Title: A METHOD FOR THE MANAGEMENT OF FAECAL CONSISTENCY IN DOGS

Abstract: The present invention relates to means for defecation and/or faeces management in a pet comprising feeding the pet with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil or similar vehicle.
A METHOD FOR THE MANAGEMENT OF FAECAL CONSISTENCY IN DOGS

Technical field of the invention

The present invention relates to defecation management and improvements of the faeces consistency and faeces condition in pets. The vacuum infused pet food kibbles of the present invention changes and optimises the various scores of the PURINA system surprisingly well under high volume manufacturing and modern logistics.

Background of the invention

The well-being of domestic animals is closely related to their feeding. Correct feeding should result in a fit and healthy pet. In addition to providing nutritional value, food composition influences the intestinal microflora equilibrium and may lead to or prevent gastrointestinal disorders. Therefore, knowledge on the gastrointestinal tract and digestion processes of healthy animals is integral to the understanding of a practical feeding practice. As carnivores, cats and dogs are e.g. characterized by a short digestive tract and a rapid flow rate of the bolus of food.

The number and composition of this endogenous flora tend to be rather stable, although age and, to a lesser degree, food may modify it. Gastric acidity, bile, intestinal peristalsis and local immunity are factors thought to be important in the regulation of bacterial flora in the small intestine of human beings and various other mammals.

Often canine and feline gastrointestinal disorders are linked to bacterial overgrowth and the production of enterotoxins produced by pathogenic bacteria.

During the last few years, research has focused on some valuable strains of Lactic Acid Bacteria (LAB) and their potential use as probiotic agents. Probiotics are considered to be viable microbial preparations which promote mammalian health by preserving the natural microflora in the intestine. Probiotics are believed to attach to the intestinal mucosa, colonize the intestinal tract and thereby prevent attachment of harmful micro-organisms thereon. A prerequisite for their action
resides in that they have to reach the gut's mucosa in a proper and viable form and especially do not get destroyed by the influence of the low pH prevailing in the stomach. In particular, the physiology of the digestive tract of cats and dogs differs from humans. For example, the average pH in the stomach is about 3.4 for dogs and 4.2 for cats.

Although many references discloses the inclusion of a probiotic micro-organism in a pet food, the available art is does not disclose the effects of products with sufficient CFUs (Colonies Forming Units) in the feed which comply with high volume manufacturing and modern logistics.

Consequently, there is a need to provide pet food compositions that are still after shelf life of months able to quickly levelling the faeces conditions of the pets. Rapid levelling of defecation and diarrhoea to more neutral stool conditions is of both great health and general nutritional concern.

**Summary of the invention**

Thus, an object of the present invention relates to means for defecation and/or faeces management in a pet comprising feeding the pet with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil or similar vehicle.

In particular, it is an object of the present invention to provide a method for defecation management in a dog comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil that solves the above mentioned problems of the prior art with high volume manufacturing and modern logistics and still retaining the beneficial effects of the product.

Other aspects and preferred embodiments will be apparent from the following detailed description of the invention.
**Brief description of the figures**

Figure 1
Shows the Purina score system. Figure IA-G corresponds to a Purina score of 1-7, respectively.

Figure 2
Shows a Bristol Stool Chart. The Bristol Stool Chart uses a scoring system that is similar to the Purina score with type 1-7.

Figure 3
Shows the Purina score of dog no. 1 - Elodi during the 5 week testing period.

Figure 4
Shows the Purina score of dog no. 2 - Galicia during the 5 week testing period.

Figure 5
Shows the Purina score of dog no. 3 - Georges during the 5 week testing period.

Figure 6
Shows the Purina score of dog no. 4 - Esteban during the 5 week testing period.

Figure 7
Shows the Purina score of dog no. 5 - Doyka during the 5 week testing period.

Figure 8
Shows the Purina score of dog no. 6 - Grisa during the 5 week testing period.

Figure 9
Shows the Purina score of dog no. 7 - Gripala during the 5 week testing period.
Figure 10
Shows the Purina score of dog no. 8 - Weimie during the 5 week testing period.

Figure 11
Table 1. Viscosity of selected oil types vs temperature. The viscosity was measured using a rheometer. Delta viscosity between 20°C and 25°C is indicated. For further details see Example 11.

Figure 12
The figure displays the viscosity of selected oil types versus temperature within the temperature interval of 20-25°C. 1: Crude fish oil, 2: Salmon oil A, 4: Cod liver oil, 5: Salmon oil B. For further details see Example 11.

Figure 13
Figure 13 shows the viscosity of selected oil types versus temperature within the temperature interval of 20-25°C.

Figure 14
Figure 14 shows the viscosity of selected vegetable oil types versus temperature within the temperature interval of 20-25°C.

Figure 15
Figure 15 shows the viscosity of linseed oil versus temperature within the temperature interval of 15-35°C.

Figure 16
Figure 16 shows the viscosity of salmon oil A with (susp) or without (raw oil). Suspension comprises probiotics at a concentration/inclusion rate 1.2 kg/ton of final product. Data are shown for an increasing temperature from 5 to 50°C (arrow pointing to the right) and for a decreasing temperature from 50 to 5°C.
The present invention will now be described in more detail in the following.

**Detailed description of the invention**

The present invention describes numerous applications of defecation management or faeces management in pets.

It is an object of preferred embodiments of the present invention to provide means for defecation and/or faeces management in a pet comprising feeding the pet with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil or similar vehicle.

**Vacuum infused pet food**

Vacuum infusion

The terms "vacuum infusion" refers to inclusion of a substance into an object by means on vacuum. On limiting examples of vacuum infusion are infusion of a suspension (comprising a vehicle and at least one probiotic micro-organism) in a of porous food matrices such as a pet food kibble by means of a vacuum infusion process, vacuum infusion of a fat, and vacuum infusion of a digest in e.g. a pet food product such as a pet food kibble.

**Food product**

The term "food product" as used herein refers to any food product to which the beneficial function of probiotics is wished to be added. For example, it may be a breakfast cereals, pet food, treats. However, it may be any food, intended for any humans and/or animals. For example, the food product may be a particulate food or food ingredient, such as extruded snack products, tortilla chips, breakfast cereal, cookies, crisp bread, food foams, Rice broken, blend of peanut, soybean and corn, puffed wheat, low density foamed corn and rice breakfast, Co-extruded products, muesli bars and any other extruded products that are formed by
extrusion process. "Pet food" in the context of the present invention refers to food products obtained by methods of extrusion. For example, a food kibble such as a dog food kibble.

5 Kibble
As used herein refers kibble to pellets of dry dog food that are produced by one of two methods, extrusion and baking. During the extrusion process, cut dough or a mixture of raw materials is fed into an expander, while pressurized steam or hot water is added. When removed from the high pressure that results, the pellets puff up like popcorn. The resultant kibble is allowed to dry, and is then sprayed with vitamins, fats and oils, or any other ingredients that are not heat-tolerant. The exact procedure for the production of kibble can vary according to the desired product.

15 Probiotic micro-organisms
Probiotics, which means, "for life," have been used for centuries as natural components in health-promoting foods. Probiotic micro-organisms or simply probiotics refers to dietary supplements of live bacteria thought to be healthy for the host organism. According to the currently adopted definition by FAO/WHO, probiotics are: 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'.

Lactic acid bacteria (LAB) are the most common type of microbes used as probiotics. LAB has been used in the food industry for many years, because they are able to convert sugars (including lactose) and other carbohydrates into lactic acid. This not only provides the characteristic sour taste of fermented dairy foods such as yogurt, but also, by lowering the pH, may create fewer opportunities for spoilage organisms to grow, hence creating possible health benefits by preventing gastrointestinal infections.

30 The term "probiotic" as used herein is defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance.
Examples of suitable probiotic micro-organisms include yeasts such as Saccharomyces, Debaromyces, Candidaw Pichia and Torulopsis, moulds such as Aspergillus, Rhizopus, Mucor, and Penicillium and Torulopsis and bacteria such as the genera Bifidobacterium, Bacteroides, Clostridium, Fusobacterium,


Strains of the genera Lactobacillus, Enterococcus and Bifidobacterium, are the most widely used probiotic bacteria, and is thus a preferred embodiment of the present invention.

The choice of probiotic micro-organism depends on the specific application in question e.g. pet food such as dog food. Enterococcus faecium is suitable for probiotic dog food. Thus, in a preferred embodiment the at least one probiotic micro-organism is Enterococcus faecium (e.g. (E1707)). The suspension may subsequently be used for the preparation of a probiotic extruded food product for dogs (e.g. a probiotic dog food kibble comprising Enterococcus faecium). In a particular embodiment, the at least one probiotic micro-organism is the NCIMB
10415 strain of *Enterococcus faecium*. The NCIMB 10415 strain may be EC No. 13 (E1707 (new classification)).

Probiotic bacterial cultures are intended to assist the naturally occurring gut flora, ecology of microbes, to re-establish themselves.

Maintenance of a healthy gut flora is, however, dependent on many factors, especially the quality of food intake. Probiotics are a valuable factor herein.

10 **Colony forming unit - CFU**

Colony-forming unit (CFU) refers to a measure of viable bacterial or fungal numbers. Unlike in direct microscopic counts where all cells, dead and living, are counted, CFU measures viable cells. By convenience the results are usually given as colony-forming units per millilitre (CFU/mL) or colony-forming units per gram (CFU/gram).

An embodiment of the present invention relates to a method wherein the kibble contains above $10^3$ CFU per gram of the kibble, such as above $10^4$ CFU per gram of the kibble, such as above $10^5$ CFU per gram of the kibble, such as above $10^6$ CFU per gram of the kibble, such as above $10^7$ CFU per gram of the kibble, such as above $10^8$ CFU per gram of the kibble, such as above $10^9$ CFU per gram of the kibble, such as above $10^{10}$ CFU per gram of the kibble, such as above $10^{11}$ CFU per gram of the kibble, such as above $10^{12}$ CFU per gram of the kibble, such as above $10^{13}$ CFU per gram of the kibble, such as above $10^{14}$ CFU per gram of the kibble, such as above $10^{15}$ CFU per gram of the kibble, such as above $10^{16}$ CFU per gram of the kibble, such as above $10^{17}$ CFU per gram of the kibble.

In another embodiment the kibble contains $10^6$. $10^9$ CFU/kg, such as $10^6$. $10^{16}$, such as $10^6$. $10^{12}$, such as $10^7$. $10^{14}$, such as $10^7$. $10^{12}$, such as $10^7$. $10^{10}$, or such as $10^8$. $10^{10}$ CFU/kg.

To produce the desirable effect a minimum concentration of micro-organisms must be able to survive ingestion and grow in the intestine. However, the minimum effective dose of live bacteria cannot be easily identified. It has been suggested that once the concentration of a particular micro-organism fell to $10^7$
per g of faeces, it does not play a role in the ecosystem provided that it remains below this level at all times. This is supported by observations that the host animal can tolerate populations less than $10^7$ Clostridia or enterobacteria per gram of intestinal contents. It is therefore postulated that a probiotic will be effective if it provides at least $10^7$ CFU and hence these levels have been adopted as a minimum dose.

Pet
The term "pet" refers to an animal kept for companionship and enjoyment or a household animal, as opposed to livestock, laboratory animals, or working animals, which are kept for economic reasons. Accordingly, pet food is a food product intended for consumption by a pet (such as a dog food or a cat food).

Extrusion, extruded
The terms "extrusion" or "extruded" refers in the present context to "cooking extrusion" which is a combination of heating of food products with the act of extrusion to create a cooked and shaped food product and is a process in which moistened, starchy, proteinaceous foods are cooked and worked into a viscous, plastic-like dough. The results of cooking the food ingredients during extrusion may be: 1) gelatinization of starch, 2) denaturation of protein, 3) inactivation of raw food enzymes, 4) destruction of naturally occurring toxic substances, and 5) diminishing of microbial counts originating from the pre-extruded product. Upon discharge through the die, the hot, plastic extrudate expands rapidly with loss of moisture and heat because of sudden decrease in pressure. After expansion cooling, and drying, the extruded product develops a rigid structure and maintains a porous texture.

Density
The term "density" of a material is defined as the mass of the material per unit volume (g/L).
Suspension

Suspension refers to a fluid (such as an oil) containing particles that will not
dissolve in the fluid and are sufficiently large for sedimentation such as freeze-
dried micro-organisms. A homogenous suspension refers to a suspension, wherein
the particles are dispersed throughout the external phase (the fluid) through
mechanical agitation (such as mixing). The suspended particles (e.g. micro-
organisms) are visible under a microscope and will settle over time if left
undisturbed.

Oil

The oil may be any to any edible vegetable and animal oils or a combination of at
least one edible vegetable and one edible animal oils. Accordingly, in one
embodiment the oil is selected from the group consisting of vegetable oils and
animal oils or a combination thereof. Animal oils include fish oil. In a further
embodiment, the oil is selected the group consisting of vegetable oils and fish oils.
In embodiment of the present invention the oil is a fish oil. The fish oils in the
context of the present invention include but are not limited to salmon oil,
mackerel oil, lake trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil,
sand eel oil \( (Ammodytes tobianus) \), and menhaden oil. Thus, in an embodiment,
the fish oil is selected from the group consisting of salmon oil, mackerel oil, lake
trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil, sand eel oil
\( (Ammodytes tobianus) \), and menhaden oil. In a further embodiment, the fish oil is
salmon oil. The oil may be refined oil, a crude oil or a mixture of oils. Thus, in one
embodiment the oil is crude fish oil.

The source of the oil may also be suitable vegetable oils. Thus in one
embodiment, the oil is a vegetable oil, such as oil of flax or flax seed (commonly
known as linseed). In another embodiment, the oil is selected from linseed oil,
olive oil, borage oil, lin oil, camelina oil, grape seed oil, chia oil, kiwifruit seeds oil,
perilla oil, lingonberry, purslane oil, seabuckthorn oil, hemp oil, refined maize oil,
soy bean oil, sunflower oil. In a further embodiment, the oil is linseed oil. Linseed
oil has unique viscosity properties as described in the present application, which
may make it a unique oil vehicle.
In the context of the present invention the term "oil" refers to any edible vegetable and animal oils. Oil in the context of the present invention is in a viscous liquid state ("oily") at room temperature. Oil includes "fatty acids", which are carboxylic acids often with a long un-branched aliphatic tail (chain), which is either saturated or unsaturated (such as monounsaturated or polyunsaturated). The ratio of saturated to unsaturated fatty acids varies among oils. For example, flaxseed oil comprises 9% of saturated fatty acids, 18% mono-unsaturated fatty acids, and 73% of polyunsaturated fatty acids. In contrast, coconut oil comprise 91% saturated fatty acids, 7% mono-unsaturated fatty acids, and 2% poly-unsaturated fatty acids. For dietary application oils rich in unsaturated fatty acids are highly preferred due to the health benefits of the unsaturated fatty acids over the saturated fatty acids. Thus, in order to sustain the key health benefits and features of the food product, the products described in this invention preferably comprises a high level of unsaturated fatty acids. Fish oils fall within the definition of oil. Fish oils include but are not limited to salmon oil, mackerel oil, lake trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil, sand eel oil \((Ammodytes tobianus)\), and menhaden oil.

**Fish oil**

Fish oil refers to oil derived from the tissues of oily fish. Fish oil is recommended for a healthy diet because it contains the omega-6 and omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

**Omega-3 fatty acids**

The term "omega-3 fatty acids" are a family of unsaturated fatty acids that have in common a final carbon-carbon double bond in the n-3 position; that is, the third bond from the methyl end of the fatty acid. Examples of important nutritionally essential omega-3 fatty acids are \(\alpha\)-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

**Omega-6 fatty acids**

The term "omega-6 fatty acids" are a family of unsaturated fatty acids which have in common a final carbon-carbon double bond in the n-6 position; that is, the
sixth bond from the end of the fatty acid. Examples of omega-6 fatty acids are linoleic acid and arachidonic acid.

Vehicle

"Vehicle" or "carrier" refer to a fluid component (such as an oil) that carries at least one substance. In the context of the present invention an oil is used as vehicle for vacuum infusion of at least one probiotic micro-organism into an extruded food product. The vehicle may have the additional function of preserving the at least one probiotic micro-organism embedded in the extruded food product.

Accordingly, at least one oil used by the present invention functions as vehicle for infusion of probiotic micro-organisms in the manufacturing of an extruded food product. The manufacturing is performed at room temperature in order to optimize the probiotic count (CFU) in the final food product. In this respect the viscosity properties of the oil (e.g. dynamic viscosity) influence whether or not the oil is suitable for the vacuum infusion of the food product. Oils having an optimal viscosity at a temperature above room temperature may not be applicable at room temperature due to the change in viscosity.

Viscosity

The term "viscosity" refers to a measure of the resistance of a fluid which is being deformed by either shear stress or extensional stress. In everyday terms (and for fluids only), viscosity is "thickness". The coefficient of viscosity is most often used as a value for viscosity. The shear viscosity and dynamic viscosity are most frequently used. "Dynamic viscosity" (or absolute viscosity) is a unit of measuring viscosity. The SI physical unit of dynamic viscosity is the pascal-second (Pa-s), which is identical to kg-mT^-1-s^-1. If a fluid with a viscosity of one Pa-s is placed between two plates, and one plate is pushed sideways with a shear stress of one pascal, it moves a distance equal to the thickness of the layer between the plates in one second. The cgs physical unit for dynamic viscosity is the poise. It is more commonly expressed, particularly in ASTM standards, as centipoise (cP). The relation between poise and pascal-seconds is: 1 cP = 0.001 Pa-s = 1 mPa-s.

Water at 20 °C has a viscosity of 1.0020 cP. Dynamic viscosity is measured with various types of rheometer, for example Physica MCR 301. The temperature
dependence of the viscosity of the fluid is the phenomenon by which fluid viscosity generally decrease (or, alternatively, its fluidity generally increases) as its temperature increases. Thus, close temperature control of the fluid is essential to accurate measurements, particularly in materials like lubricants, whose viscosity can double with a change of only 5 °C. The dynamic viscosity referred to in the context of the present invention is the dynamic viscosity at 20°C if noting else is stated. In the context of the present invention the change in dynamic viscosity of an oil is expressed as \(\Delta \text{Pa-s/}^0\text{C}\). Alternatively, the change in dynamic viscosity of an oil is described as the difference between the dynamic viscosity at 25°C and 20°C (\(\text{Pa-s at 25°C - Pa-s at 20°C} = \Delta \text{Pa-s}\)).

Room temperature
The term "room temperature" or (also referred to as ambient temperature) is denoting the temperature within enclosed space at which humans are accustomed. The room temperature (RT) in the context of the present invention is defined by the range of 5°C to 30°C.

Solid fat index (SFI)
The term "Solid fat index" (SFI) is used herein (SFI) is a measure of the percentage of fat in crystalline (solid) phase to total fat (the remainder being in liquid phase) across a temperature gradient.

Peroxide value
The best test for autoxidation (oxidative rancidity) is determination of the "peroxide value". Peroxides are intermediates in the autoxidation reaction. The number of peroxides present in edible fats and oils is an index of their primary oxidative level and consequently of its tendency to go rancid. The lower is the peroxide value, the better is fat or oil quality and its status of preservation. Other methods are available but peroxide value is the most widely used. The double bonds found in fats and oils play a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. Autoxidation is a free radical reaction involving oxygen that leads to deterioration of fats and oils which form off-flavours and off-odours. Peroxide value, concentration of peroxide in an oil or fat, is useful for assessing the extent to which spoilage has advanced.
The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. Typically this is expressed in units of milliequivalents (mequiv or meq). If SI units are used the appropriate unit is millimoles per kilogram (1 millimole = 2 milliequivalents).

The peroxide value of the oil also affects the preservation of the probiotic micro-organisms for which the oil is used as vehicle in the vacuum inclusion of the probiotic micro-organisms in an extruded food product. An oil with a low peroxide value is preferred as vehicle due to the better probiotic preservative properties over an oil with a higher peroxide value.

Preservative
"Preservative" refers to a natural or synthetic substance that is added to the food product to preserve the product. "Probiotic preservative" refers to a substance that preserves the probiotic micro-organism in the sense of the ability of the micro-organism to establish and populate the gastro-intestinal system of the host (e.g. a human being or an animal such as a pet animal). The preservation is reflected in the colony-forming units (CFU) of the final food product and/or the sustained CFU of the final food product over time of storage.

Antioxidant
The term "antioxidant" refers to a substance capable of slowing or preventing the oxidation of other substances. Antioxidants are frequently used as food additives to reduce food deterioration. Both synthetic and natural antioxidants are used. Natural antioxidants have been identified among a wide range of classes of compounds such as flavanoids, cartonoids, tocotrienol, tocopherol and terpenes (such as astaxanthin). In one embodiment of the invention the synthetic antioxidant is selected from the group consisting of BHA and BHT and natural antioxidant is selected from the group consisting of Vitamin E, flavonoids, and polyphenols. The natural antioxidant may be provided in the form of an extract for example rosemary or grape seed extracts (comprising resveratrol).
Suspension of probiotic micro-organism and vehicle oil

One aspect of the present invention relates a suspension for vacuum infusion of an extruded probiotic food product, wherein said suspension comprises an oil and at least one probiotic micro-organism in the concentration of $10^6$-$10^{16}$ CFU/kg of said oil and said suspension having a dynamic viscosity of less than 0.08 pascal-second (Pa-s) at 20°C. The suspension is used in the preparation of an extruded food product and serves as a mean of obtaining a probiotic food extruded product characterized by homogenously distribution of the probiotic micro-organisms throughout the porous matrices of the food product. In order to accomplish this object, the substances for the preparation of the suspension should be carefully selected. The suspension in the final form ready for use in the manufacturing of the probiotic food extruded product should enable an efficient vacuum infusion process without interfering with the manufacturing process such as clotting various parts of the apparatus used in the manufacturing. For example, the inventors have experienced that the usage of probiotic/oil suspension may clot the fluidic system e.g. by clotting the nozzle used for spaying the suspension on the product in a vacuum coater. The accumulation of matter from the suspension in the system leading to clotting of the fluidics such clotting of the spraying nozzle may results in premature termination of the production in order to clean and eventually repair the line of production. One key parameter is the viscosity of the probiotic oil suspension for the vacuum infusion process. In their effort to avoid the very unfortunate terminations of the production of the manufacturing of the probiotic food extruded product, the inventors discovered that the importance of the viscosity of the oil used as vehicle in the suspension. Further, the inventors discovered that although the oil may be suitable as such for vacuum infusion, the physical properties of the probiotic/oil suspension based on the oil may be different and the suspension may not be suitable for the vacuum infusion process due to a suboptimal viscosity.

The probiotic/oil suspension of the invention comprises at least one oil and at least one probiotic micro-organism. In one embodiment, the suspension comprises additionally at least one additive. The present invention provides a suspension comprising at least one oil and at least one probiotic micro-organism
for application in a vacuum having a dynamic viscosity of less than 0.08 pascal-second (Pa-s) at 20°C.

Properties of oil used in the suspension

5 Dynamic viscosity of the vehicle oil
The oil component of the suspension serves the purpose of a vehicle. In one embodiment of the invention, the oil of the suspension has a dynamic viscosity of less than 0.08 pascal-second (Pa-s) at 20°C, such as less than 0.075 pascal-second (Pa-s) at 20°C, for example less than 0.070 pascal-second (Pa-s) at 20°C, such as less than 0.065 pascal-second (Pa-s) at 20°C, for example less than 0.060 pascal-second (Pa-s) at 20°C, such as less than 0.055 pascal-second (Pa-s) at 20°C, such as less than 0.050 pascal-second (Pa-s) at 20°C, such as less than 0.045 pascal-second (Pa-s) at 20°C, such as less than 0.040 pascal-second (Pa-s) at 20°C. In one embodiment, the dynamic viscosity of the vehicle oil is less than 0.060 pascal-second (Pa-s) at 20°C. In a further embodiment, the dynamic viscosity of the vehicle oil within the range of 0.050 to 0.07 pascal-second (Pa-s) at 20°C, such as the range of 0.053 to 0.066 pascal-second (Pa-s) at 20°C. An example of an oil having a viscosity at 20°C of less than 0.060 pascal-second (Pa-s) is linseed oil (Vobra Special Petfoods BV, Netherlands).

Δ Pa-s of the oil vehicle between 20°C and 25°C
The change in viscosity of the oil vehicle between 20°C and 25°C may be an important feature of the oil vehicle. Thus, in an embodiment according to the invention, the Δ Pa-s between 20°C and 25°C of the oil vehicle is at least 0.009, such as such as in the range 0.009-0.05 Pa-s, such as in the range 0.01-0.05 Pa-s, such as 0.01-0.04 Pa-s, such as 0.013-0.020 Pa-s, such as in the range 0.013-0.018 Pa-s such as in the range 0.013-0.016 Pa-s. An example of an oil in these intervals is salmon oil A (see figure 11-13).

In the present context delta viscosity (Δ Pa-s) is calculated by subtracting the viscosity at 20°C from the viscosity at 25°C. Viscosity of oils is calculated using the method disclosed in example 11.
In an additional embodiment the oil vehicle has either a dynamic viscosity of less than 0.08 Pa-s or a Δ Pa-s of the oil vehicle between 25°C and 20°C of at least 0.009 Pa-s. Examples of such oils are salmon oil A and linseed oil (see figure 11 and 13).

In yet an embodiment the oil vehicle has a dynamic viscosity of less than 0.08 Pa-s and a Δ Pa-s of the oil vehicle between 25°C and 20°C in the range 0.009-0.05 Pa-s. An example of such an oil is salmon oil A (see figure 11 and 13).

It is to be understood that the intervals provided for the dynamic viscosity and the delta viscosity of the oil vehicles according to the invention also apply to the embodiments relating to the combination of the two embodiments and the embodiments which relate to an alternatives between the two embodiments.

**Classes of oils**

The oil may be any to any edible vegetable and animal oils. Accordingly, in one embodiment the oil is selected from the group consisting of vegetables oils and animal oils. Animal oils include fish oil. In a further embodiment, the oil is selected from the group consisting of vegetables oils and fish oils. In embodiment of the present invention the oil is a fish oil. The fish oils in the context of the present invention include but are not limited to salmon oil, mackerel oil, lake trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil, sand eel oil (*Ammodytes tobianus*), and menhaden oil. Thus, in one embodiment, the oil is selected from the group consisting of salmon oil, mackerel oil, lake trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil, sand eel oil (*Ammodytes tobianus*), and menhaden oil. In a further embodiment, the fish oil is salmon oil. The oil may be refined oil, a crude oil or a mixture of oils. Thus, in one embodiment the oil is crude fish oil.

The source of the oil may also be suitable vegetable oils. Thus, in one embodiment, the oil is a vegetable oil, such as oil of flax or flax seed (commonly known as linseed). In a further embodiment, the oil is linseed oil.
**Saturated versus unsaturated fatty acids**

Oils such as vegetable oils and fish oils are compositions comprising saturated and unsaturated fatty acids. The group of unsaturated fatty acids includes mono-unsaturated fatty acids as well as poly-unsaturated fatty acids. The ratio of saturated to unsaturated fatty acids varies among oils. For dietary application oils rich in unsaturated fatty acids are highly preferred due to the health benefits of the unsaturated fatty acids over the saturated fatty acids. Thus, the oil used in the suspension is preferably rich in unsaturated fatty acids. Thus, in one embodiment, the oil is rich in unsaturated fatty acids such as mono-unsaturated and/or poly-unsaturated fatty acids. Thus in one embodiment the ratio of saturated to unsaturated fatty acids varies in the oils and is less than 5 to 1, such as less than 4 to 1, such as less than 3 to 1, such as less than 2 to 1, such as less than 1 to 1. The content of unsaturated fatty acids in the oil may be higher than the content of saturated fatty such that the ratio of unsaturated to saturated fatty acids is 2 to 1 or more, such as 3 to 1 or more, such as 4 to 1 or more, such as 5 to 1 or more, such as 6 to 1 or more, such as 7 to 1 or more, such as 8 to 1 or more, such as 9 to 1 or more, such as 10 to 1 or more.

The ratio of saturated to unsaturated fatty acids varies among oils. For example, flaxseed oil comprises 9% of saturated fatty acids, 18% mono-unsaturated fatty acids, and 73% of polyunsaturated fatty acids. In contrast, coconut oil comprise 91% saturated fatty acids, 7% mono-unsaturated fatty acids, and 2% poly-unsaturated fatty acids.

In order to sustain the key health benefits and features of the food product, the product described in this invention shall comprise a high level of unsaturated fatty acids. Furthermore, the total amount of fats in the food product shall range 0.5% till 45% of net weight of the product, where preferably the ratio between saturated to unsaturated fats within the total fat content shall range 1/1 - 20/1.

Known health beneficial unsaturated fatty acids are omega-3 (n-3) fatty acids such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) and omega-6 (n-6) fatty acids such as linoleic acid and arachidonic acid.
Accordingly, in one embodiments of the invention the oil of the suspension comprises the unsaturated fatty acid selected from the group of omega-3 fatty acids consisting of \( \alpha \)-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) and omega-6 fatty acids such as linoleic acid and arachidonic acid. In one embodiment the oil of the suspension is rich in the unsaturated fatty acids, wherein the unsaturated fatty acids are n-3 fatty acids. In general it is to be understood that the group of unsaturated fatty acids includes mono-unsaturated fatty acids and poly-unsaturated fatty acids.

Thus in yet an embodiment the unsaturated fatty acids of the oil of the suspension comprises at least one of \( \alpha \)-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid and arachidonic acid.

**Peroxide level of vehicle oil**

Another important parameter of the vehicle oil of the suspension is the peroxide level of the oil. Peroxides are intermediates in the autoxidation reaction and the peroxide level of the oil reflects the degree of rancidification oil and thus the quality of the oil. Apart from deterioration of fats and oils which form off-flavours and off-odour due to rancidification, a high level of peroxide also affects the preservation of the probiotic micro-organism for which the oil is used as vehicle in the vacuum inclusion of the probiotics organism in an extruded food product. An oil with a low peroxide value is preferred as vehicle due to the better probiotic preservative properties over an oil with a higher peroxide value.

Accordingly, in one embodiment of the present invention the wherein the peroxide level of said oil is less that 6 meq 02/kg oil, such as less than 5 meq 02/kg, such as less than 4 meq 02/kg, such as less than 3 meq 02/kg. In a preferred embodiment of the present invention the peroxide level of the oil is less that 2 meq 0.2/kg.

**Additive**

The suspension of the invention may comprise at least one additive. Thus in one embodiment of the invention the suspension for vacuum infusion of an extruded food product comprises an additive such as an antioxidant. The additive may
serve at least the function of preserving the oil vehicle component for example by reducing the accumulation of peroxide in the oil. By minimizing the accumulation of peroxide in the oil the quality of the oil is maintained during storage of the probiotic extruded food product. Oils with a high degree of unsaturation are most susceptible to autoxidation. The peroxide value of the oil also affects the preservation of the probiotic micro-organism for which the oil is used as vehicle in the vacuum inclusion of the probiotics in an extruded food product. Accordingly, adding an antioxidant to the suspension reduce autoxidation reaction of the thereby maintaining the quality oil the oil in terms of food quality but also in terms of preserving the probiotic comprised in the probiotic food product and a fixed level of the unsaturated fats ratio to saturated ones.

Thus, in one embodiment of the invention the suspension comprises at least one additive. In a further embodiment, the suspension comprises an antioxidant. In yet another embodiment, the antioxidant is selected from the group consisting of natural antioxidants and synthetic antioxidants. In one embodiment of the invention the synthetic antioxidant is selected from the group consisting of BHA and BHT and natural antioxidant is selected from the group consisting of Vitamin E flavonoids, and polyphenols. The natural antioxidant may be provided in the form of an extract for example rosemary or grape seed extracts (comprising resveratrol).

Preferably, natural antioxidants are used. Accordingly, in yet another embodiment the antioxidant is natural antioxidant selected from the group consisting of flavanoids, cartonoids, tocotrienol, tocopherol and terpenes. In a particular embodiment, the antioxidant is astaxanthin.

Probiotic micro-organism of the suspension
The probiotic micro-organism(s) (probiotics(s)) are added to the extruded food product as supplement in order to improving the intestinal microbial balance of the host animal (such as human being or pet). The probiotic micro-organism used by the present invention is preferably in preserved state such as freeze-dried. The size of the freeze-dried particles are from 1 µM and larger. In the freeze-dried form the probiotic micro-organism is an ametabolic state of life as a consequence of cryopreservation. However, the probiotic micro-organism will revert into a
metabolic state of life when exposed to an environment enabling the metabolic state of life and populate the environment such as the intestine of the host. Accordingly, a non-viable (dead) micro-organism is not a probiotic micro-organism.

In one embodiment of the invention, the concentration of the at least one probiotic micro-organism is $10^6-10^{16}$ CFU/kg of the oil component of the suspension such as $10^7-10^{16}$ CFU/kg, such as $10^7-10^{16}$ CFU/kg, such as $10^8-10^{16}$ CFU/kg, such as $10^9-10^{16}$ CFU/kg, such as $10^9-10^{14}$ CFU/kg, such as $10^9-10^{14}$ CFU/kg, or such as $10^{10}-10^{14}$ CFU/kg. In a further embodiment, the concentration of the probiotic micro-organism takes into account that the probiotic extruded food product obtained using the suspension should have $1 \times 10^9$ till $3,5 \times 10^{10}$ CFU/kg of complete food, and that the suspension is suitable for vacuum infusion in the view of the above such as a dog food enriched by *E. faecium*.

The state of preservation is further sustained by the use of the oil in the suspension of the invention. Thus, apart from serving the purpose of vehicle for infusion of the probiotics into the extruded food product, the oil also function as a preservation of the probiotic micro-organism embedded in the food product.

Thereby, the stability of the probiotic food product is improved and the shelf life of the final food product increased.

Product benefits

Refrigerated conditions

Refrigerated conditions refer to the conditions that apply to a conventional refrigerator. A refrigerator should stay at $5 \degree C$ or less. At a temperature of $5 \degree C$ or less is important because it slows the growth of most bacteria. The temperature will not kill the bacteria, but it will keep them from multiplying.

Hence, if a product is not kept at refrigerated conditions bacteria can possibly grow in the product. This can have both dramatic and positive effects depending on the product and the bacteria.
In an embodiment of the present invention is the kibble stored under refrigerated conditions.

In an even more specific embodiment of the present invention is the kibble not stored under refrigerated conditions.

Stability of the product

As used herein, the term "shelf life" refers to that property of the products of the invention whereby about 1% or more, alternatively about 5% or more, alternatively about 10% or more, alternatively about 25% or more, alternatively about 50% or more, alternatively about 75% or more, of the probiotic microorganisms are viable (see also definitions of CFU) at the referenced time period after exposure to ambient environmental conditions. The shelf life of the products of the invention is 6-36 month, such as 6-24 month, such as 9-20 month, and such as 12-16 month.

In a more specific embodiment is the change in faeces consistency or condition is achievable 12 months after the production date of the kibble. The pet food product of the present invention will even be stable for at least 24 months, such as at least 15 months, such as at least 12 months, such as at least 9 months, such as at least 6 months, such as at least 3 months at needed concentration to achieve sufficient levelling of score 3 and 4 of the PURINA faeces scoring system.

In a even more specific embodiment is the change in faeces consistency or condition is achievable at least 10 months, such as at least 9 months, such as at least 8 months, such as at least 7 months such as at least 6 months such as at least 5 months such as at least 4 months such as at least 3 months, such as at least 2 months such as at least 1 month after the production date of the kibble.

The consistency and condition of faeces refers to the general state of the dog's defecation. Central elements in a dog's consistency and condition are the visual characteristics - these can be evaluated in scoring systems, which are discussed below.
Shelf-life

The probiotic comprising products of the invention may have a superior shelf-life. Thus, in an embodiment of the invention the count of at least one probiotic in the food product is $10^4$-$10^{16}$ CFU/kg, such as $10^5$-$10^{16}$, such as $10^6$-$10^{16}$, such as $10^7$-$10^{16}$, such as $10^7$-$10^{14}$, such as $10^7$-$10^{12}$, such as $10^7$-$10^{10}$, or such as $10^8$-$10^{10}$ CFU/kg after at least 3 month after the date of manufacturing.

In another embodiment of the invention the count of at least one probiotic in the food product is $10^4$-$10^{16}$ CFU/kg, such as $10^5$-$10^{16}$, such as $10^6$-$10^{16}$, such as $10^7$-$10^{16}$, such as $10^7$-$10^{14}$, such as $10^7$-$10^{12}$, such as $10^7$-$10^{10}$, or such as $10^8$-$10^{10}$ CFU/kg after at least 6 month after the date of manufacturing.

In a further embodiment of the invention the count of at least one probiotic in the food product is $10^4$-$10^{16}$ CFU/kg, such as $10^5$-$10^{16}$, such as $10^6$-$10^{16}$, such as $10^7$-$10^{16}$, such as $10^7$-$10^{14}$, such as $10^7$-$10^{12}$, such as $10^7$-$10^{10}$, or such as $10^8$-$10^{10}$ CFU/kg after at least 10 month after the date of manufacturing.

In yet another embodiment of the invention the count of at least one probiotic in the food product is $10^4$-$10^{16}$ CFU/kg, such as $10^5$-$10^{16}$, such as $10^6$-$10^{16}$, such as $10^7$-$10^{16}$, such as $10^7$-$10^{14}$, such as $10^7$-$10^{12}$, such as $10^7$-$10^{10}$, or such as $10^8$-$10^{10}$ CFU/kg after at least 15 month after the date of manufacturing.

In an additional embodiment of the invention the count of at least one probiotic in the food product is $10^4$-$10^{16}$ CFU/kg, such as $10^5$-$10^{16}$, such as $10^6$-$10^{16}$, such as $10^7$-$10^{16}$, such as $10^7$-$10^{14}$, such as $10^7$-$10^{12}$, such as $10^7$-$10^{10}$, or such as $10^8$-$10^{10}$ CFU/kg after at least 20 month after the date of manufacturing.

In one embodiment, the minimal amount of the probiotic in the product is in the range of $10^5$ CFU/Kg to $10^{15}$ CFU/Kg, such as $1 \times 10^9$ CFU/Kg to $7.5 \times 10^n$, such as $2.5 \times 10^9$ CFU/Kg to $7.5 \times 10^n$. In another embodiment, the product % moisture is above 7%, preferably 8-10%.

It is to be understood that these counts may achieved following standard storing conditions (shelf-life) known to the person skilled in the art.
Defecation management and improvement

It should be understood that faeces consistency and faeces condition discussed in connection with the methods or uses according to the invention. Changes in consistency and/or condition can visually be observed when defecation management and improvement such as changes and optimization of the various scores of the PURINA system is the objective for the skilled addressee.

Timing

The pet food of the present invention can be used at anytime of the animal's life but specific points of great importance are:

- immediately after birth to establish a correct microflora
- after changes of feed or housing
- pre and post anaesthesia
- during and after antibiotic or steroid therapy
- after vaccination
- in preparation for, during and after periods of stress

As the aim with probiotics is to restore and maintain normal gut function, they should be used whenever gut balance is upset. Situations likely to upset gut balance:

- Gut infections
- Vomiting and diarrhoea
- Antibiotic therapy
- Dietary changes
- Travel
- Stress
- Athletic competition
- Following surgery and anaesthesia
- Poor appetite

Strategic use of pet food of the present invention is during times of stress (vaccination, rehoming) nutritional changes (weaning), or after antibiotic use is very beneficial and an excellent way of introducing the concept of probiotic use.
The present invention relates to a method for defecation improvement in a dog comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil.

One embodiment of the present invention relates to a method for improving defecation in a dog comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil. This dog may e.g. suffer from constipation or diarrhoea.

An embodiment of the present invention relates to a method wherein the change in faeces consistency or condition is reached in less than 3 weeks, such as less than 14 days, such as less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days, less than 6 days, less than 5 days, less than 4 days, less than 3 days, less than 2 days, or less than 1 day.

Laboratory testing of faeces
Feces will sometimes be required for microbiological testing, looking for an intestinal pathogen or other parasite or disease.

Biochemical tests done on feces include fecal elastase and fecal fat measurements, as well as tests for fecal occult blood.

It is recommended that the clinician correlate the symptoms and submit specimens according to laboratory guidelines to obtain results that are clinically significant. Formed stools often do not give satisfactory results and suggest little of actual pathological conditions.

Three main types of microbiological tests are commonly done on feces:

Antibody-antigen type tests that look for a specific virus (e.g. rotavirus).

Microscopic examination for intestinal parasites and their ova (eggs).
The pet food of the present invention reduces the parasite number.

Bristol Stool Chart
The Bristol Stool Chart or Bristol Stool Scale is a medical aid designed to classify the form of human feces into seven categories. Sometimes referred to in the UK as the "Meyers Scale," it was developed by Heaton and Lewis at the University of Bristol and was first published in the Scandinavian Journal of Gastroenterology in 1997. The form of the stool depends on the time it spends in the colon.

10 The seven types of stool are:
   Type 1: Separate hard lumps, like nuts (hard to pass)
   Type 2: Sausage-shaped, but lumpy
   Type 3: Like a sausage but with cracks on its surface
   Type 4: Like a sausage or snake, smooth and soft
   Type 5: Soft blobs with clear cut edges (passed easily)
   Type 6: Fluffy pieces with ragged edges, a mushy stool
   Type 7: Entirely liquid

Types 1 and 2 indicate constipation, with 3 and 4 being the "ideal stools" especially the latter, as they are the easiest to pass, and 5-7 being further tending towards diarrhoea or urgency.

PURINA faeces scoring system
The Purina faeces scoring system was developed by Nestle for and similar scoring systems for pets and refers to the evaluation of stool samples based on visual characteristics. The scoring is from 1 to 7 going from hard and dry at 1 to no texture and watery at 7:

1 Very hard and dry, no residue left on the ground when picked up.
2 Firm, not hard. Little residue left on the ground when picked up.
3 Log-like, moist surface, leaves residues but holds form when picked up.
4 Very moist, long shape leaves residues and loses form if picked up.
5 Very moist, present in piles, distinct shape, leaves residues and loses form if picked up.
6 No defined shape, but has texture, occurs as spot or pile, leaves residues if picked up.

7 No texture, watery, flat, occurs as puddles.

5 The optimal score is considered to be 4.

In one embodiment of the present invention, the faeces condition of the dog is levelled to score 3 and 4 of the PURINA faeces scoring system.

10 An embodiment of the present invention relates to the use of a pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil for the manufacture of a composition for improving the faeces condition of a dog to score 3 and 4 of the PURINA faeces scoring system.

15 Another embodiment of the present invention relates to the use of a pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil for the manufacture of a composition for maintaining a faeces condition of a dog at score 3 and 4 of the PURINA faeces scoring system.

20 An embodiment of the present invention relates to vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil for use in improving the faeces condition of a dog to score 3 and 4 of the PURINA faeces scoring system.

25 A more specific embodiment of the present invention relates to a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil for use in maintaining the faeces condition of a dog at score 3 and 4 of the PURINA feces scoring system.

30 An embodiment of the present invention relates to a method wherein the fish oil has dynamic viscosity of less than 0.08 pascal-second (Pa-s) at 20°C.

A vacuum infused kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil for use as a medicament for in the
improvement of the faeces condition of a dog to score 3 and 4 of the PURINA feces scoring system.

Fecal improvements or benefits

5 Food allergy
Dog foods can be specially formulated for dogs allergic to common ingredients such as chicken, wheat, or corn. These foods usually contain "novel proteins" and substitute uncommon starches for the usual grains. Meats used in allergy formulas can range from the mundane, such as lamb, beef, or whitefish, to the unusual, such as venison or duck.

Carbohydrates in allergy formulas are usually a less common grain, such as rice or barley, but such ingredients as potato and quinoa are sometimes used. Allergies are more likely to develop with consistent exposure to certain proteins (i.e. prolonged feeding of the same food).

Diets can possibly contain common ingredients that have been hydrolyzed to prevent them from triggering an immune response.

Sensitive digestive systems are often a side effect of these allergies in dogs. Hence, an embodiment of the present invention relates to a method for defecation improvement in a dog with allergy, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil.

An embodiment of the present invention relates to the use of a vacuum infused pet food kibble according to the present invention in the treatment of allergy in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of allergy in a pet.
In a specific embodiment the allergy is selected from the group consisting of food allergy, medicinal allergy, lactose intolerance, bacterial food poisoning such as but not limited to staphylo toxin, pharmacologic allergies such as but not limited to scombroid (histamine) fish poisoning.

Constipation

Constipation, costiveness, or irregularity, is a condition of the digestive system in which an animal experiences hard faeces that are difficult to expel. This usually happens because the colon absorbs too much water from the food. If the food moves through the gastro-intestinal tract too slowly, the colon may absorb too much water, resulting in faeces that are dry and hard.

Thus, an embodiment of the present invention relates to a method for increasing bowel transit time in a dog, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic microorganisms and a fish oil.

In a more specific embodiment of the present invention is the bowel transit time in a dog is increased by at least 3 days, such as at least 2 days, such as at least 1 day, such as at least 16 hours, such as at least 12 hours, such as at least 8 hours, such as at least 6 hours, such as at least 4 hours, such as at least 2 hours, such as at least 1 hour, such as at least 30 min.

One way of measuring the bowel transit time in a dog is by adding beetroot to the dog food and measuring the time until the red colour of the beetroot can be seen in the faeces of the dog. This test is known as the home test.

For a home test, the dog will drink some red vegetable dye or eat a food like corn kernels or beets. It is then possible keep track of how long it takes for the dye or vegetable to show up in its stool.

Other tests include the dye test and the pellet test.

For a dye test, the dog will swallow a pill that has dye in it and keep track of how long it takes before the dye shows up in its stool.
For a pellet test, the dog swallows small pills (pellets) before having X-rays of its belly. The pellets look like white spots or rings in the X-ray pictures. The dog will have X-rays over time, typically 2 or 3 days to keep track of how fast the pellets move through its intestines.

Defecation may be extremely painful, and in severe cases (faecal impaction) lead to symptoms of bowel obstruction. The term obstipation is used for severe constipation that prevents passage of both stools and gas.

An embodiment of the present invention relates to a method for defecation improvement in a dog with constipation, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil.

It is an object of preferred embodiments of the present invention that the term constipation relates to dietary, symptoms of bowel obstruction, bowel obstruction, hormonal, anatomical, a side effect of medications (e.g. some opiates), or an illness or disorder.

The vacuum infused pet food kibble will have a positive effect on the constipation over time. Such a positive effect can be observed when a dog with constipation - typically with a Purina score of 1-2 - shows optimization of the faeces condition e.g. where the Purina score is 3-4.

An embodiment of the present invention relates to a method wherein the positive effect on the constipation is reached in less than 3 weeks, such as less than 14 days, such as less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days, less than 6 days, less than 5 days, less than 4 days, less than 3 days, less than 2 days, or less than 1 day.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in the treatment of constipation in a pet.
Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of constipation in a pet.

Diarrhoea
The presence of diarrhoea in dogs should always be cause for concern. There are a plethora of causes of both acute and chronic diarrhoea in dogs, including infectious causes, toxins, inflammation or disease of the intestinal tract and parasites. Diarrhoea can have a devastating effect on the body due to its dehydrating effect, and left untreated it can lead to blood sugar depletion, circulatory collapse and death. Although mild cases of diarrhoea may resolve without intervention, diarrhoea accompanied by vomiting, lethargy or any other behavioural changes should be treated as a medical emergency. Because the severity of the cause of diarrhoea is not immediately present at onset, prompt medical attention must be sought to quickly diagnose and treat the underlying problem.

Thus, an embodiment of the present invention relates to a method for decreasing bowel transit time in a dog, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic microorganisms and a fish oil.

In a more specific embodiment of the present invention is the bowel transit time in a dog is decreased by at least 2 days, such as at least 1 day, such as at least 16 hours, such as at least 12 hours, such as at least 8 hours, such as at least 6 hours, such as at least 4 hours, such as at least 2 hours, such as at least 1 hour, such as at least 30 min.

Diarrhoea is always a symptom of an underlying problem, and not a disease in itself. Diarrhoea can be used to describe a varying severity of a problem, from occasional loose stools to a continuous watery stream of faeces. Unfortunately there are many potential causes of diarrhoea in dogs.
An embodiment of the present invention relates to a method for defecation improvement in a dog with diarrhoea, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil.

The vacuum infused pet food kibble will have a positive effect on the diarrhoea over time. Such a positive effect can be observed when a dog with diarrhoea - typically with a Purina score of 6-7 - shows optimization of the faeces condition e.g. where the Purina score is 3-4.

An embodiment of the present invention relates to a method wherein the positive effect on the diarrhoea is reached in less than 3 weeks, such as less than 14 days, such as less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days, less than 6 days, less than 5 days, less than 4 days, less than 3 days, less than 2 days, or less than 1 day.

In a particular preferred embodiment the positive effect on the diarrhoea is reached in less than 1 day, such as less than 16 hours, less than 12 hours, less than 8 hours, less than 4 hours, or less than 2 hours.

An embodiment of the present invention relates to the a vacuum infused pet food kibble according to the present invention for use in the treatment of diarrhoea in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of diarrhoea in a pet.

Water activity

The moisture content and water activity of faeces can be used for evaluation.

The term "Water activity (a_w)" reflects the active part of moisture content or the part which, under normal circumstances, can be exchanged between the product and its environment. The active part of moisture content and, therefore, water
activity, provide better information than the total moisture content regarding the micro-biological, chemical and enzymatic stability of perishable products such as foods and seeds. Water activity can be defined as:

\[ a_w = P / \text{ps} \quad \text{and} \quad \% \text{ERH} = 100 \times a_w \]

In these equations "\( P \)" is the partial pressure of water vapor at the surface of the product, "ps" is the saturation pressure, or the partial pressure of water vapor above pure water at the product temperature and "\%ERH" is the equilibrium relative humidity.

Typically will the water activity be low in e.g. the faeces of a constipated dog and high in e.g. a dog with diarrhoea.

Thus, it is an embodiment of the present invention to provide a method for changing the water activity in the faeces of a dog, comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and a fish oil.

An embodiment of the present invention relates to the use of an vacuum infused pet food kibble according to the present invention for the changing the water activity in the faeces of a pet.

Intestinal parasites

Intestinal parasites are a very common cause of diarrhoea in dogs. There are many types of parasites that can infect dogs, and diarrhoea is often one of the most common symptoms of a parasite infection.

Roundworms are one of the most commonly seen intestinal parasites in puppies. Although roundworms are not commonly active in most adult dogs, puppies are especially susceptible to their presence and side effects. A pot-bellied appearance, poor growth and a rough, dull hair coat are signs of a worm infestation. Diarrhoea and vomiting may be present as well, and the dog may expel worms in their stool or vomits. If allowed to continue unchecked, the worms can cause pneumonia, intestinal obstructions and death.
Hookworms can be seen in dogs of all ages, but are most common in warmer, humid climates. Transmitted by ingestion of contaminated faeces, mature hookworms attach to the lining of the intestinal tract and feed on the blood supply there. In pregnant dogs, the hookworms migrate into the foetuses, and begin to infest the puppies before they are even born. Hookworms in puppies can be devastating, as they can cause severe anaemia, weakness and bloody diarrhoea.

While not a worm, Giardia is an intestinal parasite caused by a single-celled organism that lives in the intestines of infected animals. Recent research has shown that Giardia is present in up to 11% of the general population of pets, and as many as 50% of puppies. Giardia can be transmitted from pet to pet, through contaminated feed or water, and through the soil. The most common symptom of Giardia is diarrhoea of varying severity. However, many animals who are infected with Giardia can show no symptoms for extended periods of time, which makes routine testing even more important.

Coccidia is another single-celled organism that infects the small intestine of dogs. Dogs with coccidia may show know signs of illness, and some may have severe bouts of watery stools and bloody diarrhoea, vomiting, depression and fever, and even death as a result of severe dehydration. These severe side effects of coccidia are most common in puppies and adult dogs suffering from other illnesses.

Thus, in one embodiment the present invention relates to the treatment of infection with intestinal parasites in a dog comprising feeding said dog with a vacuum infused pet food kibble of the present invention.

An embodiment of the present invention relates to the a vacuum infused pet food kibble according to the present invention for use in the treatment of intestinal parasites in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of intestinal parasites in a pet.
Bacterial infections
Other causes of acute diarrhoea in dogs can include bacterial infections, such as salmonella and *E. coli*, toxin exposure, such as from insecticide and lead, and even stress.

Treatment of a dog suffering from diarrhoea depends on what may be the cause. In cases of intestinal upset, a bland diet may be all that's needed to settle the stomach. Parasites can be treated with de-wormers, and a drug may be prescribed to help return the digestive system to working order. Treatment of severe diarrhoea will begin with intravenous fluid therapy, and balancing of electrolyte levels to combat the fluid loss caused by the diarrhoea. By combining the various treatments with vacuum infused pet food kibble of the present invention, the dog will recover much faster than with conventional pet food.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in the treatment of bacterial infections in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of bacterial infections in a pet.

Chronic problems
While acute cases of diarrhoea can be cause for great concern, chronic diarrhoea, while less common.

Diarrhoea that persists for three or more weeks is considered chronic. Often the stool may begin to firm, only to become soft and unformed again. It is not uncommon to see mucous or even small amounts of blood in the sample. Because chronic diarrhoea can lead to poor digestion and absorption of nutrients, often dogs will not eat well, have a low energy level and poor quality hair coat.
Food allergies and intolerances are a common cause of mild chronic diarrhoea. Similar to lactose intolerance in people, dogs may have or develop allergies or sensitivities to variety of ingredients in dog food, leading to chronic inflammation in the intestinal tract.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in the treatment of chronic diarrhoea in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of chronic diarrhoea in a pet.

Pancreatitis

Pancreatitis can present in dogs in both an acute form, as well as a chronic problem. The pancreatic gland is responsible for secreting hormones such as insulin and glucagons into the bloodstream to regulate blood sugar levels, as well as making the digestive enzymes that break down food for digestion. Pancreatitis, or inflammation of the pancreas, can cause these digestive enzymes to decrease, and in severe cases the enzymes may begin to digest the actual organs of the dog instead of digesta. Diarrhoea, abdominal pain, vomiting and a poor appetite are the symptoms of pancreatitis, but because these symptoms are shared with so many other gastrointestinal problems, it can be hard to diagnose.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in the treatment of pancreatitis in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of pancreatitis in a pet.
Inflammatory bowel disease (IBD)

IBD can also be a cause of chronic diarrhoea in dogs. In affected dogs, the intestine is taken over by inflammatory cells, eventually leading to scar tissue throughout the lining of the digestive system. Although the exact cause of IBD is unknown, nutrition, genetics and the immune system are thought to play a role in its development. Dogs with a long history of diarrhoea or weight loss that have been found to be free of parasites and diarrhoea causing agents should be considered for IBD. Diagnosis of IBD can be difficult, and often requires an intestinal biopsy to confirm. Treatment is aimed at reducing the inflammation, as well as dietary changes to provide a more easily digestible food source.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in the treatment of IBD in a pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for the manufacture of a medicament for treatment of IBD in a pet.

Whether chronic or acute, diarrhoea is almost always a sign of an underlying medical condition that needs to be addressed. Because diarrhoea in itself has the potential to be life threatening, any dog suffering from more than a short-term bout of diarrhoea, or dogs showing signs of other medical problems, should immediately be fed with vacuum infused pet food kibble of the present invention.

In addition, because young puppies are so susceptible to several potentially fatal viruses, the presence of diarrhoea in any puppy should be treated as a medical emergency until proven otherwise.

Probiotic micro-organisms (hereinafter: probiotics) are living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond basic nutrition. The beneficial effects that probiotics may induce are numerous and form part of the knowledge of the skilled person. As few examples one may mention the reduction of lactose intolerance, the inhibition of pathogenic bacteria and parasites, the reduction of diarrhoea, activity against Helicobacter pylori; the
prevention of colon cancer, the improvement or prevention of constipation, the in situ production of vitamins, the modulation of blood lipids, and the modulation of host immune functions.

5 General health and wellbeing

The various bowel problems described above can have a dramatic effect of the general health and wellbeing of a pet.

A pet can, in addition to the bowel problems, suffer from other accompanying problems like unrest, lack of sufficient energy uptake and decreasing effect of the immune defence system.

Thus, the use of a vacuum infused pet food kibble according to the present invention for maintaining a normal bacterial flora and restoring a balanced bacterial flora in the pet is an embodiment of the present invention.

An embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in increasing the digestive uptake from the intestinal tract in the pet.

Another embodiment of the present invention relates to a vacuum infused pet food kibble according to the present invention for use in improving the immune defence system in the pet.

25 Dosage regime

One embodiment of the present invention relates to dosage regime packaging solution wherein the content of the daily feed is comprising 1/4 of the new diet with probiotics and 3/4 of the old food for the first 3 days, then feed a half portion new food and half old food for another three days and then 1/4 old food and 3/4 new probiotic food for another three days.
Fecal management

The management of faeces is an issue of hygiene, since faeces contribute to spreading of diseases and intestinal parasites.

5 Animal waste collection

Animal waste collection as a pollution source control; involves using a combination of educational outreach and enforcement to encourage residents to clean up after their pets. The presence of pet waste in stormwater runoff has a number of implications for urban stream water quality with perhaps the greatest impact from fecal bacteria.

Non-human waste represents a significant source of bacterial contamination in urban watersheds. Genetic studies conclude that 95 percent of the fecal coliform found in urban stormwater was of non-human origin. Bacterial source tracking studies in a watershed in the Seattle, Washington area also found that nearly 20% of the bacteria isolates that could be matched with host animals were matched with dogs.

Animal waste collection programs use awareness and education, signs, and pet waste control ordinances to alert residents to the proper disposal techniques for pet droppings, however the stool condition of the faeces represent a barrier for many pet owners that refuse to collect low-viscosity fecal material. Thus, one purpose of the present invention relates to minimize the amount of low-viscosity fecal material in pets in urban areas.

25 The pet food of the present invention enables easy and hygienic animal waste collection by optimizing the frequencies of score 3 and 4 of the PURINA faeces scoring system in the average population of pets feed with the vacuum infused pet food of the present invention.

30 General

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention.
All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety. Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In addition, singular reference do not exclude a plurality. Thus, references to "a", "an", "first", "second" etc. do not preclude a plurality.

The invention will now be described in further details in the following non-limiting examples.
Examples

Example 1

Highly digestible functional probiotic food, for dogs of all ages with sensitive digestion, allergies and adverse reaction to other foods.

Ingredients

Lamb, rice, maize, chicken, animal fat, chicken protein, vegetable pulp, maize gluten, egg, yeast, linseed, fish oil, yucca extract, hydrolysed crustaceans, hydrolysed cartilage, FOS, lecithin, vitamins & minerals, no colorants. Antioxidant: according to EU regulations.

Product contains *Enterococcus faecium* NCIMB 10415 EC No. 13 (E1707 (new classification)). (registered in EU for use in dogs) viable probiotic strain with average concentration of $10^9$ CFU/kg respectively in complete feed.

*Enterococcus faecium* is a scientifically investigated and widely used probiotic culture that is added to food to exert beneficial effects on the host by regulating the host intestinal microbial balance.

Effects and benefits

Balances sensitive gut flora

Special balanced content of the SENSITIVE helps to establish balanced and beneficial microflora in dog intestine with sensitive digestion.

Antimicrobial effect

Competitive exclusion for space with bacteriocins and lactic acid production by *Enterococcus faecium* helps to avoid contamination and reproduction of harmful and pathogenic bacteria.

Reduces stress

Lactic acid produced by *Enterococcus faecium* lowers pH level and as a result reduces the effects of stress.
Reduces diarrhoea and other digestive upsets

Metabolic activity of *Enterococcus faecium* bacteria increases digestibility rate of intestinal tract by producing enzymes, vitamins and lactic acid.

Average nutrient content

<table>
<thead>
<tr>
<th><strong>Composition</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>23%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>12%</td>
</tr>
<tr>
<td>Crude ashes</td>
<td>7%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3%</td>
</tr>
<tr>
<td>Humidity</td>
<td>9%</td>
</tr>
<tr>
<td>Ca</td>
<td>1.0%</td>
</tr>
<tr>
<td>P</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Vitamins</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>20000 IU/kg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>2000 IU/kg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150 mg/kg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>6 mg/kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Minerals</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Co</td>
<td>1.5 mg/kg</td>
</tr>
<tr>
<td>Se</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>Cu</td>
<td>20 mg/kg</td>
</tr>
<tr>
<td>Zn</td>
<td>110 mg/kg</td>
</tr>
<tr>
<td>Fe</td>
<td>100 mg/kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Fatty acids</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega 3</td>
<td>1.8 g/kg</td>
</tr>
</tbody>
</table>
Metabolic energy value 3800 kcal/kg

Daily feeding amounts (gram per day)

<table>
<thead>
<tr>
<th>Dog weight, kg</th>
<th>2-5</th>
<th>5-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
</tr>
</thead>
</table>

Example 2

Functional probiotic food for ageing dogs.

Ingredients

Chicken, maize, rice, animal fat, chicken protein, maize gluten, vegetable pulp, yeast, egg, fish oil, linseed, hydrolyzed crustaceans, hydrolyzed cartilage, yucca extract, FOS, lecithin, vitamins & minerals, no colorants. Antioxidant: according to EU regulations.

Product contains *Enterococcus faecium* NCIMB 10415 EC No. 13 (E1707 (new classification)) (registered in EU for use in dogs) viable probiotic strain with average concentration of $10^9$ CFU/kg respectively in complete feed.

*Enterococcus faecium* is a scientifically investigated and widely used probiotic culture that is added to food to exert beneficial effects on the host by regulating the host intestinal microbial balance.
Effects and benefits

Soothes the gut
Balanced content of SENIOR helps to soothe the ageing dog intestine.

5 Improves digestion
Metabolic activity of Enterococcus faecium bacteria increases digestibility rate of intestinal tract by production of enzymes, vitamins and lactic acid.

Antimicrobial effect
10 Competitive exclusion for space with bacteriocins and lactic acid production by Enterococcus faecium helps to avoid contamination and reproduction of harmful and pathogenic bacteria.

Reduces stress
15 Lactic acid produced by Enterococcus faecium lowers pH level and as a result reduces the effects of stress.

Average nutrient content

<table>
<thead>
<tr>
<th>Composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>22%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>10%</td>
</tr>
<tr>
<td>Crude ashes</td>
<td>6.5%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3%</td>
</tr>
<tr>
<td>Humidity</td>
<td>9%</td>
</tr>
<tr>
<td>Ca</td>
<td>1.1%</td>
</tr>
<tr>
<td>P</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>15000 IU/kg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>1500 IU/kg</td>
</tr>
</tbody>
</table>
Metabolic energy value 3600 kcal/kg

### Daily feeding amounts (gram per day)

<table>
<thead>
<tr>
<th>Dog weight, kg</th>
<th>2-5</th>
<th>5-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>50-100</td>
<td>100-160</td>
<td>160-260</td>
<td>160-260</td>
<td>360-460</td>
<td>460-560</td>
<td>560-660</td>
<td>660-760</td>
<td>760-860</td>
</tr>
</tbody>
</table>

Example 3

Functional probiotic food for puppies from 5 weeks to 6-9 months of age.

### Ingredients

Chicken, lamb, fish, rice, maize, animal fat (chicken), maize gluten, egg, vegetable pulp, chicken protein, yeast, fish oil, yucca extract, inulin, hydrolyzed crustaceans, hydrolyzed cartilage, lecithin, FOS, vitamins & minerals, no colorants.
Antioxidant: according to EU regulations. Product contains Enterococcus faecium NCIMB 10415 EC No. 13 (E1707 (new classification)) (registered in EU for use in dogs) viable probiotic strain with average concentration of 10^9 CFU/kg respectively in complete feed.

Enterococcus faecium is a scientifically investigated and widely used probiotic culture that is added to food to exert beneficial effects on the host by regulating the host intestinal microbial balance.

Effects and benefits

Establishes balanced microflora
Since early age, while puppies intestine is relatively sterile PUPPY establishes the balanced and beneficial microflora in the gut.

Develops immunity
Enterococcus faecium stimulates antibody production and increases macrophage activity that helps to develop immunity.

Improves digestion
Metabolic activity of Enterococcus faecium bacteria increases digestibility rate of intestinal tract by production of enzymes, vitamins and lactic acid.

Reduces stress
Lactic acid produced by Enterococcus faecium lowers pH level and as a result reduces the effects of stress.

Reduces diarrhoea and other digestive upsets
Metabolic activity of Enterococcus faecium bacteria increases digestibility rate of intestinal tract by producing enzymes, vitamins and lactic acid.

Average nutrient content
## Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>28%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>16%</td>
</tr>
<tr>
<td>Crude ashes</td>
<td>7%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.5%</td>
</tr>
<tr>
<td>Humidity</td>
<td>9%</td>
</tr>
<tr>
<td>Ca</td>
<td>1%</td>
</tr>
<tr>
<td>P</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

## Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>20000 IU/kg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>2000 IU/kg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150 mg/kg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>6 mg/kg</td>
</tr>
</tbody>
</table>

## Minerals

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Co</td>
<td>1.5 mg/kg</td>
</tr>
<tr>
<td>Se</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>Cu</td>
<td>20 mg/kg</td>
</tr>
<tr>
<td>Zn</td>
<td>110 mg/kg</td>
</tr>
<tr>
<td>Fe</td>
<td>100 mg/kg</td>
</tr>
</tbody>
</table>

## Fatty acids

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega 3</td>
<td>1.61 g/kg</td>
</tr>
<tr>
<td>Omega 6</td>
<td>17 g/kg</td>
</tr>
</tbody>
</table>

Metabolic energy value: 4100 kcal/kg

### Daily feeding amounts (gram per day)

<table>
<thead>
<tr>
<th>Breed</th>
<th>0-4 month</th>
<th>4-6 month</th>
<th>6-9 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small breeds</td>
<td>30-260</td>
<td>60-280</td>
<td>20-360</td>
</tr>
</tbody>
</table>
Example 4

5 Functional probiotic food for adult dogs with active lifestyle.

Ingredients
Chicken, maize, rice, animal fat, chicken protein, vegetable pulp, yeast, maize gluten, fish, egg, fish oil, yucca extract, hydrolyzed crustaceans, hydrolyzed cartilage, FOS, lecithin, vitamins & minerals, no colorants. Antioxidant: according to EU regulations.

Product contains *Enterococcus faecium* NCIMB 10415 EC No. 13 (E1707 (new classification)). (registered in EU for use in dogs) viable probiotic strain with average concentration of $10^9$ CFU/kg respectively in complete feed.

*Enterococcus faecium* is a scientifically investigated and widely used probiotic culture that is added to food to exert beneficial effects on the host by regulating the host intestinal microbial balance.

Effects and benefits

Restores energy levels
Probiotic activity increases the intake of nutritional compounds with extra sources of energy to meet all the demands of the dog with active lifestyle.

Increases vitality
With probiotic effect, consumption of ADULT PLUS reduces the effects of stress, maintains good condition of the animal and helps the organism to
achieve the top state of health and mood.

*Improves digestion*
Metabolic activity of *Enterococcus faecium* bacteria increases digestibility rate of intestinal tract by producing enzymes, vitamins and lactic acid.

*Maintains good skin and coat condition*
Consumption of probiotics decreases risk of irritations or allergic reactions therefore helps to maintain healthy skin and shiny coat.

10

*Average nutrient content*

<table>
<thead>
<tr>
<th>Composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>27%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>15%</td>
</tr>
<tr>
<td>Crude ashes</td>
<td>7%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.8%</td>
</tr>
<tr>
<td>Humidity</td>
<td>9%</td>
</tr>
<tr>
<td>Ca</td>
<td>1.4%</td>
</tr>
<tr>
<td>P</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

15 *Vitamins*

<table>
<thead>
<tr>
<th>Vitamin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>20000 IU/kg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>2000 IU/kg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150 mg/kg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>6 mg/kg</td>
</tr>
</tbody>
</table>

Minerals

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Co</td>
<td>1.5 mg/kg</td>
</tr>
<tr>
<td>Se</td>
<td>0.2 mg/kg</td>
</tr>
</tbody>
</table>
Fatty acids

<table>
<thead>
<tr>
<th></th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega 3</td>
<td>2</td>
</tr>
<tr>
<td>Omega 6</td>
<td>20</td>
</tr>
</tbody>
</table>

Metabolic energy value 4000 kcal/kg

**Daily feeding amounts (gram per day)**

<table>
<thead>
<tr>
<th>Dog weight, kg</th>
<th>2-5</th>
<th>5-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>50-100</td>
<td>100-160</td>
<td>160-260</td>
<td>160-260</td>
<td>360-460</td>
<td>460-560</td>
<td>560-660</td>
<td>660-760</td>
<td>760-860</td>
</tr>
</tbody>
</table>

Example 5

10 Functional probiotic food for adult dogs with normal activity to meet all needs of healthy organism.

**Ingredients**

Chicken, maize, rice, animal fat, chicken protein, vegetable pulp, maize gluten, yeast, egg, fish oil, linseed, yucca extract, hydrolyzed crustaceans, hydrolyzed cartilage, FOS, lecithin, vitamins & minerals, no colorants.

Antioxidant: according to EU regulations. Product contains *Enterococcus faecium* NCIMB 10415 EC No. 13 (E1707 (new classification)) (registered in EU for use in dogs) viable probiotic strain with average concentration of $10^9$ CFU/kg respectively in complete feed.
Enterococcus faecium is a scientifically investigated and widely used probiotic culture that is added to food to exert beneficial effects on the host by regulating the host intestinal microbial balance.

5 Effects and benefits

Increases vitality
With probiotic effect, consumption of ADULT PLUS reduces the effects of stress, maintains good condition of the animal and helps the organism to achieve the top state of health and mood.

10 Improves digestion
Metabolic activity of Enterococcus faecium bacteria increases digestibility rate of intestinal tract by producing enzymes, vitamins and lactic acid.

15 Maintains strong immunity
Enterococcus faecium helps to maintain strong immunity by increasing antibody production and stimulating macrophage activity.

Maintains good skin and coat condition
Consumption of probiotics decreases risk of irritations or allergic reactions therefore helps to maintain healthy skin and shiny coat.

Average nutrient content

<table>
<thead>
<tr>
<th>Composition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>24%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>14%</td>
</tr>
<tr>
<td>Crude ashes</td>
<td>7%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3%</td>
</tr>
<tr>
<td>Humidity</td>
<td>9%</td>
</tr>
<tr>
<td>Ca</td>
<td>1.2%</td>
</tr>
<tr>
<td>P</td>
<td>0.9%</td>
</tr>
</tbody>
</table>
### Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount (IU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>20000</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>2000</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>6</td>
</tr>
</tbody>
</table>

### Minerals

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>Co</td>
<td>1.5</td>
</tr>
<tr>
<td>Se</td>
<td>0.2</td>
</tr>
<tr>
<td>Cu</td>
<td>20</td>
</tr>
<tr>
<td>Zn</td>
<td>110</td>
</tr>
<tr>
<td>Fe</td>
<td>100</td>
</tr>
</tbody>
</table>

### Fatty acids

<table>
<thead>
<tr>
<th>Type</th>
<th>Amount (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega 3</td>
<td>1.8</td>
</tr>
<tr>
<td>Omega 6</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Metabolic energy value 3920 kcal/kg

### Daily feeding amounts (gram per day)

<table>
<thead>
<tr>
<th>Dog weight, kg</th>
<th>2-5</th>
<th>5-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
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Example 6

*Trial information*
Setup of the trial-panel

Eight dogs were selected based on specific individual health problems. The nature of their disease, which was determined by the veterinary, was the main criteria of the selection of these dogs.

Time schedule of the trials

The selected panel received only the kibbles described in example 1 for the entire duration of the 5 week testing period.

Controls during trials

For the entire duration of the trials the following people have checked that the protocol was exactly executed:

Veterinarian

- Full check-up of all the dogs before the trials started
- Determination of the possible disease
- Every week 3 complete health check-ups have been done on all dogs
- Evaluation of the faeces during the entire period of the trials
- Control of the check-lists

Official investigators

- Feeding-method was checked daily by official investigators
- Weighing of feed and faeces was done by official controllers under supervision of the veterinarian.
- All data is filled into the appropriate forms by the investigators.

Statistical analysis:

The following aspects were checked during this test

- Faeces quality: based on the quotation system of Proplan - Purina. The Bristol Stool Chart is similar to this quotation system (figure 1 and 2)
- General health improvement
- Skin condition
- Coat condition
- Behaviour
- Vitality

5

Results chart
- Charts are made from all the obtained faeces scores per dog (figures 3-10)
- Logarithmic trend line represents the faeces consistency change in time.

10 Dog no. 1 - Elodi

Pre-trial period
Breed: Boxer
Gender: female
15 Age: 3 years
Weight: 22 kg
Stools rating: 6 - 7
Standard food: Crok 23/11 United Petfood
General condition: Good

20

Veterinarian comment
This dog had a very poor faeces quality. The dog had a normal weight and has no
problems maintaining it. From time to time the dog has blood in the faeces.
After the check-up the cause of the diarrhoea was determined as stress diarrhoea.

25

Test results (faeces quotation in figure 3)
The dog was fed with a feeding rate: 200 gr. in the morning and 200 gr. in the
evening.

30 Conclusion
Figure 3 shows a clear improvement of the quality of the faeces during the trial
period. After 20 days of feeding the Formula Probiotic Sensitive (Example 1) the
faeces of the dog was almost optimal and stayed like this until the end of the trial.

35 The weight of the dog remained the same for the entire feeding period.
The living conditions of the dog have not been altered during the test to avoid a change in the stress situation of the dog. This leads to the conclusion that the dog has overcome his problem of stress diarrhoea thanks to the Formula Probiotic Sensitive.

Additional info
Sample IgA in µg/g faeces end of test period : 12.98

10 Dog no. 2 - Galicia

Pre-trial period
Breed : Boxer
Gender: female
Age: 9 months
Weight: 23 kg
Stools rating : 5 - 6
Standard food : Crok 23/11 United Petfood
General condition : Very large dog with a good health although it is a little too skinny.

Veterinarian comment
This dog eats an average of 800 gr per day of her standard food and is still underweight. Until the age of 8 months this dog was eating Europremium Junior. The dog had almost continuously diarrhoea. From the 8th month on the breeder switched to the adult food which the dog was still receiving up to the date of the trial. Since the switch, the dog was taking on weight but still had very frequent diarrhoea.

The general diagnose of the dog : bad digestive system and possible food intolerance which causes problems to take the energy out of the food.

Test results (faeces quotation in figure 4)
The dog was fed with a feeding rate: 250 gr in the morning and 250 gr in the evening. The feeding quantity per day was changed on the 11th day, since the
dog was still losing weight. From the 11th day the dog received 2 times 300 gr/day instead of 2 times 250 gr/day.

Conclusion

Figure 4 shows that the quality of the faeces improved from 5 to 3 in 16 days. After the 16th day it reaches the 3 level, with a few exceptions. The feeding with Formula Probiotic Sensitive (Example 1) can be given as the reason of this improvement since the living situations of this dog did not change during the trial period. The change in the result can also be linked to the fact that the dog was given a larger quantity of food per day since the improvement started to show on the 12th day, or one day after the changing of the daily feeding quantity.

After the trial period the weight of the dog went up to 25 kg, which gives the dog a better general condition. This leads to conclude that the digestive problem was solved thanks to the use of Formula Probiotic Sensitive (Example 1).

Additional information

Sample IgA in µg/g faeces end of test period: 44,18

20 Dog no. 3 - Georges

Pre-trial period

Breed: Boxer
Gender: Male
Age: 9 months
Weight: 24 kg
Stools rating: 5
Standard food: Crok 25/12 United Petfood + Duck (frozen meat)
General conditions: Good when the dog receives the additional frozen meat.

Veterinarian comment:
The dog had chronic diarrhoea before he received the additional frozen meat. The additional food gave the dog a more stable stool, but still at a level 5. The dog received before the test 1 kg of frozen meat and 200 gr Crok 25/12 per day.
The diagnoses showed stress diarrhoea and food intolerance. The dog is also underweight. His optimal weight should be 28 to 30 kg.

Test results (faeces quotations in figure 5)

The dog was fed with a feeding rate: 250 gr/day in the morning and 250 gr/day in the evening.

Conclusion

Figure 5 shows that after only 4 days an improvement of the faeces quality was established. The dog went from level 5 to level 4. But even more spectacular was the fact the dog went from a daily feeding of 1.25 kg (frozen meat and dry petfood combined) to a 500 gr portion per day of the Formula Probiotic Sensitive (Example 1). Even with such a big difference of daily feeding the dog did not lose weight during the trial period. In fact, he even improved his weight with 1.5 kg.

The living conditions of the dog were not altered during the trial period. This allows us to conclude that the problem of stress diarrhoea and food intolerance was solved thanks to the feeding with Formula Probiotic. As a result of the Formula Probiotic feeding the dog also gained weight, which shows that the product is well digested.

Additional information

Sample IgA in µg/g feces end of test period : 40,18

25 Dog no. 4 - Esteban

Pre-trial period
Breed: Boxer
Gender: male
Age: 3 years
Weight: 33 kg
Stools rating: 4
Standard food: Crok 25/12 United Petfood
General condition: Good
Veterinarian comment:
This dog ate 900 gr/day of his standard food to maintain a good weight according to his size. The quality of the faeces is acceptable. Esteban is a large size dog, which partially explains his bigger nutritional needs compared to other Boxer dogs. Reducing his feeding quantity affects his general condition.

The diagnoses showed a possible digestive problem, which causes the dog to need large quantities of food to be able to meet his nutritional requirements. The dog is very active even when he is in his kennel.

Test results (faeces quotation in figure 6)
The dog was fed with a feeding rate: 250 gr in the morning and 250 gr in the evening.

The feeding quantity was dropped from 900 gr/day of his standard food to 500 gr/day of the Formula Probiotic Sensitive (Example 1). On the 15th day the feeding quantity was changed to 600 gr/day since the dog was losing weight. From that moment on the dog went back to his normal weight after a period of one week.

Conclusion
Figure 6 shows no difference in the faeces quality during the first 15 days. Its only when the feeding quantity is set to a higher level that an improvement of the faeces quality can be seen.

The feeding with the Formula Probiotic Sensitive (Example 1) solved the digestive problem of this dog, since he can now meet his nutritional requirements with 30% less food. The level of activity of this dog changed also during the trials, the dog was calmer at the end of the trial. This could mean that the dog was active before the trial due to a feeling of hunger.

Additional information
Sample IgA in µg/g feces end of test period: 207,58
Dog no. 5 - Doyka

Pre-trial period
Breed: Malinois Sheppard
Gender: female
Age: 4 years
Weight: 25 kg
Stools rating: 4
Standard food: Proplan Salmon & Rice

General condition: good

Veterinarian comment
The diagnoses showed possible small intestine diarrhoea, which causes the dog to have soft stools. Also in case of stress the dog has stress diarrhoea. The breeder was aware of this problem and was able to reduce it with Proplan Salmon & Rice, if the dog receives another food he has diarrhoea.

Test results (faeces quotation in figure 7)
The dog was fed with a feeding rate: 400 gr in the morning and 400 gr in the evening.

During the first 4 days of the feeding with Formula Probiotic Sensitive (Example 1), the dog has a very light reaction to the food. Comments from the breeder indicate that such a light reaction is exceptional for this dog.

After 18 days of feeding we see slight improvement from level 4 to level 3.

Conclusion
The data in figure 7 show no difference in the faeces quality during the first 15 days. The consistency is completely stable with a mild improvement form the 18th day.

The feeding with the Formula Probiotic Sensitive gave the same result as with the Proplan Salmon & Rice. The living conditions of this dog didn't change during the trials, so we can conclude that Formula Probiotic Sensitive provides the adequate solution for the small intestine diarrhoea.
Additional information
Sample IgA in µg/g feces end of test period: 20.28

Dog no. 6 - Grisa

Pre-trial period
Breed: Malinois Sheppard
Gender: female
Age: 2 years
Weight: 26 kg
Stools rating: 6
Standard food: Duck frozen meat
General condition: good

Veterinarian comment:
The diagnoses showed a possible small intestine diarrhoea, which causes the dog to have very soft stools. Also in case of stress the dog has stress diarrhoea. The breeder was aware of this problem and tried to solve the problem with Proplan Salmon & Rice, but he changed it to Duck frozen meat. Even with this type of food he still had level 6 stools.

The fact that the breeder switched to frozen meat as diet for this dog also indicates bad digestive system which causes the dog to have problems digesting dry petfood.

Test results (faeces quotation in figure 8)
The dog was fed with a feeding rate: 400 gr in the morning and 400 gr in the evening.

Transferring a dog from a frozen meat diet to a dry petfood diet can lead to digestive problems. But in the case of this dog we see no reaction. After 4 days of feeding the dog with Formula Probiotic we can even see a small improvement on the feces quality, from level 6 to level 5.
Conclusion

The transfer of the frozen meat diet to the Formula Probiotic Sensitive was done successfully (figure 8). The product had a better result than when the dog was given Proplan Salmon & Rice. Even with Formula Probiotic Sensitive it was not possible to improve the stools to an acceptable level. The living conditions of this dog did not change during the trials, so it can be concluded that Formula Probiotic Sensitive provides a solution for the small intestine diarrhoea.

That the stools improved only with one level can be due to the bad digestive system of the dog. An extra reason for the less good stool quality can be the fact that the dogs eat very quickly, which leads to a bad intake of the food.

As general conclusion it can be stated that the Formula Probiotic Sensitive was the first dry petfood which gave an acceptable result on the dog.

Additional information

Sample IgA in µg/g feces end of test period: 167.38

*Dog no. 7 - Gripala*

Pre-trial period

Breed: Malinois Sheppard
Gender: male
Age: 1 year
Weight: 24 kg
Stools rating: 6-7
Standard food: Duck frozen meat + rice
General condition: Good, occasional diarrhoea

Veterinarian comment:

The diagnoses showed a possible small intestine diarrhoea, which is occurring occasionally. It is also linked to stress situations, since the dog is only in the kennel since 2 months, stress diarrhoea. The weight of this dog is correct according to his size. This dog received the frozen meat diet as a precaution.
Test results (faeces quotation in figure 9)
The dog was fed with a feeding rate: 400 gr in the morning and 400 gr in the evening.

Transferring a dog from a frozen meat diet to a dry petfood diet can lead to digestive problems. But in the case of this dog we see no reaction. After 2 days of feeding the dog with Formula Probiotic we can even see a small improvement on the faeces quality, from level 7 to level 6 and even further to level 5.

Conclusion
The transfer of the frozen meat diet to the Formula Probiotic Sensitive was done successfully (figure 9).
The living conditions of this dog didn't change during the trials, so we can conclude that Formula Probiotic Sensitive provides a solution for the small intestine diarrhoea.

That the stools improved only with one or two levels can be due to the fact that the dog is very nervous. According to the veterinarian the dog has also problems linked to his status within his new living environment.

As a general conclusion we can state the Formula Probiotic Sensitive offers the necessary comfort to the dog to provide with stable faeces during his adapting period.

Additional information
Sample IgA in µg/g feces end of test period: 23.88

Dog no. 8 - Weimie

Pre-trial period
Breed: Weimaraner
Gender: female
Age: 1 year
Weight: 22 kg
Stools rating: 6
Standard food: Crok 23/11 United Petfood

General condition: good

Veterinarian comment:

5 The diagnoses showed chronically stress diarrhoea. The dog has a very nervous character. The weight of the dog is a little low, the perfect weight for this type of dog should be 26 to 28 kg. This can indicate also a bad digestive system due to stress.

10 Skin and coat condition is rather low. The dog has no shiny coat; this can be explained with the bad digestive system, which causes a loss of the nutritional values of the food.

Test results (faeces quotation in figure 10)

15 The dog was feed with a feeding rate: 350 gr per day

These data show a very quick response to the Formula Probiotic Sensitive. Starting from the first day, the dog shows a clear improvement of his faeces quality. From the 5th day on the dog comes to level 5 stools. From the 20th day on we can register an even better stool quality with a best of level 3.

Conclusion

The transfer from his normal diet to Formula Probiotic Sensitive was done without any problems (figure 10). The improvement of the stools started from the first day of admission. From the 20th day we also see an improvement of the stools quality towards level 3. If the dog continues the Formula Probiotic diet, we feel that the level 3 stools could become the normal stools.

The weight of the dog increased after 15 days, to end after the complete trial at a weight of 24,5 kg. We can conclude that if the feeding with Formula Probiotic Sensitive would continue, the dog could reach his perfect weight (26 kg) within a period an extra month. The gain in weight shows also that the dog is now able to take all the nutritional value out of his diet. Skin and coat quality improved drastically. The dog had a shiny coat after 20 days of feeding him with Formula Probiotic Sensitive. The living conditions of this dog didn't change during the
trials, so we can conclude that Formula Probiotic Sensitive provides a solution for all the problems which this dog had.

Additional information
5 Sample IgA in µg/g faeces end of test period: not tested

**General conclusions**

In the attempt to prove the health effect of the Formula Probiotic these trials were based on the following diseases and symptoms:

Diarrhoea
Several dogs which were used in these trials were diagnosed with diarrhoea. The purpose was to prove the effect of the probiotic ingredient on the intestinal flora of these dogs and so prove that we could improve the faeces quality.

Stress diarrhoea
The effect of Formula Probiotic on stress diarrhoea has been proven through different dogs, where we were able to improve drastically the faeces quality.

Stress diarrhoea is very common with dogs which are living in breeding farms or dogs which are participating in competitions.

Chronic diarrhoea
The reason of chronic diarrhoea is often a food intolerance which leads to a bad digestive system. These are very common problems with modern dogs, especially when they are pure breed. During these trials we solved almost all the problem cases.

Food intolerance
It is only when we have a food intolerance towards one ingredient that the probiotic ingredient is not sufficient. To be able to prove the effect on food intolerance we advice to make a selection of dogs, where the allergy has been determined without any doubt.

Bad digestive system
There are several breeds of dogs which have a bad digestive system. The main breeds where this problem occurs are for example the Sheppard breeds, the Bulldog and Boxers. For this reason these dogs were all included in the trials. These dogs have a short digestive system, which causes them to have problems to digest completely the given food.

As we have seen through these test results, the probiotic ingredient helps these dogs to digest better their food. It also allows the dog owner to give lesser quantities of the food. So we can conclude that the nutritional rentability of the food given to the dog is much higher.

Skin problems
The main cause of skin problems is immunity deficiency. The first result on a dog with a low immunity level is the poor skin quality.

The results of these trials show that the probiotic ingredient cannot take away an actual disease such as demodex. But we were able to reduce the symptoms and the inconvenience for the dog.

When we look to the overall state of the skin and coat of the different dogs which participated in these trials, we can conclude that all dogs showed a better skin and coat quality at the end of the trials.

Vitality problems
Dogs with a low immunity level show a low vitality level. The problem is here that it is close to impossible to measure the level of vitality of a dog. For this result we had to rely on the opinion of the breeder.

The overall opinion of the several breeders which participated in these trials was that the vitality of the dogs was good. On the dog which received only half of his daily feeding, thanks to the Formula Probiotic, we were able to see a significant improvement of his vitality.
### Trial results table

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<th>ESTEBAN score</th>
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Scoring system (figure 1)

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<thead>
<tr>
<th>Score</th>
<th>Feces quality</th>
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<tbody>
<tr>
<td>1</td>
<td>Very hard and dry, no residue left on the ground when picked up.</td>
</tr>
<tr>
<td>2</td>
<td>Firm, not hard. Little residue left on the ground when picked up.</td>
</tr>
<tr>
<td>3</td>
<td>Log-like, moist surface, leaves residues but holds form when picked up</td>
</tr>
<tr>
<td>4</td>
<td>Very moist, long shape leaves residues and loses form if picked up.</td>
</tr>
<tr>
<td>5</td>
<td>Very moist, present in piles, distinct shape, leaves residues and loses form if picked up.</td>
</tr>
<tr>
<td>6</td>
<td>No defined shape, but has texture, occurs as spot or pile, leaves residues if picked up.</td>
</tr>
<tr>
<td>7</td>
<td>No texture, watery, flat, occurs as puddles</td>
</tr>
</tbody>
</table>

Example 7

A dog was fed with a kibble of the present invention in a trail and the following was stated:

"The dog has a very sensitive stomach and has only been fed moist intestinal products".

"The dog is doing much better with your dry food now and likes it very much."

Example 8

Two dogs were fed with a kibble of the present invention in a trail and the following was stated:

"I have two dogs, both German Shorthaired Pointers, the younger one will be four in November has had digestive problems since birth. Over the years, he has suffered from skin problems, chewing his pawls, licking his skin a lot, bad body odour, bad breath, a lot of gas in his stomach and diarrhoea. With some dog food, he had such a volume of waste that he had to use his bowels several times a day. We have had a lot of problems with
him. With the Formula Probiotic for the younger dog, the following result was achieved:

1. No body odor
2. No bad breath
3. No chewing paws or licking himself
4. No diarrhoea or large bowel movements
5. No gas problems
6. His energy levels are good"

Example 9

A dog was fed with kibbles of the present invention in a trial and the following was stated:

"For our Labrador (3 years), we have tried different types of food, but the allergy on the skin did not disappear. Once we fed our dog with your probiotic food, the allergy disappeared. We were very happy."

"Now we are back to the old food and the allergies are back as well..."

Example 10

Comparative studies of changes in PURINA Score

An embodiment of the present invention relates to a method wherein the positive effect on the constipation is reached in less than 3 weeks, such as less than 14 days, such as less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days, less than 6 days, less than 5 days, less than 4 days, less than 3 days, less than 2 days, or less than 1 day.

Example 11

Measuring the viscosity of selected oils

Equipment: Dynamic rheometers Physica MCR 301 (Anton Paar GmbH, Germany), C-PTD200 Peltie temperature control and CC27 coaxial cylinder measuring system (in/out diameter 26.66 and 28.92 mm)
Method: The viscosity of the oils was measured at turning speed of 180 rpm; at temperature range of 5 to 50°C, heating rate was 0.5°C/min, viscosity was registered after each 1°C. Two parallels of samples were measured. The table of Figure 11 lists the average viscosity (Pa-s) of the oils.

UPP, Belgium supplied:
1. Crude fish oil
2. Salmon oil A
3. Refined maize oil
4. Cod liver oil

Vobra Special Petfoods BV, Netherlands supplied:
5. Salmon oil B
6. Soybean oil (with antioxidant)
7. Sunflower oil (with antioxidant)
8. Linseed oil
9. Borage oil

Results: One of the oils, Salmon oil A (supplied by United Petfoods (UPP) Belgium), displays unique viscosity properties over the remaining oils tested in the present experiment. Although the viscosity of Salmon oil A at refrigerating temperatures is higher than the remaining fish oils, in the temperature range of range 20-25°C Salmon oil A loose viscosity much faster with increasing temperature than the remaining oils tested. Accordingly, the change in the viscosity (ΔPa-s/°C) of Salmon oil A with temperature (within the temperature range 20-25°C) of Salmon oil A is different from the remaining oils tested in the experiment. The change in the viscosity (ΔPa-s/°C) of crude fish oil (supplied by United Petfoods (UPP) Belgium), cod liver oil (supplied by United Petfoods (UPP) Belgium) and salmon oil B (Vobra Special Petfoods BV, Netherlands) is basically the same within the temperature range of 20-25°C.

Salmon oil A was chosen as carrier oil (vehicle) for preparation of a probiotic/oil suspension for manufacturing a probiotic extrusion product by vacuum inclusion of the suspension. Salmon oil A was preferred over the remaining oils due to the unique viscosity properties in the temperature range 20-25°C. The manufacturing
process is performed in temperature range 20-25 °C and the use of Salmon A oil will avoid the clotting of a spraying tip (nozzle) of a vacuum coater and improve homogenous distribution of probiotics in the carrier oil. Additionally oil/probiotic mixture is constantly mixed in the tank before introduction into a vacuum coater, thus formation of a probiotic flakes (non suitable for a vacuum coating) is avoided during the bacteria addition to the oil.

The viscosity of the analysed oils are equal at high temperatures (starting from 40 °C), but such high temperatures have severe effects on the viability of probiotic bacteria, and consequently on the CFU/kg of the final food product.

Taken together, viscosity of oils is influenced by the source of the oil and substances added to the oil. The substances added to the oil affects the properties of the oils such as the viscosity. Accordingly, the properties of the oil have to be taken into account when choosing an oil as a vehicle for infusion of probiotic micro-organism. Since, care should also be taken to ensure that the substances added to the oil in the preparation of the oil/probiotic suspension does not severely affect important parameters of the suspension such as the viscosity.

Example 12
Mixing of probiotics and an oil solution to obtain a probiotic suspension.

The suspension can be obtained by mixing one probiotic micro-organism, in a dry powder form having a total concentration of 10^9-10^16 CFU/kg dry powder, into an oil. The inclusion rate for the final suspension should be 0.3-15 kg of the probiotic powder per 100 kg oil. When the probiotics are mixed into an oil the probiotics may precipitate if the powder is not mixed slowly into oil. Thus, not all of the freeze-dried powder should be added at once. To maintain the viability of the probiotics, the temperature of the suspension should not exceed 30 °C. The mixing may be performed in a mixing tank, such as an IBC container, under continuously stirring. This mixing may be performed manually. Preferably the obtained suspension is transferred to a storage tank comprising mixing means. The transfer from the mixing tank to the storage tank is preferably done through a bottom outlet in the mixing tank into the storage tank (thus the mixing tank is physically
positioned above the storage tank). The suspension is then mixed in the storage tank at a temperature of 15-29°C, not exceeding 30°C (the mixing may be performed by rotation at 5-350 RPM) to obtain a suspension of homogenously dispersed probiotic micro-organism. The suspension should not be stored for longer than 3 hours in the storage tank before it is used in a vacuum infusion. If the suspension is stored for a longer time the suspension may become contaminated.

Example 13

10 Suspension/oil vehicle for dog food

The right choice of an oil as a probiotic compound carrier (oil vehicle) is based on the viscosity of the specific oil and the temperature which is needed to be implemented to achieve a particular viscosity. Together with the physical/chemical parameters of the oil which can have an influence on the viability of the probiotics, the organoleptic parameter of the specific oil also is a dramatic factor on an overall product taste and odor. In addition nutritional parameters also need to be considered. Thus, to find an oil vehicle which fulfils all these parameters is not an easy task.

20 Organoleptic parameters:

In case of a probiotic dog food, a suspension with a salmon oil carrier is used to produce an extruded dry dog food, the choice of the salmon oil was based on a fact that dogs eat for 90 % with his smell and have a smell 30 times more than humans. Thus, it is very crucial to find the particular oil vehicle for a probiotic compound which will not have an influence on a palatability of the final product (dog food) based on a smell as major organoleptic parameter (especially for a dogs).

Nutritional parameters:

30 Together with above mentioned parameters, an oil used as an oil vehicle for probiotics needs to be „healthy“. High content of a saturated fatty acids, trans fatty acids and etc are generally considered as "unhealthy". The high concentration of such fats furthermore minimizes the probiotic effect of the ready product and increases the risk of coronary heart disease by raising levels of "bad"
LDL cholesterol and lowering levels of "good" HDL cholesterol. Salmon oil out of the animal fats is well known for its unique composition of poly unsaturated fatty acids (omega 3 and omega 6) and thus is generally considered as „healthy" fat. To be able to provide a product having the above mentioned properties and the same be optimal for vacuum infusion it has been discovered that the viscosity of the oil vehicle is important.

Viscosity:
To find a salmon oil which also fulfils the criteria for being suited for vacuum infusion, viscosity of different salmon oils were compared. As shown in figures 11 and 12 not all salmon oils have the same viscosity properties. The viscosity of salmon A decreases faster between 20°C and 25°C than does salmon oil B giving an extra advantage of usage of salmon oil A as a carrier (oil vehicle) of a probiotic compound. Salmon oils with such viscosity behaviour improve the mixing ability of the suspension together with equalized dispersal of the probiotic compound in the ready product and reduces sedimentation/wastes during the manufacturing stage with improvement of stability of the probiotic compound within the suspension and thus within the ready product.
Taken together salmon oil A becomes a suited oil vehicle for vacuum infusion of probiotics for animal food such as dog food.
It is to be understood that although the present example refers to dog food it does not mean that salmon oil A cannot be used in other animal products or human products.

Example 14
Oil vehicle/suspension for human food products

The right choice of an oil as a probiotic compound carrier (oil vehicle) is based on the viscosity of the specific oil and the temperature which is needed to be implemented to achieve a particular viscosity. Together with the physical/chemical parameters of the oil which can have an influence on the viability of the probiotics, the organoleptic parameter of the specific oil also is a dramatic factor on an overall product taste and odor. In addition nutritional parameters also need
to be considered. Thus, to find an oil vehicle which fulfils all these parameters is not an easy task.

Organoleptic parameters
5 Usage of animal fats/oils in a human product is limited because of the organoleptic parameters which can have an overall effect on a palatability of the ready product. Thus, such animal oils, like different type of fish oils, may lead to resistance by the end consumer towards such products, even if oil meets the health criterias (e.g. as described in example 3). Thus, the oil used as a probiotic oil vehicle in a human product needs to meet the viscosity criteria required for optimal vacuum infusion but with different organoleptic parameters than the oils used for animal products. Vegetable oils may be suitable candidates.

Nutritional parameters:
15 Instead of using animal oil it may be advantageous also to be able to have a suitable oil vehicle with vegetable origin. Several vegetable oils have positive health parameters. Linseed oil (Vobra Special Petfoods BV, Netherlands) compared with soy bean oil, maize oil and sunflower oil is considered as "healthy" oil with high concentration of poly unsaturated fatty acids (omega 3 and omega 6) and mild nutty taste. These parameters make linseed oil a suitable candidate as an oil vehicle for human product manufacturing.

Viscosity:
When comparing the viscosity of different oils with vegetable origin in the range of 20°C and 25°C, it becomes apparent that linseed oil has unique properties for being used as an oil vehicle for vacuum infusion of probiotics (Figure 11 and 14). Linseed oil has the lowest viscosity at both 20°C and 25°C out of the vegetable oils analyzed. The curve of the linseed has got a small slope (low delta viscosity) but a low viscosity when compared to the other oils. Even when compared to the animal oils (Figures 11 and 13), linseed oils has the lowest viscosity at both 20°C and 25°C.

Taken together, the viscosity of the linseed oil together with its unique physical/chemical and organoleptic parameters makes linseed oil a good candidate for usage as a probiotic oil vehicle for human product manufacturing.
It is to be understood that although the present example refers to human products it does not mean that linseed oil cannot be used in animal products.

5 Example 15

Viscosity of suspension
Since the viscosity of the final suspension is a key parameter when the suspension is going to be vacuum infused, the influence of the bacteria on the viscosity of the oil should be tested. Figure 11 (lines 10-13) and figure 16 clearly show that the influence of the bacteria on the final viscosity at different temperatures is minimal. "Susp" (solid line) is salmon oil A with probiotics with a concentration/inclusion rate 1.2 kg/ton of final product. Raw oil (dashed line) is salmon oil A without probiotics. Top lines show the viscosity when the temperature is increased from 5-50°C, whereas the bottom lines show the viscosity when the temperature is decreased from 50-5°C. In the bottom lines the dashed and solid lines are practically positioned on top of each other. The difference is between the cooling and heating is likely due to residual heat in the analyzed samples.

Figure 11 (lines 10-13) shows the viscosity of the raw salmon oil vs suspension viscosity at heating from 5°C to 50°C and backwards cooling from 50°C to 5°C. At current inclusion rate which was used the viscosity difference between both samples is minor with average of 0.001 Pa-s at each temperature step between both samples.

Δ vise. (20°C -25°C) of raw oil is 0.011 Pa-s at heating phase and 0.009 Pa-s at cooling phase.

Δ vise. (20°C -25°C) of suspension is 0.011 Pa-s at heating phase and 0.010 Pa-s at cooling phase.

Overall conclusion can be made that change of Δ vise. (20°C -25°C) of both samples at cooling and heating phases are minor and makes a 0.01 Pa-s in average.
In general there will be a difference between different measurements of the viscosity of a specific type of oil. This is likely due to the precise batch used and small variation in the way the samples are handled. Though such small variations are unavoidable the current invention clearly shows that the viscosity of the oil/suspension is indeed important for the viability of the probiotics in the final product.
Claims

1. A method for defecation management in a dog comprising feeding the dog with a vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil.

2. The method according to claim 1, wherein the faeces condition of the dog is levelled to score 3 and 4 of the PURINA feces scoring system.

3. The method according to claim 1 or 2, wherein the oil has dynamic viscosity of less than 0.08 pascal-second (Pa-s) at 20°C.

4. The method according to any of the preceding claims, wherein the kibble contains $10^6$-$10^{19}$ CFU/kg.

5. The method according to any of the preceding claims, wherein the change in feces consistency is reached in less than 14 days.

6. The method according to any of the preceding claims, wherein the change in faeces consistency is achievable 12 months after the production date of the kibble.

7. The method according to any of the preceding claims, wherein the kibble is not stored under refrigerated conditions.

8. Use of a pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil for the manufacture of a composition for improving the faeces condition of a dog to score 3 and 4 of the PURINA feces scoring system.

9. Use of a pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil for the manufacture of a composition for maintaining a faeces condition of a dog at score 3 and 4 of the PURINA faeces scoring system.
10. A vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil for use in improving the faeces condition of a dog to score 3 and 4 of the PURINA faeces scoring system.

11. A vacuum infused pet food kibble comprising within the kibble structure a mixture of probiotic micro-organisms and an oil for use in maintaining the faeces condition of a dog at score 3 and 4 of the PURINA faeces scoring system.

12. The method according to any of the preceding claims, wherein the oil is selected from the group consisting of fish oils, vegetable oils, and a combination thereof.

13. The method according to any of the preceding claims, wherein the fish oil is selected from the group consisting of salmon oil, mackerel oil, lake trout oil, herring oil, sardine oil, albacore tuna oil, cod liver oil, sand eel oil (Ammodytes tobianus), and menhaden oil.

14. The method according to claim 12, wherein the oil is a vegetable oil.

15. The method according to claim 14, wherein the oil is selected from the group consisting of linseed oil, olive oil, borage oil, lin oil, camelina oil, grape seed oil, chia oil, kiwifruit seeds oil, perilla oil, lingonberry, purslane oil, seabuckthorn oil, hemp oil and soybean oil.
## Bristol Stool Chart

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<tr>
<th>Type</th>
<th>Description</th>
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<tr>
<td>Type 1</td>
<td>Separate hard lumps, like nuts (hard to pass)</td>
</tr>
<tr>
<td>Type 2</td>
<td>Sausage-shaped but lumpy</td>
</tr>
<tr>
<td>Type 3</td>
<td>Like a sausage but with cracks on its surface</td>
</tr>
<tr>
<td>Type 4</td>
<td>Like a sausage or snake, smooth and soft</td>
</tr>
<tr>
<td>Type 5</td>
<td>Soft blobs with clear-cut edges (passed easily)</td>
</tr>
<tr>
<td>Type 6</td>
<td>Fluffy pieces with ragged edges, a mushy stool</td>
</tr>
<tr>
<td>Type 7</td>
<td>Watery, no solid pieces. <strong>Entirely Liquid</strong></td>
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</table>

**Fig. 2**

SUBSTITUTE SHEET (RULE 26)
5/16

Fig. 5
SUBSTITUTE SHEET (RULE 26)
<table>
<thead>
<tr>
<th>Oil</th>
<th>5 °C</th>
<th>10 °C</th>
<th>15 °C</th>
<th>20 °C</th>
<th>25 °C</th>
<th>30 °C</th>
<th>35 °C</th>
<th>40 °C</th>
<th>45 °C</th>
<th>50 °C</th>
<th>Δ visc. (20-25°C)</th>
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<td>0.049</td>
<td>0.04</td>
<td>0.034</td>
<td>0.028</td>
<td>0.024</td>
<td>0.021</td>
<td>0.011</td>
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<tr>
<td>2. Salmon oil A</td>
<td>0.119</td>
<td>0.097</td>
<td>0.077</td>
<td>0.061</td>
<td>0.048</td>
<td>0.04</td>
<td>0.033</td>
<td>0.028</td>
<td>0.024</td>
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<td>3. Refined maize oil</td>
<td>0.133</td>
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<td>0.066</td>
<td>0.053</td>
<td>0.044</td>
<td>0.036</td>
<td>0.031</td>
<td>0.026</td>
<td>0.022</td>
<td>0.013</td>
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<tr>
<td>4. Cod liver oil</td>
<td>0.119</td>
<td>0.096</td>
<td>0.075</td>
<td>0.061</td>
<td>0.049</td>
<td>0.041</td>
<td>0.034</td>
<td>0.029</td>
<td>0.025</td>
<td>0.021</td>
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<td>5. Salmon oil B</td>
<td>0.116</td>
<td>0.093</td>
<td>0.074</td>
<td>0.059</td>
<td>0.048</td>
<td>0.04</td>
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<td>0.024</td>
<td>0.021</td>
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<tr>
<td>6. Soy bean oil (with antioxidant)</td>
<td>0.115</td>
<td>0.092</td>
<td>0.073</td>
<td>0.059</td>
<td>0.048</td>
<td>0.04</td>
<td>0.033</td>
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<td>0.024</td>
<td>0.021</td>
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<td>7. Sunflower oil (with antioxidant)</td>
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<td>0.053</td>
<td>0.043</td>
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<td>8. Linseed oil</td>
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<td>0.065</td>
<td>0.053</td>
<td>0.043</td>
<td>0.036</td>
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<td>0.026</td>
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<td>9. Borage oil</td>
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<td>0.092</td>
<td>0.072</td>
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<td>0.047</td>
<td>0.039</td>
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<td>10. suspension (temp. up)</td>
<td>0.119</td>
<td>0.097</td>
<td>0.075</td>
<td>0.059</td>
<td>0.048</td>
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<td>0.033</td>
<td>0.028</td>
<td>0.024</td>
<td>0.02</td>
<td>0.011</td>
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<tr>
<td>11. suspension (temp. down)</td>
<td>0.107</td>
<td>0.084</td>
<td>0.067</td>
<td>0.054</td>
<td>0.044</td>
<td>0.037</td>
<td>0.031</td>
<td>0.026</td>
<td>0.023</td>
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<tr>
<td>12. Raw oil (temp. up)</td>
<td>0.115</td>
<td>0.095</td>
<td>0.073</td>
<td>0.058</td>
<td>0.047</td>
<td>0.039</td>
<td>0.032</td>
<td>0.027</td>
<td>0.023</td>
<td>0.02</td>
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<tr>
<td>13. Raw oil (temp. down)</td>
<td>0.106</td>
<td>0.083</td>
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Fig. 13

SUBSTITUTE SHEET (RULE 26)
Fig. 14

SUBSTITUTE SHEET (RULE 26)
Fig. 16
SUBSTITUTE SHEET (RULE 26)
A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K1/00 A23K1/18
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)
EPO-Internal, BIOSIS, FSTA, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C

Date of the actual completion of the international search
16 June 2010

Name and mailing address of the ISA/
European Patent Office, P B 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel (+31-70) 340-2040,
Fax (+31-70) 340-3016

Authorized officer
Couzy, François

Date of mailing of the international search report
29/06/2010

Form PCT/ISA/2.10 (second sheet) (April 2005)
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Form PCT/ISA/210 (patent family annex) (April 2005)