

The term "goat" as used in the context of the present invention should be understood to mean ruminant herbivores of the genus Capra. In particular, "goat" includes the nanny goat, the billy goat, the kid and the ibex.

The term "cervid", as used in the context of the present invention should be understood to mean antler-bearing ruminants of the Cervidae family. In particular, "cervids" includes the deer, the stag, the brocket deer, the hind, the fawn, the roe deer, the pricket, the she-reindeer, the reindeer, the fallow deer buck, the fallow deer doe, and the moose.

The term "camelid", as used in the context of the present invention should be understood to mean artiodactyl mammals from the Camelidae family. In particular, "camelids" includes the dromedary, the camel, the she-camel, the llama, and the alpaca.

In an advantageous embodiment, the ruminant of the invention is a bovine; in particular, it is selected from the cow, in particular the dairy cow, the calf, the weanling, the suckled calf, the steer, and the fattening steer, and more particularly preferably it is the dairy cow.

In a particularly advantageous embodiment, the ruminant is a farm animal.

The composition of the invention may have any appropriate form for the nutrition of non-human mammals, such as herbivores (for example ruminants, horses, lagomorphs), in particular a solid form, a powdered form or a granulated form.

It may be added directly to the ration of non-human mammals, in particular herbivores (such as ruminants, horses, lagomorphs), or it may be added as a premix or feed supplement or to nutritional blocks or buckets.

In an advantageous embodiment, it is added to the daily ration for the non-human mammal, advantageously in an amount of 40 grams (g) to 50 g of composition per kilogram (kg) of ration.

This ration may, for example, be composed of fodder of any type and in any of its forms (green, dehydrated, silaged, agglomerated, etc), such as grass and other forage grasses, forage cereals (barley, corn, oats, wheat, sorghum, soya, rye), legumes (peas, beans, lupins, soya, alfalfa, sainfoin, clover), roots, tubers and their by-products (beet, beet pulp, potato, potato pulp, etc), cabbage, rape, sunflower, plant waste (tops, stalks, cereal husks, bran, hulled corn cobs, bagasse) and starches, by-products from the agro-alimentary industry (starch manufacture, distillers, brewing, milling, etc), as well as oilseed cake, syrups, and nitrogenous feed material such as urea and its derivatives (biuret, ureides), and ammoniacal salts.

Preferably, in accordance with the invention, the appropriate ration for the ruminants comprises fodder, preferably corn silage typically representing 10% to 50% of the ingested dry matter, preferably daily, preferably associated with other fodder such as hay, straw or grass silage or cereal silage and preferably supplemented with concentrated feeds such as cereals, oilseed cake or compound feeds.

In particular, it is a mixture of corn silage, hay and concentrates (constituted, for example, by wheat, corn, barley, beet pulp, wheat bran and molasses), for example in a ratio of 50:30:20, or of hay, concentrate and corn starch, for example in a ratio of 77:9:12:2.

In another advantageous embodiment, the composition of the present invention is added to a feed bucket.

In an advantageous embodiment, according to field tests on dairy cows, the daily consumption of the mammal is in an amount of 120 g to 150 g of composition per mammal.

The present invention thus also concerns a feed bucket for a non-human mammal, in particular a ruminant as described hereinabove, comprising 20% to 30% by weight of the composition of the invention relative to the total weight of the bucket.

The feed bucket may furthermore comprise additives that are well known to the person skilled in the art, such as monocalcium phosphate, a source of sodium (sodium chloride and/or sodium sulfate), a source of calcium (for example calcium carbonate or calcium and magnesium carbonate), a source of magnesium (for example magnesium oxide and/or calcium and magnesium carbonate, magnesium sulfate), oligo-elements, and optionally vitamins.

In particular, vitamin or the mixture of vitamins is selected from the group consisting of vitamin A, vitamin D2, 25-hydroxycalciferol, vitamin D3, beta-carotene, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, pantothenic acid, vitamin PP, vitamin B9, vitamin H2, B7 or BW, choline, inositol, carnitine, betaine and taurine, more particularly vitamin A, vitamin E and vitamin D3.

Advantageously, the oligoelement or the mixture of oligoelements is selected from the group consisting of ferrous carbonate, ferrous chloride tetrahydrate, ferric chloride hexahydrate, ferrous citrate hexahydrate, ferrous fumarate, ferrous lactate tetrahydrate, ferric oxide, ferrous sulfate monohydrate, ferrous sulfate heptahydrate, ferrous chelate of amino acids, hydrate, ferrous chelate of glycine, hydrate, iron pidolate, calcium iodate hexahydrate, anhydrous calcium iodate, sodium iodide, potassium iodide, cobalt acetate tetrahydrate, basic cobalt carbonate monohydrate, cobalt carbonate hexahydrate, cobalt chloride hexahydrate, cobalt sulfate heptahydrate, cobalt sulfate monohydrate, cobalt nitrate hexahydrate, cobalt pidolate, cupric acetate monohydrate, basic copper carbonate monohydrate, cupric chloride dihydrate, copper methionate, cupric oxide, cupric sulfate pentahydrate, cuprous chelate of amino acids, hydrate, cuprous chelate of glycine, hydrate, copper methionine hydroxy analog chelate, copper pidolate, manganese carbonate, manganese chloride tetrahydrate, acidic manganese phosphate trihydrate, manganese oxide, manganic oxide, manganese sulfate tetrahydrate, manganese

sulfate monohydrate, manganese chelate of amino acids, hydrate, manganese chelate of glycine, hydrate, manganese methionine hydroxy analog chelate, manganese pidolate, zinc lactate trihydrate, zinc acetate dihydrate, zinc carbonate, zinc chloride monohydrate, zinc oxide, zinc sulfate heptahydrate, zinc sulfate monohydrate, zinc chelate of amino acids, hydrate, zinc chelate of glycine, hydrate, zinc methionine hydroxy analog chelate, zinc pidolate, ammonium molybdate, sodium molybdate, sodium selenite, sodium selenate and/or a mixture thereof.

Advantageously, the feed bucket of the invention does not contain molasses.

Particularly advantageously, the feed bucket of the invention comprises in the range 4% to 9% of co-products from cereal distillation or from beer production with or without solubles, advantageously in the range 5% to 7%, by weight relative to the total weight of the bucket, and in the range 12% to 24% of concentrated distiller's solubles, advantageously in the range 15% to 20%, by weight relative to the total weight of the bucket.

The present invention furthermore concerns the use of the composition of the invention or of a feed bucket of the invention, to improve the zootechnical performances of a non-human mammal, in particular herbivores (such as ruminants, horses, lagomorphs), more particularly of a ruminant.

The invention furthermore concerns the use of the composition of the invention or of a feed bucket of the invention, to increase the digestibility of the dry matter and fibers, in particular fodder and concentrates, and/or to improve the consumption index and/or the nutritional efficiency and/or to promote weight gain and/or to increase the intensity of fermentation and of microbial metabolism and/or to reduce the emission of gas, in particular methane, and/or to inhibit protozoa of the flora and/or to reduce the degradation of the proteins and/or to direct the fermentations towards the production of volatile fatty acids (VFA), in particular towards propionic acid in a non-human mammal, advantageously in a ruminant.

Finally, the invention concerns the use of the composition of the invention or of a feed bucket of the invention, to increase the productivity, the production quality and to keep ruminant type animals in good health.

The invention can be better understood in the light of the following description of the figures and examples, which are given purely by way of indication.

Figure 1 shows the total volume of gas produced in milliliters per gram (mL/g) of dry matter (DM), as a function of fermentation time (in hours (h)) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 2).

Figure 2 shows the total volume of gas produced in mL/g of dry matter (DM), as a function of fermentation time (in hours) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 3 shows the volume of $CH_4$ produced after 24 h of fermentation, in mL/g of dry matter (DM) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 2).

Figure 4 shows the volume of $CH_4$ produced after 24 h of fermentation, in mL/g of dry matter (DM) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 5 shows the change in the pH during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 6 shows the change in the pH during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 7 shows the change in the population of protozoa ($10^5$/mL) during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 8 shows the change in the population of protozoa ($10^5$/mL) during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 9 shows the change in the concentration of propionic acid (C3) in g/L during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 10 shows the change in the concentration of propionic acid (C3) in g/L during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 11 shows the change in the ratio C2/C3 during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 12 shows the change in the ratio C2/C3 during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 13 shows the change in the concentration of ammoniacal nitrogen (N-$NH_3$) in mg/L during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 14 shows the change in the concentration of ammoniacal nitrogen (N-$NH_3$) in mg/L during fermentation (after 6 h or 24 h of fermentation) for a pasture ration

for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 15 shows monitoring of the digestibility of the dry matter (dDM) as a % during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 16 shows monitoring of the digestibility of the dry matter (dDM) as a % during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 17 shows monitoring of the digestibility of the ADF (dADF) as a % during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 18 shows monitoring of the digestibility of the ADF (dADF) as a % during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 19 shows monitoring of the digestibility of NDF (dNDF) as a % during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the positive control, the negative control and for the composition of the invention (Mix 3.0: Example 1 after 6 h and Example 2 after 24 h).

Figure 20 shows monitoring of the digestibility of NDF (dNDF) as a % during fermentation (after 6 h or 24 h of fermentation) for a pasture ration for the negative control and for the composition of the invention (Mix 3.0 + Fix N: Example 3).

Figure 21 shows the change in the concentration of total VFA (g/L) during fermentation (after 6 h or 24 h or 48 h of fermentation) for a Brazilian style ration for the negative control and for the composition of the invention (Amaferm 1g/d + Mix 3.0: Example 4 and Amaferm 2g/d + Mix 3.0: Example 5).

Figure 22 shows the volume of $CH_4$ produced after 24 h of fermentation, in mL/g of dry matter (DM) for a test ration with humates for the control and for the composition of the invention (HA + Mix 3.0 40 g/L: Example 6; HA + Mix 3.0 80 g/L: Example 7 and HA + Mix 3.0 120 g/L: Example 8).

Figure 23 shows the change in the concentration of total VFA (g/L) during fermentation (after 6 h or 24 h of fermentation) for a test ration with humates for the control and for the composition of the invention (HA + Mix 3.0 40 g/L: Example 6; HA + Mix 3.0 80 g/L: Example 7 and HA + Mix 3.0 120 g/L: Example 8).

Figure 24 shows the change in the concentration of ammoniacal nitrogen (N-$NH_3$) in mg/L during fermentation (after 6 h or 24 h of fermentation) for a winter ration for the control and for a comparative composition (Mix 2.0: Comparative Example 1).

Figure 25 shows the change in the pH during fermentation (after 6 h or 24 h of fermentation) for a winter ration or a spring ration for the control and for a comparative composition (Mix 2.0: Comparative Example 1).

**Study of the effect of the composition of the invention on fermentations in the rumen and the degradation of the ration**

The compositions of the invention comprising the following were tested:

· 71% of CDS + 25% of DDGS + 4% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* (Example 1);

· 71% of CDS + 26% of DDGS + 3% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* (Example 2);

· 41% of CDS + 40% of DDGS + 5.5% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* + 13.5% of saponins (FIX'N) (Example 3);

· 42.94% of CDS + 46.075% of DDGS + 5.985% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* + 5% of the product of the fermentation of *Aspergillus oryzae* (AMAFERM 1g/d) (Example 4).

· 41.132% of CDS + 44.135% of DDGS + 5.733% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* + 9% of the product of the fermentation of *Aspergillus oryzae* (AMAFERM 2g/d) (Example 5).

· 14.916% of CDS + 16.005% of DDGS + 2.079% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* + 67% of humates *(*leonardite: HA 40 g/L*)* (Example 6).

· 9.04% of CDS + 9.7% of DDGS + 1.26% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the feed yeast *Saccharomyces cerevisiae* + 80% of humates *(*leonardite: HA 80 g/L*)* (Example 7).

· 6.328% of CDS + 6.79% of DDGS + 0.882% of a mixture of prebiotic fibers, probiotics, amino acids, proteins, omega 3, lignans, and digestive enzymes obtained from wheat, oats, barley malt, flax, flax meal, fermented with the aid of the nutritional yeast *Saccharomyces cerevisiae* + 86% of humates *(*leonardite: HA 120 g/L*)* (Example 8).

· 48.7% of CDS + 51.3% of DDGS (Comparative Example 1).

APPARATUS AND METHOD

The approach is based on the technique of *in vitro* fermentations developed by the Laboratoire de Nutrition Animale du Center Mondial de l'Innovation Roullier [Animal Nutrition Laboratory of the Global Innovation Center, Roullier]. The principle is based on incubating fresh rumen fluid mixed with artificial saliva prepared in the laboratory, the additives to be tested and the rations (spring and winter) under conditions that resemble reality (anaerobic at 39.5°C) and with continuous stirring (in order to imitate peristaltic movements). The bacterial flora present on the adapted substrate reproduced ruminal fermentations, which meant that the production of gas as well as the final products such as VFA, $N\text{-}NH_3$ could be monitored as a function of the aims of the study.

In order to evaluate the effect of the composition of the invention on the fermentations of the rumen and the degradation of the ration, rumen fluid was removed from 2 fistulated cows fed with a ration based on corn silage, hay and concentrates (cereals and soybean cake). In order to reproduce the physiological conditions, a ration based on corn silage, hay and concentrate (50:30:20) was incubated in the inoculum composed of rumen fluid and artificial saliva.

Each 100 milliliter (mL) flask for incubation was composed of:

· 20 mL of filtered rumen fluid;

· 40 mL of artificial saliva (buffer capacity: buffer substances: Na and ammonium carbonate; macro-elements: Na, P, K, and Mg; micro-elements: Ca, Mn, Co, and Fe);

· 0.5 g of substrate ground to 1mm: the substrate could be a winter ration or a pasture ration or a Brazilian style ration or a spring ration or a ration for the tests with humates:

o composition of rations:

o winter ration = 50% corn silage + 30% hay + 20% concentrate (constituted by 20% wheat, 20% corn, 20% barley, 20% beet pulp, 15% wheat bran and 5% molasses);

o pasture ration = 77% hay + 9% concentrate + 12% corn starch + 2% urea;

o Brazilian style ration: ground to 1 mm and composed of 30% corn silage + 40% hay + 20% concentrate (constituted by 20% wheat, 20% corn, 20% barley, 20% beet pulp, 15% wheat bran and 5% molasses) + 10% soybean cake;

o spring ration: frozen fresh grass (March 2016);

o test rations with humates: ground to 1mm and composed of 50% corn silage + 30% hay + 20% soybean cake;

· Adding starch and urea meant that the food values of the basic ration could be adjusted to obtain the values for a grassland at the start of spring, namely 1UFL/kg and 135g of PDIN/kg.

The pasture ration and the spring ration are very much richer in fiber than the winter ration, the Brazilian style ration and the test rations with humates.

· 0.5g of additives or of test composition.

The device for the study was composed of flasks divided up as indicated in Table 2 below. After 6 to 24 h and optionally 48 h of incubation at 39.5°C, the flasks were placed in ice to stop the reactions. The pH was measured directly at the outlet from the incubator before filtration.

The substrate was separated from the rumen fluid by centrifuging (20 min at 4000 rpm) in order to recover 50 mL of fluid intended for assay of the ammoniacal nitrogen and the volatile fatty acids (VFA) in the medium. The recovered substrate was freeze dried for 48 h then ground in order to assay the fibers (neutral detergent fiber (NDF) and acid detergent fiber (ADF)).

In order to count the population of protozoa, a sample was diluted by half in a solution of 18.75% formaldehyde stained with 2% methylene blue. Counting was carried out with the aid of a Malassez cell and a x20 microscope.

Continuously recording the pressure in the flasks allowed the volume of gas produced to be determined in mL/g of dry matter (DM). Finally, a collecting bag was fitted to the outlet from each module for recording gas production, in order to recover a sample and allow the composition of the gases produced after 24 h of incubation to be analyzed.

**Table 2: Experimental test design**

| Conditions | 6 h of incubation<br><br>4 repetitions | 24 h of incubation<br><br>4 repetitions | 48 h of incubation<br><br>4 repetitions |
|---|---|---|---|
| **Negative control** | 60 mL of inoculum + 0.5 g of substrate | 60 mL of inoculum + 0.5 g of substrate | 60 mL of inoculum + 0.5 g of substrate |

| | | | |
|---|---|---|---|
| Composition of the invention | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 1 (Mix 3.0)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 2 (Mix 3.0)** | |
| Composition of the invention + saponins | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 3 (Mix 3.0 + Fix'N)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 3 (Mix 3.0 + Fix'N)** | |
| Composition of the invention + *Aspergillus oryzae* fermentation product | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 4 (Amaferm 1g/d + Mix 3.0)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 4 (Amaferm 1g/d + Mix 3.0)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 4 (Amaferm 1g/d + Mix 3.0)** |
| | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 5 (Amaferm 2g/d + Mix 3.0)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 5 (Amaferm 2g/d + Mix 3.0)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 5 (Amaferm 2g/d + Mix 3.0)** |
| Composition of the invention + leonardite | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 6 (HA + Mix 3.0 40 g/L)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 6 (HA + Mix 3.0 40 g/L)** | |
| | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 7 (HA + Mix 3.0 80 g/L)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 7 (HA + Mix 3.0 80 g/L)** | |

| | | |
|---|---|---|
| | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 8 (HA + Mix 3.0 120 g/L)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex 8 (HA + Mix 3.0 120 g/L)** |
| Comparative Example | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex Comp 1 (Mix 2.0 18.5g/d)** | 60 mL of inoculum + 0.5 g of substrate + 0.5g **Ex Comp 1 (Mix 2.0 18.5g/d)** |

The results were processed using GraphPad Prism 6 statistical software.

**Monitoring gas production**

Fermentation of the substrate by the rumen flora causes the production of gas, principally $CO_2$ and methane ($CH_4$). Figures 1 to 4 and 22 present the profile of the total production of gas and the $CH_4$ levels after 24 h or even 48 h of fermentation. The gas profiles for the 3 conditions were identical and in agreement with the fermentation of a basic ration of corn silage and hay (Pell and Schofield, 1993). The supplement in Mix 3.0 of Example 2 or of Example 3 (Mix 3.0 + Fix N) reduced the total gas production ($p < 0.001$). These treatments inhibit the fermentation activity of a portion of the flora of the rumen.

During fermentation, the production of $CH_4$ was reduced by adding the compositions of the invention (Figure 3: $p < 0.001$, Figure 4: $p < 0.0335$ and Figure 22: $p = 0.0650$). This reduction confirms the direct inhibition of methanogenic bacteria and of protozoa. In fact, the combination of these 2 families is responsible for 9% to 25% of the production of $CH_4$. Adding the composition of the invention should limit energy losses because the production of $CH_4$ causes approximately 10% of the energy consumed by the animal to be lost.

**Rumen fluid parameters and fermentation products**

**pH monitoring**

The pH of the medium was measured after 6 h and 24 h of incubation (Figures 5, 6 and 25). For all of the conditions, the fermentation was carried out under physiological conditions with values normally situated in the range 6 to 6.9. The composition of the invention enabled the pH to be maintained better despite the production of VFAs during fermentation of the substrate by the rumen flora ($p < 0.001$). In fact, VFAs are weak acids that reduce the pH of the medium. Under these *in vitro*

conditions, the composition of the invention reduced the drop in pH and contributed to equilibrium in the rumen, thereby enabling optimized function of flora and avoiding the risk of acidosis. This was not the case with the comparative composition containing CDS and DDGS alone (Figure 25: Mix 2.0: Comparative Example 1), where the effects on the pH were not significant.

**Monitoring protozoa population**

The protozoa could easily be counted by counting on a Malassez cell. These cells have a grid pattern that can be used to determine the number of microorganisms in a known volume. In order to facilitate counting, the sample was diluted by half in 18.75% formaldehyde solution stained with methylene blue, with a minimum residence time of 4 h. This solution could be used to stain the various protozoa.

Protozoa are a family of microorganisms that are vital to ruminal equilibrium, but which participate in energy losses due to the production of $CH_4$. In fact, methanogenic bacteria bind to protozoa. One of the strategies used to reduce $CH_4$ emissions in the ruminant is based on defaunation of the protozoan population. The composition of the invention significantly reduced the number of protozoa at 6 h and 24 h ($p < 0.01$), as can be seen in Figures 7 and 8. The reduction in $CH_4$ (see Figures 3 and 4) was due to the direct effect of the product on the protozoa, and thus also on the associated methanogenic bacteria.

**Monitoring VFA production**

Figures 9 to 12 present the change in the concentrations of propionic acid and the ratio C2/C3 during fermentation. Figures 21 and 23 present the change in the total VFA concentrations during fermentation. The composition of the invention significantly increased the production of VFA after 6 h and 24 h of fermentation, with Examples 6 to 8 ($p = 0.1384$) and after 48 h of fermentation, with Examples 4 and 5 ($p = 0.0043$), which meant that the energy available for production (milk, meat, growth, etc) was increased.

After 6 h and 24 h of fermentation, the production of C3 for the composition of the invention significantly increased ($p = 0.0014$ or $p < 0.001$) respectively by 15.6%, 3.3% and 7.4% relative to the negative control (Figures 9 and 10). The tested compositions of the invention direct the C3 fermentation routes. This directing was confirmed by the reduction in the ratio C2/C3 with the composition of the invention ($p < 0.001$ and $p < 0.0001$), see Figures 11 and 12. By promoting the production of C3, the composition of the invention could be used to supply more energy to the animal. In addition, with dairy cows, C3 is an essential precursor to glycogenesis that should improve production performances.

**Monitoring N-$NH_3$ levels**

The composition of the invention had a significant effect on the N-$NH_3$ levels in the medium ($p < 0.001$). Adding the composition of the invention represented a dose of 9.7 mg of N-$NH_3$/L, which is a negligible fraction compared with the ration. However, the composition of the invention reduces the nitrogen levels in the medium (Figures 13 and 14), which could demonstrate inhibition of the rumen flora that use the amino acids and peptides as a source of energy. They are protozoa the reduction of which improves the efficiency of the nitrogen by recycling N and by the synthesis of microbial proteins. This was not the case with the comparative composition containing only CDS and DDGS (Figure 24: Mix 2.0: Comparative Example 1), for which the effects on the N-$NH_3$ levels in the medium were not significant.

**Parameters for the ration**

Figures 15 to 20 present the digestibilites of dry matter (dDM), of ADF (dADF) and of NDF (dNDF) during fermentation. Table 3 below presents the digestibilites of the ADF (dADF) and of NDF (dNDF) during fermentation with Examples 6 to 8.

Table 3

| | Control | Ex 6 | Ex 7 | Ex 8 |
|---|---|---|---|---|
| **dNDF 6 h** | 20.77% | **33.49%** | **24.08%** | **28.42%** |
| **dADF 6 h** | 11.22% | **19.42%** | - | **21.90%** |

The composition of the invention can be used to improve the dDM ($p = 0.003$ or $p < 0.001$). The ration is therefore consumed better by the rumen flora irrespective of whether it is cellulolytic or amylolytic. The composition of the invention also increases the dADF ($p = 0.0058$) and the dNDF ($p = 0.005$) (figures and Table 3). It promotes the degradation and the utilization of fibers of the ration in this fodder-rich case (80%) with corn silage and hay.

**CONCLUSION**

The effect of the composition of the invention on ruminal fermentation and the degradation of the ration under physiological conditions has thus been able to be evaluated. This composition can therefore enable optimized function of the rumen. It reduces the production of gas, in particular of $CH_4$. It inhibits a portion of the flora, the protozoa, which are associated with methanogenic bacteria. This defaunation can be used to limit $CH_4$ emissions, thereby reducing energy losses for the animal. In fact, it improves the production of propionic acid (C3). This fermentation pathway produces

one of the essential precursors for the performance of dairy cows. The reduction in the N-$NH_3$ levels results in a reduction in degradation of the proteins in the ration that is in part due to the reduction of protozoa. In fact, defaunation contributes to improving the nitrogen efficiency by recycling N and increasing the synthesis of microbial proteins. The composition of the invention significantly improves degradation of the ration. In fact, the digestibilities of the dry matter (DM) and fibers (NDF and ADF) increase compared with the control without supplementation. This product can be used to improve the utilization and degradation of fibers by the ruminal flora. In contrast, the use of CDS and of DDGS alone (Comparative Example 1) has a non-significant effect on the pH and the N-$NH_3$ levels, which clearly demonstrates that the use of these compounds alone is not sufficient to significantly improve microbial metabolism and thus utilization of the rations.

## PATENTKRAV

1. Sammensætning til ernæring af et ikke-humant pattedyr, fortrinsvis en planteæder, omfattende en blanding af biprodukter fra korndestillation eller fra ølproduktion med eller uden opløselige stoffer (DDG og DDGS), koncentreret Distillers solubles (CDS) og en blanding af præbiotiske fibre, probiotika, aminosyrer, proteiner, omega-3, lignan og fordøjelsesenzymer opnået fra hvede, havre, bygmalt, hør, hørfrømel og næringsgær Saccharomyces cerevisiae, hvilke kornarter fermenteres ved hjælp af den nævnte næringsgær Saccharomyces cerevisiae.

2. Sammensætning ifølge krav 1, **kendetegnet ved, at** vægtforholdet imellem den koncentrerede Distillers solubles og biprodukterne fra korndestillation eller fra ølproduktion med eller uden opløselige elementer ligger i området 1/1 til 5/1, fortrinsvis i området 2/1 til 4/1.

3. Sammensætning ifølge ethvert af kravene 1 eller 2, **kendetegnet ved, at** den koncentrerede Distillers solubles repræsenterer 41 til 80, fortrinsvis 65 til 75 vægt-% af den totale vægt af sammensætningen.

4. Sammensætning ifølge ethvert af kravene 1 til 3, **kendetegnet ved, at** biprodukterne fra korndestillation eller fra ølproduktion med eller uden opløselige elementer repræsenterer fra 15 til 40, fortrinsvis 21 til 28 vægt-% af den totale vægt af sammensætningen.

5. Sammensætning ifølge ethvert af kravene 1 til 4, **kendetegnet ved, at** den yderligere omfatter én eller flere ingredienser valgt blandt essentielle olier, planteekstrakter, humater, såsom leonardit, tørv, aluminosilikater, tannin, hørfrø, polyphenoler, saponiner, alger og ekstrakter af alger, såsom mikroalger, eksempelvis spirulina eller makroalger, eksempelvis grøn-, rød-, eller brun-alger, produktet af fermenteringen af Aspergillus oryzae og blandinger deraf.

6. Sammensætning ifølge ethvert af kravene 1-5, **kendetegnet ved, at** de præbiotiske fibre er valgt blandt fructo-oligosaccharider, linolin, mannan-oligosaccharider, beta-glucaner, arabino-xylo-oligosaccharider, xylo-oligosaccharider og blandinger deraf.

7. Sammensætning ifølge ethvert af kravene 1-6, **kendetegnet ved, at** fordøjelsesenzymerne er en blanding af amylolytiske, fibrolytiske, proteolytiske og phospholytiske enzymer.

8. Sammensætning ifølge ethvert af kravene 1-7, **kendetegnet ved, at** probiotikaene er Saccharomyces cerevisiae.

9. Sammensætning ifølge ethvert af kravene 1-7, **kendetegnet ved, at** protetinerne er en blanding af hvedeproteiner, havreproteiner, hørproteiner og bygmaltproteiner.

10. Sammensætning ifølge ethvert af kravene 1-9, **kendetegnet ved, at** pattedyret er en drøvtygger.

11. Sammensætning ifølge ethvert af kravene 1-5, **kendetegnet ved, at** den tilføjes til den daglige ration for pattedyret i en mængde på 40 g til 50 g af sammensætningen pr. 1 kg ration.

12. Foderspandfuld til et ikke-humant pattedyr, omfattende 20 til 30 vægt-% af sammensætningen ifølge ethvert af kravene 1 til 11 i forhold til den totale vægt af denne spandfuld.

13. Foderspandfuld ifølge krav 12, **kendetegnet ved, at** den indeholder monocalciumphosphat, en kilde for natrium såsom natriumchlorid, en kilde for calcium, en kilde for magnesium og/eller oligo-elementer og eventuelt vitaminer.

14. Foderspandfuld ifølge ethvert af kravene 12 eller 13, **kendetegnet ved, at** den ikke indeholder melasse.

15. Foderspandfuld ifølge ethvert af kravene 12 til 14, **kendetegnet ved, at** den omfatter i området 4 til 9 vægt-% biprodukter fra korndestillation eller fra ølproduktion med eller uden opløselige stoffer, fortrinsvis området 5 til 7 vægt-% i forhold til den tottale vægt af denne spandfuld, og i området 12 til 24 vægt-% koncentreret Distillers solubles, fortrinsvis i området 15 til 20 vægt-% i forhold til den totale vægt af denne spandfuld.

16. Anvendelse af en sammensætning ifølge ethvert af kravene 1 til 11 eller af en foderspandfuld ifølge ethvert af kravene 12 til 15, for forbedring af den zootekniske funktion for et ikke-humant pattedyr, især en drøvtygger.

17. Anvendelse af en sammensætning ifølge ethvert af kravene 1 til 11 eller en foderspandfuld ifølge ethvert af kravene 12 til 15, for forøgelse af fordøjeligheden af tørstoffet og fibre, især foder og koncentrater, og/eller forbedring af optagelsesindeks og/eller næringseffektiviteten og/eller for at forbedre vægtforøgelse og/eller at forbedre intensiteten af fermentering og af mikrobiel metabolisme og/eller til reduktion af afgivelsen af gas, især methan, og/eller til at inhibere protozoer i floraen og/eller for at reducere nedbrydningen af proteiner og/eller for at dirigere fermenteringerne imod produktionen af flygtige fedtsyrer, især imod propionsyre, i et ikke-humant pattedyr, fortrinsvis i en drøvtygger.

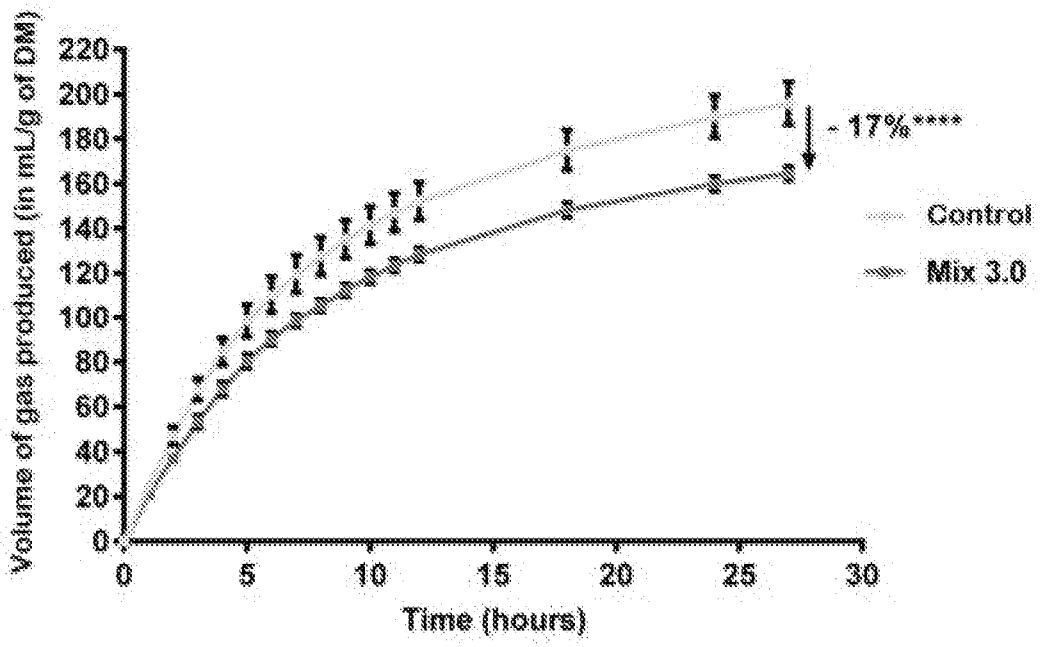
Volume of gas produced (in mL/g of DM)
220
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (hours)
- 17%****
Control
Mix 3.0

FIG 1

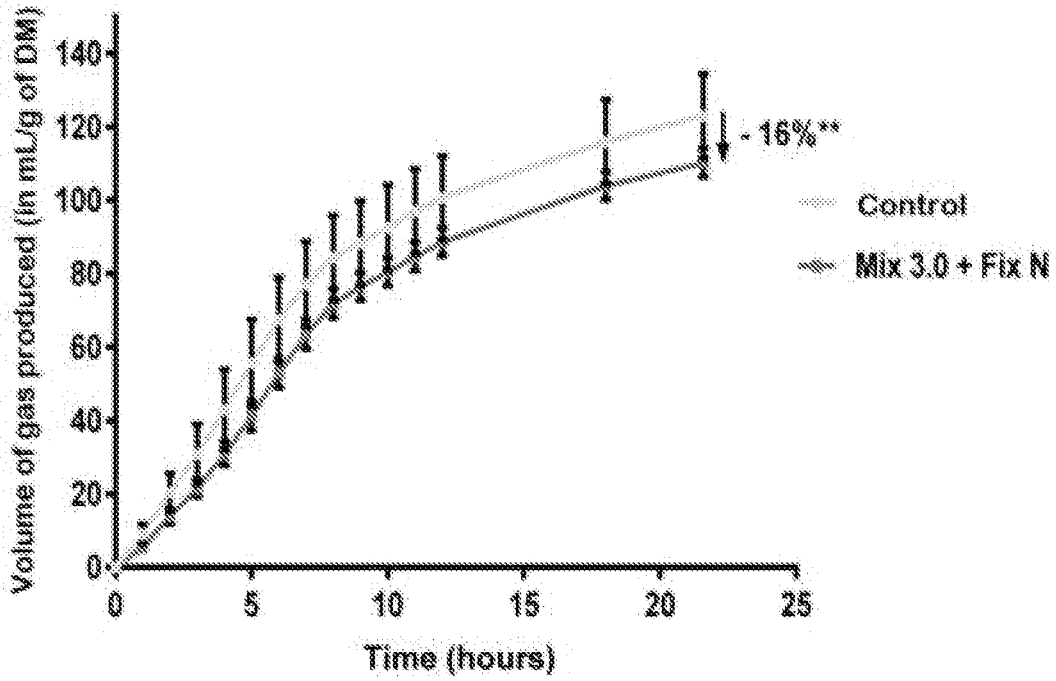
Volume of gas produced (in mL/g of DM)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (hours)
- 16%**
Control
Mix 3.0 + Fix N

FIG 2

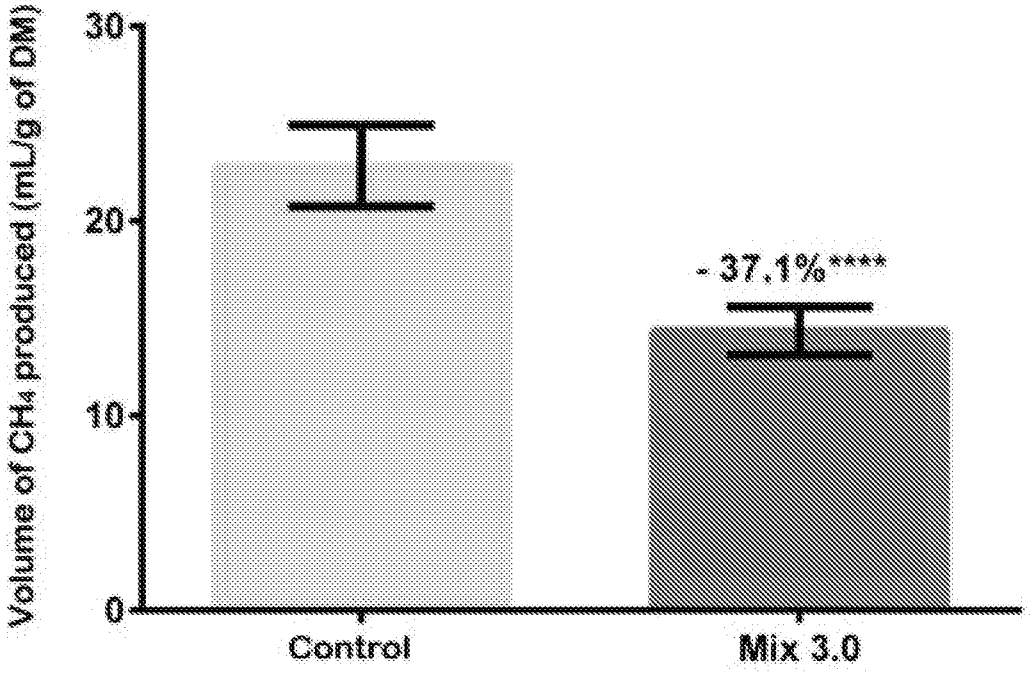
Volume of $CH_4$ produced (mL/g of DM)
30
20
10
0
- 37.1%****
Control
Mix 3.0

FIG 3

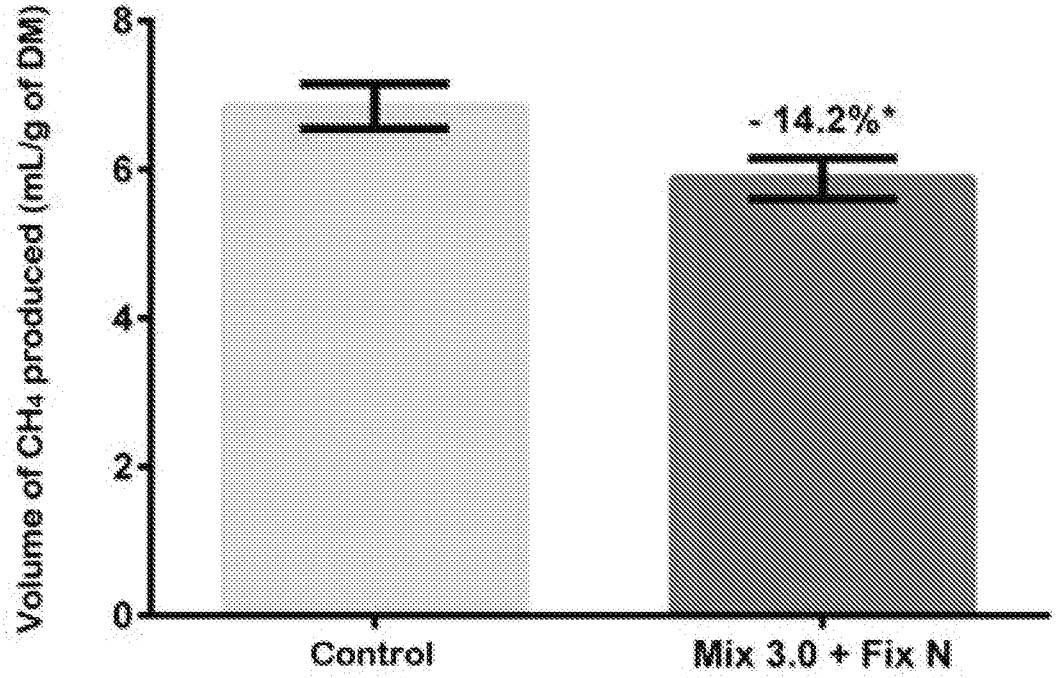
Volume of $CH_4$ produced (mL/g of DM)
8
6
4
2
0
- 14.2%*
Control
Mix 3.0 + Fix N

FIG 4

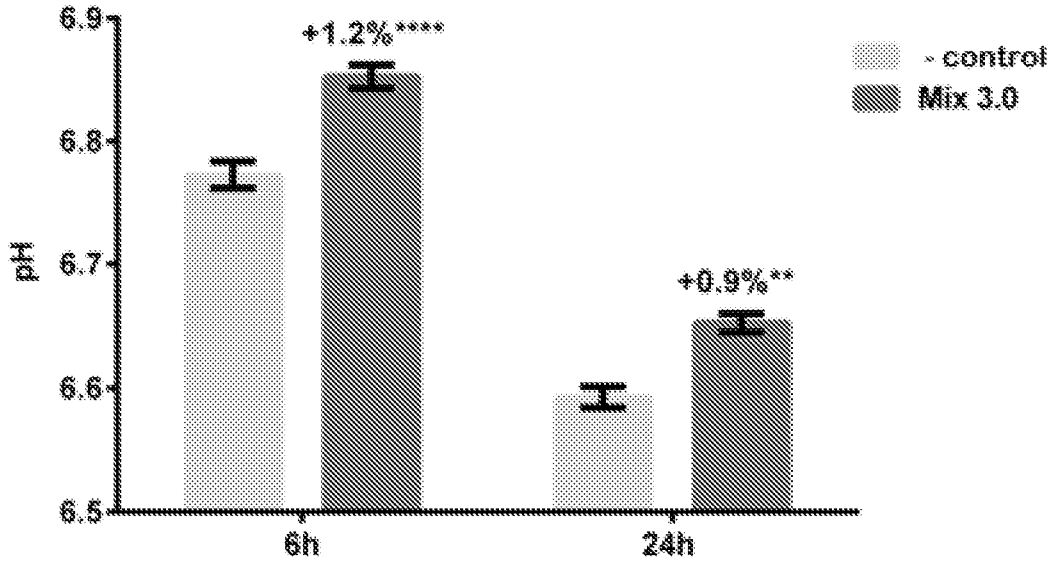
6.9
6.8
6.7
6.6
6.5
pH
+1.2%****
+0.9%**
- control
Mix 3.0
6h
24h

FIG 5

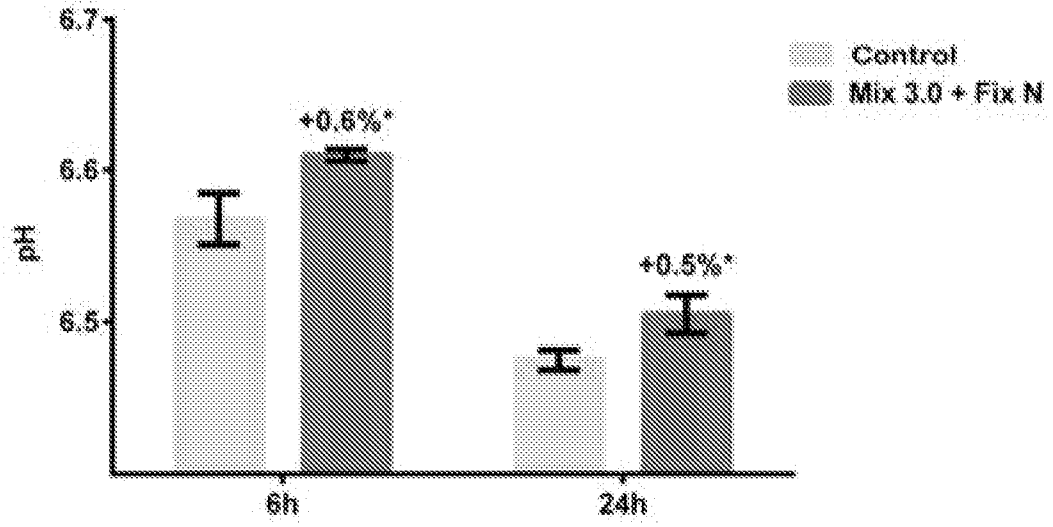
6.7
6.6
6.5
pH
+0.6%*
+0.5%*
Control
Mix 3.0 + Fix N
6h
24h

FIG 6

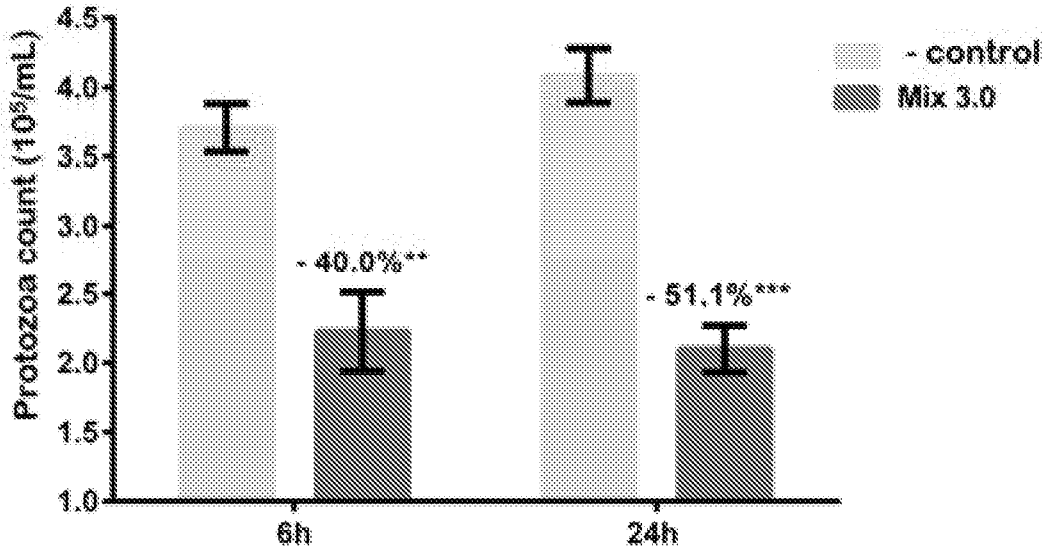
Protozoa count ($10^5$/mL)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
- 40.0%**
- 51.1%***
6h
24h
- control
Mix 3.0

FIG 7

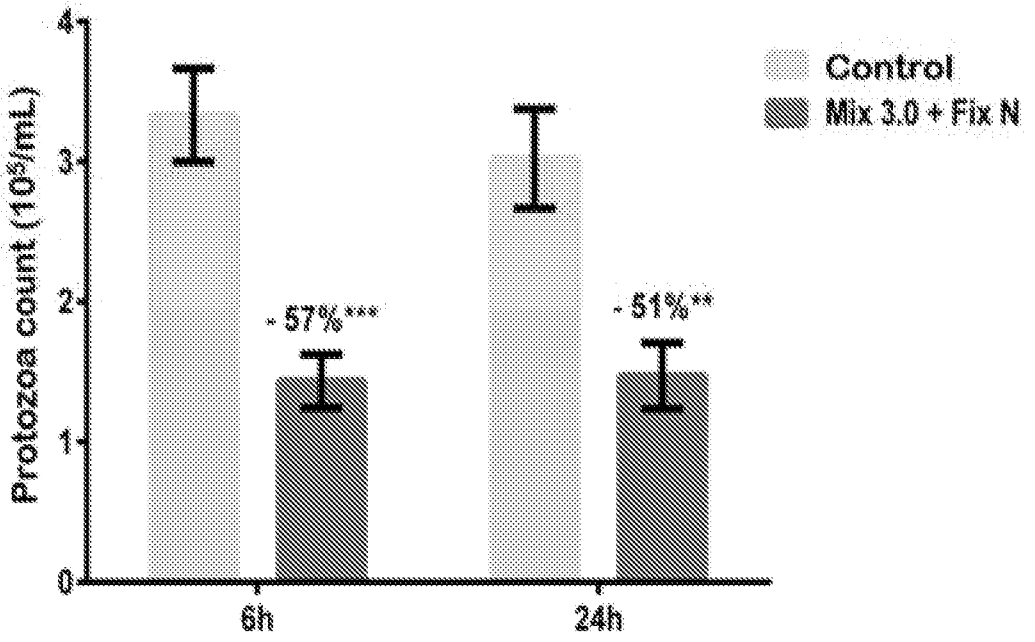
Protozoa count ($10^5$/mL)
4
3
2
1
0
- 57%***
- 51%**
6h
24h
Control
Mix 3.0 + Fix N

FIG 8

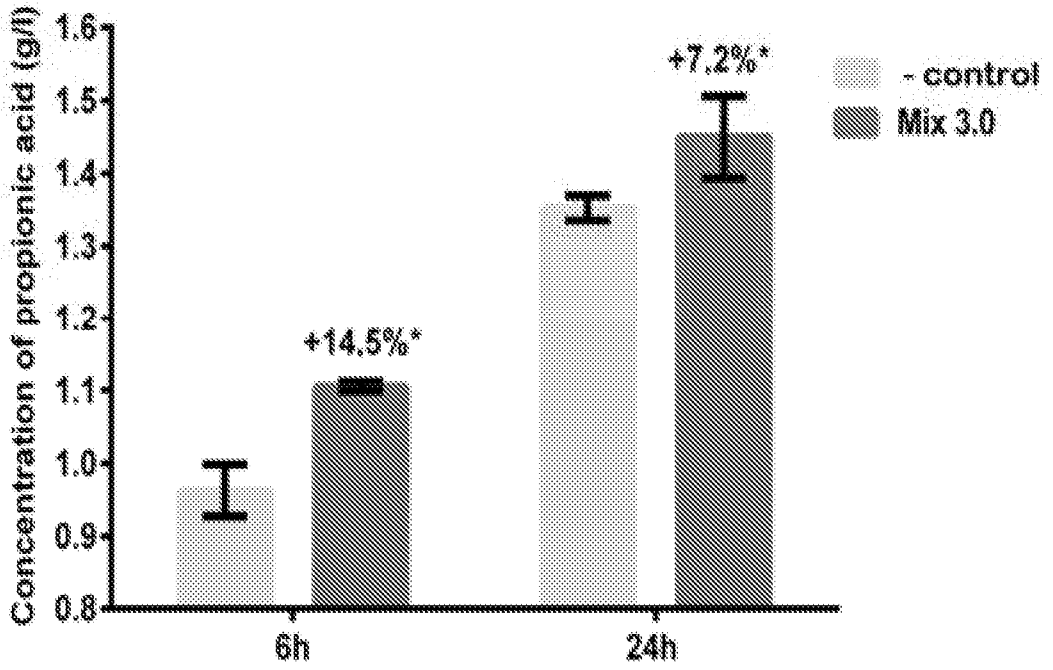
Concentration of propionic acid (g/l)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
+14.5%*
+7.2%*
- control
Mix 3.0
6h
24h

FIG 9

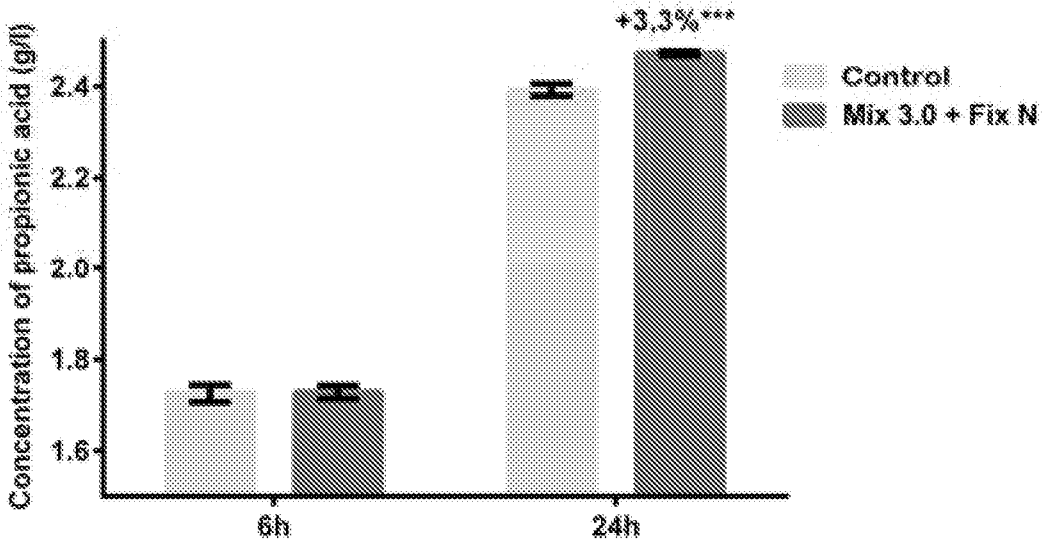
Concentration of propionic acid (g/l)
2.4
2.2
2.0
1.8
1.6
+3.3%***
Control
Mix 3.0 + Fix N
6h
24h

FIG 10

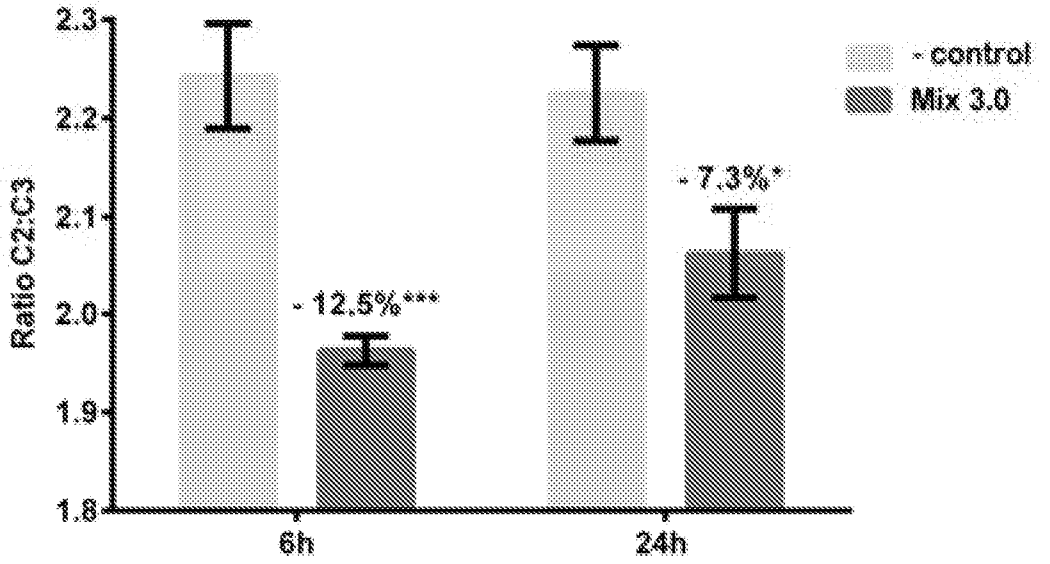
2.3
2.2
2.1
2.0
1.9
1.8
Ratio C2:C3
- 12.5%***
- 7.3%*
6h
24h
- control
Mix 3.0

FIG 11

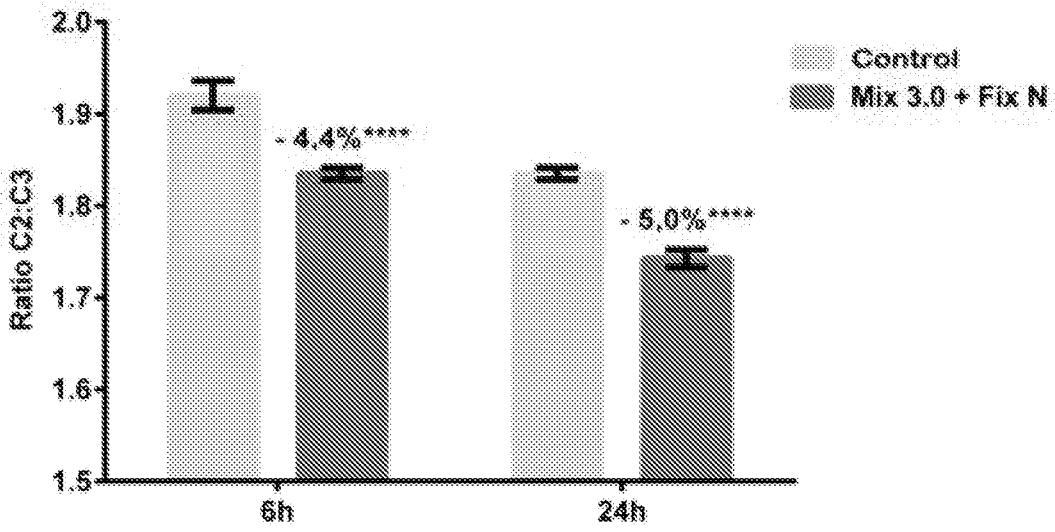
2.0
1.9
1.8
1.7
1.6
1.5
Ratio C2:C3
- 4.4%****
- 5.0%****
6h
24h
Control
Mix 3.0 + Fix N

FIG 12

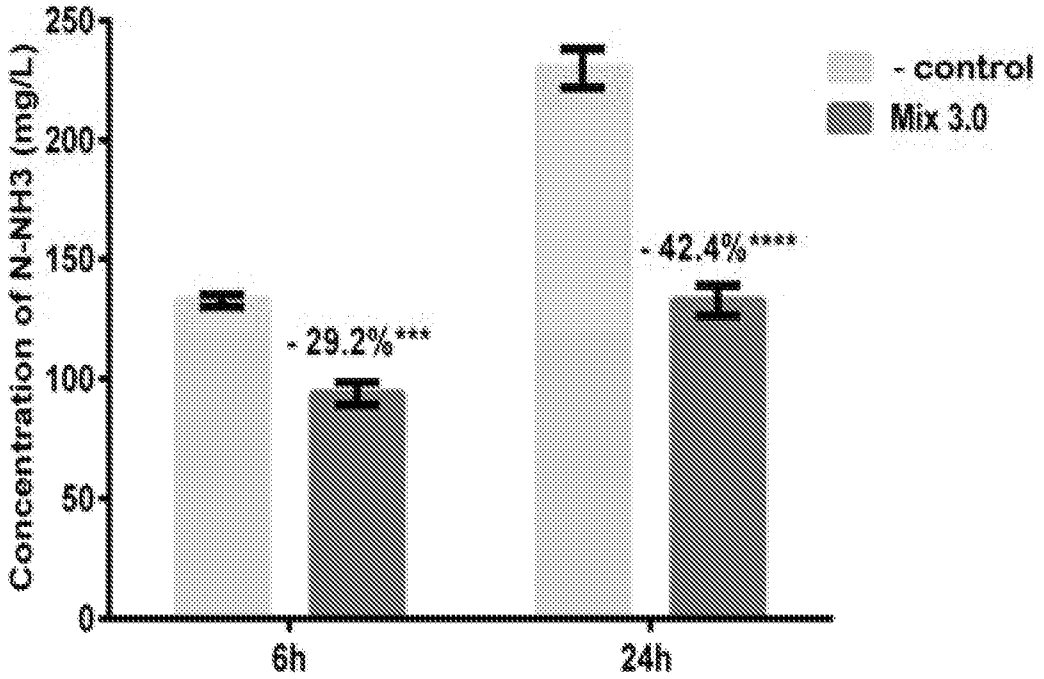
Concentration of N-NH3 (mg/L)
250
200
150
100
50
0
- control
Mix 3.0
- 29.2%***
- 42.4%****
6h
24h

FIG 13

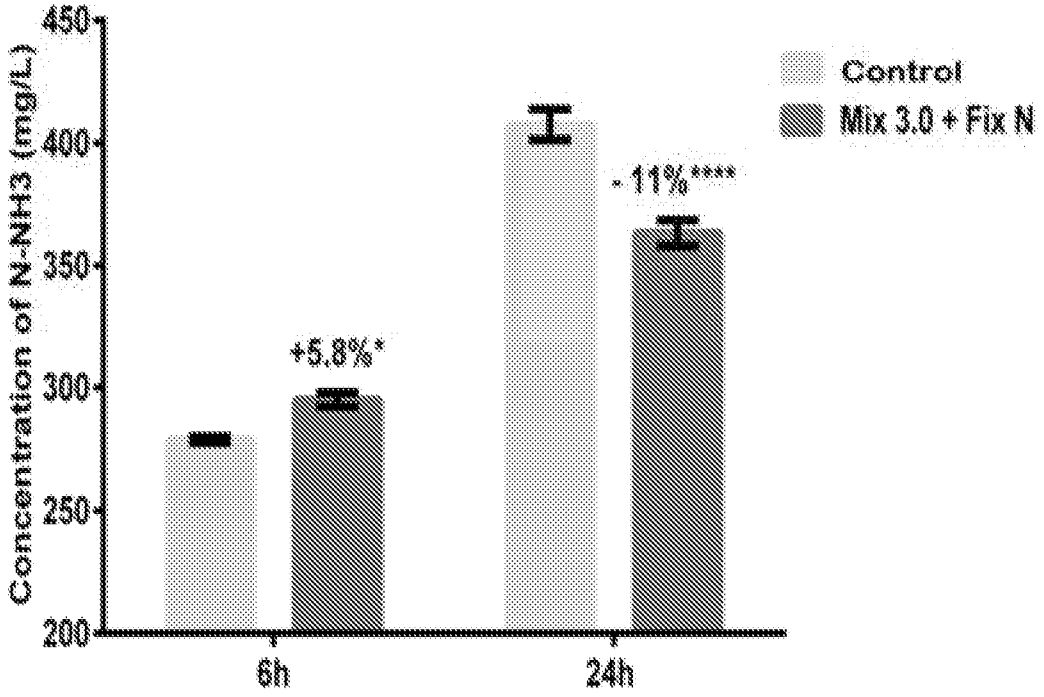
Concentration of N-NH3 (mg/L)
450
400
350
300
250
200
Control
Mix 3.0 + Fix N
+5.8%*
- 11%****
6h
24h

FIG 14

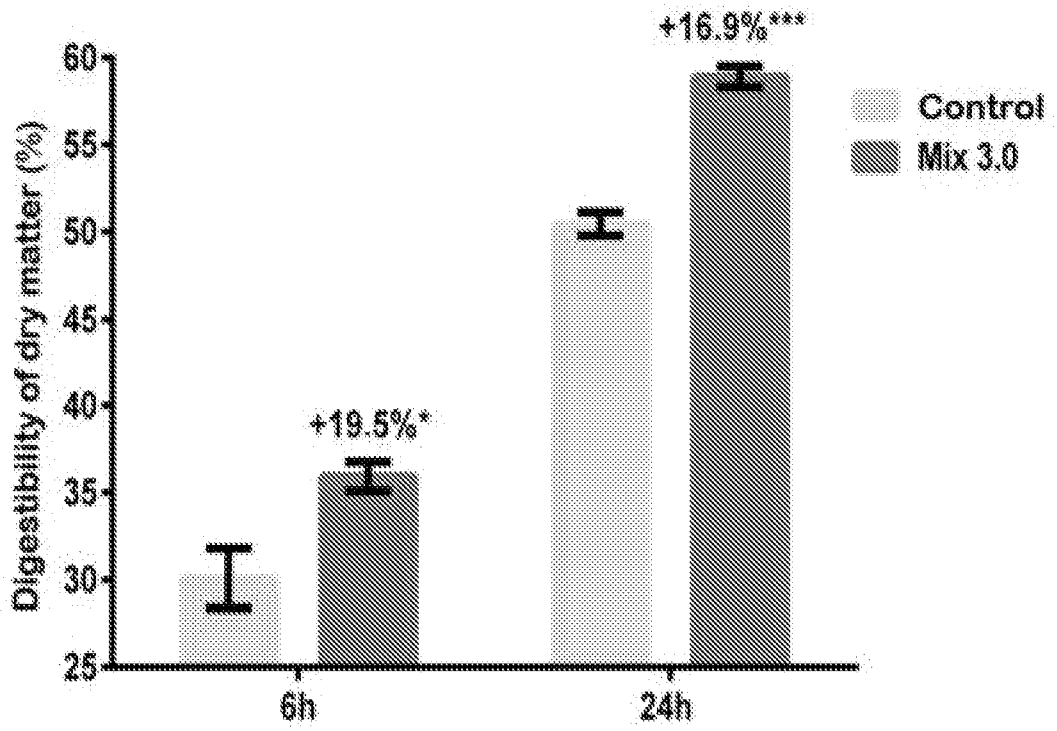
+16.9%***
Control
Mix 3.0
Digestibility of dry matter (%)
60
55
50
45
40
35
30
25
+19.5%*
6h
24h

FIG 15

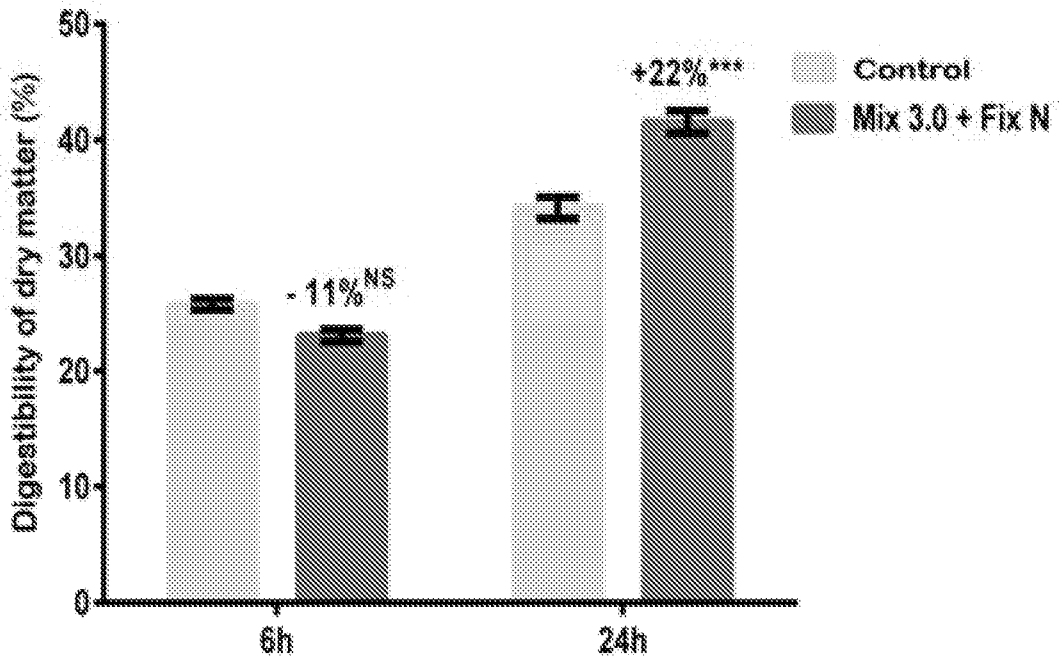
+22%***
Control
Mix 3.0 + Fix N
Digestibility of dry matter (%)
50
40
30
20
10
0
- 11%NS
6h
24h

FIG 16

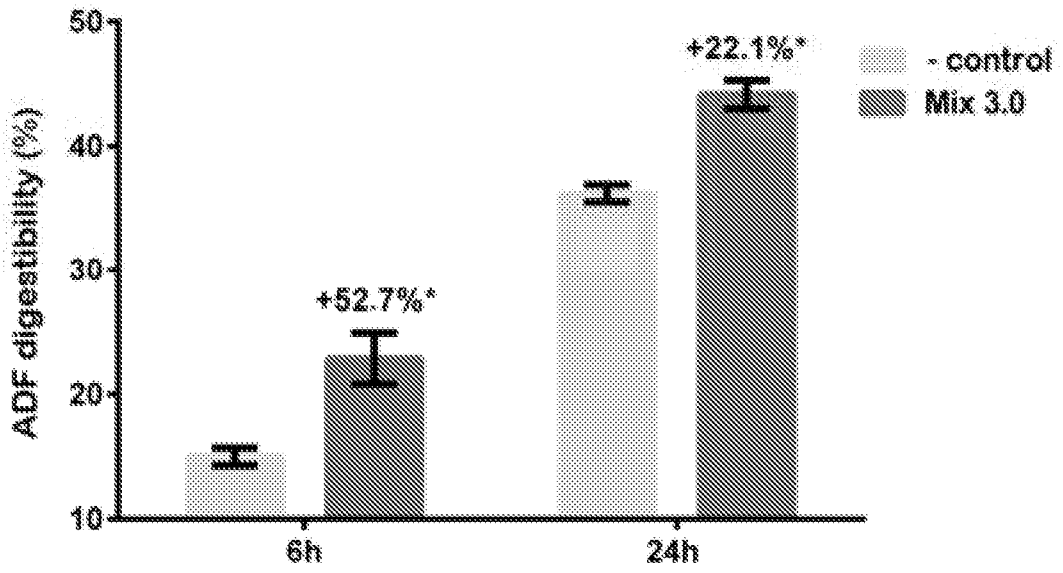
ADF digestibility (%)
50
40
30
20
10
+52.7%*
+22.1%*
- control
Mix 3.0
6h
24h

FIG 17

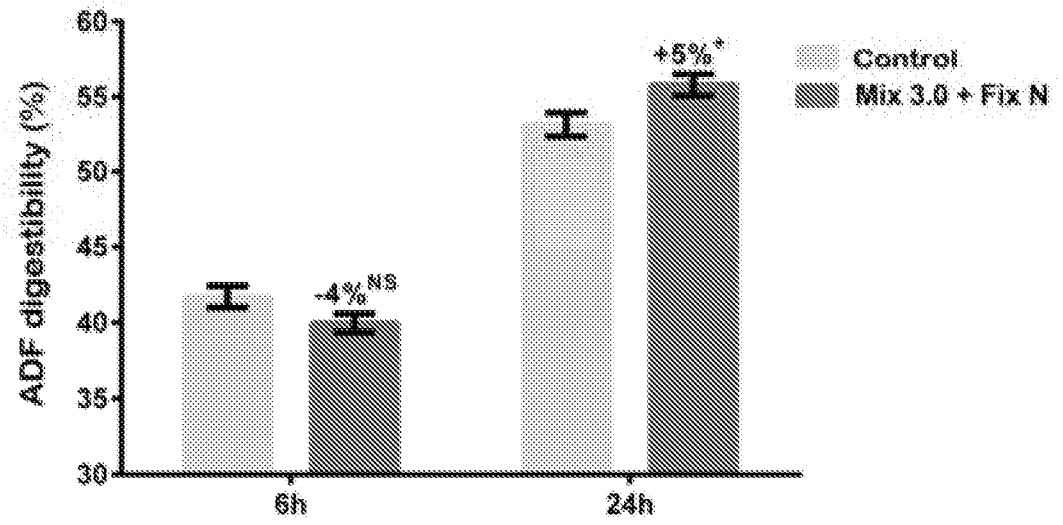
ADF digestibility (%)
60
55
50
45
40
35
30
-4%NS
+5%*
Control
Mix 3.0 + Fix N
6h
24h

FIG 18

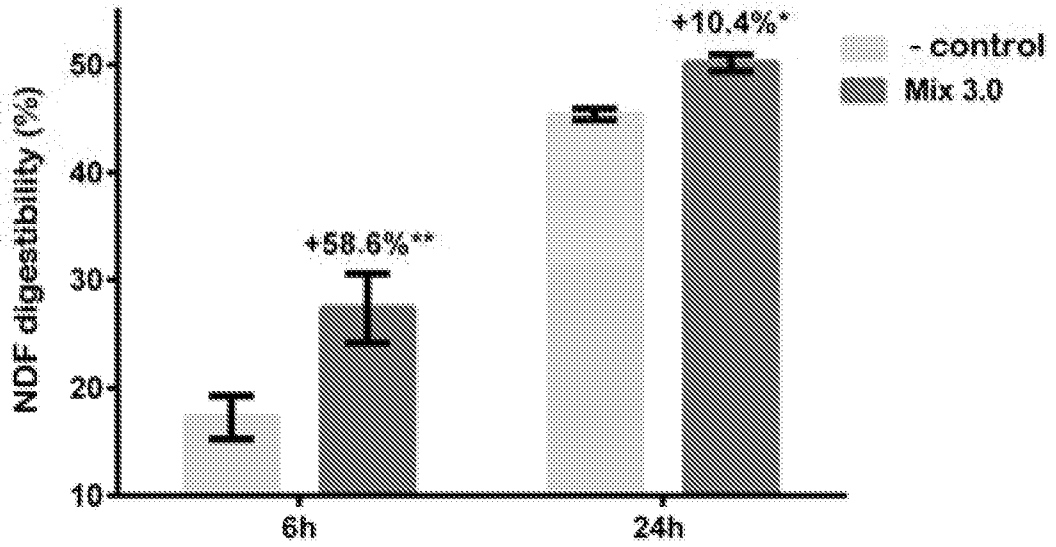
NDF digestibility (%)
50
40
30
20
10
+58.6%**
+10.4%*
- control
Mix 3.0
6h
24h

FIG 19

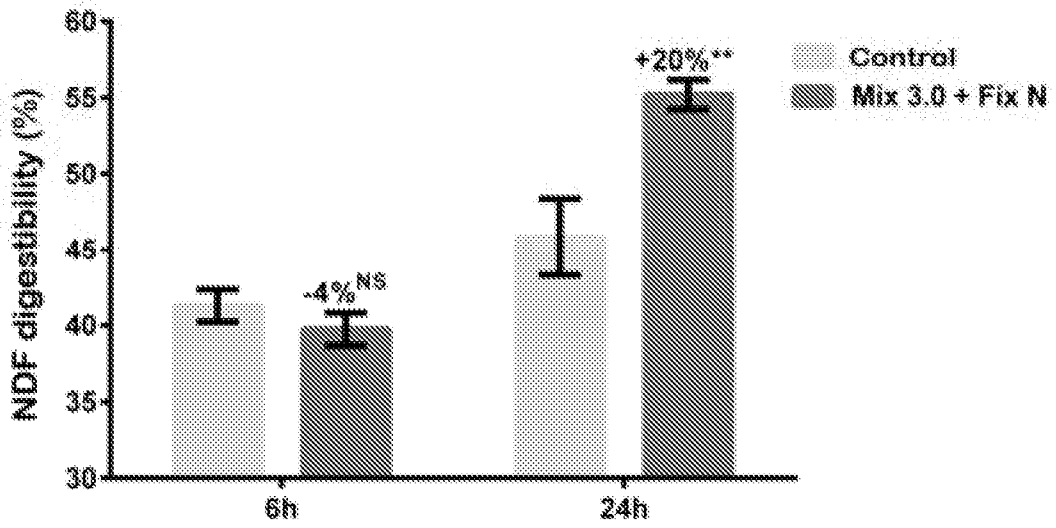
NDF digestibility (%)
60
55
50
45
40
35
30
-4%NS
+20%**
Control
Mix 3.0 + Fix N
6h
24h

FIG 20

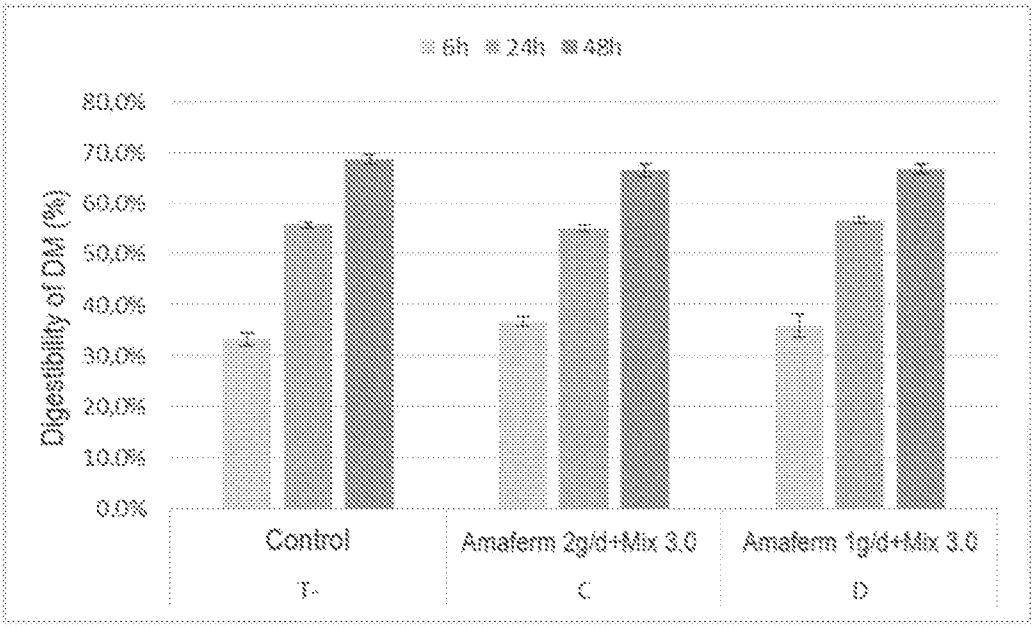
6h
24h
48h
80,0%
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
Digestibility of DM (%)
Control
Amaferm 2g/d+Mix 3.0
Amaferm 1g/d+Mix 3.0
C
D

**FIG 21**

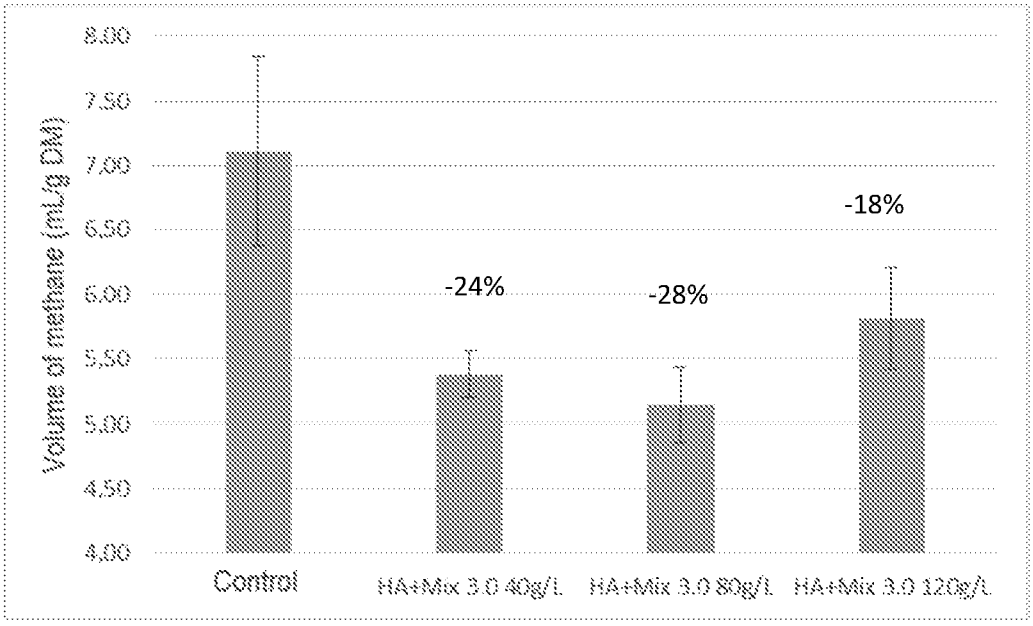
8,00
7,50
7,00
6,50
6,00
5,50
5,00
4,50
4,00
Volume of methane (mL/g DM)
-24%
-28%
-18%
Control
HA+Mix 3.0 40g/L
HA+Mix 3.0 80g/L
HA+Mix 3.0 120g/L

**FIG 22**

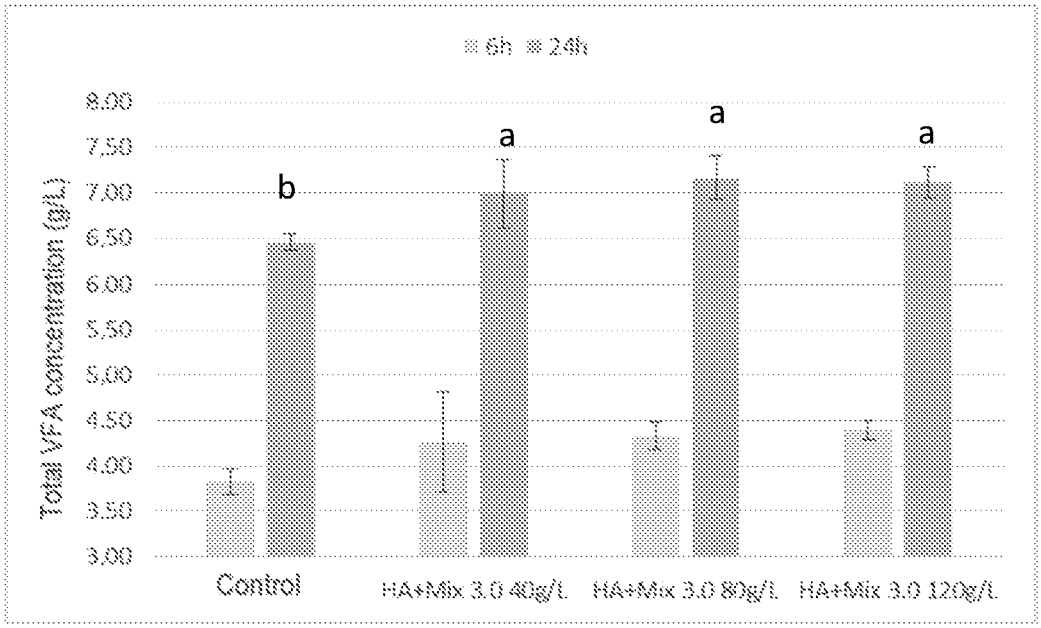
6h
24h
Total VFA concentration (g/L)
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
b
a
a
a
Control
HA+Mix 3.0 40g/L
HA+Mix 3.0 80g/L
HA+Mix 3.0 120g/L

**FIG 23**

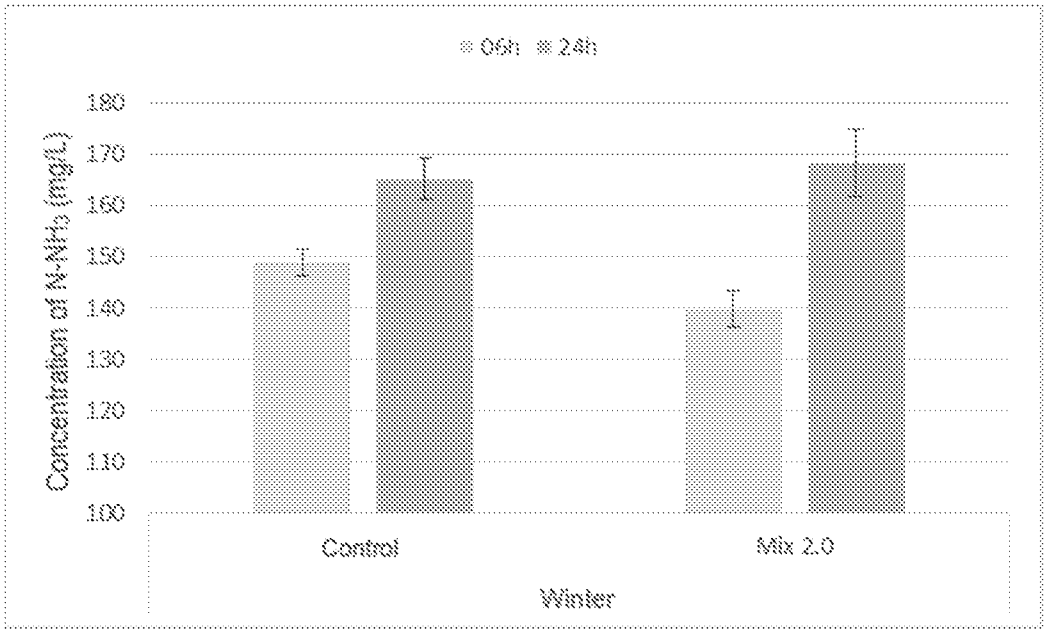
06h
24h
Concentration of N-NH3 (mg/L)
180
170
160
150
140
130
120
110
100
Control
Mix 2.0
Winter

**FIG 24**

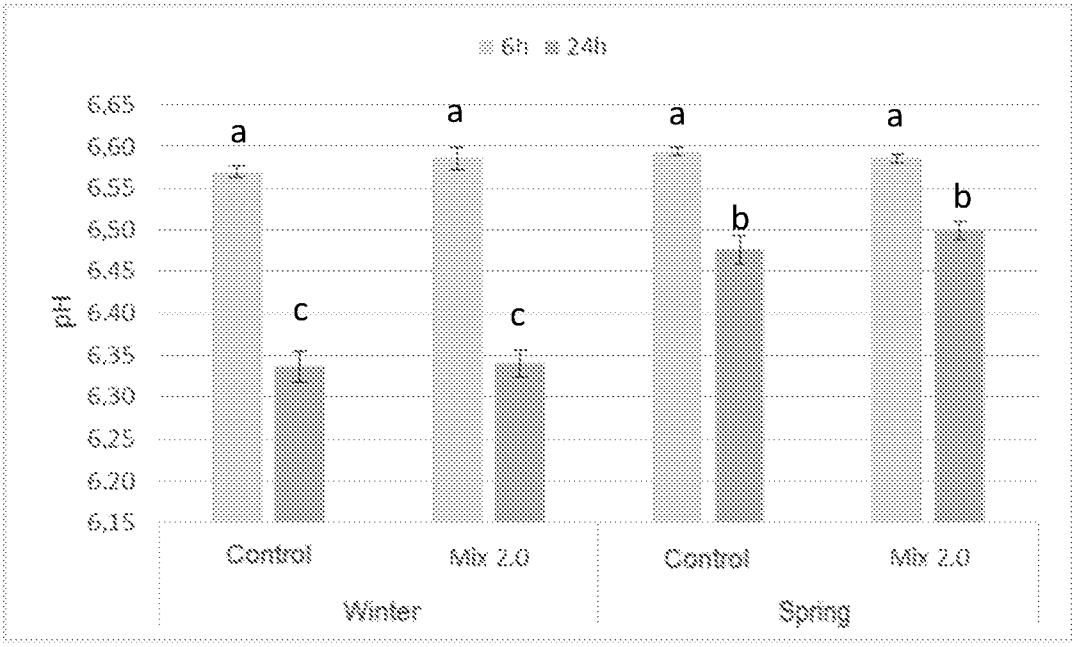
6h
24h
6,65
6,60
6,55
6,50
6,45
6,40
6,35
6,30
6,25
6,20
6,15
pH
a
c
a
c
a
b
a
b
Control
Mix 2.0
Control
Mix 2.0
Winter
Spring

**FIG 25**