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(54) Title: ORAL COMPOSITION COMPRISING SACCHARIDES FOR THE DIAGNOSIS OF INSULIN DYSREGULATION IN EQUINES AND METHODS OF USE THEREOF

(57) Abstract: The invention relates to novel palatable, well-tolerated, accurate, oral glycemic challenge compositions comprising saccharides and methods for use in equines to diagnose insulin dysregulation.

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ORAL COMPOSITION COMPRISING SACCHARIDES FOR THE DIAGNOSIS OF INSULIN DYSREGULATION IN EQUINES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

5 The present invention relates to the field of medicine, particularly veterinary medicine. In particular, the invention relates to a novel palatable, well-tolerated, accurate, oral glycemic challenge composition comprising saccharides and methods for use in equines to diagnose insulin dysregulation.

BACKGROUND OF THE INVENTION

10 The two major equine endocrinopathies are pituitary pars intermedia dysfunction (PPID) and equine metabolic syndrome (EMS). EMS is a collection of risk factors for endocrinopathic laminitis with insulin dysregulation (ID) being the key central and consistent feature. Endocrinopathic laminitis is also associated with a significant subset of PPID cases, which mostly also experience concurrent ID. Laminitis has a severe and life-threatening impact on the health of equine animals. It is a multifactorial condition causing structural and mechanical changes
15 in the supporting tissues within the hoof, resulting in acute and chronic pain, lameness, and potentially euthanasia. The link between ID and laminitis risk has been already recognized in the 1980s and since then evidence has been further increased for example by studies from Treiber et al., Asplin et al. and de Laat et al. The American College of Veterinary Internal Medicine consensus statement on EMS from 2010 (Frank et al.) and more recently the European College of Equine Internal Medicine (ECEIM) consensus statement on EMS
20 from 2018 (Durham et al.) further highlight and describe the importance of diagnosing ID early in order to manage ID and hence reduce the risk of laminitis. In this context Durham et al. discuss various oral challenge tests, such as the Oral Glucose Test (OGT) and the Oral Sugar Test (OST). Several different variants of tests for diagnosis of ID have been described. So far, there is agreement, that oral glycemic challenge tests are preferred, because they mirror the complete sequence of events a herbivore experiences with food ingestion and digestion
25 of carbohydrates (https://sites.tufts.edu/equineendogroup/files/2018/09/2018-Final-EMS_Recommendations_Web.pdf; Bertin and de Laat; Durham et al., ECEIM).

Oral sugar tests (OST) employ sugar syrups to be administered via syringes directly into the oral cavity. Schuver et al. compared the results of an OST based on Karo light corn syrup (0.15 mL syrup per kg body weight - BW) from ACH Food Companies Inc. (Memphis, TN, USA) in the diagnosis of insulin dysregulation in horses with
30 those with an intravenous glucose tolerance test (ivGTT). They concluded that both OST and ivGTT insulin results are closely correlated rendering the OST a promising field test for ID in horses.

Jocelyn et al. studied the effect of the dose (0.15, 0.3 and 0.45 mL/kg BW) of the Karo light corn syrup from ACH Food Companies Inc. (Memphis, TN, USA) on the insulin and glucose response to the OST and concluded that a dose of 0.45 mL/kg BW of such corn syrup may be preferable to differentiate between ponies with a
35 history of laminitis and without laminitis.

The composition of Karo light corn syrup is not given by the manufacture, the personal analysis by Jocelyn et al., revealed a variable dextrin, maltotriose, maltose (15.8%) and glucose (19.8%) content of this corn syrup (see

Supplementary Item 2). A further analysis of the Karo light syrup that is available in the public domain, found substantially different maltose (11.6%) and glucose (13.3%) content (Anresco analysis).

Lindåse and co-workers reported a modified OST for the evaluation of insulin and glucose dynamics in horses which is based on a Scandinavian commercially available glucose syrup (Dan Sukker Glykossirap, Nordic Sugar A/S, Copenhagen, Denmark) with a partly available sugar composition (glucose and maltose). However, for “other sugars” it is not further disclosed of what kind of saccharides they are comprised (Lindase et al., Table 2). Nevertheless, they concluded that this modified OST appears to be a practical and useful diagnostic tool for the assessment of insulin dysregulation in horses.

Consequently, a drawback of these OSTs based on Karo light corn syrup or Dan Sukker Glykossirap or likely other sugar syrups available for human food consumption, is that the exact sugar composition is not disclosed by the manufacturer and/or may vary with different batches, as it is not marketed as a diagnostic tool but as a food component. In addition, they are not marketed in all territories worldwide.

Smith et al. compared the in-feed OGT and the OST and concluded that the results of the OGT and OST in the identification of ID in equines are not comparable in all cases and that further work is required to establish which test more accurately diagnoses ID in horses and ponies.

Oral glucose tests (OGT) employ dextrose powder to be administered orally or to be mixed into a feed. Warnken and co-workers described variations in insulin response to OGT via naso-gastric tubing and aimed at providing a clinical useful cut-off value for ID when using the insulin quantification performed with an equine-optimized insulin ELISA. They concluded that the OGT performed with 1,000 mg glucose per kg BW and administration via naso-gastric tubing can easily be performed under clinical settings and allows to diagnose ID in horses. In subsequent studies the group provided evidence that also a lower dose of glucose (500 mg/kg BW) is sufficient to reliably assess ID [Warnken – 2018 abstract].

Another variation of an OGT is to provide the dextrose powder in feed (in-feed OGT). E.g. de Laat et al. (2016) employed D-glucose dissolved in 500 ml warm water and mixed with 200 g wheat bran, before being mixed with 0.3% of BW lucerne chaff and reported reasonable repeatability. However, partial feed refusals (up to 70%) were recorded for 5 of the 12 ponies during the first OGT.

Also meal challenges have been tested to characterize insulin dysregulation. To investigating glucose and insulin responses to meals has been attempted by Rapson et al. with a so called “standardized meal challenge” (SMC) and by Meier et al. in a “diet challenge test” (DCT). The tests were able to describe e.g. changes of insulin responses with age and / or showed a pronounced insulin dysregulation in ponies that later developed laminitis in comparison to others that did not. However, the compositions obviously depend on availability of feed components, this makes these tests useful for a defined experiment, but it is unlikely that they can be reproducibly employed as a diagnostic test worldwide.

The main limitation of all prior art variations of diet challenges (in-feed OGT, SMC, DCT) is the incomplete intake of the challenge by a number of animals at the first instance of test. With training, i.e. some days of tasting/getting used to the challenge or a sequence of several tests, the acceptance improves; however, these procedures are unsuitable for field conditions where only a single test is required.

Further prior art is as follows:

De Laat MA et al. examine the repeatability of an oral glucose test in ponies.

Durham AE et al. disclose the ECEIM consensus statement on Equine Metabolic Syndrome (EMS).

Knowles EJ et al. describe the use of an oral sugar test in ponies when performed with or without prior fastening.

- 5 WO 99/51108 discloses a composition to improve glucose and insulin metabolism in companion animals by controlling the postprandial glycemic and/or insulin response in those animals, wherein such composition includes a source of protein, a source of fat, and a source of carbohydrates from a multiple grain source.

WO 2014/161836 relates to the treatment of metabolic disorders in equine animals.

- 10 WO 2005/017532 describes a solid oral diagnostic test meal that contains a polysaccharide, wherein the oral diagnostic test meal provides a medically controlled quantity of glycemic carbohydrate after being ingested by a vertebrate subject.

Disadvantages of the above and further prior art are in summary as follows:

- Basal insulin concentration (without a stimulation with a glycemic challenge):
 - are not recommended due to low diagnostic sensitivity
- 15 • Intra-venous insulin tolerance test (ivITT):
 - tests tissue insulin sensitivity – not recommended to test for insulin dysregulation
- Intra-venous test employing glucose i.e. intra-venous glucose tolerance test (ivGTT); frequently sampled intra-venous glucose tolerance test (FSIGTT); combined intra-venous glucose-insulin test (CGIT) or euglycemic hyperinsulinemic clamp procedures (EHC):
 - 20 ○ IV tests, even if comprising a glucose challenge, omit a possibly important element of ID, i.e. the entero-insular axis e.g. effects of intestinal incretins on stimulated insulin secretion.
 - in addition, these tests are time and labour intensive, i.e. complex and costly (see definitions). They can be employed in a clinical study setting, but are not suitable for routine use in the field.
- Oral glycemic challenge tests are recommended, however:
 - 25 ○ OSTs use sugar syrups, which are not developed and marketed for diagnostic use; the exact sugar composition is not disclosed by the manufacturer and/or may vary with different batches. In addition, they are not marketed in all territories worldwide.
 - OGTs with dextrose administered as a large volume solution via a naso-gastric tubing are semi invasive procedures, which are not well perceived by the horse owner.
 - 30 ○ OGTs as in-feed or also other feed/meal tests have clear limitations for field use, i.e. low acceptance/palatability and thus, incomplete voluntary intake in horses when offered for the first time. An oral glycemic challenge to evaluate e.g. the endogenous blood glucose and insulin response, should be taken up fast (i.e. within 5 – 10 minutes). Thus, this is not achievable in many horses with in-feed OGTs or feed/meal tests.
- 35 There is an urgent need for an oral glycemic challenge composition for the diagnosis of insulin dysregulation in equines under field conditions which overcomes the problems of the prior art as described above.

SUMMARY OF THE INVENTION

The present invention concerns a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for use in a method of diagnosing insulin dysregulation in equine animals. In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a
5 corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

10 The present invention also concerns the composition for use as herein described and claimed, wherein such diagnosing insulin dysregulation in equine animals or method of diagnosing insulin dysregulation in equine animals comprises the following steps:

- (a) determining the insulin concentration in at least one biological ex-vivo sample taken from an equine
15 animal; and
- (b) comparing the insulin concentration determined according to step (a) with a reference insulin concentration of a reference population of equine animals;

wherein a difference in the insulin concentration determined according to step (a) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

20 In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals comprising such steps (a) and (b) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals comprising such steps (a) and (b) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in
25 equine animals comprising such steps (a) and (b) is also intended to be comprised by the spirit and disclosure of the underlying invention.

The present invention further concerns the composition for use as herein described and claimed, wherein prior to step (a) such equine animal has orally been administered a composition, preferably a solid or liquid oral
30 glycemic challenge composition, comprising saccharides, more preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge
35 composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

The present invention also concerns the composition for use as herein described and claimed, wherein such diagnosing insulin dysregulation in equine animals or method of diagnosing insulin dysregulation in equine animals comprises the following steps:

- 5 (a) determining the insulin concentration in at least one biological ex-vivo sample taken from an equine animal; and
(b) comparing the insulin concentration determined according to step (a) with a reference insulin concentration of a reference population of equine animals;

10 wherein a difference in the insulin concentration determined according to step (a) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation; and wherein prior to step (a) such equine animal has orally been administered a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides, more preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration.
15 In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals comprising such steps (a) and (b) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals comprising such steps (a) and (b) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in
20 equine animals comprising such steps (a) and (b) is also intended to be comprised by the spirit and disclosure of the underlying invention.

The present invention further concerns a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for use in a method of diagnosing insulin dysregulation in equine animals
25 comprising the steps:

- (a) optionally, taking a first biological ex-vivo sample, preferably a blood sample, from an equine animal; which has preferably fastened overnight, more preferably for 8 to 14 hours, or which preferably has been in a resting prandial status;
(b) orally administering to such equine animal the composition comprising saccharides, wherein preferably
30 such composition is administered to the equine animal over a period of time of less than 10 minutes, more preferably 5 minutes; preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration;
(c) taking a second biological ex-vivo sample, preferably a blood sample, from such equine animal after
35 having administered such composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of the composition by such equine animal;
(d) determining the insulin concentration, preferably the blood and/or plasma insulin concentration, in the first and second biological ex-vivo sample, preferably blood sample;

(e) comparing the insulin concentration in such first and second biological ex-vivo samples with a reference insulin concentration of a reference population of equine animals;

wherein a difference in the insulin concentration obtained according to step (e) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

5 In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals comprising such steps (a) to (e) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals comprising such steps (a) to (e) or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in
10 equine animals comprising such steps (a) to (e) is also intended to be comprised by the spirit and disclosure of the underlying invention.

The present invention further concerns a composition, preferably a solid or liquid oral glycemic challenge composition, comprising, preferably consisting of:

- 15 (a) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:
- (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-
20 saccharides; the oligo-saccharides consisting of glucose units;
- (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple,
25 honey, banana, salt, curcuma, vanilla, beet pulp;

or

- (b) water and/or fruit juice, preferably in the form of a syrup, comprising:
- (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-
30 saccharides; the oligo-saccharides consisting of glucose units;
- (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple,
35 honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (b) is further diluted with water, e.g. 1:1;

or

(c) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:

(i) from 5% to 15% by weight of water soluble oligo-saccharides; more preferably, 10% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerisation larger or equal to 10;

(ii) from 20% to 30% by weight of water soluble oligo-saccharides; more preferably, 25% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerisation larger or equal 3, but smaller than 10;

(iii) from 25% to 35% by weight mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;

(iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp

or

(d) water and/or fruit juice, preferably in the form of a syrup, comprising:

(i) from 0% to 5% by weight of water soluble oligo-saccharides; more preferably, 1% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerisation larger or equal to 10;

(ii) from 30% to 40% by weight of water soluble oligo-saccharides; more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerisation larger or equal 3, but smaller than 10;

(iii) from 25% to 35% of mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;

(iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (d) is further diluted with water, e.g. 1:1.

The present invention further concerns the composition as herein described and claimed for use in the methods as herein described and claimed.

In the course of the present invention corresponding methods of diagnosing insulin dysregulation in equine

animals or corresponding uses for diagnosing insulin dysregulation in equine animals or corresponding uses in a

method of diagnosing insulin dysregulation in equine animals are also intended to be comprised by the spirit and disclosure of the underlying invention.

The present invention further concerns a diagnostic kit comprising the composition as herein described and claimed and instructions for performing diagnosing insulin dysregulation in equine or the method of diagnosing insulin dysregulation in equine animals as herein described and claimed, and, optionally, an applicator for administering the composition to the equine animals, preferably a syringe.

The advantages of the oral glycemic challenge composition according to the present invention are as follows:

- defined and reproducible compositions of the oral glycemic challenge test, designated for use as a diagnostic tool in equines
- high acceptance/palatability of the oral diagnostic glycemic challenge compositions as described above by the equines when offered for free intake (solid, pelleted or syrup diluted in water) – making an intake of the glycemic challenge composition within 5-10 minutes feasible
- non-invasive procedures of administering the oral glycemic challenge compositions as described above, being well accepted by the equines when given directly into the oral cavity with a syringe as syrup
- diagnosis can be performed evaluating one or more biomarker in a basal and one or more stimulated sample (after the challenge), or evaluating the area under the curve, i.e. the excursion of one or more biomarker in response to the glycemic challenge – a biomarker being a blood concentration selected from: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP)
- in addition, diagnosis can be performed in a single sample – such ease of use enables a more flexible test procedure, a simple “one sampling” test, i.e. the horse owner can easily administer the glycemic challenge of the present invention and the veterinarian can take a blood sample for analysis of one or more biomarker, i.e. blood concentrations selected from: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP) on any time point between 15 min and 240 min after the challenge
- In any way – single or stimulated sample or two sample analysis - the test allows a distinct separation between healthy and insulin dysregulated equines based on the blood concentrations of the one or more biomarkers as described above, thereby enabling veterinarians to easier diagnose insulin dysregulation in equines.

DETAILED DESCRIPTION OF THE INVENTION

Before the embodiments of the present invention are described in further details it shall be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. All given ranges and values may vary by 1 to 5 % unless indicated otherwise or known otherwise by the person skilled in the art, therefore, the term “about” was usually omitted from the description and claims. In particular, however, in the course of the present invention, the variability of concentrations of mono-, di- and oligosaccharides consisting of glucose units – being sourced from natural products – may vary from 10 to 20%. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the substances, excipients, carriers, and methodologies as reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

In the course of the present invention the term “**saccharides**” refers to glucose (mono-saccharide) and/or maltose (di-saccharide, consisting of glucose units) and/or water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$.

Herein, **glucose** refers to D-glucose; also interchangeably the older term dextrose is used.

Herein, **palatability** refers to the voluntary intake in a certain number of animals. Similar to the definition of regulatory authorities – if tested in a larger cohort at least 70% of the equines should show voluntary intake.

The term “**equine**”, herein also interchangeably referred to as “horse”, in the course of the present invention encompasses any member of the genus *Equus*. It encompasses, e.g., any horse, pony and/or donkey, the taxonomic designations *Equus ferus* and/or *Equus caballus*, and/or the subspecies *Equus ferus caballus*. The equine may, e.g., be a domestic horse.

Herein, **insulin dysregulation (ID)** refers to a disturbance of the balanced inter-relationship between plasma concentrations of insulin, glucose, and lipids. ID can manifest in several possible ways including basal hyperinsulinemia, and/or an excessive or prolonged hyperinsulinemia in response to a carbohydrate challenge, with or without an excessive or prolonged hyperglycaemia, and/or tissue insulin resistance.

Herein, **hyperinsulinemia** refers to excess levels of insulin circulating in the blood. It can be caused by increased insulin secretion or delayed insulin clearance, which may be cause or consequence of insulin resistance.

Herein, **insulin resistance** refers to the failure of insulin-sensitive tissue, such as skeletal muscle, adipose and liver tissue, to respond to insulin.

Herein, an **excursion**, e.g. an insulin excursions or glucose excursion, designates a change in concentration or level in blood over time. The magnitude of excursions, e.g. insulin excursions or glucose excursions may be expressed as area-under-curve (AUC) values.

Herein, **ivITT** refers to an intravenous insulin tolerance test. In an ivITT, 0.03 U insulin per kg body mass may typically be employed.

Herein, **ivGTT** refers to an intravenous glucose tolerance test. In an ivGTT, 0.2 g dextrose per kg body mass may typically be employed.

Herein, **FSIGTT** refers to a frequently sampled intra-venous glucose tolerance test. In an FSIGTT 150 mg glucose per kg BW may typically be given intravenously. Blood samples are taken frequently thereafter,
5 typically at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after glucose infusion.

Herein, **CGIT** refers to an intravenous combined glucose-insulin tolerance test. In a CGIT, 0.15 mg glucose per kg BW and 0.1 U insulin per kg body mass may typically be given intravenously.

Herein, **EHC** refers to a euglycemic hyperinsulinemic clamp procedures. Typically, a continuous rate infusion of
10 glucose and recombinant human insulin is initiated through 1 of 2 jugular catheters, with a multichannel volumetric infusion pump. The infusion rate for insulin is held constant e.g. at 3 mIU/kg/min, and a variable rate of glucose is infused to maintain blood glucose concentration at euglycemia (approx. 5 mmol/L) during the 3-hour infusion. The glucose infusion rate is adjusted if the concentration deviated by more than 0.2 mmol/L from euglycemia. Blood samples are obtained from the 2nd jugular catheter every 5 minutes for immediate analysis of
15 blood glucose concentration with a handheld glucometer and every 10 minutes throughout the EHC for subsequent determination of plasma glucose (10-minutes intervals) and plasma insulin (20-minutes intervals) concentrations.

Herein, **OST** refers to an oral sugar test typically based on administration of Karo light corn syrup or Dan Sukker Glykossirap. Doses of 0.15 to 0.45 mL/kg BW are employed typically.

Herein, an **OGT** refers to an oral glucose test based on administration of typically 0.5 to 1 g glucose / kg BW dissolved in 2 liter of water and administered via a nasogastric tubing.

Herein, an **in-feed OGT** refers to an oral glucose test based of 0.5 to 1 g glucose / kg BW mixed with water and wheat bran and/or chaff or other non-sweet feed.

Herein, a **meal challenge** refers to variable compositions of food stuff containing high amounts of non-
25 structured carbohydrates employed in a specific experiment.

Herein, a **resting prandial status** of the equine to be tested refers to a non-fasted feeding status, i.e. status in the morning prior to regular feeding of an equine that did have only restricted access to small amounts of hay or other forages overnight.

Herein, **adipokines** are biologically active substances produced in adipose tissue that act in an
30 autocrine/paracrine or endocrine fashion, e.g. leptin or adiponectin.

Herein, **incretins** are biologically active substances produced in gastrointestinal mucosa that act in an autocrine/paracrine or endocrine fashion, e.g. Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP).

Herein, a **biomarker** is any measurable blood concentration of a characteristic involved in the physiology of
35 insulin pathways and/or the pathophysiology of insulin dysregulation e.g. selected from: glucose, insulin,

glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP).

In one aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein such composition has been administered over a period of time of less than 10 minutes, preferably 5 minutes.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In another aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein prior to administering such composition to the equine animal, such equine animal has fastened overnight, preferably for 8 to 14 hours, or has been in a resting prandial status.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In yet another aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein the at least one biological ex-vivo sample are two biological ex-vivo samples, and wherein the first of the two biological ex-vivo samples is taken from the equine animal prior to orally administering the composition; and wherein the second of the two biological ex-vivo samples is taken from the equine animal after consumption of the composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of such composition by such equine animal.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein such at least one biological ex-vivo sample taken from the equine animal is selected from the group consisting of: blood sample, blood plasma sample; preferably is a blood sample.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein the insulin concentration is selected from the group consisting of: blood insulin concentration, blood plasma insulin concentration; preferably is blood insulin concentration.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein in addition and parallel to the insulin concentration the following one or more additional biomarkers are measured: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP), wherein the one or more additional biomarker concentration is determined and compared with the respective reference biomarker concentration of said reference population of equine animals, wherein preferably blood sampling for measuring such one or more additional biomarkers is performed on one or more time points selected from: prior to the administration of the composition and/or in the stimulated status, e.g. 30, 60, 120, 180 and/or 240 minutes after the administration of the composition.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein such composition is a liquid composition, preferably a "ready-to-use" liquid composition

or a liquid composition that was generated by diluting dry powder with water, most preferably a syrup, or wherein such composition is a solid composition, preferably a solid composition consisting of pellets, more preferably a solid "ready-to-use" composition consisting of pellets and/or wherein such composition is offer to the equine animals for free intake or is given directly into the oral cavity with a syringe (as for the liquid composition).

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein such saccharides comprise, preferably consist of: glucose (mono-saccharide) and/or maltose (di-saccharide, consisting of glucose units) and/or water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$; more preferably glucose (mono-saccharide) and maltose (di-saccharide, consisting of glucose units) and water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

In a further aspect, the present invention relates to the composition for use in the methods as herein described and claimed, wherein such method is characterized by one or more of the following technical features:

- (1) defined and reproducible compositions of the oral glycemic challenge test – designated for use as a diagnostic tool in equines;
- (2) high acceptance/palatability of the oral diagnostic glycemic challenge compositions by the equines when offered for free intake (solid, pelleted or syrup diluted in water) – making an intake of the glycemic challenge composition within 5-10 minutes feasible;
- (3) non-invasive procedures of administering the oral glycemic challenge compositions – being e.g. well accepted by the equines when given directly into the oral cavity with a syringe as syrup;
- (4) diagnosis can be performed evaluating one or more biomarker in a basal sample and stimulated sample (after the challenge) or evaluating the area under the curve, i.e. the excursion of one or more biomarker in response to the glycemic challenge;

- (5) diagnosis can be performed in a single sample – such ease of use enables a more flexible test procedure, a simple “one sampling” test, i.e. the horse owner can easily administer the oral glycemic challenge composition and the veterinarian can take a blood sample for analysis of one or more biomarker;
- (6) single or stimulated sample or two sample analysis – the test allows a distinct separation between healthy and insulin dysregulated equines based on the blood concentrations of the one or more biomarkers, thereby enabling veterinarians to easier diagnose insulin dysregulation in equines.

In the course of the present invention a corresponding method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals or a corresponding use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals is also intended to be comprised by the spirit and disclosure of the underlying invention.

BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1: Insulin [mU/L] AUC after a glycemic challenge with either syrup A (administered directly into the mouth via syringe), pelleted “glycemic challenger” composition (pellets), or glucose solution via nasogastric tubing administration (dextrose) in healthy and EMS horses (n = 18). The correlation between individual results of dextrose and syrup A (empty symbols and dotted line) or pellets (filled symbols and solid line) is shown.
- Figure 2: Insulin [mU/L] at 120 min after a glycemic challenge with either syrup A (administered directly into the mouth via syringe), pelleted “glycemic challenger” composition (pellets), or glucose solution via nasogastric tubing administration (dextrose) in healthy and EMS horses (n=4 EMS and n=4 healthy).

EXAMPLES

The following examples serve to further illustrate the present invention; but the same should not be construed as a limitation of the scope of the invention disclosed herein.

Example 1: Palatability / acceptance of the liquid “glycemic challenger” compositions

In a partly cross over design eight healthy equines received two different liquid “glycemic challenger” compositions (syrup A and syrup B) according to the present invention and a typically used standard in-feed glucose tolerance test composition (in-feed OGT). A dose equivalent to a total dextrose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight has been given. The composition of syrup A was about 37% oligo-saccharide (consisting of dextrose units), about 30% dextrose and maltose, 2% salt, and about 30% water. The composition of syrup B was about 37% oligo-saccharide (consisting of dextrose units), about 28% dextrose and maltose, about 15% apple juice, about 5% apple sugar extract, about 0.05% apple

aroma, and about 15% water. For the in-feed OGT dextrose (0.5 g/kg BW) was mixed with 100-200 mL water, 200 g wheat bran and a handful of lucerne or alfalfa hay. The mixture was prepared freshly on the day of use.

The syrup has been administered directly into the mouth via syringe whereas the standard in feed glucose was offered as free feed in a trough. For use as an oral glycemic challenge to evaluate e.g. the endogenous blood glucose and insulin response the uptake of the glycemic challenge should be fast (i.e. within 5 – 10 minutes).

The palatability / acceptance has been recorded according to the following scoring system and the individual results are summarized in Table 1 below.

Table 1: Acceptance and discomfort scoring of two different liquid “glycemic challenger” compositions (syrup A and syrup B) and a standard in feed glucose tolerance test composition (dextrose in feed).

Results of testing	Dextrose in feed		Syrup A syringed		Syrup B syringed	
	Acceptance	Discomfort	Acceptance	Discomfort	Acceptance	Discomfort
median	4.0	1.0	3.0	2.0	2.0	1.5
mean	3.8	1.1	2.8	2.0	2.0	1.5

Acceptance score

Score 1 = very good (test item was accepted without problems) to Score 4 = unacceptable (test item was hardly taken up freely—less than 1/2 of the feed portion – in case of liquid - administered by syringe with mayor reinforcement, or impossible)

Discomfort Score

Score 1 = none (no salivation or other signs of discomfort after administration) to Score 3 = high (saliva dripping or running from the oral cavity or pronounced signs of discomfort noted)

The in feed glucose tolerance test composition was largely unacceptable. None of the animals consumed the whole test meal within the given time frame of 10 min. In contrast, the syrup formulations were mostly well accepted and most animals had a discomfort score of 1 (no discomfort) or 2 (slight to moderate).

Example 2: Palatability / acceptance of liquid and pelleted “glycemic challenger” compositions

In a cross over design eighteen equines (healthy or insulin dysregulated) received a liquid “glycemic challenger” composition (syrup A) and a pelleted “glycemic challenger” composition (pellets) according to the present invention. A dose equivalent to a total dextrose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight has been given. The composition of syrup A was about 37% oligo-saccharide (consisting of glucose units), about 30% glucose and maltose, 2% salt, and about 30% water. The composition of the pellets was about 35% oligo-saccharide (consisting of glucose units), about 32% glucose and maltose; there were about 35% flour of linseed, about 22% flour of maize germ, about 3% of linoil, and about 0.4% of Curcuma/Vanilla.

The syrup has been administered directly into the mouth via syringe, whereas the pellets were offered as free feed in a trough. For use as an oral glycemic challenge to evaluate e.g. the endogenous blood glucose and insulin response the uptake of the glycemic challenge should be fast (i.e. within 5 – 10 minutes).

The palatability / acceptance has been recorded according to the scoring system as described in Example 1 and the individual results are summarized in Table 2 below

Table 2: Acceptance and discomfort scoring of a liquid “glycemic challenger” compositions (syrup A) and a pelleted “glycemic challenger” composition (pellets).

Results of testing	pellets		Syrup A syringed	
	Acceptance	Discomfort	Acceptance	Discomfort
median	1.0	1.0	2.0	1.5
mean	1.0	1.3	2.1	1.7

The pelleted “glycemic challenger” composition was highly palatable (score 1). All eighteen animals consumed the whole test meal within the given time frame of 10 min. Also the syrup formulation was mostly well accepted with a median acceptance and discomfort score of 2 and 1.5, respectively.

In addition to the described testing procedure, the syrup formulation (with different flavors e.g. honey, banana, apple, salty) was offered diluted 1:1 in water to selected animals. The diluted syrup was taken up freely within the time frame of 10 min by most animals.

Example 3: Palatability / acceptance and pharmacodynamics of liquid “glycemic challenger” compositions

As described in example 1, standard “in feed glucose” is not taken up completely by many horses in the required dose equivalent to 0.5 g glucose per kg body weight. Consequently, nasogastric tubing administration of glucose solution was employed to demonstrate the comparability of the pharmacodynamics effects with those of the liquid “glycemic challenger” compositions according to the present invention. Sixteen healthy horses were employed in a cross over design receiving the following “glycemic challenger” composition after fasting overnight:

- 0.5 g glucose per kg body weight dissolved in 2 L water and administered via nasogastric tubing
- syrup A (about 37% oligo-saccharide (consisting of glucose units), about 30% glucose and maltose, 2% salt, and about 30% water), dose equivalent to a total glucose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, administered directly into the mouth via syringe
- syrup B (about 37% oligo-saccharide (consisting of glucose units), about 28% glucose and maltose, about 15% apple juice, about 5% apple sugar extract, about 0.05% apple aroma, and about 15% water), dose equivalent to a total glucose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, administered directly into the mouth via syringe

The palatability / acceptance was recorded as described in Example 1 and the individual results are summarized in Table 3.

Table 3: Acceptance and discomfort scoring of two different liquid “glycemic challenger” compositions (syrup A and syrup B).

Results of testing	Syrup A syringed		Syrup B syringed	
	Acceptance	Discomfort	Acceptance	Discomfort

median	2.0	1.5	2.0	2.0
mean	2.2	1.5	2.4	1.8

The syrup formulations were mostly well accepted and most animals had a discomfort score of 1 (no discomfort) or 2 (slight to moderate salivation or other signs of discomfort).

To assess the pharmacodynamics, blood samples were taken prior to the administration of the “glycemic challenger” compositions and at 60 min and 120 min after dosing. Directly after sampling, blood glucose concentrations were recorded with a hand-held glucometer in a blood spot (Alphatrak 2, test strip lot 1606819, Abbott). EDTA plasma insulin was analyzed in samples that had been frozen at $\leq -15^{\circ}\text{C}$ until analysis with an Enzyme-Linked Immunosorbent Assay (Equine optimized Insulin, #10-1205-01, Mercodia AB).

The analysis of glucose and insulin response to the challenges showed no significant difference between the different compositions (Table 4)

Table 4: Glucose [mM] and Insulin (mU/L) before (basal), after 60 min and 120 min of a glycemic challenge with either syrup A or syrup B (both administered directly into the mouth via syringe), or glucose solution via nasogastric tubing administration (dextrose) in healthy horses and ponies (n = 16), mean $\pm$ SD

Results of testing	Dextrose (tubing)		Syrup A (syringed)		Syrup B (syringed)	
	Glucose mM	Insulin mU/L	Glucose mM	Insulin mU/L	Glucose mM	Insulin mU/L
before	5.5 $\pm$ 0.5	4.2 $\pm$ 1.9	5.6 $\pm$ 0.6	6.3 $\pm$ 3.6	6.0 $\pm$ 1.0	6.0 $\pm$ 2.9
60 min	10.3 $\pm$ 0.9	31.1 $\pm$ 20.0	9.9 $\pm$ 1.5	31.9 $\pm$ 18.5	10.6 $\pm$ 1.4	34.6 $\pm$ 24.6
120 min	9.5 $\pm$ 1.7	28.6 $\pm$ 19.3	11.2 $\pm$ 1.1	50.0 $\pm$ 35.0	10.8 $\pm$ 1.8	45.9 $\pm$ 36.0

In conclusion, the results show that the “glycemic challenger” compositions according to the present invention elicits glucose and insulin excursions, that are comparable to “gold standard” OGT results, but the “glycemic challenger” according to the present invention are easier to administer.

Example 4: Pharmacodynamics of liquid and pelleted “glycemic challenger” compositions

As described in example 1, standard “in feed glucose” is not taken up completely by many horses in the required dose equivalent to 0.5 g glucose per kg body weight. Consequently, nasogastric tubing administration of glucose solution was employed to demonstrate the comparability of the pharmacodynamics effects with those of the liquid and pelleted “glycemic challenger” compositions according to the present invention. Eighteen healthy or insulin dysregulated horses were employed in a cross over design receiving the following “glycemic challenger” composition after fasting overnight:

- 0.5 g glucose per kg body weight dissolved in 2 L water and administered via nasogastric tubing
- syrup A (about 37% oligo-saccharide (consisting of glucose units), about 30% glucose and maltose, 2% salt, and about 30% water), dose equivalent to a total glucose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g glucose per kg body weight, administered directly into the mouth via syringe

- pellets (about 35% oligo-saccharide (consisting of glucose units), about 32% glucose and maltose, about 35% flour of linseed, about 22% flour of maize germ, about 3% of linoil, and about 0.4% of Curcuma/Vanilla), dose equivalent to 0.5 g glucose per kg body weight, offered as free feed in a trough

5 To assess the pharmacodynamics, blood samples were taken prior to the administration of the “glycemic challenger” compositions and at 30 min, 60 min and 120 min after dosing. Blood glucose concentrations were measured in sodium-fluoride plasma and insulin in EDTA plasma. EDTA plasma insulin was analyzed with an Enzyme-Linked Immunosorbent Assay (Equine optimized Insulin, #10-1205-01, Mercodia AB).

10 The analysis of glucose and insulin response to the challenges showed no significant difference between the different compositions (Table 5).

Table 5: Glucose [mM] and Insulin (mU/L) before (basal), after 30 min, 60 min and 120 min of a glycemic challenge with either syrup A (administered directly into the mouth via syringe), pelleted "glycemic challenger" composition (pellets) offered in a trough for free intake, or glucose solution via nasogastric tubing administration (dextrose) in healthy and EMS horses; n=18, mean $\pm$ SD

Results of testing	Syrup A (synged)		Pellets (trough)		Dextrose (tubing)	
	Glucose mM	Insulin mU/L	Glucose mM	Insulin mU/L	Glucose mM	Insulin mU/L
before	4.7 $\pm$ 0.3	11.7 $\pm$ 11.6	4.8 $\pm$ 0.3	8.8 $\pm$ 4.6	4.6 $\pm$ 0.2	7.1 $\pm$ 4.1
30 min	5.9 $\pm$ 0.6	39.8 $\pm$ 37.8	5.9 $\pm$ 0.6	95.5 $\pm$ 69.5	5.9 $\pm$ 0.8	49.2 $\pm$ 41.2
60 min	6.7 $\pm$ 0.7	60.6 $\pm$ 69.7	6.7 $\pm$ 0.6	140.5 $\pm$ 135.7	7.1 $\pm$ 1.0	82.1 $\pm$ 70.5
120 min	7.1 $\pm$ 1.2	76.0 $\pm$ 104.2	7.7 $\pm$ 1.3	123.9 $\pm$ 120.2	7.7 $\pm$ 1.2	102.4 $\pm$ 119.9

15 In conclusion, the results show that the “glycemic challenger” compositions according to the present invention elicits glucose and insulin excursions, that are comparable to “gold standard” OGT results, but the “glycemic challenger” according to the present invention are easier to administer.

20 **Example 5: Pharmacodynamics of liquid and pelleted “glycemic challenger” compositions – accuracy of diagnosing insulin dysregulation in equines**

As described in Example 4, eighteen healthy or insulin dysregulated horses were employed in a cross over design receiving the following “glycemic challenger” composition after fasting overnight:

- 0.5 g glucose per kg body weight dissolved in 2 L water and administered via nasogastric tubing
 - 25 • syrup A (about 37% oligo-saccharide (consisting of glucose units), about 30% glucose and maltose, 2% salt, and about 30% water), dose equivalent to a total glucose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, administered directly into the mouth via syringe
 - pellets (about 35% oligo-saccharide (consisting of glucose units), about 32% glucose and maltose, about 35% flour of linseed, about 22% flour of maize germ, about 3% of linoil, and about 0.4% of Curcuma/Vanilla), dose equivalent to a total glucose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, offered as free feed in a trough
- 30

The diagnosis of insulin dysregulation can be done evaluating the area under the curve (AUC) of the excursion of insulin in response to the challenge. In this herd healthy and insulin dysregulated horses were present, Figure 1 clearly shows that there is a very good correlation between the dextrose (tubing) results and the response to the syrup A ($r^2 = 0.87$) as well as with the response to the pellets ($r^2 = 0.74$).

- 5 In conclusion, the pelleted as well as the liquid “glycemic challenger” compositions according to the present invention can be employed as a palatable, well-tolerated, accurate, oral glycemic challenge test enabling veterinarians to diagnose insulin dysregulation easier in an equine subject.

10 **Example 6: Pharmacodynamics of liquid and pelleted “glycemic challenger” compositions – accuracy of diagnosing insulin dysregulation in equines employing a single sample analysis**

As described in Example 4, healthy or insulin dysregulated horses were employed in a cross over design receiving the following “glycemic challenger” composition after fasting overnight:

- 0.5 g glucose per kg body weight dissolved in 2 L water and administered via nasogastric tubing
- syrup A (about 37% oligo-saccharide (consisting of glucose units), about 30% dextrose and maltose, 2% salt, and about 30% water), dose equivalent to a total dextrose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, administered directly into the mouth via syringe
- pellets (about 35% oligo-saccharide (consisting of glucose units), about 32% dextrose and maltose, about 35% flour of linseed, about 22% flour of maize germ, about 3% of linoil, and about 0.4% of Curcuma/Vanilla), dose equivalent to a total dextrose, maltose and oligo-saccharide (consisting of glucose units) of 0.5 g per kg body weight, offered as free feed in a trough

The diagnosis of insulin dysregulation can be done evaluating the insulin response at any single time point between 30 and 240 minutes. Figure 2 shows the results at 120 min of $n=4$ healthy and $n=4$ insulin dysregulated horses. Figure 2 shows that employing the gold standard - dextrose (tubing) - as well as the syrup A or the pelleted “glycemic challenger” according to the present invention the insulin response at 120 minutes after administering the challenge can distinguish clearly between healthy and insulin dysregulated horses.

In conclusion, the pelleted as well as the liquid “glycemic challenger” compositions according to the present invention can be employed as a palatable, well-tolerated, accurate, oral glycemic challenge test enabling veterinarians to diagnose insulin dysregulation in a single sample after administering the “glycemic challenger” compositions according to the present invention in an equine subject.

Example 7: Pharmacodynamics of liquid and pelleted “glycemic challenger” compositions in a resting feeding status

The liquid or pelleted “glycemic challenger” compositions according to the present invention can also be employed in horses or ponies which are in a resting feeding status, i.e. a challenge test for the diagnosis of insulin dysregulation as described in Example 3-6 can be performed in the morning prior to regular feeding in an

equine that did have only restricted access to small amounts of hay or other forages overnight. Also in this non-fasted feeding status the liquid or pelleted “glycemic challenger” compositions according to the present invention provides a palatable, well-tolerated, accurate, oral glycemic challenge test enabling veterinarians to diagnose insulin dysregulation in an equine subject.

5

Twelve Icelandic horses with variable metabolic status, sex, age and body condition score are either offered 0.2 kg/100 kg BW (fasted, “F”) or 1.0 kg/100 kg BW (no-fasted “NF”) hay overnight (~12 h) prior to a test employing an oral pelleted “glycemic challenger” composition according to the present invention an amount equivalent to 0.5 g/kg BW glucose or a standard OGT with 0.5g/kg BW glucose administered via nasogastric tubing. All horses are tested in all 3 procedures in a randomized cross-over design during the winter hay feeding period. Blood samples are collected prior and until 4 h after the challenge and analyzed for insulin and glucose, as described in Example 4.

10

The different fasting / no-fasting procedures does not affect the basal blood glucose and insulin concentrations. And, fasting or no-fasting does not alter the glucose and insulin response to the different challenges. As in previous studies, the results of the pelleted “glycemic challenger” (F and NF) are comparable to the OGT. The AUC_{insulin} as well as the insulin concentrations at +120 min after the challenge are strongly correlated (Table 6).

15

Table 6: Pearson correlation analysis results of “AUC Insulin” or “+120 min Insulin”. F, fasted, NF, no-fasted. Healthy and EMS horses; n=12.

Pearson r	AUC Insulin				+ 120 min Insulin		
	Pellets -F	Pellets -NF	OGT		Pellets -F	Pellets -NF	OGT
Pellets -F	1				1		
Pellets -NF	0,85	1			0,96	1	
OGT	0,89	0,87	1		0,87	0,90	1

In conclusion, horses can be tested in various feeding status (fed, resting or fasted) without falsifying the clinical relevant results for insulin dysregulation with a “glycemic challenger” composition according to the present invention.

20

Example 8: Analysis of the composition of saccharides in the oral glycemic challenge compositions (liquid or pelleted)

The liquid or pelleted “glycemic challenger” compositions are analyzed according to standard feed stuff analysis methods. The pelleted “glycemic challenger” compositions according to the present invention contain 67% of carbohydrates in two different analysis performed in the same production batch. The composition of the carbohydrates is given in the following Table 7, clearly showing that the oligosaccharides in the liquid or pelleted “glycemic challenger” compositions comprise glucose and oligo-saccharides consisting of glucose units.

25

Table 7: Composition of the carbohydrates in the liquid or pelleted “glycemic challenger” composition

30

	Syrup	Pellets
	[%]	[%]
glucose	22	14

galactose	0	< 1
fructose	0	< 1
maltose	8	13
lactose	0	< 1
sucrose	0	< 1
maltodextrin	37	30
starch	0	10
sum		
carbohydrates	67%	67%

In addition, High Performance Anion Exchange Chromatography (HPAEC)-coupled to Pulsed Amperometric Detection (PAD) is employed to determine the “malto-oligosaccharides fingerprint” and elucidate the relative amounts of different degrees of polymerization (DP) of glucose units – see Table 8:

5 **Table 8:** “Malto-oligosaccharides fingerprint” (HPAEC-PAD) analysis of the maltodextrin component of the liquid or pelleted “glycemic challenger” composition (DP18 being the limit of detection)

	Syrup	Pellets
	[of 100 %]	[of 100 %]
Maltose DP2	17%	17%
DP3	23%	23%
DP4	11%	11%
DP5	11%	11%
DP6	17%	17%
DP7	12%	12%
DP8	5%	5%
DP9	2%	2%
DP10	1%	1%
DP11	<1%	<1%
DP12	<1%	<1%
DP13	<1%	<1%
DP14	<1%	<1%
DP15	<1%	<1%
DP16	<1%	<1%
DP17	<1%	<1%
DP18	<1%	<1%

The following clauses are also comprised by the scope and spirit of the present invention:

1. A composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides
10 for use in a method of diagnosing insulin dysregulation in equine animals.
2. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to clause 1, wherein such method of diagnosing insulin dysregulation in equine animals comprises the following steps:
 - 15 (a) determining the insulin concentration in at least one biological ex-vivo sample taken from an equine animal; and
 - (b) comparing the insulin concentration determined according to step (a) with a reference insulin concentration of a reference population of equine animals;

wherein a difference in the insulin concentration determined according to step (a) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

3. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to clause 2, wherein prior to step (a) such equine animal has orally been administered a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides, more preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration.

4. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to clause 3, wherein such composition has been administered over a period of time of less than 10 minutes, preferably 5 minutes.

5. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 3 to 4, wherein prior to administering such composition to the equine animal, such equine animal has fastened overnight, preferably for 8 to 14 hours, or has been in a resting prandial status.

6. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to clause 5, wherein the at least one biological ex-vivo sample are two biological ex-vivo samples, and wherein the first of the two biological ex-vivo samples is taken from the equine animal after fastening or after having been in a resting prandial status, but prior to orally administering the composition; and wherein the second of the two biological ex-vivo samples is taken from the equine animal after consumption of the composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of such composition by such equine animal.

7. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 2 to 6, wherein such at least one biological ex-vivo sample taken from the equine animal is selected from the group consisting of: blood sample, blood plasma sample; preferably is a blood sample.

8. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 2 to 7, wherein the insulin concentration is selected from the group consisting of: blood insulin concentration, blood plasma insulin concentration; preferably is blood insulin concentration.

9. A composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for use in a method of diagnosing insulin dysregulation in equine animals comprising the steps:
- (a) optionally, taking a first biological ex-vivo sample, preferably a blood sample, from an equine animal; which has preferably fastened overnight, more preferably for 8 to 14 hours, or which preferably has been in a resting prandial status;

- (b) orally administering to such equine animal the composition comprising saccharides, wherein preferably such composition is administered to the equine animal over a period of time of less than 10 minutes, more preferably 5 minutes; preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration;
 - (c) taking a second biological ex-vivo sample, preferably a blood sample, from such equine animal after having administered such composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of the composition by such equine animal;
 - (d) determining the insulin concentration, preferably the blood and/or plasma insulin concentration, in the first and second biological ex-vivo sample, preferably blood sample;
 - (e) comparing the insulin concentration in such first and second biological ex-vivo samples with a reference insulin concentration of a reference population of equine animals;
- wherein a difference in the insulin concentration obtained according to step (e) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

10. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 2 to 9, wherein in addition and parallel to the insulin concentration the following one or more additional biomarkers are measured: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP), wherein the one or more additional biomarker concentration is determined and compared with the respective reference biomarker concentration of said reference population of equine animals, wherein preferably blood sampling for measuring such one or more additional biomarkers is performed on one or more time points selected from: prior to the administration of the composition and/or in the stimulated status, e.g. 30, 60, 120, 180 and/or 240 minutes after the administration of the composition.

11. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 1 to 10, wherein such composition is a liquid composition, preferably a "ready-to-use" liquid composition or a liquid composition that was generated by diluting syrup with water, most preferably a syrup or wherein such composition is a solid composition, preferably a solid composition consisting of pellets, more preferably a solid "ready-to-use" composition consisting of pellets and/or wherein such composition is offered to the equine animals for free intake or is given directly into the oral cavity with a syringe (as for the liquid composition).

12. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 1 to 11, wherein such saccharides comprise, preferably consist of: glucose (mono-saccharide) and/or maltose (di-saccharide, consisting of glucose units) and/or water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$; more preferably glucose (mono-saccharide) and maltose (di-saccharide, consisting of glucose units) and water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$.

13. The composition for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 1 to 12, wherein such method is characterized by one or more of the following technical features:

- (1) defined and reproducible compositions of the oral glycemic challenge test – designated for use as a diagnostic tool in equines;
- (2) high acceptance/palatability of the oral diagnostic glycemic challenge compositions by the equines when offered for free intake (solid, pelleted or syrup diluted in water) – making an intake of the glycemic challenge composition within 5-10 minutes feasible;
- (3) non-invasive procedures of administering the oral glycemic challenge compositions – being e.g. well accepted by the equines when given directly into the oral cavity with a syringe as syrup;
- (4) diagnosis can be performed evaluating one or more biomarker in a basal sample and stimulated sample (after the challenge) or evaluating the area under the curve, i.e. the excursion of one or more biomarker in response to the glycemic challenge;
- (5) diagnosis can be performed in a single sample – such ease of use enables a more flexible test procedure, a simple “one sampling” test, i.e. the horse owner can easily administer the oral glycemic challenge composition and the veterinarian can take a blood sample for analysis of one or more biomarker;
- (6) single or stimulated sample or two sample analysis – the test allows a distinct separation between healthy and insulin dysregulated equines based on the blood concentrations of the one or more biomarkers, thereby enabling veterinarians to easier diagnose insulin dysregulation in equines.

14. A composition, preferably a solid or liquid oral glycemic challenge composition, comprising, preferably consisting of:

- (a) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:
 - (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;
 - (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
 - (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

- (b) water and/or fruit juice, preferably in the form of a syrup, comprising:

- (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;
- (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (b) is further diluted with water, e.g. 1:1;

or

- (c) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:

- (i) from 5% to 15% by weight of water soluble oligo-saccharides; more preferably, 10% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;
- (ii) from 20% to 30% by weight of water soluble oligo-saccharides; more preferably, 25% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% by weight mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

- (d) water and/or fruit juice, preferably in the form of a syrup, comprising:

- (i) from 0% to 5% by weight of water soluble oligo-saccharides; more preferably, 1% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;
- (ii) from 30% to 40% by weight of water soluble oligo-saccharides; more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% of mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;

- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of:
apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (d) is further diluted with water, e.g. 1:1.

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15. The composition according to clause 14 for use in a method of diagnosing insulin dysregulation in equine animals according to any of clauses 1 to 13.

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16. A diagnostic kit comprising the composition according to clause 14 and instructions for performing the method of diagnosing insulin dysregulation in equine animals according to any of clauses 1 to 13, and, optionally, an applicator for administering the composition to the equine animals, preferably a syringe.

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17. Use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals.

18. Use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals.

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19. The use according to any one of clauses 17 to 18, wherein such diagnosing insulin dysregulation in equine animals or method of diagnosing insulin dysregulation in equine animals comprises the following steps:

(a) determining the insulin concentration in at least one biological ex-vivo sample taken from an equine animal; and

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(b) comparing the insulin concentration determined according to step (a) with a reference insulin concentration of a reference population of equine animals;

wherein a difference in the insulin concentration determined according to step (a) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

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20. The use according to clause 19, wherein prior to step (a) such equine animal has orally been administered a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides, more preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration.

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21. The use according to clause 20, wherein such composition has been administered over a period of time of less than 10 minutes, preferably 5 minutes.

22. The use according to any of clauses 20 to 21, wherein prior to administering such composition to the equine animal, such equine animal has fastened overnight, preferably for 8 to 14 hours, or has been in a resting prandial status.

23. The use according to clause 22, wherein the at least one biological ex-vivo sample are two biological ex-vivo samples, and wherein the first of the two biological ex-vivo samples is taken from the equine animal after fastening or after having been in a resting prandial status, but prior to orally administering the composition; and wherein the second of the two biological ex-vivo samples is taken from the equine animal after consumption of the composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of such composition by such equine animal.

24. The use according to any of clauses 19 to 23, wherein such at least one biological ex-vivo sample taken from the equine animal is selected from the group consisting of: blood sample, blood plasma sample; preferably is a blood sample.

25. The use according to any of clauses 19 to 24, wherein the insulin concentration is selected from the group consisting of: blood insulin concentration, blood plasma insulin concentration; preferably is blood insulin concentration.

26. Use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides for diagnosing insulin dysregulation in equine animals comprising the steps:

- (a) optionally, taking a first biological ex-vivo sample, preferably a blood sample, from an equine animal; which has preferably fastened overnight, more preferably for 8 to 14 hours, or which preferably has been in a resting prandial status;
- (b) orally administering to such equine animal the composition comprising saccharides, wherein preferably such composition is administered to the equine animal over a period of time of less than 10 minutes, more preferably 5 minutes; preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration;
- (c) taking a second biological ex-vivo sample, preferably a blood sample, from such equine animal after having administered such composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of the composition by such equine animal;
- (d) determining the insulin concentration, preferably the blood and/or plasma insulin concentration, in the first and second biological ex-vivo sample, preferably blood sample;
- (e) comparing the insulin concentration in such first and second biological ex-vivo samples with a reference insulin concentration of a reference population of equine animals;

wherein a difference in the insulin concentration obtained according to step (e) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

27. Use of a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides in a method of diagnosing insulin dysregulation in equine animals comprising the steps:
- (a) optionally, taking a first biological ex-vivo sample, preferably a blood sample, from an equine animal; which has preferably fastened overnight, more preferably for 8 to 14 hours, or which preferably has been in a resting prandial status;
- (b) orally administering to such equine animal the composition comprising saccharides, wherein preferably such composition is administered to the equine animal over a period of time of less than 10 minutes, more preferably 5 minutes; preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration;
- (c) taking a second biological ex-vivo sample, preferably a blood sample, from such equine animal after having administered such composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of the composition by such equine animal;
- (d) determining the insulin concentration, preferably the blood and/or plasma insulin concentration, in the first and second biological ex-vivo sample, preferably blood sample;
- (e) comparing the insulin concentration in such first and second biological ex-vivo samples with a reference insulin concentration of a reference population of equine animals;
- wherein a difference in the insulin concentration obtained according to step (e) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.
28. The use according to any of clauses 19 to 27, wherein in addition and parallel to the insulin concentration the following one or more additional biomarkers are measured: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP), wherein the one or more additional biomarker concentration is determined and compared with the respective reference biomarker concentration of said reference population of equine animals, wherein preferably blood sampling for measuring such one or more additional biomarkers is performed on one or more time points selected from: prior to the administration of the composition and/or in the stimulated status, e.g. 30, 60, 120, 180 and/or 240 minutes after the administration of the composition.
29. The use according to any of clauses 17 to 28, wherein such composition is a liquid composition, preferably a “ready-to-use” liquid composition or a liquid composition that was generated by diluting syrup with water, most preferably a syrup or wherein such composition is a solid composition, preferably a solid composition consisting of pellets, more preferably a solid “ready-to-use” composition consisting of pellets and/or wherein such composition is offered to the equine animals for free intake or is given directly into the oral cavity with a syringe (as for the liquid composition).
30. The use according to any of clauses 17 to 29, wherein such saccharides comprise, preferably consist of: glucose (mono-saccharide) and/or maltose (di-saccharide, consisting of glucose units) and/or water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably

with a chain length of $3 \leq n \leq 10$; more preferably glucose (mono-saccharide) and maltose (di-saccharide, consisting of glucose units) and water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$.

5 31. A composition, preferably a solid or liquid oral glycemic challenge composition, comprising, preferably consisting of:

(a) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:

10 (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;

(ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the
15 mono- and di-saccharides being glucose and maltose;

(iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

(b) water and/or fruit juice, preferably in the form of a syrup, comprising:

20 (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;

(ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the
25 mono- and di-saccharides being glucose and maltose;

(iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (b) is further diluted with water, e.g. 1:1;

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(c) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:

35 (i) from 5% to 15% by weight of water soluble oligo-saccharides; more preferably, 10% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;

- (ii) from 20% to 30% by weight of water soluble oligo-saccharides; more preferably, 25% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% by weight mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

- (d) water and/or fruit juice, preferably in the form of a syrup, comprising:

- (i) from 0% to 5% by weight of water soluble oligo-saccharides; more preferably, 1% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;
- (ii) from 30% to 40% by weight of water soluble oligo-saccharides; more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% of mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (d) is further diluted with water, e.g. 1:1.

32. A diagnostic kit comprising the composition according to clause 31 and instructions for performing diagnosing insulin dysregulation in equine animals or the method of diagnosing insulin dysregulation in equine animals according to any of clauses 17 to 30, and, optionally, an applicator for administering the composition to the equine animals, preferably a syringe.

REFERENCES

- (1) Treiber KH et al., J Am Vet Med Assoc 2006, 228: 1538-1545
- (2) Asplin KE et al., Vet J 2007, 174: 530-535
- (3) de Laat MA et al., Equine Vet J 2010, 42: 129-135
- (4) de Laat MA et al., Equine Vet J 2016, 49(2): 238-243
- (5) Frank N et al. J Vet Internal Med 2010, 24: 467-475
- (6) Durham AE et al, 2018, ems-consensus-statement-
<https://www.eceim.info/sites/default/files/documents/2018-ems-consensus-statement-draft.pdf>
- (7) Durham AE et al., J Vet Internal Med 2019, 33(2): 335-349

- (8) https://sites.tufts.edu/equineendogroup/files/2018/09/2018-Final-EMS_Recommendations_Web.pdf
- (9) Bertin and De Laat *Equine Vet J.* 2017;49(5):570-576
- (10) Warnken T et al., *Acta Veterinaria Scandinavica* 2018, 60: 4; <https://doi.org/10.1186/s13028-018-0358-8>
- (11) Warnken T et al. *ACVIM2018 JVIM* DOI: 10.1002/jvim. 15314
- 5 (12) Meier et al. *Domestic Animal Endocrinology* 2018, 63: 1–9
- (13) Schuver A et al., *Journal of Equine Veterinary Science* 2014, 34: 465-470
- (14) Jocelyn NA et al., *Equine Vet J.* 2018, 50: 836-841
- (15) Anresco analysis <http://bmedia.fooducate.com/wp-content/uploads/2012/09/Anresco-820120322-Report.pdf>
- 10 (16) Lindåse S et al., *Acta Vet Scand* 2016, 58(Suppl 1): 55-63
- (17) Smith S et al., *Equine Vet J* 2016, 48(2): 224-227
- (18) Rapson JL et al., *Equine Vet J.* 2018, 50(5): 690-696
- (19) Knowles EJ et al. *Equine Vet J* 2017, 49(4): 519-524
- (20) WO 99/51108
- 15 (21) WO 2005/017532
- (22) WO 2014/161836

CLAIMS

1. A method of diagnosing insulin dysregulation in equine animals comprising the steps:
 - (a) determining the insulin concentration in at least one biological ex-vivo sample taken from an equine animal; and
 - (b) comparing the insulin concentration determined according to step (a) with a reference insulin concentration of a reference population of equine animals;wherein a difference in the insulin concentration determined according to step (a) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.
2. The method according to claim 1, wherein prior to step (a) such equine animal has orally been administered a composition, preferably a solid or liquid oral glycemic challenge composition, comprising saccharides, more preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration.
3. The method according to claim 2, wherein such composition has been administered over a period of time of less than 10 minutes, preferably 5 minutes.
4. The method according to any one of claims 2 to 3, wherein prior to administering such composition to the equine animal, such equine animal has fastened overnight, preferably for 8 to 14 hours, or has been in a resting prandial status.
5. The method according to claim 4, wherein the at least one biological ex-vivo sample are two biological ex-vivo samples, and wherein the first of the two biological ex-vivo samples is taken from the equine animal after fastening or after having been in a resting prandial status, but prior to orally administering the composition; and wherein the second of the two biological ex-vivo samples is taken from the equine animal after consumption of the composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of such composition by such equine animal.
6. The method according to any one of claims 1 to 5, wherein such at least one biological ex-vivo sample taken from the equine animal is selected from the group consisting of: blood sample, blood plasma sample; preferably is a blood sample.
7. The method according to any one of claims 1 to 6, wherein the insulin concentration is selected from the group consisting of: blood insulin concentration, blood plasma insulin concentration; preferably is blood insulin concentration.
8. A method of diagnosing insulin dysregulation in equine animals comprising the steps:

- (a) optionally, taking a first biological ex-vivo sample, preferably a blood sample, from an equine animal; which has preferably fastened overnight, more preferably for 8 to 14 hours, or which preferably has been in a resting prandial status;
 - (b) orally administering to such equine animal a composition comprising saccharides, preferably a solid or liquid oral glycemic challenge composition, wherein preferably such composition is administered to the equine animal over a period of time of less than 10 minutes, more preferably 5 minutes; preferably in dosages equivalent to a total saccharides content of from 0.25 to 1.0 g/kg body weight per administration, even more preferably from 0.4 to 0.6 g/kg body weight per administration, most preferably 0.5 g/kg body weight per administration;
 - (c) taking a second biological ex-vivo sample, preferably a blood sample, from such equine animal after having administered such composition, preferably 0.25 hours to 4 hours, more preferably 0.5 hours to 3 hours after consumption of the composition by such equine animal;
 - (d) determining the insulin concentration, preferably the blood and/or plasma insulin concentration, in the first and second biological ex-vivo sample, preferably blood sample;
 - (e) comparing the insulin concentration in such first and second biological ex-vivo samples with a reference insulin concentration of a reference population of equine animals;
- wherein a difference in the insulin concentration obtained according to step (e) with the reference insulin concentration of said reference population of equine animals is indicative of insulin dysregulation.

9. The method according to any one of claims 1 to 8, wherein in addition and parallel to the insulin concentration the following one or more additional biomarkers are measured: glucose, insulin, glucagon, leptin or adiponectin, triglycerides or non-esterified fatty acids, Glucagon Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP), wherein the one or more additional biomarker concentration is determined and compared with the respective reference biomarker concentration of said reference population of equine animals, wherein preferably blood sampling for measuring such one or more additional biomarkers is performed on one or more time points selected from: prior to the administration of the composition and/or in the stimulated status, e.g. 30, 60, 120, 180 and/or 240 minutes after the administration of the composition.

10. The method according to any one of claims 1 to 9, wherein such composition is a liquid composition, preferably a "ready-to-use" liquid composition or a liquid composition that was generated by diluting syrup with water, most preferably a syrup or wherein such composition is a solid composition, preferably a solid composition consisting of pellets, more preferably a solid "ready-to-use" composition consisting of pellets and/or wherein such composition is offered to the equine animals for free intake or is given directly into the oral cavity with a syringe (as for the liquid composition).

11. The method according to any one of claims 1 to 10, wherein such saccharides comprise, preferably consist of: glucose (mono-saccharide) and/or maltose (di-saccharide, consisting of glucose units) and/or water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$; more preferably glucose (mono-saccharide) and maltose (di-

saccharide, consisting of glucose units) and water-soluble oligo-saccharides consisting of glucose units connected in chains of variable length, preferably with a chain length of $3 \leq n \leq 10$.

12. A composition, preferably a solid or liquid oral glycemic challenge composition, comprising, preferably consisting of:

- (a) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:
- (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;
- (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

- (b) water and/or fruit juice, preferably in the form of a syrup, comprising:
- (i) from 25% to 45% by weight of water soluble oligo-saccharides; more preferably, from 30% to 40% of water soluble oligo-saccharides; even more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units;
- (ii) from 20% to 40% by weight mono- and di-saccharides; more preferably, from 25% to 35% of mono- and di-saccharides; even more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iii) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (b) is further diluted with water, e.g. 1:1;

or

- (c) a mixture of various grounded/milled seeds and/or grains, preferably selected from the group consisting of: linseed, maize germ, corn, oats, barley, wheat, soybeans, more preferably being compressed to pellets, comprising:
- (i) from 5% to 15% by weight of water soluble oligo-saccharides; more preferably, 10% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;

- (ii) from 20% to 30% by weight of water soluble oligo-saccharides; more preferably, 25% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% by weight mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

or

(d) water and/or fruit juice, preferably in the form of a syrup, comprising:

- (i) from 0% to 5% by weight of water soluble oligo-saccharides; more preferably, 1% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal to 10;
- (ii) from 30% to 40% by weight of water soluble oligo-saccharides; more preferably, 35% of water soluble oligo-saccharides; the oligo-saccharides consisting of glucose units with a degree of polymerization larger or equal 3, but smaller than 10;
- (iii) from 25% to 35% of mono- and di-saccharides; more preferably, 30% of mono- and di-saccharides; the mono- and di-saccharides being glucose and maltose;
- (iv) from 0.05% to 3% by weight flavors, preferably selected from the group consisting of: apple, honey, banana, salt, curcuma, vanilla, beet pulp;

wherein preferably the resulting liquid oral glycemic challenge composition (d) is further diluted with water, e.g. 1:1.

13. The method according to any one of claims 2 to 11, wherein such composition is the composition, preferably the solid or liquid oral glycemic challenge composition, according to claim 12.

14. A diagnostic kit comprising the composition according to claim 12 and instructions for performing the method of diagnosing insulin dysregulation in equine animals according to any one of claims 1 to 11 and 13, and, optionally, an applicator for administering the composition to the equine animals, preferably a syringe.

Figure 1

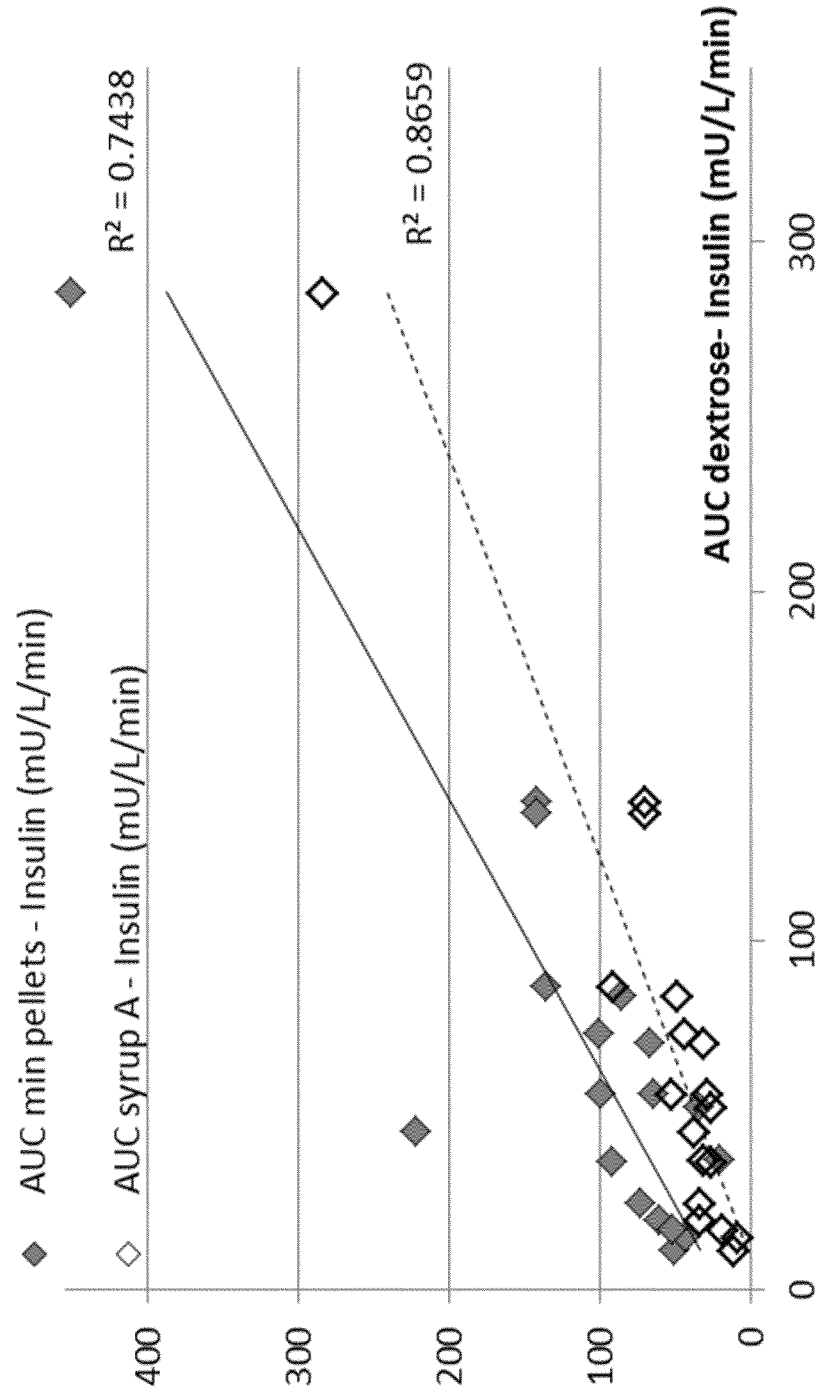
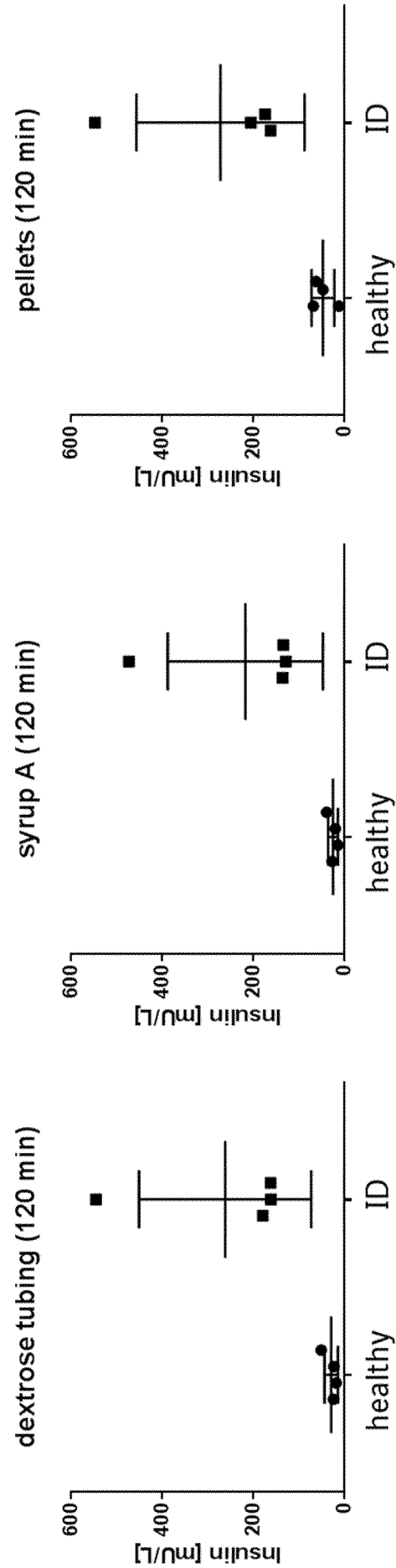


Figure 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/050968

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/74 A23K20/00 A23K50/20 A23L33/125
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)

G01N A23L C11C A23K

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, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N. A. JOCELYN ET AL: "Effect of varying the dose of corn syrup on the insulin and glucose response to the oral sugar test", EQUINE VETERINARY JOURNAL., vol. 50, no. 6, 22 March 2018 (2018-03-22), pages 836-841, XP055570470, GB	1-11
Y	ISSN: 0425-1644, DOI: 10.1111/evj.12826 abstract page 836 to 837, sections : study design, sample analysis, data analysis and results. Supplementary item 2 ----- -/--	12-14



Further documents are listed in the continuation of Box C.



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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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

3 March 2020

Date of mailing of the international search report

19/03/2020

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/050968

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANNA LINDÅSE ET AL: "A modified oral sugar test for evaluation of insulin and glucose dynamics in horses", ACTA VETERINARIA SCANDINAVICA, vol. 58, no. S1, 1 October 2016 (2016-10-01), XP055570559, DOI: 10.1186/s13028-016-0246-z	1-11
Y	abstract page 56, section "experimental design" to page 58, section "results".	12-14
X	SCHUVER AMY ET AL: "Assessment of Insulin and Glucose Dynamics by Using an Oral Sugar Test in Horses", JOURNAL OF EQUINE VETERINARY SCIENCE, vol. 34, no. 4, 1 November 2013 (2013-11-01), pages 465-470, XP028649930, ISSN: 0737-0806, DOI: 10.1016/J.JEVS.2013.09.006	1-11
Y	abstract page 466-467, "Materials and Methods section".	12-14
X	E. J. KNOWLES ET AL: "Use of the oral sugar test in ponies when performed with or without prior fasting", EQUINE VETERINARY JOURNAL., vol. 49, no. 4, 5 September 2016 (2016-09-05), pages 519-524, XP055570675, GB	1-11
Y	ISSN: 0425-1644, DOI: 10.1111/evj.12607 abstract pages 519-520, "Materials and Methods" section	12-14
X	WO 2014/161836 A1 (BOEHRINGER INGELHEIM VETMED [DE]) 9 October 2014 (2014-10-09) example 4	1-11
Y	WO 2005/017532 A1 (CEAPRO INC [CA]; REDMOND MARK J [CA]; SHAW DIANA F [CA]) 24 February 2005 (2005-02-24) page 21, last paragraph.	12-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2020/050968

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014161836 A1	09-10-2014	EP 2981269 A1	10-02-2016
		US 2014303096 A1	09-10-2014
		US 2017071969 A1	16-03-2017
		WO 2014161836 A1	09-10-2014

WO 2005017532 A1	24-02-2005	AT 509276 T	15-05-2011
		CN 1836167 A	20-09-2006
		EP 1660889 A1	31-05-2006
		ES 2365138 T3	23-09-2011
		RU 2348040 C2	27-02-2009
		US 2006188443 A1	24-08-2006
		WO 2005017532 A1	24-02-2005
