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(54) Title: MEANS AND METHODS FOR MODULATING BEHAVIOR

(57) Abstract: The present invention relates to a method for modulating behavior of an animal, improving animal welfare, and for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin improving animal performance, using an oligosaccharide preparation.

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MEANS AND METHODS FOR MODULATING BEHAVIOR

[001] The present invention relates to a method for modulating behavior of an animal, improving animal welfare, and for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin improving animal performance, using an oligosaccharide preparation.

[002] Secondary metabolites are molecules produced by an organism, and exert their function in interactions of the producing organism with its environment. Some well known secondary metabolites are caffeine, taxol, nicotine, artemisinin, morphine, botulinum toxin etc. Notably, many secondary metabolites may serve several purposes. For instance, caffeine is believed to be produced by plants as a pesticide, while humans consume caffeine to increase vigilance. Other secondary metabolites produced by one organism may act as a neurotransmitter in another organism. Neurotransmitters are signaling molecules which allow communication among cells of an organism. Receptors for neurotransmitters may be present e.g. on muscle cells or neurons. Gamma-aminobutyric acid (GABA; CAS-No. 56-12-2; also referred to e.g. as 5-aminobutanoic acid) is known to be a main inhibitory neurotransmitter in the brain and the spinal cord. GABA slows or blocks specific nerve signals in the brain, thereby modulating levels of anxiety, fear and/or stress as perceivable by humans and animals. Accordingly, imbalanced GABA activity in the body can lead to associated disorders, such as anxiety disorders.

[003] Kynurenine (KYN) is known as a neuromodulator of stress. In particular the ratio of KYN over tryptophan (TRP) was found to specifically relate to aggressive behavior such as feather pecking, and to social disturbance in laying hens in general. It was found that a lower KYN/TRP ratio is linked to higher social disturbance profile. It is hypothesized that an increase of the kynurenine/tryptophan ratio is responsible for reducing social disturbance behavior, thus improving animal welfare.

[004] Serotonin within the central nervous system cannot cross the blood/brain barrier, while tryptophan is capable of crossing the blood/brain barrier. Therefore, higher tryptophan in the gut means more tryptophan will cross the blood/brain barrier, where it can be transformed to central serotonin. Serotonin is the precursor of melatonin. An increase in serotonin level will thus cause an increase in melatonin level. It is known that melatonin and its precursor serotonin can impact the production of insulin and glucagon. An increase in the melatonin concentration can enhance the level of insulin and glucagon in animal body. It is also known that increased levels of insulin and glucagon enhance the synthesis of fat. Both insulin and melatonin are further involved in regulating circadian rhythm. Changes in the light cycle affect the levels of insulin and melatonin produced by an animal. The changed level of insulin and melatonin in the body of the animal in turn regulates the animal's physiological response to the light cycle change. Poultry production in

general, and broiler rearing process is now going to long light time, as much as 23 hours a day. This illumination regimen strongly impacts production performance such as faster fat gain but is detrimental to animal welfare.

[005] Tail biting (TB) is an abnormal, pathological behavior frequently observed in weaner and grower-finisher in commercial pig production, and thus a major health and welfare problem due to the risk of infection, the pain and stress experienced by the bitten animal as well as the additional stress experienced by the entire animal group. Previous studies have shown that pigs that perform tail biting have a different concentration of neurotransmitters than pigs that do not develop that aggressive behavior. It is increasingly being recognized that neurochemicals or neurotransmitters produced from either or both, an animal and/or its microbiome represent a common language for host-microbe communication. It has been described that microbial endocrinology plays a role in animal behavior through the microbiota-gut-brain axis (MGBA) (Lyte et al. 2019. Animal. 13(11):2689-2698). It is known that gut microbiota are capable of synthesizing neuroactive molecules that are structurally similarly to host-derived neurotransmitters including γ-aminobutyric acid (GABA), indole-derivatives and catecholamines such as dopamine and norepinephrine. Many of these co-synthesized neurotransmitters are involved in regulation of the host mood, behavior and cognition.

[006] The GABAergic system is widely distributed in the hippocampus of piglets and has an inhibitory action on the neurons in the hippocampus. In general, GABA is synthesized through the decarboxylation of glutamate, catalyzed by glutamic acid decarboxylase (GAD), and is widely distributed in the nervous system to reduce the activity of neurons by binding to GABA receptors. Brain levels of GABA are low in mice and rats that exhibit aggressive behaviors, which have been interpreted as being concordant with the proposed inhibitory role of GABA in aggression (Clement et al. 1987. Pharmacol Biochem Behav. 26(1):83-8). Furthermore, direct supplementation with GABA can regulate stress, which is reflected in reducing aggressive behaviors and improving growth performance. Additionally, GABA supplementation could improve the transport stress of growing-finishing pigs (Bi et al. 2020. J Anim Physiol Anim Nutr. 104: 590-596). Notably however, direct external supply of GABA yielded conflicting results with limited efficacy so far. Also, the half-life of GABA in the body is short (e.g. less than 17 min in mice), thus further reducing efficacy and efficiency of direct GABA administration.

[007] Despite the indisputable relevance of such secondary metabolites on human and animal wellbeing, welfare and performance, convenient and reliable means and methods for modulating the levels of these secondary metabolites in the body, to modulate behavior, and to improve welfare, performance and wellbeing remain to be identified.

[008] In view of the prior art as outlined above, it is an objective of the present invention to provide means and methods for modulating behavior of an animal, for improving animal welfare, and for improving animal performance of non-stressed as well as of stressed animals.

[009] Surprisingly, this objective is achieved by providing a method for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; iv) for improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or v) for reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the steps of 1) providing an oligosaccharide preparation produced by or producible by a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; 2) feeding the oligosaccharide preparation to the animal; and 3) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[010] In some embodiments, the method according to the invention for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; relates to the improvement of animal performance of animals facing stress, which animals facing stress received the oligosaccharide preparation, compared to animals facing stress not having received the oligosaccharide preparation. In some embodiments, the method according to the invention for v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; relates to the reduction of cortisol in plasma, saliva and/or hair of animals facing stress, which animals facing stress received the oligosaccharide preparation, compared to animals facing stress not having received the oligosaccharide preparation.

[011] In another aspect, the invention relates to a method for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or iv) for improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; the method comprising the steps of 1) providing an oligosaccharide preparation, comprising a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization

(DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; 2) feeding the oligosaccharide preparation to the animal; and 3) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[012] The term "condensing" or "condensation" as used herein are to be interpreted as the steps necessary for allowing combination of feed sugars or starting sugars, e.g. simple sugars (monosaccharides), with one another to form larger sugars (disaccharides, oligo- or polysaccharides). For instance, two monosaccharides may be combined with one another to form a disaccharide. Analogously, one monosaccharide may be condensed with a disaccharide to form a trisaccharide etc. In the course of such a condensation reaction, water molecule is released. Herein, condensation is initiated by applying heat to a mixture of feed sugar(s) in the presence of a catalyst.

[013] In some embodiments, the invention relates to a method for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; iv) for improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or v) for reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the steps of 1) providing an oligosaccharide preparation produced by or producible by condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; 2) feeding the oligosaccharide preparation to the animal; and 3) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[014] In some embodiments, the invention relates to a method for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; iv) for improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or v) for reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the steps of 1) providing an oligosaccharide preparation produced by or producible by condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction

having a DP of more than 2; 2) feeding the oligosaccharide preparation to the animal; and 3) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[015] In some embodiments, the invention relates to a method for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; iv) for improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or v) for reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the steps of 1) providing an oligosaccharide preparation produced by or producible by condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine, and wherein the catalyst is methylphosphonic acid; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; 2) feeding the oligosaccharide preparation to the animal; and 3) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[016] In some embodiments, the at least second sugar in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is also N-acetylglucosamine. In other embodiments, the at least second sugar in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is selected from glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

[017] In some embodiments, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) are not the same sugar(s) as in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine). In some embodiments, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) are both glucose. In some embodiments, the at least first sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) is glucose; and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from arabinose, sucrose, xylose, mannose, lactose and fructose.

[018] In some embodiments, the at least first sugar and the at least second sugar of a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from lactose. In some embodiments, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) is condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from lactose. In some embodiments, a method according to the invention comprises the step of condensing at least a first sugar with at least a second sugar using a catalyst, preferably wherein the catalyst is methylphosphonic acid, wherein the at least first sugar is N-acetylglucosamine, wherein the at least second sugar is glucose, and wherein the at least third sugar is selected from lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

[019] In some embodiments, at least one of the sugars of a) is/are glucose; wherein said glucose is used at an amount from 50-99% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments, the at least second sugar is used at an amount from 1-50% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments, the at least third sugar is used at an amount from 1-50% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments, a) is performed by condensing at least a first sugar with at least a second sugar and at least a third sugar using a catalyst, wherein the at least first sugar, the at least second sugar and the at least third sugar are used at a ratio of 50-99 : 1-50 : 1-50, preferably wherein at least one of the at least first sugar, the at least second sugar and the at least third sugar is glucose.

[020] In some embodiments of a method according to the invention, at least one of the sugars of b) is/are glucose; wherein said glucose is used at an amount from 50-99% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments of a method according to the invention, the at least second sugar is used at an amount from 1-50% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments of a method according to the invention, the at least third sugar is used at an amount from 1-50% (e.g. wt%) of all sugars that are being used in the condensation step. In some embodiments of a method according to the invention, b) is performed by condensing at least a first sugar with at least a second sugar and at least a third sugar using a catalyst, wherein the catalyst is methylphosphonic acid, wherein the at least first sugar, the at least second sugar and the at least third sugar are used at a ratio of 50-99 : 1-50 : 1-50, preferably wherein at least one of the at least first sugar, the at least second sugar and the at least third sugar is glucose.

[021] In another aspect, the invention relates to a method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community; the method comprising the steps of 1) providing an oligosaccharide preparation produced or producible by a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; and contacting the microbial community with the oligosaccharide preparation.

[022] In another aspect, the invention relates to a method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community; the method comprising the steps of 1) providing an oligosaccharide preparation, comprising a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2; and contacting the microbial community with the oligosaccharide preparation.

[023] Hereby, production of gamma-aminobutyric acid, kynurenine and/or serotonin can be achieved independent from an animal hosting the microbial community. In some instances, such a method can be used e.g. in screening studies on substances capable of enhancing or inhibiting microbial production of gamma-aminobutyric acid, kynurenine and/or serotonin.

[024] A person skilled in the art will understand that the production of gamma-aminobutyric acid, kynurenine and/or serotonin (e.g. by a microbial community) will start upon contacting one or more cell(s) (e.g. of the microbial community) with the oligosaccharide preparation, and the amount(s) of gamma-aminobutyric acid, kynurenine and/or serotonin produced will increase with ongoing incubation or fermentation time.

[025] In some embodiments, the microbial community is an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably an animal intestinal microbial community, e.g. pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow.

[026] In another aspect, the present invention relates to a method for producing an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress

and/or fear disorders; for iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal; and/or for iv) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the step of: a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[027] In some embodiments, the invention relates to a method for producing an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal; and/or for iv) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; the method comprising the step of: condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine, and wherein the catalyst is methylphosphonic acid; thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[028] In a further aspect, the invention relates to an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or for v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; wherein the oligosaccharide preparation is produced or producible by a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[029] In some embodiments, the invention relates to an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving

animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or for v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; wherein the oligosaccharide preparation is produced or producible by condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine, wherein the catalyst is methylphosphonic acid; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[030] In some embodiments, the at least second sugar in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is also N-acetylglucosamine. In other embodiments, the at least second sugar in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is selected from glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

[031] In some embodiments of the oligosaccharide preparation, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) are not the same sugar(s) as in a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine). In some embodiments of the oligosaccharide preparation, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) are both glucose. In some embodiments of the oligosaccharide preparation, the at least first sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) is glucose; and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from arabinose, sucrose, xylose, mannose, lactose and fructose.

[032] In some embodiments of the oligosaccharide preparation, the at least first sugar and the at least second sugar of a) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine) is condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from lactose. In some embodiments of the oligosaccharide preparation, the at least first sugar and the at least second sugar of b) (condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst

is methylphosphonic acid) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from lactose. In some embodiments of the oligosaccharide preparation, the oligosaccharide preparation is produced or producible by condensing at least a first sugar with at least a second sugar using a catalyst, preferably wherein the catalyst is methylphosphonic acid, wherein the at least first sugar is N-acetylglucosamine, wherein the at least second sugar is glucose, and wherein the at least third sugar is selected from lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

[033] In some aspects, the invention relates to an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or for v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; wherein the oligosaccharide preparation comprises at least two distinct monosaccharide units selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from glucose and N-acetylglucosamine; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2. In some embodiments, the invention relates to an oligosaccharide preparation for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or for v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress; wherein the oligosaccharide preparation comprises at least two distinct monosaccharide units selected from glucose, N-acetylglucosamine, and at least a third distinct monosaccharide unit selected from lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[034] In some embodiments, the oligosaccharide preparation according to the invention comprises at least 10 wt%, (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 wt% etc.) of the at least first fraction; preferably at least 20 wt%.

[035] In some embodiments, the oligosaccharide preparation according to the invention is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

[036] In some embodiments, the oligosaccharide preparation according to the invention comprises at least 4 fractions (e.g. at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 etc. fractions) each having a distinct DP.

[037] In some embodiments, the oligosaccharide preparation according to the invention comprises not more than three distinct monosaccharide units.

[038] In some embodiments, the oligosaccharide preparation according to the invention comprises less than 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2% anhydro-subunit containing oligosaccharides by relative abundance; and/or wherein each fraction of the oligosaccharide preparation comprises greater than 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% anhydro-subunit containing oligosaccharides by relative abundance.

[039] In some embodiments, the oligosaccharide preparation according to the invention has a weight averaged molecular mass from 300 to 5000 g/mol (e.g. from about 2000 to 2800 g/mol, 2100 to 2700 g/mol, 2200 to 2600 g/mol, 2300 to 2500 g/mol, or 2320 to 2420 g/mol), 500 to 5000 g/mol, 700 to 5000 g/mol, 500 to 2000 g/mol, 700 to 2000 g/mol, 700 to 1500 g/mol, 300 to 1500 g/mol, 300 to 2000 g/mol, 300 to 700 g/mol, 400 to 1300 g/mol, 400 to 1200 g/mol, 400 to 1100 g/mol, 500 to 1300 g/mol, 500 to 1200 g/mol, 500 to 1100 g/mol, 600 to 1300 g/mol, 600 to 1200 g/mol, or 600 to 1100 g/mol); and/or wherein the oligosaccharide preparation has a number averaged molecular mass from 1000 to 2000 g/mol (e.g. from 1100 to 1900 g/mol, 1200 to 1800 g/mol, 1300 to 1700 g/mol, 1400 to 1600 g/mol, 300 to 1050 g/mol, 300 to 1020 g/mol, 300 to 1010 g/mol, 400 to 1050 g/mol, 400 to 1020 g/mol, 400 to 1010 g/mol, 500 to 1050 g/mol, 500 to 1020 g/mol, 500 to 1010 g/mol, or 1450 to 1550 g/mol).

[040] In a further aspect, the invention relates to a use of an oligosaccharide preparation according to the invention and as referred to herein, for i) modulating behavior of an animal, in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; for v) increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community; and/or for vi) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress.

[041] In particular embodiments, the invention relates to a use of an oligosaccharide preparation according to the invention and as referred to herein, for i) modulating behavior of a healthy animal,

in particular for reducing aggressive behavior; for ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders of a healthy animal; for iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio of a healthy animal; for iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio of a healthy animal facing stress; for v) increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community; and/or for vi) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress.

[042] In another aspect, the invention relates to an oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid, kynurenine and/or cortisol, wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation, wherein the oligosaccharide preparation is produced or producible by a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[043] In some embodiments, the invention relates to an oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid, kynurenine and/or cortisol, wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation, in an animal suffering from or at risk at suffering from one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine, wherein the oligosaccharide preparation is produced or producible by a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by b) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the catalyst is methylphosphonic acid; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2. Animals suffering from one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine, may be animals (e.g. swine) e.g. being subjected to a situation prone to cause social unease for said animals, e.g. mixing of individuals amongst groups of animals. Analogously, e.g. animals about to being subjected to such situation(s) may be considered to be at risk at suffering from one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine.

[044] In some embodiments, the oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis is an oligosaccharide preparation according to the invention and as described herein.

[045] In some embodiments, the invention relates to an oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid, kynurenine and/or cortisol, wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation, wherein the oligosaccharide preparation is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

[046] In some embodiments of the invention, the animal referred to herein is selected from the group consisting of pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna; preferably, the animal is selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow; preferably swine.

[047] It has been observed that the health of the host animal, animal welfare and/or animal performance are improved in four aspects by the means, methods and uses of the invention. First, welfare of the group of production animals is improved. It is a common problem for monogastric animals such as pigs, chicken or ducks raised in a confined space to develop social disturbance behaviors such as feather pecking or tail biting. Disturbance behaviors like this cause poor welfare of the production animal and thus has been a persisting problem for animal farmers. The means, methods and uses according to the invention help to improve the welfare of animals.

[048] Second, the health of the host animal can be improved by way of decreasing systemic inflammation of the animal. Systemic inflammation is the result of release of pro-inflammatory cytokines from immune-related cells and the chronic activation of the innate immune system. It contributes to the development of chronical disease conditions in animals. The method according to the invention helps to reduce systemic inflammation of the animal.

[049] Third, the health of the host animal can be improved by way of decreasing local inflammation of the animal. Local inflammation occurs within the area affected by the harmful stimulus. Acute local inflammation develops within minutes or hours following a harmful stimulus, has a short duration, and primarily involves the innate immune system. The method according to the invention helps to reduce local inflammation of the animal.

[050] Fourth, the health of the host animal can be improved by way of reducing the light regimen/duration into the daily circadian rhythm of the animal. The circadian rhythms associated with light have important effects on the growth of production animals. In the production animal farming business, one way for increasing the growth rate and meat production is by prolongation

of the illumination. In some extreme cases, the illumination on poultry is extended to 23 hours a day, leaving the poultry under darkness for only one hour a day. Although such a method may increase productivity, it has negative impacts on the health as well as the welfare of the animal. It has been observed that the melatonin level of chicken under the 23 hours light and 1 hour darkness period treatment was lowered to less than half of the amount of melatonin of the chicken which are under the 16 hours light and 8 hours darkness period treatment. The means, methods and uses according to the present invention help to increase the amount of melatonin and its precursor serotonin and thus restore the level of melatonin in animals which are subjected to prolonged illumination. Since artificially prolonged photoperiod leads to abnormal behavior such as aggressive interactions (tail biting, feather pecking, mobility/motility issues etc.) in poultry, restoring of melatonin level in such animals helps to improve the welfare of the animals. The inventors of the present application have discovered that by compensating melatonin production by applying the means, methods and uses according to the invention, a stronger serotonergic flux is going into more melatonin and thus a reduction of the illumination regimen and a better animal welfare can be achieved.

[051] The means and methods and uses of the present invention are inter alia applicable to production animals in general and may be provided to any suitable animal. In some embodiments, the animal is monogastric. It is generally understood that a monogastric animal has a single-chambered stomach. In other embodiments, the animal is a ruminant. It is generally understood that a ruminant has a multi-chambered stomach. In some embodiments, the animal is a ruminant in the pre-ruminant phase. Examples of such ruminants in the pre-ruminant phase include nursery calves.

[052] In some embodiments, the animal is a poultry (e.g. chicken, turkey), seafood (e.g. shrimp), sheep, cow, cattle, buffalo, bison, pig (e.g. nursery pig, grower/finisher pig), cat, dog, rabbit, goat, guinea pig, donkey, camel, horse, pigeon, ferret, gerbil, hamster, mouse, rat, bird, or human.

[053] In some embodiments, the animal is livestock. In some embodiments, the animal is a companion animal. In some embodiments, the animal is poultry. Examples of poultry include chicken, duck, turkey, goose, quail, or Cornish game hen. In one variation, the animal is a chicken. In some embodiments, the poultry is a layer hen, a broiler chicken, or a turkey.

[054] In other embodiments, the animal is a mammal, including, for example, a cow, a pig, a goat, a sheep, a deer, a bison, a rabbit, an, a llama, a mule, a horse, a reindeer, a water buffalo, a yak, a guinea pig, a rat, a mouse, an alpaca, a dog, or a cat. In one variation, the animal is a cow. In another variation, the animal is a pig. In another variation, the animal is a sow.

[055] The invention is further characterized by the following items:

[056] Item 1: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[057] Item 2: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[058] Item 3: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  - b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[059] Item 4: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  - a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or comprising
  - b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[060] Item 5: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[061] Item 6: Method for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- feeding the oligosaccharide preparation to the animal; and
- increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

[062] Item 7: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam,

trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- and contacting the microbial community with the oligosaccharide preparation.

[063] Item 8: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- and contacting the microbial community with the oligosaccharide preparation.

[064] Item 9: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation produced or producible by
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;

- and contacting the microbial community with the oligosaccharide preparation.

[065] Item 10: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or comprising
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- and contacting the microbial community with the oligosaccharide preparation.

[066] Item 11: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- and contacting the microbial community with the oligosaccharide preparation.

[067] Item 12: Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community (e.g. an intestinal microbial community (i.e. as comprised in or obtainable from an intestinal sample, e.g. from caecum, ileum), preferably from an animal intestinal microbial community, e.g. from pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow); the method comprising the steps of

- providing an oligosaccharide preparation, comprising
  b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

  wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
- and contacting the microbial community with the oligosaccharide preparation.

[068] Item 13: Method for producing an oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

the method comprising the steps of

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or
b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[069] Item 14: Method for producing an oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

the method comprising the step of

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine;

thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[070] Item 15: Method for producing an oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or

iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

the method comprising the step of

b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[071] Item 16: The method according to any of the preceding items, wherein the at least second sugar of step a) is selected from N-acetylglucosamine, glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

[072] Item 17: The method according to any of the preceding items, wherein the at least first sugar of step b) is glucose, and wherein the at least second sugar of step b) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

[073] Item 18: The method according to any of the preceding items, wherein the at least first sugar of step a), and the at least second sugar of step a) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose.

[074] Item 19: The method according to any of the preceding items, wherein the at least first sugar of step b) and the at least second sugar of step b) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose.

[075] Item 20: The method according to any of the preceding items, wherein the at least second sugar of step a) is glucose; and wherein the catalyst is selected from (+)-camphor-10-sulfonic acid, and methylphosphonic acid.

[076] Item 21: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar of step a) is selected from lactose,

mannose, arabinose, fructose, sucrose, and xylose; and optionally wherein the catalyst is selected from (+)-camphor-10-sulfonic acid, methylphosphonic acid, and phosphoric acid.

[077] Item 22: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is lactose and wherein the catalyst is (+)-camphor-10-sulfonic acid.

[078] Item 23: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is lactose and wherein the catalyst is methylphosphonic acid.

[079] Item 24: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is mannose and wherein the catalyst is methylphosphonic acid.

[080] Item 25: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is arabinose and wherein the catalyst is (+)-camphor-10-sulfonic acid.

[081] Item 26: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is arabinose and wherein the catalyst is methylphosphonic acid.

[082] Item 27: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is arabinose and wherein the catalyst is phosphoric acid.

[083] Item 28: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is fructose and wherein the catalyst is (+)-camphor-10-sulfonic acid.

[084] Item 29: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is fructose and wherein the catalyst is methylphosphonic acid.

[085] Item 30: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is fructose and wherein the catalyst is methylphosphonic acid.

[086] Item 31: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is fructose and wherein the catalyst is phosphoric acid.

[087] Item 32: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is (+)-camphor-10-sulfonic acid.

[088] Item 33: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is (+)-camphor-10-sulfonic acid.

[089] Item 34: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is methylphosphonic acid.

[090] Item 35: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is methylphosphonic acid.

[091] Item 36: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is methylphosphonic acid.

[092] Item 37: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is sucrose and wherein the catalyst is phosphoric acid.

[093] Item 38: The method according to any one of items 1-15, wherein the at least second sugar of step a) is glucose; wherein the at least third sugar is xylose and wherein the catalyst is methylphosphonic acid.

[094] Item 39: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose and wherein the at least second sugar of step b) is arabinose.

[095] Item 40: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose and wherein the at least second sugar of step b) is lactose.

[096] Item 41: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose and wherein the at least second sugar of step b) is N-acetylglucosamine.

[097] Item 42: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose and wherein the at least second sugar of step b) is xylose.

[098] Item 43: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is arabinose.

[099] Item 44: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is arabinose.

[100] Item 45: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is xylose; and wherein the at least third sugar of step b) is arabinose.

[101] Item 46: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is arabinose; and wherein the at least third sugar of step b) is lactose.

[102] Item 47: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is lactose.

[103] Item 48: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is N-acetylglucosamine; and wherein the at least third sugar of step b) is lactose.

[104] Item 49: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is lactose.

[105] Item 50: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is xylose; and wherein the at least third sugar of step b) is lactose.

[106] Item 51: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is mannose.

[107] Item 52: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is N-acetylglucosamine; and wherein the at least third sugar of step b) is mannose.

[108] Item 53: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is mannose.

[109] Item 54: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is xylose; and wherein the at least third sugar of step b) is mannose.

[110] Item 55: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is arabinose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[111] Item 56: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[112] Item 57: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[113] Item 58: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[114] Item 59: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[115] Item 60: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[116] Item 61: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is xylose; and wherein the at least third sugar of step b) is N-acetylglucosamine.

[117] Item 62: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is fructose; and wherein the at least third sugar of step b) is xylose.

[118] Item 63: The method according to any one of items 1-15, wherein the at least first sugar of step b) is glucose; wherein the at least second sugar of step b) is sucrose; and wherein the at least third sugar of step b) is xylose.

[119] Item 64: An oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

wherein the oligosaccharide preparation is produced or producible by

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or
b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[120] Item 65: The oligosaccharide preparation according to item 64, wherein the at least second sugar of step a) is selected from glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

[121] Item 66: The oligosaccharide preparation according to item 64 or 65, wherein the at least first sugar of step b) is glucose, and wherein the at least second sugar of step b) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

[122] Item 67: The oligosaccharide preparation according to any one of items 64-66, wherein the at least first sugar of any one of step a) or b) and the at least second sugar of any one of step a) or b) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from lactose.

[123] Item 68: An oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

wherein the oligosaccharide preparation comprises at least two distinct monosaccharide units selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably selected from glucose and N-acetylglucosamine; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[124] Item 69: An oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

wherein the oligosaccharide preparation comprises at least two distinct monosaccharide units selected from glucose, N-acetylglucosamine, and at least a third distinct monosaccharide unit selected from lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose; and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[125] Item 70: The oligosaccharide preparation according to any one of items 64-69, wherein the oligosaccharide composition comprises at least 10 wt%, (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 wt% etc.) of the at least first fraction; preferably at least 20 wt%.

[126] Item 72: The oligosaccharide preparation according to any one of items 64-71, wherein the oligosaccharide preparation is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

[127] Item 73: An oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;

ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders; and/or

iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal;

wherein the oligosaccharide preparation is produced by or producible by the method according to any one of items 13-63.

[128] Item 74: The oligosaccharide preparation according to any one of items 64-73, wherein the animal is selected from the group consisting of pet animals, dog, cat, canary, guinea pig, hamster, rabbit, mouse, rat, deer, boar, zoo animals, horse, donkey, poultry, swine, ruminant, chicken, cow, sheep, goat, pig, piglet, turkey, aquaculture, fish, shrimp, prawn, crayfish, crab, oyster, mussel, clam, trout, tilapia, salmon, carp, catfish, tuna, preferably, the animal is selected from the group consisting of poultry such as chicken, swine, and ruminant such as cow.

[129] Item 75: The oligosaccharide preparation according to any one of items 64-74, wherein the oligosaccharide preparation comprises at least 4 fractions (e.g. at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 etc. fractions) each having a distinct DP.

[130] Item 76: The oligosaccharide preparation according to any one of items 64-75, wherein the oligosaccharide preparation comprises not more than three distinct monosaccharide units.

[131] Item 77: The oligosaccharide preparation according to any one of items 64-76, wherein at least one fraction of the oligosaccharide preparation comprises less than 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2% anhydro-subunit containing oligosaccharides by relative abundance; and/or wherein each fraction of the oligosaccharide preparation comprises greater than 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% anhydro-subunit containing oligosaccharides by relative abundance.

[132] Item 78: The oligosaccharide preparation according to any one of items 64-77, wherein the oligosaccharide preparation has a weight averaged molecular mass from 300 to 5000 g/mol (e.g. from about 2000 to 2800 g/mol, 2100 to 2700 g/mol, 2200 to 2600 g/mol, 2300 to 2500 g/mol, or 2320 to 2420 g/mol), 500 to 5000 g/mol, 700 to 5000 g/mol, 500 to 2000 g/mol, 700 to 2000 g/mol, 700 to 1500 g/mol, 300 to 1500 g/mol, 300 to 2000 g/mol, 300 to 700 g/mol, 400 to 1300 g/mol, 400 to 1200 g/mol, 400 to 1100 g/mol, 500 to 1300 g/mol, 500 to 1200 g/mol, 500 to 1100 g/mol, 600 to 1300 g/mol, 600 to 1200 g/mol, or 600 to 1100 g/mol); and/or wherein the

oligosaccharide preparation has a number averaged molecular mass from 1000 to 2000 g/mol (e.g. from 1100 to 1900 g/mol, 1200 to 1800 g/mol, 1300 to 1700 g/mol, 1400 to 1600 g/mol, 300 to 1050 g/mol, 300 to 1020 g/mol, 300 to 1010 g/mol, 400 to 1050 g/mol, 400 to 1020 g/mol, 400 to 1010 g/mol, 500 to 1050 g/mol, 500 to 1020 g/mol, 500 to 1010 g/mol, or 1450 to 1550 g/mol).

[133] Item 79: Use of an oligosaccharide preparation according to any one of items 64-78 for

i) modulating behavior of an animal (e.g. a healthy animal), in particular for reducing aggressive behavior;

ii) improving animal welfare (e.g. of a healthy animal), in particular for reducing anxiety, stress and/or fear disorders;

iii) improving animal performance (e.g. of a healthy animal), in particular for improving average body weight gain and/or reducing feed conversion ratio;

iv) improving animal performance of animals (e.g. of healthy animals) facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or

v) increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community.

[134] Item 80: An oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine, wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation, wherein the oligosaccharide preparation is produced or producible by

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or

b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[135] Item 81: An oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine, wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation, in an animal suffering from or at risk at suffering from one or more disorders associated with an imbalanced level of gamma-aminobutyric acid and/or kynurenine, wherein the oligosaccharide preparation is produced or producible by

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or

b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

[136] Item 82: The oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis according to item 80 or 81, wherein the oligosaccharide preparation is the oligosaccharide preparation according to any one of items 64-78.

[137] Item 83: The oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis according to any one of items 80-82, wherein the oligosaccharide preparation is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

[138] It is also considered that the oligosaccharide preparation may be provided in the form of a powderous formulation comprising at least 20% (w/w) of the oligosaccharide preparation as referred to herein; at least 25% (wt/wt) of a silica-based adsorbate (e.g. diatomaceous earth, amorphous precipitated silica) having an average particle size D of less than or equal to 3000 µm (e.g. 100-500, 200-500, 200-300 µm); and optionally 0-25% (wt/wt) of water and/or an auxiliary substance; wherein the % are based on the total weight of the powderous formulation. For instance, such a powderous formulation may comprise 30-70% (wt/wt) of the oligosaccharide preparation as referred to herein; 30-70% (wt/wt) of a silica based adsorbate (e.g. having an average particle size of at least 50 µm); and 0-21% (wt/wt) of water; wherein the % are based on the total weight of the powderous formulation. In some embodiments the oligosaccharide preparation is formulated analogous to the protocol as described in any one of Examples 22-26 and 33 of WO 2020/097458.

[139] As used herein, an “oligosaccharide preparation” may comprise one or more monosaccharide(s) or sugar(s), and/or anhydro-monosaccharide(s), and/or one or more compound molecule(s) such as di-, tri-, tetra-saccharides or higher polymerized saccharides, containing two or more monosaccharide subunits, which are linked by glycosidic bonds to form the oligosaccharide(s). An “oligosaccharide” may also refer to a compound molecule containing two or more monosaccharide subunits, where at least one monosaccharide unit is replaced by an anhydro-subunit. An “oligosaccharide” may be optionally functionalized. As used herein, the term “oligosaccharide” encompasses all species of the oligosaccharide, wherein each of the monosaccharide subunits in the oligosaccharide is independently and optionally functionalized and/or replaced with its corresponding anhydro-monosaccharide subunit.

[140] An “anhydro-subunit” may be a product of reversible thermal dehydration of a monosaccharide or monosaccharide subunit, or a sugar caramelization product. For example, an “anhydro-subunit” may be an anhydro-monosaccharide such as anhydro-glucose. As another

example, an “anhydro-subunit” may be linked with one or more regular monosaccharide subunits and/or with one or more anhydro-monosaccharide subunits via glycosidic linkage(s).

[141] An oligosaccharide may be characterized to contain two or more monosaccharide subunits linked by glycosidic bonds. In this regard, a “gluco-oligosaccharide” may refer to a compound containing two or more glucose monosaccharide subunits linked by glycosidic bonds. A “gluco-oligosaccharide” may also refer to compound containing two or more glucose monosaccharide subunits linked by glycosidic bonds, wherein at least one monosaccharide subunit is replaced by an anhydro-glucose subunit. Similarly, a “galacto-oligosaccharide” may refer to a compound containing two or more galactose monosaccharide subunits linked by glycosidic bonds. A “galacto-oligosaccharide” may also refer to a compound containing two or more galactose monosaccharide subunits linked by glycosidic bonds, wherein at least one monosaccharide subunit is replaced by an anhydro-galactose subunit. Analogously, a gluco-galactose-oligosaccharide may be a compound comprising one or more glucose monosaccharide subunits and one or more galactose monosaccharide subunits linked by glycosidic bonds. A gluco-galactose oligosaccharide may also refer to a compound, wherein at least one of the monosaccharide subunits is replaced with its respective anhydro-monosaccharide subunit. A gluco-galacto-xylo-oligosaccharide may refer to a compound produced by the condensation reaction of glucose, galactose, and xylose. An oligosaccharide preparation comprising gluco-galacto-xylo-oligosaccharides may comprise gluco-galactose-oligosaccharides, gluco-xylo-oligosaccharides, galacto-xylo-oligosaccharides, gluco-galacto-xylo-oligosaccharides, and compounds containing one or more glucose monosaccharide subunits, one or more xylose monosaccharide subunits, and one or more galactose monosaccharide subunits linked by glycosidic bonds, as well as anhydro-monosaccharide subunit(s) thereof.

[142] As used herein, the term “monosaccharide unit” and “monosaccharide subunit” may be used interchangeably, unless suggested otherwise. A “monosaccharide subunit” may refer to a monosaccharide monomer in an oligosaccharide. For an oligosaccharide having a degree of polymerization (DP) of 1, the oligosaccharide may be referred to as a monosaccharide subunit or monosaccharide. For an oligosaccharide having a degree of polymerization higher than 1, its monosaccharide subunits are linked via glycosidic bonds.

[143] As used herein, the term “regular monosaccharide” may refer to a monosaccharide that does not contain an anhydro-subunit. The term “regular disaccharide” may refer to a disaccharide that does not contain an anhydro-subunit. Accordingly, the term “regular subunit” may refer to a subunit that is not an anhydro-subunit.

[144] The term “relative abundance” or “abundance” as used herein, may refer to the abundance of a species in terms of how common or rare the species exists. For example, a DP1 fraction (i.e. a fraction having a DP of 1) comprising 10% anhydro-subunit containing oligosaccharides by

relative abundance may refer to a plurality of DP1 oligosaccharides, wherein 10%, by number, of the DP1 oligosaccharides are anhydro-monosaccharides.

[145] Degree of Polymerization (DP) distribution: A distribution of the degree of polymerization of the oligosaccharide preparation may be determined by any suitable analytical method and instrumentation, including but not limited to end group method, osmotic pressure (osmometry), ultracentrifugation, viscosity measurements, light scattering method, size exclusion chromatography (SEC), SEC-MALLS, field flow fractionation (FFF), asymmetric flow field flow fractionation (A4F), high-performance liquid chromatography (HPLC), and mass spectrometry (MS). For example, the distribution of the degree of polymerization may be determined and/or detected by mass spectrometry, such as MALDI-MS, LC-MS, or GC-MS. For another example, the distribution of the degree of polymerization may be determined and/or detected by SEC, such as gel permeation chromatography (GPC). As yet another example, the distribution of the degree of polymerization may be determined and/or detected by HPLC, FFF, or A4F. In another example, the degree of polymerization of the oligosaccharide preparation may be determined based on its molecular weight and molecular weight distribution (for a more detailed description see WO 2020/097458).

[146] Anhydro-subunit level: An oligosaccharide preparation may comprise n distinct fractions, wherein each fraction has a distinct DP, and wherein n is any integer, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 etc. In some embodiments, each of the n fractions of oligosaccharides of the oligosaccharide preparation as described herein independently comprises an anhydro-subunit level. For instance, in some embodiments, the DP1 fraction comprises 10% anhydro-subunit containing oligosaccharides by relative abundance, and the DP2 fraction comprises 15% anhydro-subunit containing oligosaccharides by relative abundance. For another example, in some embodiments, DP1, DP2, and DP3 fraction each comprises 5%, 10%, and 2% anhydro-subunit containing oligosaccharides by relative abundance, respectively. In other embodiments, two or more fractions of oligosaccharides may comprise similar level of anhydro-subunit containing oligosaccharides. For example, in some embodiments, the DP1 and DP3 fraction each comprises about 5 % anhydro-subunit containing oligosaccharides by relative abundance.

[147] The level of anhydro-subunits may be determined by any suitable analytical methods, such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, HPLC, FFF, A4F, or any combination thereof. In some embodiments, the level of anhydro-subunits is determined, at least in part, by mass spectrometry such as MALDI-MS. In some embodiments, the level of anhydro-subunits may be determined, at least in part, by NMR. In some embodiments, the level of anhydro-subunits may be determined, at least in part, by HPLC. For example, in some embodiments, the level of anhydro-subunits may be determined by MALDI-MS, as illustrated in more detail in WO 2020/097458.

[148] Glycosidic Linkages: In some embodiments, the oligosaccharide preparation described herein comprise a variety of glycosidic linkages. The type and distribution of the glycosidic linkages may depend on the source and manufacturing method of the oligosaccharide preparation. In some embodiments, the type and distribution of various glycosidic linkages may be determined and/or detected by any suitable methods known in the art such as NMR. For example, in some embodiments, the glycosidic linkages are determined and/or detected by proton NMR, carbon NMR, 2D NMR such as 2D JRES, HSQC, HMBC, DOSY, COSY, ECOSY, TOCSY, NOESY, or ROESY, or any combination thereof. In some embodiments, the glycosidic linkages are determined and/or detected, at least in part, by proton NMR. In some embodiments, the glycosidic linkages are determined and/or detected, at least in part, by carbon NMR. In some embodiments, the glycosidic linkages are determined and/or detected, at least in part, by 2D HSQC NMR.

[149] In some embodiments, an oligosaccharide preparation may comprise one or more α-(1,2) glycosidic linkages, α-(1,3) glycosidic linkages, α-(1,4) glycosidic linkages, α-(1,6) glycosidic linkages, β-(1,2) glycosidic linkages, β-(1,3) glycosidic linkages, β-(1,4) glycosidic linkages, β-(1,6) glycosidic linkages, α-(1,1)-α glycosidic linkages, α-(1,1)-β glycosidic linkages, β-(1,1)-β glycosidic linkages, or any combination thereof.

[150] In some embodiments, the oligosaccharide preparations may have a glycosidic bond type distribution of about from 0 to 60 mol%, 5 to 55 mol%, 5 to 50 mol%, 5 to 45 mol%, 5 to 40 mol%, 5 to 35 mol%, 5 to 30 mol%, 5 to 25 mol%, 10 to 60 mol%, 10 to 55 mol%, 10 to 50 mol%, 10 to 45 mol%, 10 to 40 mol%, 10 to 35 mol%, 15 to 60 mol%, 15 to 55 mol%, 15 to 50 mol%, 15 to 45 mol%, 15 to 40 mol%, 15 to 35 mol%, 20 to 60 mol%, 20 to 55 mol%, 20 to 50 mol%, 20 to 45 mol%, 20 to 40 mol%, 20 to 35 mol%, 25 to 60 mol%, 25 to 55 mol%, 25 to 50 mol%, 25 to 45 mol%, 25 to 40 mol%, or 25 to 35 mol% of α-(1,6) glycosidic linkages.

[151] Molecular Weight: The molecular weight and molecular weight distribution of the oligosaccharide preparation may be determined by any suitable analytical means and instrumentation, such as end group method, osmotic pressure (osmometry), ultracentrifugation, viscosity measurements, light scattering method, SEC, SEC-MALLS, FFF, A4F, HPLC, and mass spectrometry. In some embodiments, the molecular weight and molecular weight distribution are determined by mass spectrometry, such as MALDI-MS, LC-MS, or GC-MS. In some embodiments, the molecular weight and molecular weight distribution are determined by size exclusion chromatography (SEC), such as gel permeation chromatography (GPC). In other embodiments, the molecular weight and molecular weight distribution are determined by HPLC. In some embodiments, the molecular weight and molecular weight distribution are determined by MALDI-MS.

[152] In some embodiments, the oligosaccharide preparation has a weight averaged molecular mass from 300 to 5000 g/mol (e.g. from about 2000 to 2800 g/mol, 2100 to 2700 g/mol, 2200 to 2600 g/mol, 2300 to 2500 g/mol, or 2320 to 2420 g/mol), 500 to 5000 g/mol, 700 to 5000 g/mol,

500 to 2000 g/mol, 700 to 2000 g/mol, 700 to 1500 g/mol, 300 to 1500 g/mol, 300 to 2000 g/mol, 300 to 700 g/mol, 400 to 1300 g/mol, 400 to 1200 g/mol, 400 to 1100 g/mol, 500 to 1300 g/mol, 500 to 1200 g/mol, 500 to 1100 g/mol, 600 to 1300 g/mol, 600 to 1200 g/mol, or 600 to 1100 g/mol); and/or wherein the oligosaccharide preparation has a number averaged molecular mass from 1000 to 2000 g/mol (e.g. from 1100 to 1900 g/mol, 1200 to 1800 g/mol, 1300 to 1700 g/mol, 1400 to 1600 g/mol, 300 to 1050 g/mol, 300 to 1020 g/mol, 300 to 1010 g/mol, 400 to 1050 g/mol, 400 to 1020 g/mol, 400 to 1010 g/mol, 500 to 1050 g/mol, 500 to 1020 g/mol, 500 to 1010 g/mol, or 1450 to 1550 g/mol).

[153] Types of Oligosaccharides: In some embodiments, the species of oligosaccharides present in an oligosaccharide preparation referred to herein may depend on the type of the feed sugar(s). For example, in some embodiments, the oligosaccharide preparations comprise a gluco-oligosaccharide when the feed sugars comprise glucose. For example, in some embodiments, the oligosaccharide preparations comprise a fructose-oligosaccharide when the feed sugars comprise fructose. For another example, in some embodiments, the oligosaccharide preparations comprise gluco-fructo-oligosaccharides when the feed sugars comprise fructose and glucose.

[154] In some embodiments, the oligosaccharide preparations comprise one or more species of monosaccharide subunits. In some embodiments, the oligosaccharide preparation may comprise oligosaccharides with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more different species of monosaccharides subunits.

[155] Method of Manufacturing Oligosaccharide Preparations: An oligosaccharide preparation may be manufactured by heating an aqueous composition comprising one or more feed sugar(s) and a catalyst to a temperature and for a time sufficient to induce polymerization, wherein the catalyst is selected from the group consisting of: (+)-camphor-10-sulfonic acid; 2-pyridinesulfonic acid; 3-pyridinesulfonic acid; 8-hydroxy-5-quinolinesulfonic acid hydrate; α-hydroxy-2-pyridinemethanesulfonic acid; (β)-camphor-10-sulfonic acid; butylphosphonic acid; diphenylphosphinic acid; hexylphosphonic acid; methylphosphonic acid; phenylphosphinic acid; phenylphosphonic acid; tert-butylphosphonic acid; SS)-VAPOL hydrogenphosphate; 6-quinolinesulfonic acid, 3-(1-pyridinio)-1-propanesulfonate; 2-(2-pyridinyl)ethanesulfonic acid; 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate; 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate; bis(4-methoxyphenyl)phosphinic acid; phenyl(3,5-xylyl)phosphinic acid; L-cysteic acid monohydrate; poly(styrene sulfonic acid -co- divinylbenzene); lysine; ethanedisulfonic acid; ethanesulfonic acid; isethionic acid; homocysteic acid; HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); 2-Hydroxy-3-morpholinopropanesulfonic acid; 2-(N-morpholino)ethanesulfonic acid; methanesulfonic acid; methaniazide; naphthalene-1-sulfonic acid; naphthalene-2-sulfonic acid; perfluorobutanesulfonic acid; 6-sulfoquinovose; triflic acid; 2-

aminoethanesulfonic acid; benzoic acid; chloroacetic acid; trifluoroacetic acid; caproic acid; enanthic acid; caprylic acid; pelargonic acid; lauric acid; palmitic acid; stearic acid; arachidic acid; aspartic acid; glutamic acid; serine; threonine; glutamine; cysteine; glycine; proline; alanine; valine; isoleucine; leucine; methionine; phenylalanine; tyrosine; tryptophan.

[156] In some embodiments, the polymerization of the feed sugars is achieved by a step-growth polymerization. In some embodiments, the polymerization of the feed sugars is achieved by polycondensation.

[157] Feed Sugar: The one or more feed sugars used in the methods of manufacturing oligosaccharide preparations described herein may comprise one or more types of sugars. In some embodiments, the one or more feed sugars comprise monosaccharides, disaccharides, trisaccharides, tetrasaccharides, or any mixtures thereof. Feed sugar(s) may be e.g. one or more selected from glucose, lactose, galactose, mannose, glucosamine, N-acetylglucosamine, arabinose, xylose, sucrose, and fructose.

[158] As used herein, the singular forms “a,” “and,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an agent” includes a plurality of such agents, and reference to “the oligosaccharide” includes reference to one or more oligosaccharides (or to a plurality of oligosaccharides) and equivalents thereof known to those skilled in the art, and so forth.

[159] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included. The term “about” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range, in some instances, will vary between 1% and 15% of the stated number or numerical range.

[160] In the following, the present invention is further described by non-limiting examples.

Examples

[161] The present invention as disclosed herein is not limited to specific embodiments, figures, methodology, examples, protocols etc. described herein but solely defined by the claims.

Example 1

[162] A library of synthetic oligosaccharide preparations was prepared using different combinations of feed sugar(s) and catalysts. Feed sugars were selected from glucose, lactose, galactose, mannose, glucosamine, N-acetylglucosamine, arabinose, xylose, sucrose, and fructose. Catalysts were selected from sulfuric acid, acetic acid, propionic acid, butanoic acid, L-glutamic acid, L-lysine, phosphoric acid, (+)-camphor-10-sulfonic acid, methylphosphonic acid, L-

cysteic acid, hydrochloric acid, citric acid, lactic acid, 3-(1-pyridinio)-1-propanesulfonate, 2-pyridinesulfonic acid, 3-pyridinesulfonic acid, α-hydroxy-2-pyridinemethanesulfonic acid, (β)-camphor-10-sulfonic acid, butylphosphonic acid, diphenylphosphinic acid, hexylphosphonic acid, phenylphosphinic acid, phenylphosphonic acid, tert-butylphosphonic acid, 2-(2-pyridinyl)ethanesulfonic acid, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate, 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate, bis(4-methoxyphenyl)phosphinic acid, and phenyl(3,5-xylyl)phosphinic acid.

[163] In principle, 10 g scale reactions were performed by charging respective masses of powdered sugars (dry solids basis) into 20 mL scintillation vials, melting the contents by heating to 135 °C while stirring, and allowing for oligomerization to occur by adding catalyst and continuing heating to evolve water off of the reaction vessel for up to several hours until adequate conversion was achieved as indicated by increased viscosity, brown or amber color. Table 1 below shows exemplary combinations of feed sugars and the respective catalysts used for synthesizing oligosaccharide preparations. Feed sugars of OP IDs 1-61 as listed in the order of Table 1 below were used at ratios 98:2, 75:5:20, 85:5:10, 93:5:2, 97:3, 90:5:5, 90:5:5, 68:2:30, 90:5:5, 90:10, 65:5:30, 95:5, 90:5:5, 67:3:30, 95:5, 94:5:1, 95:5, 92:5:3, 90:5:5, 94:5:1, 90:10, 90:5:5, 90:5:5, 95:5, 95:5, 95:5, 95:5, 85:5:10, 94:5:1, 85:5:10, 94:1:5, 92:5:3, 98:2, 85:5:10, 90:5:5, 90:5:5, 97:3, 95:5, 90:5:5, 75:5:20, 90:5:5, 98:2, 99:1, 94:5:1, 93:5:2, 85:5:10, 92:5:3, 90:5:5, 92:5:3, 92:5:3, 98:2, 99:1, 90:5:5, 88:2:10, 94:5:1, 92:5:3, 92:5:3, 90:5:5, 93:2:5, 85:5:10, and 94:5:1, respectively.

Table 1: Feed sugars and catalysts used for preparing synthetic oligosaccharide preparations with the indicated oligosaccharide preparation identifier (OP ID).

OP ID	Feed sugars	Catalyst
1	glucose, glucosamine	sulfuric acid
2	glucose, fructose, lactose	(+)-camphor-10-sulfonic acid
3	glucose, arabinose, lactose	methylphosphonic acid
4	glucose, xylose, N-acetylglucosamine	methylphosphonic acid
5	glucose, N-acetylglucosamine	methylphosphonic acid
6	glucose, xylose, mannose	hydrochloric acid
7	glucose, xylose, arabinose	methylphosphonic acid
8	glucose, N-acetylglucosamine, lactose	(+)-camphor-10-sulfonic acid
9	glucose, sucrose, arabinose	methylphosphonic acid
10	glucose, lactose	methylphosphonic acid
11	glucose, sucrose, lactose	phosphoric acid
12	glucose, arabinose	(+)-camphor-10-sulfonic acid
13	glucose, sucrose, xylose	phosphoric acid
14	glucose, glucosamine, lactose	hydrochloric acid
15	glucose, xylose	methylphosphonic acid
16	glucose, sucrose, N-acetylglucosamine	methylphosphonic acid
17	glucose, arabinose	sulfuric acid
18	glucose, fructose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
19	glucose, sucrose, xylose	(+)-camphor-10-sulfonic acid
20	glucose, sucrose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
21	glucose, lactose	(+)-camphor-10-sulfonic acid

22	glucose, fructose, arabinose	methylphosphonic acid
23	glucose, fructose, xylose	(+)-camphor-10-sulfonic acid
24	glucose, fructose	(+)-camphor-10-sulfonic acid
25	glucose, xylose	(+)-camphor-10-sulfonic acid
26	glucose, xylose	sulfuric acid
27	glucose, arabinose	methylphosphonic acid
28	glucose, sucrose, mannose	methylphosphonic acid
29	glucose, fructose, N-acetylglucosamine	methylphosphonic acid
30	glucose, fructose, mannose	methylphosphonic acid
31	glucose, N-acetylglucosamine, mannose	methylphosphonic acid
32	glucose, sucrose, N-acetylglucosamine	phosphoric acid
33	glucose, N-acetylglucosamine	methylphosphonic acid
34	glucose, sucrose, lactose	methylphosphonic acid
35	glucose, sucrose, xylose	methylphosphonic acid
36	glucose, xylose, mannose	(+)-camphor-10-sulfonic acid
37	glucose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
38	glucose, fructose	sulfuric acid
39	glucose, sucrose, arabinose	(+)-camphor-10-sulfonic acid
40	glucose, fructose, lactose	methylphosphonic acid
41	glucose, fructose, arabinose	(+)-camphor-10-sulfonic acid
42	glucose, glucosamine	(+)-camphor-10-sulfonic acid
43	glucose, N-acetylglucosamine	methylphosphonic acid
44	glucose, arabinose, N-acetylglucosamine	methylphosphonic acid
45	glucose, sucrose, N-acetylglucosamine	methylphosphonic acid
46	glucose, arabinose, lactose	sulfuric acid
47	glucose, fructose, N-acetylglucosamine	methylphosphonic acid
48	glucose, fructose, xylose	methylphosphonic acid
49	glucose, fructose, N-acetylglucosamine	phosphoric acid
50	glucose, sucrose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
51	glucose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
52	glucose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid
53	glucose, xylose, mannose	methylphosphonic acid
54	glucose, N-acetylglucosamine, lactose	methylphosphonic acid
55	glucose, arabinose, N-acetylglucosamine	phosphoric acid
56	glucose, sucrose, N-acetylglucosamine	methylphosphonic acid
57	glucose, fructose, glucosamine	phosphoric acid
58	glucose, fructose, mannose	(+)-camphor-10-sulfonic acid
59	glucose, glucosamine, mannose	(+)-camphor-10-sulfonic acid
60	glucose, xylose, lactose	methylphosphonic acid
61	glucose, arabinose, N-acetylglucosamine	(+)-camphor-10-sulfonic acid

Example 2

[164] The amount of catalyst present in the oligosaccharide preparations after production was quantified by anion chromatography. The analysis was performed on a Thermo-Fisher ICS-3000 ion chromatography system equipped with EG-3000 eluent generator, AS autosampler and CD-3000 conductivity detector. Separation was achieved on an AS19 column (4.0 mm x 250 mm) protected with an AG19 precolumn (4.0 mm x 50 mm). The mobile phase was a KOH gradient (0-55 mM) produced by an eluent generator (EGCII EluGen® cartridge). The chromatographic parameters were: 1.0 mL/min flow rate, 25 µL injection volume, and 30 °C column temperature. Calculations were based on peak areas. Quantitative results were obtained by comparing peak areas with an external standard calibration curve. All catalysts applied could be quantified and were below 0.1%, i.e. 1000 ppm, after the condensation reaction.

[165] Samples of the oligosaccharide preparations as prepared in Example 1 were further analyzed by gel permeation chromatography on an Agilent 1260 Infinity system comprising degasser, binary pump, and thermostat column compartment equipped with an Agilent 1200 refractive index detector. The columns used were a PL aquagel-OH guard column, 7.5 x 50 mm, 5 µM (Agilent Part No: PL1149-1530) and a PL aquagel-OH, 7.5 x 300 mm, 5 µM (Agilent Part No: PL1120-6520), whereas the used solvent was 200 mM aqueous sodium nitrate at a flow-rate of 0.9 mL/min. and a column temperature of 40 °C. As standards, both glucose (for the quantification of total sugar content) and pullulan standards (for the size distribution) were used. Oligosaccharide preparations of OP IDs 50, 43, 57, and 54 were found to have a DP1 content (DP: degree of polymerization; DP1: degree of polymerization of 1) of 29.5 wt%, 27.3 wt%, 11.6 wt%, and 50.5 wt%, respectively; and to have a DP2+ content (oligosaccharides having a degree of polymerization of 2 or more) of 42.2 wt%, 39.2 wt%, 45.0 wt% and 22.1 wt%, respectively. Upon additional analysis of OP ID 54 via liquid chromatography with evaporative light scattering detector coupled to high-resolution mass spectrometry (LC-ELSD-HRMS) a DP1 content of 69.22 Area% was confirmed, a DP2 content of 24.64 Area% and a DP3+ content of 6.14 Area% were found. Further, the Mn (number averaged molecular mass) and Mw (weight averaged molecular mass) were determined. OP IDs 50, 43, 57, and 54 had an Mn of 477, 574, 710 and 690 g/mol, respectively; and an Mw of 578, 779, 1059, and 1008 g/mol, respectively. The higher Mn and Mw values for OP ID 57 compared to the other tested OD IDs was in agreement with the higher DP2+ content of this oligosaccharide preparation. Total dry glycan contents were 71.7, 66.5, 56.6 and 72.6 wt%, respectively.

[166] In addition, refractive indices were determined at 78.9, 79.6, 77.0 and 73.6 Brix for the oligosaccharide preparations of OP IDs 50, 43, 57, and 54, respectively.

Example 3

[167] In order to test for increased GABA production by intestinal pig microbiome, the following assay was performed under anaerobic conditions. To this end, a minimal media solution, a glycan solution (comprising the oligosaccharide preparation to be tested), a feed extract solution, and a swine cecal slurry (comprising intestinal microbiota) were prepared.

[168] The minimal media solution was prepared to contain 900 mg/L Sodium Chloride, 26 mg/L Calcium Chloride dihydrate, 20 mg/L Magnesium chloride hexahydrate, 40 mg/L Ammonium Sulfate, 300 mg/L Potassium Phosphate dibasic, 1500 mg/L Sodium Phosphate dibasic, 5000 mg/L Sodium Bicarbonate, 150 mg/L Histidine, 75 mg/L Glycine, 150 mg/L Tryptophan, 150 mg/L Arginine, 150 mg/L Methionine, 150 mg/L Threonine, 225 mg/L Valine, 225 mg/L Isoleucine, 300 mg/L Leucine, 400 mg/L Cysteine, 450 mg/L Proline, 75 mg/L Phenylalanine, 75 mg/L Tyrosine, 75 mg/L Glutamic Acid, 10 mg/L Manganese Chloride in solution, 4 mg/L Iron Sulfate in solution,

1 mg/L Cobalt Chloride in solution, 1 mg/L Pyridoxine in solution, 1 mg/L Pantothenate in solution, and 0.125 mg/L Biotin in solution.

[169] The glycan solutions were prepared to a concentration of 6 brix of each of the individual oligosaccharide preparations to be tested, diluted with distilled water. To this end, the oligosaccharide preparations OP ID 1-61 as prepared in Example 1 were used.

[170] The feed extract solution was prepared by mixing ground pig feed (as detailed in Table 2) with 20 mL of distilled water, followed by incubating at 125 °C for 15 min. Thereafter, the mixture was pelleted by centrifugation The supernatant was incubated at 121 °C for 15 min. Thereafter, sterile distilled water was added to a final volume of 20 mL.

Table 2: Pig feed composition. *Premix vitamin mineral 3144 provided per kilogram of diet: Vitamin A 6510 I.U.; Vitamin E: 75 mg.; Vitamin K: 2 mg; Vitamin D3: 2007 IU; Vitamin B1: 0.99 mg; Vitamin B2: 5.02 mg; Vitamin B6: 2.01 mg; Vitamin B12: 0.03 mg; Pantothenic acid: 17.5 mg; Folic acid: 0.51 mg; Biotin 0.1 mg; Choline: 100 mg; Mn: 40 mg; Fe: 100 mg; Cu: 15 mg; Zn: 65 mg; I: 2 mg; Se: 0.4 mg.

Ingredients (%)	**Experimental period**
	Finisher
Wheat	28.9
Corn	40
SBM 48	14.3
Rice bran	8
Premix vitamin-mineral*	3
Soy oil	3
L-Lysine	0.35
L-Methionine	0.05
NaCl	0.5
Calcium carbonate	0.8
Dicalcium phosphate	1.1
Calculated energy & nutrient contents	
Metabolizable energy (Kcal/kg)	3132
Fat (%)	6.9
Crude protein (%)	15.1
Lysine (%)	0.94
Methionine (%)	0.27
Threonine (%)	0.55
Total P total (%)	0.58
Total Ca (%)	0.64
Ratio Ca/P	0.91
Vit A (IU/kg)	6571.3
Vit E (mg/kg)	91.5
Vit D3 (IU/kg)	2007
Se (mg/kg)	0.51
Fe (mg/kg)	175.7
Zn (mg/kg)	93
Cu (mg/kg)	20.8

[171] Swine cecal slurry was prepared by mixing 3.8 g of caecal sample in 25 mL PBS (phosphate-buffered saline: 137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM

disodium hydrogenphosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4). After sedimentation of large particles within this mixture, the non-sedimented supernatant was decanted into a fresh vessel. Thereafter, this supernatant was subjected to centrifugation (500 rpm, 5 min). After centrifugation, the supernatant was recovered and mixed with 50% glycerol to a final glycerol concentration of 15% (v/v), thus forming the swine cecal slurry.

[172] In order to mimic the actual diversity of pig caecal microbiota, three caecal samples comprising different microbiome compositions were tested. As identified metagenomically, the caecal samples differed in terms of diversity as well as in microbial composition and ratio. In detail, in terms of phyla, caecal sample 1 contained 48.0%, 33.3%, 15.4% and 3.3% of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteriae, respectively. Caecal sample 2 contained 54.9%, 27.2%, 15.3% and 2.7% of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteriae, respectively. Caecal sample 3 contained 67.0%, 20.4%, 10.4%, and 2.3% of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteriae, respectively. In terms of genera, caecal sample 1 contained 15.1%, 8.5%, 14.6%, 5.2%, 3.8%, 3.6%, 4.2%, 23.4%, 6.2%, 11.1%, and 4.3% of *Bacteroides, Blautia, Clostridium, Escherichia, Faecalibacterium, Lachnoclostridium, Phocaeicola, Prevotella, Roseburia, Streptococcus*, and *Turicibacter*, respectively. Caecal sample 2 contained 10.8%. 5.5%. 3.7%. 12.3%. 6.0%. 10.3%. 4.9%. 17.9%. 4.4%. 9.9%. and 14.3% of *Bacteroides. Blautia. Campylobacter, Clostridium, Escherichia, Lactobacillus, Limosilactobacillus, Prevotella, Roseburia, Sarcina,* and *Streptococcus*, respectively. Caecal sample 3 contained 6.6%, 5.9%, 4.4%, 1.8%, 2.7%, 41.9%, 18.1%, 2.0%, 10.5%, 4.4%, and 1.8%, of *Bacteroides, Blautia, Clostridium, Faecalibacterium, Lachnoclostridium, Lactobacillus, Limosilactobacillus, Phocaeicola, Prevotella, Roseburia*, and *Ruminococcus*, respectively.

[173] Production of GABA by the microbiota was initiated upon mixing 50 µL of the swine cecal slurry with 80 µL of the feed extract solution, 70 µL of the glycan solution and 800 µL of the minimal media solution in a single well of a 96-deep well plate. The glycan solution was varied by testing several different oligosaccharide preparations as prepared in Example 1. As controls, a blank control was included, wherein the glycan solution was substituted with minimal media solution; and a glucose control was included, wherein the glycan solution contained 1% (v/v) of glucose instead of an oligosaccharide preparation as referred to herein. The glucose control was included to obtain a basal GABA response from the microbiome as a reference for fold changes calculations. Since glucose is mostly absorbed before actually reaching the caecum in the small intestine, the 1% glucose control appropriately mimicked the in vivo situation. The 96-deep well plates were sealed with a porous, breathable adhesive film, and incubated anaerobically for 24 h at 37 °C. Thereafter, the 96-deep well plates were centrifuged for pelleting the microbiota and non-dissolved matter, and the supernatants were used for further analysis, in particular for determination of production of GABA and/or KYN.

Example 4

[174] GABA was measured by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). The supernatants obtained from the in vitro fermentations of Example 3 were subjected to centrifugation. Then, 200 µL of supernatant was transferred to 96-well plates. Components comprised in these supernatants were separated on an Acquity UPLC H-class system comprising degasser, quaternary pump, sample manager and thermostat column compartment equipped with TQDetector (Waters). The columns used were a UPLC BEH C18 guard column, 2.1 x 50 mm, 1.7 µM (Waters reference: 1866003975) and an Acquity UPLC BEH C18, 2.1 x 150 mm, 1.7 µM (Waters reference: 176001048). The eluents were: A 99.8% H_2O + 0.2% formic acid; and B 99.8% ACN (LC-MS grade) + 0.2% formic acid. Flow rate was 0.3 mL/min, column temperature was 40 °C. The autosampler was set to 15 °C, run time was 7.5 minutes. Gradient profile is shown in Table 3.

Table 3: LC flow rate parameters.

Time (min)	Flow rate (mL/min)	% A
0	0.3	95.0
0.25	0.3	95.0
2.70	0.3	85.0
4.00	0.3	60.0
5.00	0.3	0.0
5.20	0.3	0.0
6.00	0.3	0.0
6.10	0.3	95.0
7.20	0.3	95.0

[175] As standards, GABA powder was used at a calibration range from 3 to 0.0003 µg/mL. The chromatographic injection volume was 2 µL, the syringe was washed after each injection. The MS method used was a multiple reaction monitoring (MRM) with a positive electrospray ionization. For GABA, the parent fragment m/z was 103.72 and the daughter fragment m/z was 85.85. The dwell time was 0.025 s, the cone voltage was 10 V, and the collision voltage was 13 V.

[176] Kynurenine (KYN) quantification was performed by ELISA kit following the supplier's recommendation (FI-EM1862; Euromedex). Briefly, 50 µL of standard, blank or samples were added to 50 µL of Biotin-labeled Antibody Working Solution and incubated for 45 min at 37 °C. The plate was washed three times with Wash Buffer, and 100 µL of HRP-streptavidin conjugate was added into each well. The plate was incubated for 30 minutes at 37 °C. The plate was then washed five times with Wash Buffer. 90 µL of TMB substrate was added to each well followed by an incubation at 37 °C in dark for 10-20 minutes. Then, 50 µL of stop solution was added to each well and the absorbance was measured at 450 nm and correlated to a standard curve of known KYN concentrations.

Example 5

[177] The synthetic oligosaccharide preparations produced in Example 1 were individually subjected to swine microbiota in in vitro fermentations as described in Example 3, and analyzed as outlined in Example 4. The effect of contacting the microbiota with any one of the synthetic oligosaccharide preparations in terms of GABA production was calculated as log2 fold change of produced GABA compared to the GABA concentration obtained from the microbiota contacted with the glucose control, see Table 4. While some oligosaccharide preparations did not or only marginally affect GABA production as compared to the glucose control (e.g. OP IDs 1, 6, 14, 17, 21, 23, 24, 38, 41, 46, 59), other oligosaccharide preparations were found capable of eliciting a significantly larger microbial production of GABA than the glucose control. In particular, oligosaccharide preparations comprising N-acetylglucosamine were found to increase GABA production. Similarly, oligosaccharide preparations produced with methylphosphonic acid as catalyst were found to increase microbial GABA production. In particular, it was found that oligosaccharide preparations produced from the same feed sugars but with a catalyst other than methylphosphonic acid, had no or a considerable lower effect on GABA than their corresponding oligosaccharide preparations with methylphosphonic acid as catalyst (compare e.g. OP IDs 30 to 58; 48 to 23; 10 to 21; 9 to 39; 15 to 25 and to 26).

Table 4: Log2 fold changes in GABA production compared to a glucose control (FC/Glu) by individual oligosaccharide preparations.

OP ID	FC/Glu	OP ID	FC/Glu	OP ID	FC/Glu	OP ID	FC/Glu
blank	0.97	15	1.48	31	2.33	47	3.19
Glu control	1.00	16	2.66	32	1.57	48	1.64
1	1.01	17	1.04	33	1.92	49	1.66
2	1.22	18	2.00	34	2.00	50	2.13
3	2.23	19	1.20	35	1.53	51	1.98
4	2.61	20	1.69	36	1.25	52	1.71
5	3.75	21	1.04	37	3.82	53	1.74
6	1.00	22	1.89	38	0.98	54	4.27
7	1.97	23	0.97	39	1.02	55	1.73
8	3.20	24	1.09	40	1.96	56	2.88
9	2.10	25	1.07	41	1.21	57	0.95
10	1.83	26	1.00	42	1.10	58	1.05
11	1.18	27	1.60	43	4.06	59	1.11
12	1.14	28	2.14	44	2.46	60	2.01
13	1.22	29	2.43	45	1.86	61	1.71
14	1.12	30	1.76	46	1.02		

Example 6

[178] To demonstrate translatability of the in vitro results of the previous examples to in vivo effects, a feeding trial in swine was performed for 14 days. To this end, five different treatments were tested, using twelve animals per treatment: As negative control, basal pig feed was used.

As positive control, the same basal pig feed was supplemented with 30 g of GABA per ton of feed. Such GABA supplementation at 30 g /MT has been described to increase growth performance, to improve stress-related parameters and to lower biting incidences (Li et al. 2015. Can. J. Anim. Sci. 95: 165-171; Bi et al. 2020. J Anim Physiol Anim Nutr. 104: 590-596). Three test treatments were prepared by spraying either one of the oligosaccharide preparations of OP IDs 50, 43, and 57 onto the basal pig feed as mash. The oligosaccharide preparations were supplied in syrup form at a concentration of 70 brix, and added to the feed at an inclusion level of 1000 mg/kg. For the negative and the positive control, the same volume of water was sprayed on the basal pig feeds as mash, instead of the syrup of any of the test treatments.

[179] The composition of the basal pig feed is shown in Table 5 below. Feeds were given for *ad libitum* consumption as mash during the experimental period. Phytase (Ronozme HiPhos) at 100 mg/kg feed was included in premix form using ground corn as carrier in the basal diet.

Table 5: Basal pig feed. *Premix vitamin mineral 3144 provided per kilogram of diet: Vitamin A 6510 I.U.; Vitamin E: 75 mg.; Vitamin K: 2 mg; Vitamin D3: 2007 IU; Vitamin B1: 0.99 mg; Vitamin B2: 5.02 mg; Vitamin B6: 2.01 mg; Vitamin B12: 0.03 mg; Pantothenic acid: 17.5 mg; Folic acid: 0.51 mg; Biotin 0.1 mg; Choline: 100 mg; Mn: 40 mg; Fe: 100 mg; Cu: 15 mg; Zn: 65 mg; I: 2 mg; Se: 0.4 mg.

Ingredients (%)	**Experimental period**
	Grower
Corn	76.00
Soybean meal	19.60
Soybean oil	0.70
NaCl	0.35
Premix vitamin-mineral 3144*	0.30
DL-Methionine	0.05
L-Lysine	0.47
L-Thr	0.20
L-Val	0.12
Limestone	1.00
Dicalcium phosphate	1.20
HiPhos 10 000 GT	0.01
Calculated energy & nutrient contents	
Metabolizable energy (Kcal/kg)	3303
Crude protein (%)	15.28
Lysine (%)	1.01
Methionine (%)	0.27
Threonine (%)	0.66
Total P total (%)	0.57
Total Ca (%)	0.68
STTD P(%)	0.37

[180] Zootechnical parameters (mortality, weight gain, final weight, FCR - feed conversion ratio) were monitored. GABA and KYN were determined in plasma and feces samples; cortisol (COR), adrenocorticotrophic hormone (ACTH), serum neuropeptide Y (NPY), and lactate were determined in plasma samples.

[181] Statistical analysis of performance: For this study the experimental unit was the pen, n = 4 pigs / pens. The data was analyzed by a one-factor ANOVA followed by the Tukey multiple comparison test. The effects of treatment was considered significant at $p < 0.05$. For analysis, JMP software (version16.0) was used. Statistical biological analysis: For GABA and KYN, and stress-related markers COR, ACTH, NPY, glucose/lactose, in this study, the experimental unit was pigs, n = 12 pigs / treatment. The data was analyzed by a one-factor ANOVA followed by the Tukey multiple comparison test. The effects of treatment was considered significant at $p < 0.05$. For analysis, JMP software (version16.0) was used.

[182] On day 12, the plasma GABA fold change compared to day 0 was 1.07 and 1.09 for the positive control and the treatment with OP ID 57, respectively; slightly increased with 1.12 for treatment with OP ID 50; and significantly increased with 1.73 for treatment with OP ID 43. Fold changes (compared to day 0) of plasma COR as stress indicator were found significantly decreased for treatment with OP IDs 50 and 43 (0.27 and 0.38, respectively), compared to the fold changes of negative control, positive control and treatment with OP ID 57 (0.66, 0.70 and 0.77, respectively). In agreement with these findings, fold changes (compared to day 0) of plasma NPY as stress-resistance indicator were found significantly increased for treatments with OP IDs 50 and 43 (0.93 and 1.60, respectively), compared to the fold changes of negative control, positive control and treatment with OP ID 57 (0.57, 0.51, and 0.50, respectively). Thus, the scalability of the in vitro results to in vivo effects was found confirmed. As a further consequence of the determined differences in GABA and stress-related markers, fewer incidences of aggressive behavior such as tail biting could be observed, along with reduced stress, fear and anxiety, and improved animal performance in terms of weight gain and FCR.

Example 7

[183] In another feeding trial, 288 pigs were used in a study for 44 days, including a 28 days growing phase and a 16 days finishing phase. Six treatments were tested, using 48 animals per treatment. As negative control, basal diet feed was provided. Basal diet feeds are shown in Table 6 below. A GABA control was prepared by supplementing the basal diet feeds with 30 mg of GABA per kg of feed. In four test treatments, the oligosaccharide preparation of OP ID 54 was added to the basal diet feeds at inclusion rates of either 50, 150, 250, or 500 mg of oligosaccharide preparation per kg of feed. To compensate for the mass difference between the treatment feeds comprising 500 mg of oligosaccharide preparation per kg of feed and the other treatment feeds, silica was added. In particular, 0.5 kg SiO_2 per 1000 kg of feed was added to treatment feeds of the negative control; 0.47 kg SiO_2 per 1000 kg of feed was added to treatment feeds of the GABA control in addition to the 0.03 kg of GABA per 1000 kg of feed; 0.45 kg, 0.35 kg and 0.25 kg of SiO_2 per 1000 kg of feed were added to the treatment feeds comprising 50, 150, and 250 mg of oligosaccharide preparation per 1000 kg of feed, respectively.

[184] As in the previous Example, feeds were given for *ad libitum* consumption as mash during the experimental period. Phytase Ronozme HiPhos at 100 mg/kg feed was included in the premix forms using ground corn as carrier in the basal diet (2*150 g). Feed main raw materials were milled in a hammer mill using a 4mm sieve and mixed with the other ingredients (minerals, vitamins and amino acids) in 1500 L mixer. The glycan products were supplied in syrup form sprayed on the mash feed. The same volume of water was sprayed for negative control feeds.

Table 6: Basal diet feeds. *Premix vitamin mineral 3144 provided per kilogram of diet: Vitamin A 6510 I.U.; Vitamin E: 75 mg.; Vitamin K: 2 mg; Vitamin D3: 2007 IU; Vitamin B1: 0.99 mg; Vitamin B2: 5.02 mg; Vitamin B6: 2.01 mg; Vitamin B12: 0.03 mg; Pantothenic acid: 17.5 mg; Folic acid: 0.51 mg; Biotin 0.1 mg; Choline: 100 mg; Mn: 40 mg; Fe: 100 mg; Cu: 15 mg; Zn: 65 mg; I: 2 mg; Se: 0.4 mg. **Premix vitamin-mineral 3145 provided per kilogram of diet: Vitamin A: 15000 I.U.; Vitamin E: 100 mg.; Vitamin K: 20.0 mg; Vitamin C: 100 mg; Vitamin B1: 3 mg; Vitamin B2: 10 mg; Vitamin B6: 6 mg; Vitamin B12: 0.04 mg; Niacin: 60.0 mg; Pantothenic acid: 25.0 mg; Folic acid: 1.5 mg; Biotin 0.2 mg; Choline: 325 mg; Mn: 60 mg; Fe: 200 mg; Cu: 140 mg; Zn: 100 mg; I: 2 mg; Se: 0.4 mg.

Ingredients (%)	**Experimental period**	
	Grower	**Finisher**
Corn	79.00	82.00
Soybean meal	19.60	14.00
Soybean oil	0.70	0.50
NaCl	0.35	0.35
Premix vitamin-mineral 3144*	0.30	0.00
Premix vitamin-mineral 3145**	0.00	0.30
DL-Methionine	0.06	0.06
L-Lysine	0.46	0.46
L-Thr	0.13	0.13
L-Val	0.07	0.07
L-Trp	0.12	0.12
Limestone	1.00	0.90
Dicaclium phosphate	1.20	1.10
HiPhos 10 000 GT	0.01	0.01
Calculated energy & nutrient contents		
Crude protein (%)	15.28	13.11
Metabolizable energy (Kcal/kg)	3303	3300
Lysine (%)	1.10	0.88
Methionine (%)	0.28	0.26
Threonine (%)	0.59	0.52
Tryptophan (%)	0.26	0.23
Total P total (%)	0.57	0.53
STTD P(%)	0.37	0.34
Total CA (%)	0.68	0.61

[185] To study the effects of providing the animals with oligosaccharide preparations according to the invention, in particular with respect to animal welfare, a mixing challenge was conducted. Agonistic behavior after mixing of individual pigs amongst groups is known to be an animal welfare concern, resulting in skin lesions on the body, decreased growth performance and increased

aggressive behavior. At the beginning of the trial, pigs were grouped by body weight. At day 28 all pigs were weighed before mixing, then one pig per pen was selected and exchanged with a pig of similar weight of same treatment group but from another pen. Performance data (weight, weight gain, FCR), social behavior and skin lesions were monitored. Metagenomic analyses were performed from feces samples. GABA, serotonin (5-HT), Trp (tryptophan) and KYN were analyzed from plasma and feces samples. COR, ACTH, NPY, chromogranin A (CgA) and haptoglobin (HPT) were analyzed from plasma samples. Statistics were performed as in the previous example.

[186] In agreement with the previous examples, plasma GABA fold change could be found increased when oligosaccharide preparations of the invention were provided to the animals with the feed, compared to negative and GABA controls. Also plasma cortisol fold change could be found significantly decreased, and plasma 5-HT fold change significantly increased when oligosaccharide preparations of the invention were provided to the animals with the feed, compared to negative control. Average daily weight gain (ADG) between days 0-28 of the trial, i.e. prior to the mixing challenge, was 3.0% higher for the test treatment group compared to the negative control group: 862 g/d compared to 837 g/d, respectively. Mixing had a negative impact on ADG, as reflected by ADGs of 769 g/d and 810 g/d for days 28-42 for negative control and test treatment group, respectively. However, despite the stress imposed on the animals upon mixing, the test treatment groups receiving the oligosaccharide preparation had a 5.3% higher ADG than the negative control. When comparing the ADGs for days 0-42, the test treatment groups were significantly (P=0.04) higher (845 g/d) than the negative control group (814 g/d). Thus, feeding an oligosaccharide preparation according to the present invention was found to improve the animals' performance in terms of ADG in general, and in particular when the animals were confronted with stress. Average daily feed intake (ADFI) was not found to be significantly affected with 2004 g/day and 1991 g/day for days 0-28; 2577 g/day and 2605 g/day for days 28-42; and 2195 g/day and 2196 g/day for days 0-42 in the negative control group and the test treatment groups, respectively. Accordingly, the feed conversion ratio for days 0-28 was 3.3% decreased in the test treatment groups (2.32) compared to the negative control (2.40); and 1.1% decreased in the test treatment groups (3.32) compared to the negative control (3.36) for days 28-42; and significantly (P=0.07) decreased by 3.5% in the test treatment groups (2.60) compared to the negative control (2.70) for days 0-42. It was thus concluded, that energy from the feed consumed during stress was not converted to animal growth by animals of the negative control group, whereas animals having received the oligosaccharide preparation were less susceptible to stress and more successful in converting the energy from the feed to growth.

Example 8

[187] A further animal trial was performed using 144 growing pigs (72 female, 72 uncastrated male), [Large White x Landrace] x Pietrain, undocked tails. Two treatments were tested: Basal diet feed as shown in Table 7 was fed as negative control. Basal diet feed supplemented with 250 g of dry OP ID 54 per MT of feed was used as test treatment. Feed was provided for *ad libitum* consumption in pellet form.

[188] The trial was performed for a total of 105 days with an initial seven-days adaptation period (d-7 to d0), followed by diet treatment start at d0. At d55 and d56, the animals were subjected to a mixing challenge analogous to Example 7. The trial was concluded at d98 with a final weight of approximately 100-105 kg.

[189] Feeding of the oligosaccharide compositions of the invention was found to improve animal performance (e.g. in terms of daily weight gain, feed intake, feed-to-gain ratio) throughout the trial, and in particular during the stress period established by the mixing challenge. Notably, during the mixing challenge week (d51-d59), animals receiving the oligosaccharide compositions of the invention showed significant improvement of growth performance (final weight, weight gain, FCR). Details are shown in Tables 8-11 below.

[190] Saliva samples were collected on d-7 (baseline), d58 (after mixing challenge) and d97 (end of trial). Hair samples were collected on d-7 and d98 (to detect potential accumulation of cortisol during the treatment period d-7 to d98). Saliva and hair samples were tested for cortisol content, as non-invasive methods to measure stress response. Notably, the cortisol concentration in saliva samples was significantly reduced by 24.9% and by 23.9% on d58 and d97, respectively, in animals of the test treatment group compared to animals of the control group. Similarly, the cortisol concentration in hair samples on d98 was reduced by 17.5% in animals of the test treatment group compared to animals of the control group. Animals receiving oligosaccharide preparations of the invention thus showed a significantly decreased stress response after the mixing challenge, and showed lower chronic stress caused by the mixing challenge, as found by lower accumulation of the stress-biomarker cortisol in hair samples. In agreement with these findings, one and two days after the mixing challenge, the amount of severe or medium-severe lesions on ears, body and/or tails was found reduced on animals receiving oligosaccharide preparations of the invention, thus indicating a reduction in aggressive behavior of these animals compared to animals receiving the control diet.

Table 7: Basal diets feeds. [1] Vitamin and mineral premix provided per kg feed: Vitamin A (E 672) 5500 UI; vitamin D3 (E 671) 1100 UI; vitamin E (alfa tocopherol) 25 mg; vitamin B1 0.5 mg; vitamin B2 1.4 mg; vitamin B6 1 mg; vitamin B12 8 µg; vitamin K3 0.5 mg; calcium panthotenate 5.6 mg; nicotinic acid 8 mg; choline 120 mg; Fe (E 1) (from $FeSO_4 \cdot 7H_2O$) 80 mg; I (E 2) (from $Ca(I_2O_3)2$) 0.5 mg; Co (E 3) (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$) 0.4 mg; Cu (E 4) (from $CuSO_4 \cdot 5H_2O$) 5 mg; Cu (E 4) (from aminoacids quelate) 5 mg; Mn (E 5) (from MnO) 40 mg; Zn (E 6) (from ZnO) 100 mg;

Se (E 8) (from Na_2SeO_3) 0.25 mg. [2] Noxyfeed is ITPSA, Barcelona, Spain. Contains BHT+ propyl galate (56%) and citric acid (14%).

Ingredients %	Grower-1	Grower-2	Finisher
Corn	20.00	10.00	-
Wheat	25.00	30.00	38.17
Barley	26.94	34.79	39.32
Soybean meal 48% CP	19.96	18.20	15.84
Sunflower meal (37% CP)	3.47	2.13	1.58
Animal fat	2.03	2.39	2.69
L-lysine HCl 98	0.19	0.16	0.15
L-threonine	0.04	0.03	0.02
DL- methionine	0.03	0.02	0.02
Calcium carbonate	0.25	0.32	0.38
Dicalcium phosphate	1.25	1.13	1.02
Salt	0.43	0.41	0.40
Vitamin and mineral premix[1]	0.40	0.40	0.40
Noxyfeed [2]	0.02	0.02	0.02
Calculated nutrient content			
ME Kcal/kg	3180	3178	3175
Crude protein %	18.00	17.50	17.00
Crude Fibre %	3.44	3.45	3.50
Fat %	4.00	4.23	4.40
Ash %	4.92	4.83	4.72
SID lysine %	0.89	0.83	0.77
SID threonine %	0.58	0.54	0.50
SID methionine %	0.28	0.26	0.24
SID Met+Cys %	0.55	0.53	0.51
SID tryptophan %	0.19	0.18	0.18
Calcium %	0.67	0.66	0.65
Total phosphorus %	0.60	0.57	0.55
Digestible phosphorus %	0.28	0.27	0.25

Table 8: Animal performance for days 0-29. "Control" animals were fed the negative control diet; "test" animals were fed the test treatment diet.

Grower-1 phase (d0-d29)	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed Intake (g/d)	Feed to gain ratio	Gain to feed ratio
Females: control	24.05	47.21	799	1518	1.903	0.526
Females: test	23.86	46.28	773	1506	1.963	0.511
Males: control	24.30	46.38	761	1459	1.927	0.520
Males: test	24.38	48.22	822	1539	1.879	0.536
Overall: control	24.17	46.80	780	1488	1.915	0.523
Overall: test	24.12	47.25	798	1523	1.921	0.524
Root MSE	0.794	1.628	48.9	95.3	0.119	0.033
Sex Effect (Pr>F)	0.164	0.317	0.721	0.703	0.462	0.375
Treatment Effect (Pr>F)	0.833	0.415	0.293	0.293	0.883	0.956
Sex*Treatment Effect (Pr>F)	0.612	0.017	0.014	0.160	0.188	0.164

Table 9: Animal performance for days 29-64. "Control" animals were fed the negative control diet; "test" animals were fed the test treatment diet.

Grower-2 phase (d29-d64)	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed Intake (g/d)	Feed to gain ratio	Gain to feed ratio
Females: control	47.21	72.86 b	733 b	1851	2.528 c	0.396 c
Females: test	46.28	72.52 b	750 b	1847	2.464 bc	0.407 bc

Males: control	46.38	74.01 ab	789 ab	1822	2.312 a	0.435 a
Males: test	48.22	77.39 a	833 a	1945	2.340 ab	0.429 ab
Overall: control	46.80	73.44	761	1837	2.420	0.415
Overall: test	47.25	74.95	792	1896	2.402	0.418
Root MSE	1.628	3.108	61.5	118.1	0.112	0.020
Sex Effect (Pr>F)	0.317	0.008	0.002	0.390	<0.001	<0.001
Treatment Effect (Pr>F)	0.415	0.157	0.152	0.145	0.636	0.728
Sex*Treatment Effect (Pr>F)	0.017	0.086	0.517	0.120	0.230	0.234

Table 10: Animal performance for days 64-98. "Control" animals were fed the negative control diet; "test" animals were fed the test treatment diet.

Finisher phase (d64-d98)	**Initial weight (kg)**	**Final weight (kg)**	**Weight gain (g/d)**	**Feed Intake (g/d)**	**Feed to gain ratio**	**Gain to feed ratio**
Females: control	72.86 b	99.94 b	796 b	2181	2.760	0.365
Females: test	72.52 b	100.27 b	816 ab	2246	2.759	0.364
Males: control	74.01 ab	103.14 ab	857 ab	2291	2.676	0.375
Males: test	77.39 a	107.88 a	897 a	2348	2.622	0.382
Overall: control	73.44	101.54	827	2236	2.718	0.370
Overall: test	74.95	104.08	857	2297	2.691	0.373
Root MSE	3.108	4.597	65.0	173.7	0.154	0.020
Sex Effect (Pr>F)	0.008	0.002	0.003	0.079	0.042	0.053
Treatment Effect (Pr>F)	0.157	0.111	0.178	0.303	0.599	0.620
Sex*Treatment Effect (Pr>F)	0.086	0.163	0.638	0.944	0.603	0.565

Table 11: Animal performance for days 51-59. "Control" animals were fed the negative control diet; "test" animals were fed the test treatment diet.

Mixin challenge week (d51-d59)	**Initial weight (kg)**	**Final weight (kg)**	**Weight gain (g/d)**	**Feed Intake (g/d)**	**Feed to gain ratio**	**Gain to feed ratio**
Females: control	65.60	70.00 ab	550 ab	1674	3.171 ab	0.328 ab
Females: test	64.80	70.06 ab	657 a	1710	2.634 a	0.382 a
Males: control	65.61	69.19 b	449 b	1584	3.992 b	0.271 b
Males: test	68.19	73.66 a	684 a	1762	2.698 a	0.384 a
Overall: control	65.60	69.60 b	499 b	1629	3.582 b	0.300 b
Overall: test	66.50	71.86 a	671 a	1736	2.667 a	0.383 a
Root MSE	2.774	3.280	156.1	223.3	0.771	0.065
Sex Effect (Pr>F)	0.079	0.213	0.478	0.802	0.098	0.208
Treatment Effect (Pr>F)	0.343	0.049	0.003	0.164	0.002	<0.001
Sex*Treatment Effect (Pr>F)	0.080	0.055	0.232	0.347	0.154	0.192

Claims

1. Method for
   i) modulating behavior of an animal, in particular for reducing aggressive behavior;
   ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;
   iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio;
   iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio; and/or
   v) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress;
   the method comprising the steps of:
   providing an oligosaccharide preparation produced or producible by
      a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by
      b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;
      wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
   feeding the oligosaccharide preparation to the animal; and
   increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal.

2. Method for increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community;
   the method comprising the steps of:
   providing an oligosaccharide preparation produced or producible by
      a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by
      b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;
      wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2;
   and contacting the microbial community with the oligosaccharide preparation.

3. Method for producing an oligosaccharide preparation for

i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal; and/or
iv) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress;

the method comprising the steps of:

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or
b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

thus producing the oligosaccharide preparation, wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

4. The method according to any of the preceding claims, wherein the at least second sugar of step a) is selected from glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

5. The method according to any of the preceding claims, wherein the at least first sugar of step b) is glucose, and wherein the at least second sugar of step b) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

6. The method according to any of the preceding claims, wherein the at least first sugar of any one of step a) or b); and the at least second sugar of any one of step a) or b) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose.

7. An oligosaccharide preparation for
i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;
iii) increasing a level of gamma-aminobutyric acid, kynurenine and/or serotonin in the body of the animal, in particular in the gastrointestinal tract and/or in the blood of the animal; and/or
iv) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress;

wherein the oligosaccharide preparation is produced or producible by

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by
b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

8. The oligosaccharide preparation according to claim 7, wherein the at least second sugar of step a) is selected from glucose, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably glucose.

9. The oligosaccharide preparation according to claim 7 or 8, wherein the at least first sugar of step b) is glucose, and wherein the at least second sugar of step b) is selected from N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose.

10. The oligosaccharide preparation according to any one of claims 7-9, wherein the at least first sugar of any one of step a) or b), and the at least second sugar of any one of step a) or b) are condensed with at least a third sugar selected from glucose, N-acetylglucosamine, lactose, mannose, glucosamine, fructose, sucrose, xylose, and arabinose; preferably lactose.

11. The oligosaccharide preparation according to any one of claims 7-10, wherein the oligosaccharide composition comprises at least 10 wt%, (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 wt% etc.) of the at least first fraction; preferably at least 20 wt%.

12. The oligosaccharide preparation according to any one of claims 7-11, wherein the oligosaccharide preparation is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

13. Use of an oligosaccharide preparation according to any one of claims 7-12 for
i) modulating behavior of an animal, in particular for reducing aggressive behavior;
ii) improving animal welfare, in particular for reducing anxiety, stress and/or fear disorders;
iii) improving animal performance, in particular for improving average body weight gain and/or reducing feed conversion ratio;
iv) improving animal performance of animals facing stress, in particular for improving average body weight gain and/or reducing feed conversion ratio;

v) increasing production of gamma-aminobutyric acid, kynurenine and/or serotonin by a microbial community; and/or

vi) reducing cortisol concentration in plasma, saliva and/or hair of animals facing stress.

14. An oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid, kynurenine, and/or cortisol; wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation; wherein the oligosaccharide preparation is produced or producible by

a) condensing at least a first sugar with at least a second sugar using a catalyst, wherein the at least first sugar is N-acetylglucosamine; or by

b) condensing at least a first sugar with at least a second sugar using a catalyst; wherein the catalyst is methylphosphonic acid;

and wherein the oligosaccharide preparation comprises at least a first fraction having a degree of polymerization (DP) of 1, at least a second fraction having a DP of 2, and at least a third fraction having a DP of more than 2.

15. The oligosaccharide preparation for use in treatment, amelioration, prevention and/or prophylaxis of one or more disorders associated with an imbalanced level of gamma-aminobutyric acid, kynurenine, and/or cortisol; wherein the one or more disorders are selected from the group of anxiety, stress, fear disorders, systemic inflammation, and local inflammation according to claim 14, wherein the oligosaccharide preparation is comprised in a nutritional composition at an inclusion rate of at least 50 ppm (e.g. at least 50, 70, 100, 150, 200, 300, 400, 500 ppm).

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/054484

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K20/163 A23K50/30 C07H1/00 A23K10/30
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 C11C C07H

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/097443 A1 (DSM IP ASSETS BV [NL]; MIDORI USA INC [US]) 14 May 2020 (2020-05-14) paragraphs [0008], [00928], [00945], [00951], [001348]; figures 25A-25C; examples 3, 25,35,38 A. feed sugar; paragraph [00915] - paragraph [00922]; table 14 ----- -/--	1-15

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2024	08/05/2024

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI WEI ET AL: "Synthesis of oligosaccharides with lactose and N-acetylglucosamine as substrates by using [beta]-d-galactosidase from Bacillus circulans", EUROPEAN FOOD RESEARCH AND TECHNOLOGY , vol. 231, no. 1 12 March 2010 (2010-03-12), pages 55-63, XP093067580, Berlin/Heidelberg ISSN: 1438-2377, DOI: 10.1007/s00217-010-1254-2 Retrieved from the Internet: URL:http://link.springer.com/article/10.1007/s00217-010-1254-2/fulltext.html [retrieved on 2023-04-06] abstract	1-15
A	BI CHONGPENG ET AL: "Effects of dietary [gamma]-aminobutyric acid supplementation on antioxidant status, blood hormones and meat quality in growing-finishing pigs undergoing transport stress", JOURNAL OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION. , vol. 104, no. 2 22 December 2019 (2019-12-22), pages 590-596, XP093047638, DE ISSN: 0931-2439, DOI: 10.1111/jpn.13280 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1111/jpn.13280 the whole document	1-15
A	WO 2022/175265 A1 (DSM IP ASSETS BV [NL]) 25 August 2022 (2022-08-25) the whole document	1-15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/054484

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		EP 4294204 A1	27-12-2023
		EP 4294205 A2	27-12-2023
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