

-Bachelor Thesis-

**Investigating the Potential and Feasibility of
Conducting Scientific Research on the
Impact of Phytonics 'Gluco balance' on
Insulin Resistant Horses**



by

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Statutory Declaration

I declare that I have written and completed the enclosed thesis entirely by myself and only the defined sources and study aids were used. Any thoughts or quotations which were inferred from these sources are clearly marked as such. This thesis was not submitted in the same or in a substantially similar version, not even partially, to any other authority to achieve an academic grading and was not published elsewhere.

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ABBREVEATIONS

IR	Insulin Resistance
EMS	Equine Metabolic Syndrome
ECS	Equine Cushing Syndrome
BCS	Body Condition Score
NCS	Neck Crest Score
FFA	Free fatty acids
EHC	Euglycemic Hyperinsulinemic Clamp
FSIGT	Frequently Sampled Intravenous Glucose Tolerance
CIGT	Combined Insulin Glucose Tolerance
IVGTT	Intravenous Glucose Tolerance Test
IST	Insulin Sensitivity Test
scFOS	Short-chain Fructo-Oligosaccharides

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SUMMARY

Insulin resistance in horses is a rather new, but evolving to a serious concern in the equine industry. Obesity is a major related factor, although it is not clear yet whether it is causal or a consequence of insulin resistance; a combination of both is most likely. During the last years the relation between pathological hyperinsulinaemia and laminitis has been investigated with serious interest. Strong correlations with so called pasture associated laminitis could be established. Since the concern and awareness about this hormonal disorder is increasing, scientific research in this field is strongly needed. While complaints related to insulin resistance are diverse and more factors and potentially related symptoms are still unknown than known, proper diagnosis and effective treatment are limited.

The company Phytonics has developed a product called 'Gluco balance' to support horses with symptoms of insulin resistance. It is claimed that insulin cell receptor sensitivity improves and with that the insulin and glucose concentrations are balanced. Although being successfully in the market since more than four years, the effect of this product has not been investigated on a scientific level; this mainly because of the difficult diagnosis, reliable and practicable comparable measurements and the general challenge to set up a proper research method. Therefore the objective of this study was to investigate the potential and feasibility of conducting scientific research on the impact of 'Gluco balance' on insulin resistant horses.

Extensive literature review aimed to gain insight in the current state of knowledge with regards to the theory behind insulin resistance, the related consequences, different diagnostic tools and treatments. Besides, a quasi-experimental, placebo controlled, double blinded clinical field study has been conducted as a pilot study. In this study the practicability and feasibility of the developed method have been tested and indicative results on the effect of 'Gluco balance' on external indicators of horses being supposedly insulin resistant have been collected.

Information of 15 horses, belonging to a risk group of insulin resistance, has been collected in the field trial during a period of five weeks. The sample has been split randomly into either the experimental group ('Gluco balance') or the control group (placebo). Pre and post assessment of the horses' condition in terms of measuring the weight, heart girth, abdominal girth, neck circumference, neck thickness and the general body condition and neck crest score has been applied. Besides that, observations and impressions of the owners have been recorded and assessed whether at the end of the trial improvement could be seen or not. Most commonly observed indicators where local fat accumulations, especially at the neck and behind the shoulder, easily gaining weight and losing it hardly, a hard neck, sensitive skin, (fat) bulbs over the whole body, being easily irritated and eczema related problems during the previous year.

Horses from the experimental group showed significant changes in weight, heart girth and neck circumference. These results support the experiences made in the field that 'Gluco balance' indeed can have a positive impact on weight loss and with that on insulin sensitivity. Significant differences between the two groups in an improvement of the symptoms and health problems after the trial could be observed in less sensitivity of the skin, local fat accumulations, (fat) bulbs over the body, getting fat easily and hardly losing it, better getting into heat, less easily beginning to sweat a lot, improved shedding and less easily irritated behaviour. No significance, but strong indications on the improvement of stiffness and willingness/ability to work were observed.

Not all applied measurements turned out to be applicable on such short term trials; especially the general body and neck condition scores were not applicable, same as the measurements of the neck thickness. All turned out to be too inaccurate and failed to determine changes during this trial.

Generally the applied method proofed to be feasible for indicating the effect of 'Gluco balance' on horses showing symptoms of insulin resistance, belonging to a risk group. It however, does not provide the tools to specifically determine insulin resistance and an improvement of insulin sensitivity. To be able to effectively support insulin resistance in horses the trial thus suggests that 'Gluco balance' indeed has beneficial impacts, but more specific testing is required for more precise statements about the impact of 'Gluco balance' on (obese and/or) insulin resistant horses. It is advisable to continue more specific research in this field to be able to increase reliability of the preliminary results and to gain more acceptance in the (veterinarian) field.

This study thus enables the company to get insight in the current state of knowledge, in the possibility and feasibility of conducting continuative scientific research and it supports the claims and experience made that 'Gluco balance' indeed can have positive effects on horses showing potential indicators of insulin resistance. Being able to indicate such positive impact in combination with the generally limited possibilities of effective treatment, the company is with this product able to further strengthen its unique role in the management of insulin resistance in horses.

SAMENVATTING

Insulineresistentie bij paarden is een nog vrij onbekend, maar steeds vaker serieus genomen probleem in de paardenindustrie. Obesitas speelt hierbij een belangrijke rol, hoewel het nog niet duidelijk is of het een oorzaak of juist een consequentie van insuline resistentie is, maar waarschijnlijk is het een combinatie van beide. Tijdens de afgelopen jaren stond voor de wetenschap de verbinding tussen hyperinsulinemie en hoefbevangenheid centraal. Inmiddels is het duidelijk geworden dat gras gerelateerde hoefbevangenheid een nauw verband heeft met chronisch verhoogde insuline waarden staat.

De bewustwording van en zorgen over dit hormonale probleem worden steeds groter, en de wetenschap gaat steeds meer op zoek naar potentiële oorzaken, symptomen en behandelingen. Terwijl de aan insulineresistentie gerelateerde klachten divers zijn, en het merendeel van de factoren en potentiële symptomen onbekend zijn, is een gefundeerde diagnose meestal moeilijk en effectieve behandelingsmogelijkheden zijn beperkt.

Het bedrijf Phytonics heeft het natuurlijke gezondheidsproduct, "Gluco balance" ontwikkeld, wat paarden met symptomen van insulineresistentie ondersteunt. Voor dit product wordt geclaimd, dat het de gevoeligheid van de insuline cel receptoren verbetert, en hiermee de insuline en glucose concentraties beter gebalanceerd kunnen worden. Alhoewel het product al meer dan vier jaar met succes op de markt is, is het effect van dit product nog niet op een wetenschappelijk verantwoordelijke manier aangetoond. Dit heeft er vooral mee te maken, dat een goede diagnose en betrouwbare en accurate vergelijkende metingen moeilijk zijn, evenals de algemene uitdaging een gefundeerd methode te ontwikkelen. Daarom was het doel van dit onderzoek om naar het potentiaal en de haalbaarheid te kijken, om wetenschappelijk onderzoek naar het effect van 'Gluco balance' op insulineresistente paarden uit te kunnen oefenen.

Uitgebreid literatuur onderzoek geeft inzicht in de huidig beschikbare kennis met betrekking tot de theorie achter het probleem, gerelateerde consequenties, verschillende diagnostische mogelijkheden en behandelingen. Hiernaast werd een quasi-experimenteel, placebo gecontroleerd, dubbel blind veld onderzoek ontwikkeld en als een pilot studie uitgeoefend. In dit onderzoek is naar de uitvoerbaarheid en haalbaarheid van de ontwikkelde methode gekeken. Daarnaast zijn er ook indicatieve resultaten over het effect van 'Gluco balance' op paarden met uiterlijke kenmerken van insulineresistentie verzameld.

De informatie van 15 paarden, welke tot een risico groep voor insulineresistentie behoorden, werd tijdens een periode van vijf weken verzameld. De groep werd per toeval in òf de experimentele groep ('Gluco balance'), òf de controle groep (placebo) ingedeeld. Pre en post beoordeling van de gezondheidsconditie van de paarden werd toegepast door het meten van het gewicht, omvang achter de schoft, buikomvang, halsomvang, halsdikte en de algemene body condition en crest neck score. Hiernaast zijn de observaties en indrukken van de eigenaren opgenomen en is er beoordeeld of er een verbetering tijdens het onderzoek heeft plaatsgevonden. De meest voorkomende indicaties waren vetophopingen vooral bij de nek en achter de schouder, makkelijk dik worden en moeilijk afvallen, een harde nek, gevoelige huid, (vet) bulten over het hele lichaam, geïrriteerd gedrag en een verandering van eczeem gerelateerde klachten in vergelijking tot afgelopen jaar.

Paarden uit de experimentele groep lieten een significant verschil van het gewicht, omvang achter de schoft en halsomvang zien. In de experimentele groep werd een duidelijke verbetering van symptomen en gezondheidsklachten na het onderzoek vastgesteld. De resultaten zijn in overeenkomst met de ervaringen uit de praktijk, dat 'Gluco balance' positieve effecten op het gewichtsverlies kan hebben.

Significante verschillen met betrekking tot verbetering van de klachten werden gevonden bij huidgevoeligheid, lokale vetophopingen, (vet) bulten over het hele lichaam, makkelijk dik worden en moeilijk afvallen, problemen met hengstigheid, makkelijk en veel beginnen te zweten, verharen en geïrriteerd gedrag. Geen significantie, maar sterke aanwijzingen op een verbetering van stijfheid en willigheid om te werken waren aanwezig.

Niet alle toegepaste metingen bleken van toepassing voor kort durende onderzoeken te zijn. Vooral de algemene body condition scores waren niet betrouwbaar, evenals de metingen van de manenkam dikte. De metingen bleken te onnauwkeurig te zijn en zijn zodoende niet betrouwbaar genoeg geweest om veranderingen tijdens het onderzoek vast te stellen.

In het algemeen blijkt het met de toegepaste methode mogelijk te zijn om een positief effect van 'Gluco balance' op paarden met symptomen van insulineresistentie aan te kunnen tonen. Echter biedt de toegepaste methode geen mogelijkheden om specifiek insulineresistentie of een verbetering van de receptor gevoeligheid te kunnen bepalen. Het onderzoek suggereert dus dat om paarden effectief in de strijd tegen insulineresistentie te kunnen helpen, 'Gluco balance' inderdaad positieve uitwerkingen kan hebben, maar specifiekere methodes zijn nodig om nauwkeurigere uitspraken over de uitwerkingen van 'Gluco balance' op paarden met (een vermoeden op) insulineresistentie te kunnen maken. Het is derhalve raadzaam om meer specifiek onderzoek op dit gebied te doen om de betrouwbaarheid van dit onderzoek te ondersteunen en de aanvaarding uit de (veterinaire) paardenwereld te vergroten.

Dit onderzoek geeft het bedrijf dus inzicht in de huidige kennis, in de mogelijkheid en haalbaarheid om een vervolgonderzoek op te zetten, en het ondersteunt de claims en ervaring dat 'Gluco balance' inderdaad een positief effect op paarden die potentiële symptomen van insulineresistentie laten zien kan hebben. Door in staat te zijn om zulke positieve uitwerkingen aan te kunnen tonen, in combinatie met de beperkte mogelijkheden op effectieve behandeling, geeft dit onderzoek het bedrijf dus de kans om nog meer de unieke rol in het management van insuline resistente paarden in te nemen.

INTRODUCTION

Insulin resistance (IR) in horses is becoming a serious concern within the equine industry; this especially since for example in the Netherlands more than 50% of the horses are overweight (PKC, 2010) and adiposity is strongly associated with insulin resistance (Hoffman et al., 2003; Frank et al., 2006b; Vick et al., 2007). The clinical signs of insulin resistance can be diverse and individual and until now it appears that a proper diagnosis is either not highly accurate or impractical and effective treatment and support is limited (Firshman et al., 2007).

The Dutch company Phytonics has developed the natural health product "*Gluco balance*" supporting horses being insulin resistant (Phytonics, 2011). 'Gluco balance' is composed of herbal agents, minerals, vitamins and other essential nutrients (see appendix I for detailed description). 'Gluco balance' claims to support the management of IR in horses and along going health problems/symptoms such as obesity, laminitis, stiffness and hormonal and metabolic problems. It is claimed that cell sensitivity towards insulin improves and the blood glucose level and the glucose metabolism in general are balanced. Although having gained considerable publicity and success during recent years, the impact of 'Gluco balance' has not been scientifically investigated. Major reasons are the difficult diagnosis, the diverse clinical picture and the general challenge to set up a proper research method.

For this reason the objective of this study is to investigate the potential and feasibility of conducting scientific research on the impact of Phytonics 'Gluco balance' on insulin resistant horses. To be able to meet the objective, extensive literature review in the field of insulin resistance is conducted and a preliminary method is developed and executed in a test trial. The literature review and development of a preliminary research method and the execution of a test trail aim to give the company valuable insights in the feasibility and practicability of conducting an extensive continuative research and delivering indicative results on the impact of the product on horses showing symptoms of insulin resistance.

Since the related health problems to insulin resistance become more and more of a serious concern scientifically investigating the impact of products such as 'Gluco balance' on horses being (prone to be) insulin resistant could offer supportive treatment for affected horses on a larger scale.

The research questions addressed in this paper are:

1. What is the current state of knowledge with regards to insulin resistance in horses, its diagnosis and possible treatment?
2. How can an appropriate method be developed to be able to scientifically investigate the impact of 'Gluco balance' on insulin resistant horses?
 - 2.1 What methods and tools are applicable for reliable measurements?
 - 2.2 Which factors might influence the measurements and how can they be controlled?
3. How does the test trial contribute to the investigation of the potential and feasibility of conducting scientific research?
 - 3.1 Is the applied method and especially the assessment tool practicable and feasible?
 - 3.2 Can trends be found in relation to the impact of 'Gluco balance'?
4. How feasible is the execution of a continuative extensive scientific research to test the effect of 'Gluco balance'?

Report outline

Extensive background information including the theory behind insulin resistance, its diagnosis and possible treatments is presented in the very beginning of this paper. The following chapter includes a detailed description and explanation of the preliminary research method applied for the test trial. Subsequently the test trial results are presented; both with regards to the practicability of the developed method as well as on indicative results of the impact of 'Gluco balance'. A discussion about the gained results and the general method set up, its potential and feasibility follows. Conclusions on general potential and feasibility for continuative research are drawn. The knowledge and information gathered during these chapters are combined in the chapter of the recommendations. In this chapter recommendations are presented on how to and whether to continue with an extensive research and how the gathered information can be applied in the field in general.

1. INSULIN RESISTANCE IN HORSES

Insulin is a hormone produced and stored in the pancreas and is the key modulator for the glucose homeostasis, ensuring no elevated blood glucose concentration for elongated periods.

Insulin is secreted when the glucose concentration in the blood rises. It binds to insulin receptors especially on skeletal muscle, fat and liver cells. Through this binding the cells activate a cascade of events and eventually allow glucose to stream in where it is used for energy storage and utilisation, the cells are thus insulin sensitive. The uptake of glucose in the cells allows the glucose concentration in the blood to drop to its basal level again and with this the secretion of insulin decreases as well. Even though the blood glucose concentration is the major stimulus for the secretion of insulin other physical or sensory stimuli, such as stress and with this the production of cortisol, stimulate the secretion of insulin as well. Besides the regulative function of the blood glucose concentration insulin also plays a vital role in other metabolic responses, such as the fat, protein and mineral metabolism.

1.1 Insulin Resistance

Insulin resistance is generally defined as insulin not being able to induce an adequate response in the target tissue under normal concentrations or as diminished ability of cells responding appropriately to the insulin signalling (Kahn, 1978). Cells can become insulin insensitive/resistant on the cell surface, a diminished transport capability of glucose into the cell, and/or may become ineffective, a disrupted glucose metabolism inside the cell (Kronfeld et al., 2005b). Especially liver, skeletal and adipose tissues are prone to become insulin resistant. It has been investigated that within a given tissue different degrees of insulin resistance can exist (Cusi et al., 2000).

As shown in figure 1 does a glucose challenge result in a short term increase of the blood glucose concentration, which is regulated through increased secretion of insulin, binding to cell receptors, activating glucose transporters and enabling glucose to enter the cells. The insulin will adjust to the blood glucose supply, so that glucose homeostasis is ensured. With the down regulation of glucose also secretion of insulin diminishes and clearance is promoted. In insulin resistant horses the same peak of glucose will appear. However, more insulin is secreted to induce the same regulative effect. (Sillence et al., 2007)

In the beginning phase of IR the horse compensates for this developing resistance to avoid a long term increase of the blood glucose concentration through producing and secreting more insulin, leading to pathological hyperinsulinaemia. Compensated IR thus means that the insulin level is (chronically) highly elevated, but still sufficient glucose is transported into the cells keeping the blood glucose on a normal level, often towards the higher end of the reference range (reviewed by Frank, 2009; Frank, 2010).

However, in affected horses it will take more time to lower the glucose concentration. Both high insulin and elevated glucose concentrations will thus be present for an elongated period (Eiler et al., 2005). Another factor contributing to this situation can be a reduced clearance of insulin through a compromised liver function (Frank et al, 2009).

In such a situation a horse having received a glucose challenge and being affected with IR, might even reach a state of hypoglycaemia; this because even though glucose homeostasis would be achieved, insulin would still be too high allowing too much glucose entering the cells.

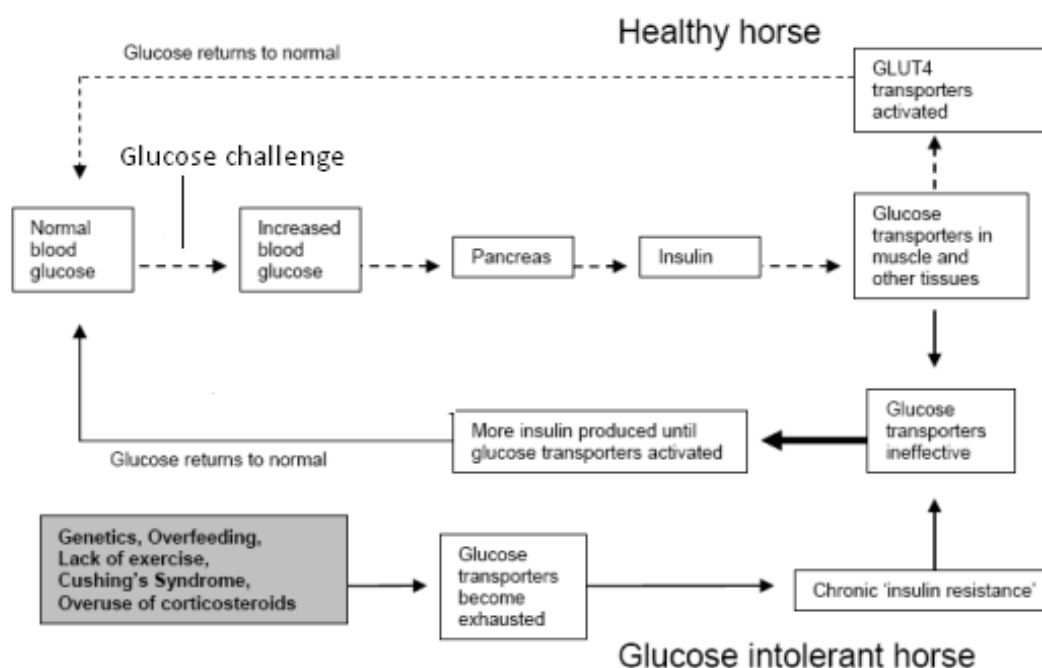


Figure 1: Course of events for healthy and glucose intolerant horses when receiving a glucose challenge. Dashed arrows represent the response of a healthy horse to an increase in blood glucose. Solid arrows represent the response of a horse or pony that is insulin resistant. (derived and adapted from Sillence et al., 2007)

On the long term however, insulin resistance may, if conditions stay unchanged, become uncompensated. This then implies that the cells become even more insensitive and insulin cannot compensate for this situation anymore and the pancreas loses its ability to produce sufficient insulin. The insulin mediated glucose cannot enter the cells and is not converted into energy anymore, leading to an elevated blood glucose and a variable insulin concentration (Frank, 2009). In this state the pancreatic function might decrease and less insulin is produced and secreted and the glucose level will increase further (Treiber et al., 2005b).

Continuous hyperglycaemia from reduced insulin secretion and/or insulin resistance is referred to as diabetes mellitus (Menziés-Gow, 2009). A considerable decrease of the pancreatic function and a highly elevated glucose level has been assumed to rarely occur in horses (Johnson et al., 2009), but diabetes type II might actually be more present in horses than previously thought (Menziés-Gow, 2009). In view of the fact that different cells might display different degrees of resistance, it is likely that while in some cells insulin still can compensate for this resistance and enabling the glucose to stream into the cells, where other cells might for the same have reached the state that insulin cannot compensate for the resistance and with that not being able to regulate the glucose metabolism anymore.

Since skeletal muscle cells make up about 80% of the stimulated glucose uptake, they are most prone of becoming insulin resistant and on the long term not being able to take up and convert glucose anymore (reviewed by Schmidt et al., 2009). An impaired uptake of glucose results in energy deficiency what than might lead to muscle tension, “unwillingness” to work lethargy and eventually to muscle mass waist. General disruption of cellular processes, intracellular disorders, and related health problems and diseases could be associated with altered glucose metabolism and thus IR (Kronfeld, 2005a). Further critical investigation is needed, but it seems to be complicated to discover the whole range of possible related effects in which IR may be involved in.

Until now the specific mechanisms of the development of insulin resistance are not fully investigated and understood. However, several factors are likely to be involved in the development of IR, such as a density reduction of insulin receptors or a malfunction of these receptors. Also internal signal pathways and a disturbed cell function in general can lead to a reduced binding of insulin and to a defective activation of glucose transporters. (Kitamura et al, 2004)

Molecular biology sciences often observe that a malfunction of receptors and especially the reduction of those receptors may build a protection of the body's system not to overload the cells with in this case glucose and run danger of cell poisoning and general damage on the short term. However, pathological hyperinsulinaemia will on the long term result in tissue and organ damage as well and might bring other indirectly related problems to the horse, often due to production of counter-regulatory hormones (reviewed by Wilcox, 2005).

When reaching the state that insulin cannot compensate for glucose intolerance anymore in the first instance both the glucose as well as the insulin level will rise leading to capillary damage as well as possible consequences for the cardiovascular system, the kidneys and damage on neurological level (reviewed by Valberg et al., 2009).

The actual onset of insulin resistance is not fully understood yet, but as Kronfeld reviewed in 2005 it is commonly agreed on that factors such as diet, activity, chronic stress, general body condition and sepsis, but also breed and genetic disposition can contribute to the development of insulin resistance. Especially modern feeding regimes, being rich in soluble carbohydrates, and too little movement/exercise often collide with the physiology of the horse, which is most likely one of the major reasons for the development of insulin resistance.

Obesity in general (Hoffman et al., 2003; Frank et al., 2006b; Vick et al., 2007; Carter et al. 2010) and local accumulation of adipose tissue especially in the neck, shoulder and tail regions (Johnson, 2002; Treiber et al., 2006) have been associated with insulin resistance. However, although obesity is a major indicator and factor for the horse becoming and being insulin resistant, also lean horses can become insulin resistant (Bailey et al., 2007). Obese horses do have considerably higher numbers of fat cells than non-obese horses. Higher numbers of adipocytes imply elevated free fatty acid (FFA) concentrations as well as elevated cytokine levels; therefore obesity nowadays is also classified as mild, but chronic inflammatory state (Das, 2001). It has been shown that high concentrations of FFA's and cytokines have a direct influence on insulin and suggestively could set of IR (Vick et al., 2007). Furthermore, Wilcox reviewed in 2005 that insulin promotes fat synthesis (lipogenesis) in adipose tissue. Besides this, it has been shown that adipocytes can become resistant to the anti-lipolytic effects of insulin and thus secreting high concentrations of FFA's into the bloodstream (DeFronzo, 2004). This thus means that in conditions of pathological hyperinsulinaemia fat synthesis is increased, FFA and cytokine concentrations become elevated and the horse will most likely gain even more weight, which both in turn increase IR and with that insulin secretion. A viscous circle has

developed. On top of that comes that affected fat cells have a diminished fat storage capacity which means that at a certain point fat will be stored visceral around the organs, exacerbating insulin resistance even more (DeFronzo, 2004).

A major focus of medical equine research currently is the relation of high insulin concentrations and pasture associated laminitis (Asplin et al., 2007; De Laat et al., 2010). In 2005 Treiber et al. described horses diagnosed being insulin resistant and with that being, under specific pasture conditions, at high risk of developing laminitis as having the pre-laminitic metabolic syndrome.

Hypertension, hypertriglyceridemia and hyperleptinemia (Bailey et al., 2008; Forhead et al., 1994; Frank et al., 2006b), but also more obvious signs such as chronic lethargy, muscle stiffness and cramping and altered reproductive cycling with a prolonged interovulatory period in mares (Vick et al. 2006) are all suggestively associated with and linked to IR. The Equine Metabolic Syndrome (EMS) (Johnson, 2002) as well as the Equine Cushing Syndrome (ECS) both are strongly related to insulin resistance.

The risk factors, short and long term consequences and symptoms of insulin resistance are thus highly diverse and very individual per affected horse, laminitis being one of the most concerning developing diseases. Since insulin is not only a fundamental hormone in the energy metabolism, but in other metabolic processes as well, pathological hyperinsulinaemia will have consequences on other processes such as the protein and mineral metabolism. On top of that are the exact mechanisms behind the onset of IR and its related symptoms still not fully understood yet.

1.2 Diagnosis

Insulin resistance in horses and the related health issues is a rather modern problem, but is becoming of increasing interest and concern for equine health (Geor, 2008). Since there is tremendous diversity of clinical signs and symptoms this health problem is until now often not very well recognized neither by veterinarians nor by horse owners and a sound and reliable diagnosis is still very complicated and complex

Clinical Signs

In advanced stages of this health problem often a visual diagnosis based on clinical signs may be sufficient. Below the most typically linked signs to insulin resistance are presented:

Body Condition Score

Since obesity is associated with insulin resistance, a general tool to assess the horses' condition is the body condition scoring (BCS) developed by Henneke et al. (1983). This scoring system can be a helpful tool to estimate the degree of general obesity, but it misses the assessment of local fat accumulation (Carter et al. 2009). It is advisable to divide the horse in several sections, assess these individually and calculating the mean of the different sections. With this division more focus can be put on local fat accumulations and more detailed body condition score can be established. Although this system is often regarded as an objective tool, in fact it is rather a subjective tool and the evaluation may strongly depend on the observers' skills and experience handling this tool. It has been suggested that when horses get scored with >6 (from a scale of 1 till 9) it can be indicated that the horse belongs to the risk group of being insulin resistant and an increased risk for laminitis (Treiber et al., 2005a).

Cresty Neck Score

Since local fat accumulations especially in the neck region are strongly associated with insulin resistance and the increased risk of laminitis (Johnson, 2002; Treiber et al., 2006, Carter et al., 2007) a more specific scoring system has currently been developed (Carter et al. 2009). This system is called cresty neck scoring (CNS). Scores have been determined from a range from 0 till 5, 0 showing no signs of fat accumulation on the neck and 5 showing obvious signs of severe fat accumulation on the neck. A score of >3 is within this scoring system described as “cresty neck” (Carter et al. 2009). In 2007 a CNS ≥ 3 has been associated with increased risk of laminitis and insulin resistance (Carter et al. 2007). The CNS may thus be a valuable tool to estimate local fat accumulation and could give a more substantiated estimation on local adiposity than the BCS does. However, also this scoring system is a subjective tool and the outcome strongly depends on the observers' skills and experience with this tool.

Laminitis

Hyperinsulinaemia and along going glucose intolerance have been suggested to be strongly related to pasture associated laminitis. It has been shown that high concentrations of insulin can directly cause laminitis in horses, although the hoof tissue itself is not depended on glucose uptake mediated through insulin. It is suggested that insulin toxicity is the major causative factor for this kind of laminitis. (Aspilin et al., 2007, De Laat et al., 2010)

In 2005 Treiber et al. suggested the labelling of the pre-laminitic metabolic syndrome for horses being at risk developing laminitis during specific pasture conditions. For clinical examination of horses being insulin resistant the diagnosis of pasture associated laminitis is thus a suggestive tool for horses having elevated insulin concentrations in the blood. Typical examination of laminitis will include walking on different grounds, sensitivity tests and examination of the general hoof condition (founder rings, convex sole, seedy toe, bruising).

Blood Pressure

Blood pressure measurements are not a commonly applied diagnostic tool in general in horses. However, since hypertension is associated with laminitis and IR (Bailey et al. 2008) it could be another helpful indicative measurement for the diagnosis of horses being insulin resistant or belonging to a risk group. Reference values are from considerable importance here, however until now only very few studies have dealt with assessing the blood pressure in horses.

Since measuring the blood pressure is a non specific tool, it could rather be supportive for clinical studies when wanting to observe and assess the effect of management adjustments such as change of diet, exercise and the application of a dietary supplement or drug.

Endocrinological Diagnosis

There are several ways to test a horse for insulin resistance, which however are until now either technically challenging and cost expensive, or not highly accurate and reliable (Firshman et al., 2007). The four most commonly applied tests are described in more detail in the following paragraphs.

For all tests applies that when testing for insulin resistance both the insulin and the glucose level need to be measured to derive accurate data (Firshman et al., 2007), measuring only one value may lead to inaccurate findings and misinterpretations.

The two most reliable, but most challenging tests are the euglycemic hyperinsulinemic clamp (EHC) test and the frequently sampled IV glucose tolerance test (FSIGT).

Euglycemic Hyperinsulinemic Clamp (EHC) test

The EHC is one of the two applied glucose clamp techniques and is accepted as the gold standard in human and equine science. The second technique is the hyperglycaemic clamp test and measures pancreatic insulin production. These glucose clamp techniques have first been developed by Andres et al. in 1966 and the EHC test has firstly been applied on horses by Elmahdi in 1998. The EHC's principle is to maintain euglycemic concentrations, to clamp glucose on a fixed level, while inducing a steady hyperinsulinemic state and observing the glucose rate required maintaining the euglycemic state. The average glucose disposal rate is calculated in the last 60 minutes of the test (DeFronzo et al., 1979). The EHC serves as a measure for insulin sensitivity of skeletal and adipose tissue. Horses being insulin resistant will thus require less glucose to maintain their euglycemic state and thus have a lower disposal rate, than horse being insulin sensitive.

For the EHC both insulin and glucose are injected intravenously and regular blood samples are drawn to administer insulin and glucose levels. This test requires special equipment, skills and staff and is therefore not commonly used in equine science yet (Kim et al., 2003). Furthermore, horses are required to retain in a clinic for at least two days. During the investigation the horses are attached to catheters for 120-180 minutes and they are fasted the night before and during the test, increasing chances of stress and with possible disturbances on the outcomes. The costs for this test are approximately 300-400€ (van der Kolk, 2011). These are all factors aggravating the application of these techniques. Since this test is not applied on a large scale, reference values are also not yet obtained in an extensive way (Kim et al., 2003; Kronfeld et al., 2005) indicating the need for further procedure standardisation to be able to apply this method more frequently in equine science (Kronfeld et al, 2005). Lacking reference values is detrimental for a proper diagnosis, but for clinical studies comparing the effect of a management adjustment or a drug the EHC, even with lacking reference values, is a very valuable tool. Until now it seems that the EHC is the most accurate and reproducible test with regards to testing for insulin sensitivity (Kim et al., 2003; Pratt et al., 2005; Firshman et al., 2007). A study of Powell et al. in 2002 reviewed that an increase of the glucose disposal rate after short term exercise could very well be investigated with the EHC, but the increase was not evident in plasma glucose and insulin measurements.

Although this test is the most reliable test for testing horses for insulin resistance it is rather difficult to be used for either private horse owners or for (large scale) clinical studies yet.

Frequently Sampled IV Glucose Tolerance Test (FSIGT) - Minimal model analysis

The FSIGT and the minimal model analysis were primarily developed by Bergman et al. 1979. The FSIGT is another endocrinological test applied to test for both insulin sensitivity and pancreatic β -cell response. Being able to test for both factors gives the opportunity to differentiate between compensated and uncompensated insulin resistance.

The data obtained from the FSIGT are inserted into a software program and values for insulin sensitivity and pancreatic function are calculated. However, these calculations are partly based on assumptions of insulin and glucose kinematics and might therefore result in estimation errors (reviewed by Pratt et al., 2005). The FSIGT and minimal model analysis have successfully been applied in horses in several studies, from which Treiber et al. (2005) tried to obtain reference values for this test, but also this test requires more practical application to obtain more reliable values of healthy and affected horses (Firshman et al., 2007).

The principle of the FSIGT is that the horse is attached to a catheter and glucose is injected intravenously. After twenty minutes a small dose of insulin is injected intravenously. The glucose and insulin levels are administered via blood samples for 180 minutes in regular frequencies. The data obtained is then inserted in a specific software, which is very complex and can only be handled and interpreted by experts, making the test complicated to execute (Treiber et al., 2005b; Kronfeld et al., 2005c).

The FSIGT is technically less challenging than the EHC and has the advantage of being able to differentiate between compensated and uncompensated IR (Kronfeld et al., 2005c). However, this approach requires specific software and is based on several assumptions. Both tests lack frequent application and thus comparable data and reliable reference values. It has been shown that the outcomes of the minimal model approach are less repeatable than the EHC which makes this approach even less applicable for clinical studies (Pratt et al., 2005). No specific cost calculations have been found, but since costs are often highly related to the blood samples and the effort required it can be assumed that the costs of this diagnostic approach will be close to the costs of the EHC. Factors which make this approach rather unpractical are similar to the ones of the EHC, namely required retention in the clinic for one to two days and frequent blood sampling and eventually related stress for the horse. An advantage of the FSIGT is that no fasting is needed the night before (Firshman et al., 2007).

The two previously mentioned tests are technically challenging and not applicable for the individual horse owner. For practical reasons two common tests are often advised to be used from veterinarians to indirectly diagnose insulin resistance, namely the iv combined glucose-insulin test (CGIT) or the measurement of resting serum insulin and glucose concentrations. (Frank, 2006a)

Intravenous Combined Glucose-Insulin Test (CGIT)

The CGIT is a combination of two traditionally applied tests, the intravenous glucose tolerance test (IV GTT) and the insulin sensitivity test (IST). The combination provides the possibility to simultaneously inject glucose and insulin at the very beginning of the test and thus testing for insulin sensitivity and glucose tolerance. The CGIT has been developed by Eiler et al. in 2005.

The principle of the CGIT is that both glucose and insulin are provided intravenously at the very beginning of the test. On beforehand basal glucose and insulin samples are taken. During a timeframe of 150 minutes blood is drawn in regular frequencies, with in total 14 blood samples. In healthy horses the blood glucose, measured with a glucometer, will be below the baseline value after 45 minutes. Horses with insulin levels of >100mU/mL at 45 minutes have higher concentrations than healthy horses, showing that insulin sensitivity is compromised and/or insulin clearance is slowed down. The longer the glucose concentration stays above the baseline value concentration the more compromised the cells are for insulin sensitivity and/or compromised clearance and thus the higher the degree of insulin resistance (Eiler et al., 2005; Frank, 2006b; Frank et al., 2010).

Compared to previously named tests, the CGIT is relatively easy to perform and shows high repeatability (Eiler et al., 2005), this since it is not technically challenging and in principle executable by every veterinarian. However, also this test requires retention in a clinic and the horse is fasted during the night before. The CGIT is same as all endocrinological tests susceptible to stress and pain. Besides, also here reference values for factors such as breed and age would be beneficial. Horses might be at risk of developing hypoglycaemia during the test. This needs careful supervision, but is a relatively small risk factor. (Eiler et al., 2005)

Resting Serum Insulin and Glucose Measurements

Taking samples of both resting serum insulin and glucose concentrations to detect the possibility of IR in horses has initially been applied in the field by Kronfeld et al. (2005a) and Treiber et al. (2006a). These measurements are commonly described as a screening test (Frank, 2006a). For this test blood will be drawn after the horse has fasted for about 12 hours. Sampling the resting serum insulin and glucose concentrations turns out to be a very practicable and most commonly applied test. This because it is easy to execute (can be done at home), it is with around 100-150€ relatively cheap, it is rather stress less, easy to understand and executable by each veterinarian.

However, it is mentioned that the accuracy of this test is rather questionable (Powell et al., 2002, Treiber, 2005, 2006; Pratt et al. 2009) and that such nonspecific indicators may be non conclusive (Kronfeld et al., 2005c); this mainly because the individual basal levels can vary widely within a short period of time, glucose up to about 15% and insulin even up to 70% (van der Kolk, 2011). Furthermore, glucose and insulin concentrations strongly depend on external factors as well, such as the time of the day, the stress level and the feeding time. In table 1 commonly applied reference values to determine IR in horses are presented.

Table 1: Reference values for insulin and glucose levels and the interpretation of the results. (GD Deventer, 2011)

Glucose level	Insulin level	Result interpretation
<5,5 mmol/L	< 10 mU/L	Insulin sensitive
	10 - 20 mU/L	Slightly elevated, possibly because of recent feeding. Repeat test.
>5,5 mmol/L	20 mU/L >	Compensated insulin resistance, adapting feeding regime. Repeat test after 4 weeks.
	< 10 of 10 - 20 mU/L	Investigating time of sampling in relation to feeding, possibly the sample has not been chilled properly
	20 mU/L >	Insulin resistance with diminished compensation

A resting insulin concentration of >20 mU/L is considered to be suggestive for IR (Frank, 2008c). However, when being in the reference values of below 20mU/L this screening test may not be appropriate for a profound diagnosis (Frank, 2006a). A horse might have IR in a mild stage and still might not show a significantly elevated resting insulin concentration, but only under a glucose challenge a disproportional increase of insulin could be observed. Besides, since the serum concentrations show a large variability it could mean that one measurement would identify the horses as being insulin resistant, whereas on another day under same circumstances the horse might fall in the reference values. A negative diagnosis with this test might thus give a wrong impression. However, if executed properly a positive test is strongly suggestive that the horse is being insulin resistant indeed. Another factor which might enhance this misinterpretation of reference values is that currently no differentiation between factors such as breed, age, nutrition and general body condition are made (Firshman et al., 2007). It has however been shown that ponies seem to be less insulin sensitive than horses and still for both the same reference values are used (Jeffcott et al., 1986, Kim et al, 2003).

Taking basal insulin and glucose measurements should thus be seen as a screening test, but not as proper diagnostic testing. However, even when using it as screening tests the testing procedure needs to be standardized to guarantee as little influence as possible. In practise however, it seems that application of this procedure is still rather diverse among veterinarians. Although certain factors can be standardized, the large variations and (unknown) factors having an influence on the outcomes make this test rather inappropriate for clinical studies, but still it is a commonly applied test. An important factor to consider is that already in 2002 it has been investigated by Powell et al. that an increase in insulin sensitivity could be detected with the EHC, but not with basal glucose and insulin measurements, which makes this method even more unattractive for clinical studies (Kronfeld et al., 2005).

It has been suggested that the mean value of three samples on three following days under the same circumstances might be comparable in its accurateness with that of the EHC (Pratt et al., 2009). However, the practicability and cost factor than become comparable with that of the EHC as well. Also so called proxies or ratios have been developed for the resting levels of glucose and insulin, although they are still applied, they might not represent high accuracy and an increasing number of veterinarians disclaims the application of such proxies/ratios (Frank et al., 2008c).

So far it seems that until now there is no ideal test existing meeting both accurateness and practicability, which challenges veterinarians and practitioners in their diagnosis for insulin resistance (Valberg et al., 2009). Especially for clinical studies it is a real challenge, since very accurate, but yet practical methods are necessary.

A perfect test would need to be easy to execute and performed with a single blood sample. Besides insulin and glucose concentrations, also adipocyte, lipid and cytokine levels and liver values should be added in the diagnosis as well (Frank et al., 2010).

1.3 Treatment

Once diagnosed as being insulin resistant it becomes necessary to apply an individually balanced treatment. For so far this mainly includes adjustments of feeding and exercising regimes. The current stage of medical treatment is that until now no registered medication exists and often medical treatment for humans is applied on horses (Durham et al., 2008; Frank et al., 2008b).

Insulin Resistance and Nutrition

Since IR is strongly associated with obesity, the major goal of treating obesity related insulin resistant horses is avoidance of gaining weight, promoting losing weight and the reduction of glycaemic and insulinaemic responses to meals. The avoidance strategy includes the reduction/avoidance of feedstuff with a high glycaemic index and thus a high percentage of non-structural carbohydrates, which also includes (young) grasses (reviewed by Geor, 2010). It has been investigated that horses fed with a diet high in soluble carbohydrates, (compared to horses fed a diet supplemented with fat), show a decrease in insulin sensitivity and horses chronically adapted to sweet feed have a decreased insulin sensitivity as well. (Hoffman et al., 2003; Treiber et al. 2005a; Pratt et al. 2006; Carter et al., 2010; Stewart-Hunt et al., 2010). In general obese horses do not require any concentrated feed and should be kept on a simple, low-carbohydrate based roughage diet and eventual vitamin/mineral supplementation and thus lower the risk of becoming or exacerbating insulin resistance (Hoffman et al., 2003, Pratt et al., 2006).

Losing weight is the other important factor and it has been shown that intensive weight loss of 1% body mass per week improves insulin sensitivity (van Weyenberg et al., 2008). However, in studies investigating weight loss and the relation to insulin sensitivity the horses/ponies are fasted rather extreme, from 1% dry matter intake of the body mass down to 35% of the recommended requirements of about 1.5-2% dry matter of the horses body weight (Van Weyenberg et al., 2008, Dugdale et al., 2010). Such extreme diets have shown to be beneficial for IR horses with regards to weight loss and insulin sensitivity, but other metabolic and behavioural problems might develop through this. Furthermore, horses being deprived of feed for long periods of the day might show changes of behaviour and stress, possibly even worsening insulin resistance. Geor (2010) therefore recommends a modest diet of losing 0.7-0.8% of the initial bodyweight per week over a period of three to four months.

To be able to promote weight loss, but still fulfil the horses' physiological needs several actions are recommendable:

- restrict pasture access through strip grazing or hourly grazing per day
- letting grasses flower first before the horse is allowed to graze
- use a grazing muzzle for the horse to restrict intake
- use slow feeders to slow down roughage intake
- soak hay for about 30-60 minutes in water to reduce soluble carbohydrate content
- provide roughage low in energy (NSC<10%)
- mix hay with straw to reduce and slow down intake
- provide twigs for time consumption and chewing

Insulin Resistance and Physical Activity

Besides feeding adjustments it is important to review the physical activity and whether it needs to be adjusted as well. Unfortunately until now results on the effect of physical activity on insulin sensitivity in horses are controversial (Geor, 2010). Studies in humans have shown that moderate training, for 150-200 minutes per week, significantly increases insulin sensitivity and decreases other risk factors related to IR. These studies state that through regular exercise the intracellular glucose pathways in the skeletal muscles are stimulated enabling better and more glucose uptake as well as the lipid metabolism is increased, having a direct influence on insulin resistance. (Goodyear et al., 1998, Havley, 2004; Bonen et al., 2006; Crandall et al., 2008)

In equine studies Powell et al. (2002) found that after already seven days of light training insulin sensitivity could be increased in obese horses. Steward-Hunt et al. (2010) found that during a seven week moderate training insulin sensitivity increased. However, De Graaf-Roelfsema et al. (2006) found no long term effect on insulin sensitivity after a long term trainings unit (18 weeks). In 2010 Carter et al. showed in their study that during eight weeks of moderate training no difference in insulin sensitivity between the trained and untrained horses could be found. These results thus show that it is not clear yet especially how intense a trainings regime needs to be in order to have an effect on insulin sensitivity. Differences in the research set up, use of tests, sample sizes and differently applied trainings and feeding regimes are most likely the factors for the controversial outcomes.

However, since clear positive effects have been found in humans the physiological principle stays the same. Same as for humans it is proposed that the combination of diet and activity will show the largest effects on weight loss and insulin sensitivity and more pronounced weight loss might show a more obvious effect on the training (Freestone et al., 1992; Geor, 2010; Frank et al., 2010; Carter et al., 2010). So far it is not clear what the perfect training regime would look like, especially since every horse is affected in another way and needs individual treatment. However, for now it is commonly recommended to start with 2-3 trainings sessions per week for 20-30 minutes per session. On the long term the duration and intensity should be increased. (Frank et al., 2010)

Insulin Resistance and Dietary Supplementation and Medication

Management adjustments with regards to nutrition and physical activity are certainly two important measurements to treat and control insulin resistance. However, dietary supplementation and medication might be beneficial and necessary as well. In the following paragraphs a brief overview of currently applied or potentially interesting substances is presented:

Metformin

Metformin is a medical drug originating from the French Lilac (*Galega officinalis*) and is used in humans for type-2 diabetes (Salpeter et al., 2006) to increase insulin sensitivity. The benefit for horses is still in discussion and contradictory results are obtained. Durnham et al. (2008) indicated in their study with 18 insulin resistant horses that insulin sensitivity increased and pancreatic β -cell secretion decreased significantly. However, Tinworth et al. (2011) did a similar study to test the effect of oral metformin application on the insulin and glucose response of six insulin resistant horses. In this study no effect on insulin sensitivity could be found. Recent studies also indicate that the bioavailability of metformin in horses is poor (Hustace et al., 2009; Tinworth et al., 2010a). It is suggested that metformin might only have an effect on obese insulin related horses for losing weight and for horses with hyperglycaemia (Tinworth et al, 2011).

Levothyroxine

Researches have shown that insulin sensitivity and disposal increase, blood lipid concentrations decrease and weight loss is promoted when healthy horses get supplied with levothyroxine (Frank et al., 2005; 2008a; 2008b). It has also been suggested that levothyroxine may have a protective effect on insulin sensitivity (Tóth et al., 2010). Although in the executed studies no adverse health effects could be detected, it is not yet scientifically proven what the long term, low dosage effects of levothyroxine on horses could be (Frank, 2009; Frank et al., 2010).

Chromium

Chromium is a widely applied element in humans with diabetes (Anderson, 1997) and is more commonly used in horses as well. It is assumed that chromium promotes increased insulin binding and receptor activation (reviewed by Firshman et al., 2007). However, it needs to be mentioned that the European food regulation prohibits the use of isolated chromium in food and with this also in dietary supplements for horses. Still a promising pilot study in Germany has come up with indicative results that chromium might indeed have a positive effect on insulin and glucose levels in horses (Vervuert et al., 2010). Nevertheless, this study has been criticized for its set up and repetitive studies would be needed to confirm the outcomes (Geor, 2010).

Psyllium

Psyllium, which is the seed husk of *Plantago ovata*, has been proven in human studies to have a positive effect on the glucose metabolism (Sierra et al., 2001; 2002) after meals. It is suggested that the psyllium fibres slow down the nutrient absorption and have a positive effect on the micro flora and might therefore have a beneficial effect on the glucose metabolism and insulin sensitivity (Sierra et al., 2001; 2002; Ellis et al., 2005; Robertson, 2007). Moreaux et al. (2011) were able to indicate with their study similar effects on healthy, non-obese horses when fed 90-270g of psyllium on a daily basis (for 60 days). The horses showed lower glucose and insulin concentrations after the meals and lower glucose peaks during the meals as well.

Psyllium thus could indeed be from beneficial potential for insulin resistant horses, but further research is required in this field with larger sample sizes and especially with affected horses (Moreaux et al., 2011)

Short-chain Fructo-Oligosaccharides (scFOS)

Short-chain fructo-oligosaccharides are so called prebiotics from which studies have shown that they beneficially change or activate a change of composition and activity in the intestinal micro flora of horses (Respondek et al., 2007, 2008a). Due to this modulating effect it has been proposed that scFOS might have a positive effect on insulin sensitivity (Robertson, 2007, Respondek, 2008b). In 2011 Respondek et al., investigated the effect of scFOS on eight obese horses. The study indicated that moderate increase of insulin sensitivity and a reduction of an acute insulin response to glucose as well as a lowering of plasma insulin concentrations could be observed through the supplementation of scFOS. However, mechanisms behind the improvement of insulin sensitivity have not been clear and more knowledge is needed to more specifically investigate the mechanisms (Respondek et al., 2011). More extensive research is necessary to further investigate and confirm the potential positive of scFOS on obese, insulin resistant horses.

Herbal Agents

In 2010 a number of herbs being from potential benefit for horses with insulin resistance have been identified and classified into six sub groups in relation to their effect (see table 2). These identified agents now need to be applied in scientific research to be able to measure the potential clinical effects and safety. (Tinworth et al., 2010)

Table 2: Summary of herbal agents as candidates for research to address insulin resistance in the horse (Tinworth et al., 2010)

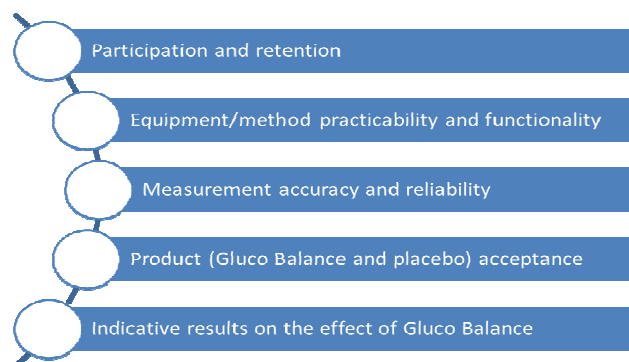
<i>Activators of the peroxisome proliferator-activated receptors</i>	-Panax (ginseng, japonicus, quinquefolius, eleutherococcus, Asian ginseng, Radix ginseng) -Linum usitatissimum (Flaxseed)
<i>Anti-obesity compounds</i>	-Glycine max (Soy Protein) -Citrus paradisi (Grapefruit) -Beta vulgaris (Beet or Beetroot) -Garcinia cambogia (Brindleberry, Brindall Berry or Malabar Tamarind)
<i>Anti-oxidants</i>	-Vitex agnus-castus (Chaste tree, Chasteberry, Monks Pepper) -Silibum marianum (Milk Thistle, St. Mary's Thistle) -Ipomoea batatas (Caiapo) -Curcumin
<i>Compounds that slow carbohydrate absorption</i>	-Trigonella foenum-graecum (Fenugreek) -Aloe vera (Aloes, Aloe vera Leaf Gel, Aloe Juice, Aloe Sap) -Amorphophallus konjac (Konjak, Konnyaku, Konjaku, Devil's Tongue, Voodoo Lily, Snake Palm or Elephant Yam) -Opuntia fuliginosa and streptacantha (Prickly Pear Cactus; Nopal)
<i>Insulin receptor activators</i>	-Cinnamomum cassia (Chinese Cinnamon or Cinnamomum aromaticum) -Grifola frondosa (Maitake, Sheep's Head, Ram's Head or Hen of the Woods)
<i>Stimulators of glucose uptake</i>	-Berberine -Mormordica charantia (Bitter Melon, Bitter Gourd) -Corosolic Acid (Glucosol, Banaba) -Pterocarpus marsupium (Indian Kino Tree)

Since the symptoms of horses being insulin resistant are very diverse and individual also the treatment needs to be very individual and what works out for one horse might not at all work out for another horse. Therefore whether the insulin resistant horse is treated with the help of management adjustments or certain drugs or supplements, or a combination of this, it is important to administer the horses' condition to be able to see whether the specific treatment actually has a positive effect on the individual horse nor not.

2. TEST TRIAL

2.1 Objective of the Test Trial

The main purpose of the test trial was to investigate the logistics, practicability and suitability of the applied method, as well as to collect and process indicative results on the impact of 'Gluco balance'. The test trial thus forms a supportive tool to investigate the potential and feasibility of conducting extensive scientific research in this field. Below a list of aspects the test trial provides information on is presented:



2.2 Materials and Methods

Desk Research

Desk research in terms of reviewing mainly primary and secondary literature has been conducted; this to be able to identify and analyse the current state of knowledge with regards to insulin resistance in horses, its diagnosis and possible treatments. Besides this, reviewing literature has been used to develop the general research method and especially the assessment tool.

Data has been collected from following scientific databases:

Scopus.com

Sciencedirect.com

Googlescholar.com

lvis.org

Pubmed.com

Field Research

Experimental Design

The test trial was designed as a quasi-experimental, placebo-controlled, double-blind clinical field study to investigate potential effects of 'Gluco balance' on horses belonging to a high risk group of being insulin resistant. This set up implies that not all variables such as the feeding and the general management have been standardized and were able to be influenced by the researcher. The study was controlled through an experimental and a control group, being supplied with 'Gluco balance' and a placebo product respectively. A clinical field trial implies that the study was done under natural conditions and the effect of a treatment, pre and post assessment, was investigated over a period of five weeks (35 days). The study was performed in the early spring (April to May) of 2011.

Subjects

16 horses participated on this trial, whereas only from 15 horses data was processed. Figure 2 shows the distribution of how many participants were found via which medium, internet forums being the biggest one.

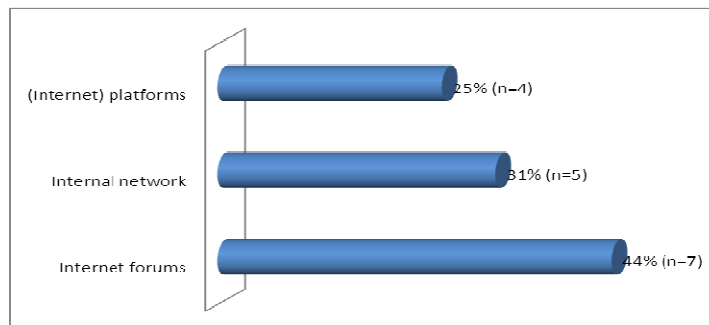


Figure 2: Medium distribution of the found participants (n=16)

-Internet forums: Bokt.nl, NHforum.nl, Ijslanderforum.nl, Paddockparadijs.nl

-Internal network: Friends, university blackboard

-(Internet) platforms: NML health newsletter, marktplaats.nl, de Hoefslag

60% (n=9) of the sample were mares and 40% (n=6) were geldings. As can be seen in figure 3 Icelandic horses were with 26.6% (n=4) the most common breed, followed by Shetland (mix) ponies and KWPN's (each 13.3%, n=2). 81% (n=12) of the total sample can be accounted to robust breeds.

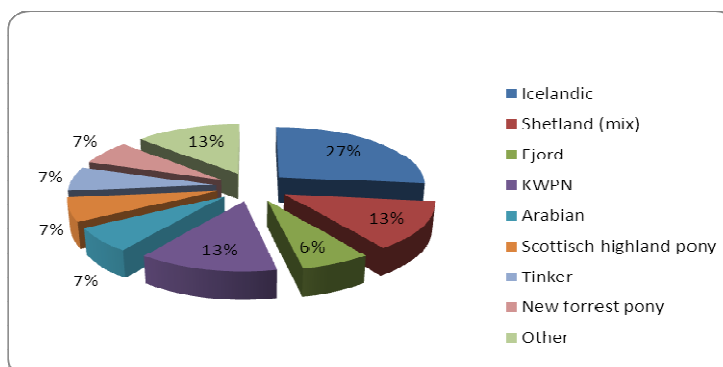


Figure 3: Breed distribution of the 15 participating horses

The average age of the 15 horses was 12.4 ±4.4 years. At the beginning of the trial the participating horses were housed on average 6.6 hours per day in the stable, 5.7 hours on the paddock and 11.7 hours on the pasture. Out of the 15 participants 68.8% (n=10) were on a diet and 31.3% (n=5) had access to roughage ad libitum.

The majority got either a mix of different roughages (53.3%) or hay/haylage (20%). However, since during the trial the weather circumstances were very dry even the horses having had access to pastures 24/7 could be regarded as being on a diet, especially when comparing the situation to previous seasons when horses normally would stand on full, flowering pastures. The average amount of roughage fed to the horses being on a diet was 5.8 ± 3.4kg, with an average body weight

of 476.6 ± 123.6 kg. However, since most horses were on pasture for some time the actual total amount including grass might differ. On average the horses were fed 356.7 ± 625.9 grams of concentrates, split in one or two meals. The most common supplied type of concentrates was muesli (46.7%). Additionally to the concentrates half of the participants (53.3%) fed supplements. 62.5% did this for appropriate mineral and vitamin supplementation. 60% (n=9) of the participants were familiar with 'Gluco balance' on beforehand, from which 40% (n=6) heard of it and 20% (n=3) had used it before.

The most common disciplines the participants were active in were recreational (46.7%) and dressage (33.3%) riding. The average training intensity per week was 4.1 ± 1.8 days, with an average training intensity of 52 ± 25 minutes per day.

80% (n=12) of the participating horses have been seriously sick during the past 5 years. 25% have had laminitis or founder and 25% suffering from (summer) eczema. 27% of the sample (n=4) were diagnosed as being insulin resistance on beforehand. 67% (n=10) of the participants belonged, solely based on a BCS of seven and higher and/or a NCS of three and higher, to an obvious risk group.

All above mentioned variables had a normal distribution and no significant differences between the experimental and control group were present.

The 16 horses were assigned into two groups; one experimental group and one control group. The horse owners received an explanatory paper about the research set up, the product and the requirements on beforehand (appendix II). During the selection phase no restrictions with regards to place, age, breed, gender and management were made. The only preconditions were that the horses either needed to be classified as being insulin resistant, since the diagnosis is rather challenging no specifications with regards to diagnosis and reference values were given, on beforehand or that they belonged to a risk group, namely general adiposity and/or along going health problems. Furthermore, the horses were not allowed to be on any other medication with regards to IR during the trial. Neither were they allowed to start another treatment during the test trial.

Product Description

The experimental group received the actual product (active treatment), 'Gluco balance' (appendix I), and the control group received a placebo product (no active treatment). Both the 'Gluco balance' and the placebo were applied as prescribed on the package; this being during the first week twice a day eight grams and during the other four weeks once a day eight grams.

As a placebo product dried and pre-chopped lucerne from the company *Subli* was used (see appendix III for a detailed description of the composition). The lucerne was grinded into a powder like substance. The reason why lucerne was chosen as a suitable placebo is that dried grasses in general do not have any "medicamentous" effect on the horse, especially not at a dose of maximum eight grams per day. Besides that, the colour and structure were comparable to the ones of 'Gluco balance'. The grinded lucerne was filled into the original package of 'Gluco balance'.

Measurements

To be able to administer and evaluate possible changes due to the impact of 'Gluco balance' an assessment tool (appendix IV) has been developed. This tool is primarily based on literature with regards to diagnosis and detection of clinical signs of insulin resistance, but also based on the experiences and observations with 'Gluco balance' made by veterinarians and practitioners so far.

General information of the horse, its management and its health condition were administered. One reason for administration was to be able to see whether significant differences in management between the two groups can be found. As major part of the tool the in total nine measurements were administered. The measurements exclusively focused on external indicators and did not include endocrinological, specific testing. This because it has been decided on beforehand that more extensive knowledge about the different tests and their current state is necessary to make a proper decision which test and whether at all to apply such test in later stages.

Since insulin resistance is highly associated with obesity and local fat accumulations the measurements had a strong focus on the assessment of the body condition. Also noticeable problems of the horses' condition in general were established with the help of the observations and interviews; this because signs of IR seem to be very diverse and individual and not all facets of the horses' condition and behaviour could be covered and established within one visit in a standardized tool. For the second visit the observations were inventoried and the owners were asked to give their impression whether at the end of the trial the particular problems staid the same or became better or worse. Below a detailed explanation measurements and observations is presented:

Weight

The weight (kg) was measured with the help of a weight measuring tape. The tape was placed behind the elbow and immediately behind the slope of the withers. The tape needed to be laid around the horse not too tight, but not too loose either. Since it was not from major importance to measure the real weight, but the actual difference before and after the trial it was more important to apply the same method during the two visits rather than identifying the real weight.

Heart Girth

The heart girth (cm) was measured at the same position with the same tape as done for the weight measurement.

Waist (abdominal circumference)

The abdominal circumference (cm) was measured with the same tape as used for measuring the weight. The abdominal circumference was measured at two-thirds of the distance between the point of the shoulder and the hip bone.

Body Condition Score

The body condition score system from 1-9 from Henneke et al., 1983 was applied be applied. For better, more detailed assessment and comparison the horse was divided into three sections, the front region (before the shoulder), the mid region (before the hip bones) and the hind region. Each subsection was assessed individually and a mean score was calculated for the overall body condition.

Neck Circumference

For the neck circumference (cm) the distance from the poll to the highest point of the withers was measured and perpendicular to this line at 0.25, 0.5 and 0.75 the neck circumference was measured and mean values were calculated (The University of Tennessee College of Veterinary Medicine, 2005). A normal measuring tape was used for this.

Neck Thickness

Neck thickness (cm) was measured at half of the distance between the poll and the highest point of the withers. The actual thickness was measured with a sliding gauge. The mane of the horses was taken to the side to guarantee the pure measurement of the neck thickness.

Neck Crest Score

To be able to identify fat accumulation on the neck the NCS (Carter et al. in 2009) was applied. This scoring system is similar to the BCS except that it score from 0 till 5, 5 showing obvious local fat accumulation.

Coat Condition

Standardized pictures before and after the trial were taken. The pictures were taken from both sides at a 90 degree angle at a distance of 1 or respectively 2 meters and a height of about 1-1.80 meters depending on the size of the horse/pony. For evaluation the pictures were placed next to each other and noticeable changes were marked and noted.

Hoof Condition

Standardized pictures before and after the trial were taken. The pictures were taken with the camera put on a level ground at a 90 degree angle to the hoof; one picture from the front view, one from the lateral view and one from the solar view. The distance to the hoof was 20cm. These three pictures were taken from the left front and left hind hoof. Most important was to at least make pictures of one front and one hind hoof.

Observations of the Owners and Researcher

In this section the researcher and owners were free to note whatever he/she thought was from importance and having observed before and after the trial.

All measurements were executed at day 0 and at day 35. During the visits each measurement was done twice and the mean value was taken as the final outcome; this was done to increase reliability and accurateness. The measurements were done by the same person to avoid inter observer bias. The experimenter got acquainted to the different measurement devices on beforehand to avoid measurement errors during the trial. The horses were handled by the owners in their familiar surrounding to avoid stress and tension, which might have influenced the measurements. For accurate measurements the horses needed to stand square and a level ground with the neck at a relaxed position of approximately 45 degrees. The coat and hooves were dry and briefly brushed to avoid misinterpretation of the information. If changes in the general management of the horses occurred during the trial they were noted in the same tool.

Statistical Analysis

The collected primary data has been processed with the help of the statistical software program SPSS; version PASW statistics 17.0. Both descriptive as well as inferential statistics have been applied. The confidence level was set for 95% and with this significance was accepted when $P < 0.05$, whereas trends were accepted when $P < 0.10$. All data is presented in mean values with ± 1 standard deviation. Normal distribution was tested with the Kolmogorov-Smirnov test. Difference testing between the experimental and control group before the trial was done with independent sample t-tests and chi square tests. Difference testing, pre and post assessment, within either of the two groups was done with paired t-tests. For difference testing for a change of variables, pre and post assessment, between both groups independent sample t-tests and Mann-Whitney tests were applied. The differences have been computed first. Due to the small sample size difference testing with scale variables has been double checked with the help of Mann-Whitney tests (for independent sample t-test) and Wilcoxon signed rank tests (for paired t-tests).

2.3 Results

The results of the test trial are divided into two parts. The first part describes the results with regards to logistics and practicability in a qualitative way. The second part describes the results with regards to the actual application of the product and the related factors to this.

Method Practicability and Logistics

Participation and Retention

In total 32 people were interested in participation, from which 13% (n=4) did already supply 'Gluco balance', 16% (n=5) where too far away and 22% (n=7) had other reasons for not participating, leaving in total 16 participating horses. Although data was collected from 16 horses, only from 15 horses the data were processed. For one horse the second measurements where highly inaccurate due to external factors. The horse belonged to the control group, so that total sample was distributed to eight and seven horses per group respectively. No participant ended the trial precociously.

Product and Placebo Response

Both the 'Gluco balance' and the placebo were accepted well by all horses and no horse refused to eat either of the products, which were normally mixed with some concentrates. From the participants having received the 'Gluco balance' 88% were aware that they received the real treatment. From the participants having received the placebo product 57% were unsure about whether they actually received the treatment or not.

Method Practicability and Usability

As can be seen in table 3 scores on a scale from 1 to 10 on six different items have been given to the individual measurements. The scores have been awarded based on the researchers experience during the trial and the outcomes of the measurements.

Usability and usefulness of the measurements of weight, heart girth, abdominal girth and neck circumference scored on average a 9. Accurateness and reliability scored on average a 7 and 7.5 respectively. Neck thickness scored on average a 5.8 with regards to reliability, accurateness, usability and usefulness. Both the BCS and the NCS scored an average of 5 in reliability and accurateness and a 7 on average for usability and usefulness. Hoof and body posture pictures were not able to be assessed in reliability and accurateness, but scored on average a 7.5 for usability and a 6 for usefulness on the short term. The owners observations scored on average a 7 in accurateness and reliability and an 8.5 for usability and usefulness.

The assessment form in general scored on average a 9 for usability and usefulness. The neck circumference, BCS and NCS, as well as the pictures taken have been classified as not being applicable for short term studies. All measurements have been classified as being applicable for long term studies.

Table 3: Assessment of the applied methods and their reliability, accurateness, usability, usefulness, short and long term benefit.

Measurements	Reliability	Accurateness	Usability	Usefulness*	Short term benefit	Long term benefit
Weight	8	7	9	10	Applicable	Applicable
Heart girth	8	7	9	8	Applicable	Applicable
Abdominal girth	7	6	9	8	Applicable	Applicable
BCS	5	5	8	7	Not applicable	Applicable
Neck circumference	7	8	9	10	Applicable	Applicable
Neck thickness	6	5	6	6	Not applicable	Applicable
CNS	5	5	7	6	Not applicable	Applicable
Body posture pictures	*	*	9	7	(Not) applicable	Applicable
Hoof pictures	*	*	6	5	(Not) applicable	Applicable
Observations	8	6	8	9	Applicable	Applicable
Assessment form	*	*	8	10	Applicable	Applicable

Legend: Scale from 1-10 (1=poor; 10= good); * usefulness on the short term

Impact of 'Gluco balance'

The potential impact of 'Gluco balance' on the participating horses has been tested with nonspecific methods with regards to insulin resistance, measuring external indicators. In table 4 an overview about the specific measurements is presented with their mean values before and after the trial. The differences of the individual measurements between and within the experimental and control group are presented below.

Table 4: Overview of the mean values of the specific measurements before and after the trial from the experimental (n=8) and control (n=7) group.

	Experimental/control	Pre-trial Mean	Post -trial Mean
Weight (kg)*	Experimental**	455,93	430,31
	Control	500,21	492,79
Heart girth (cm)*	Experimental**	177,56	172,81
	Control	181,45	180,36
Abdominal girth (cm)	Experimental**	200,01	194,78
	Control	203,39	203,82
Body condition score (front)	Experimental	7,04	6,88
	Control	7,36	7,21
Body condition score (mid)	Experimental	7,38	7,25
	Control	7,57	7,71
Body condition score (hind)	Experimental	7,06	7,06
	Control	7,36	7,21
Body condition score (mean)	Experimental	7,15	7,05
	Control	7,41	7,51
Neck circumference (cm)*	Experimental**	97,88	94,19
	Control	99,10	99,22
Neck thickness (cm)	Experimental**	6,81	6,49
	Control	6,61	6,54
Crest neck score	Experimental	2,13	1,94
	Control	2,50	2,50

Legend: * significance between the groups with 0.05%; **significance within the group with 0.05%

The distribution of having access to roughage ad libitum or restricted was not significantly different between the two groups ($p=0.0714$). Whether the participants had restricted or ad libitum access made no significant difference in the change of weight ($p=0.581$), heart girth ($p=0.629$), abdominal girth ($p=0.771$), BCS ($p=0.573$), neck circumference ($p=0.701$), neck thickness ($p=0.967$) and CNS ($p=0.698$).

Besides, no significant difference between the two groups before and after the trial, and no changes of housing, with regards to pasture access occurred during the trial (mean differences: 1.57 hours per day; $p=0.655$). Before the trial the experimental group had access to pasture for 13.4 hours per day and the control group for 9.9 hours per day ($p=0.557$). After the trial the groups had access to pasture for 16.4 and 11.3 hours per day respectively ($p=0.359$).

Measurements

Significant differences ($p=0.014$) in the change of body weight have been found between the experimental and control group with a mean difference of 18.12 kg. As can be seen in figure 4 the experimental group lost significantly more weight than the control group (25.62 ± 11.94 kg, $p=0.001$ and respectively 7.43 ± 13.05 kg, $p=0.18$). As a percentage of bodyweight the experimental group lost on average 5.62% and the control group on average 1.49% during the five week trial, resulting in an average weight loss per week of 1.12% and 0.30% respectively.

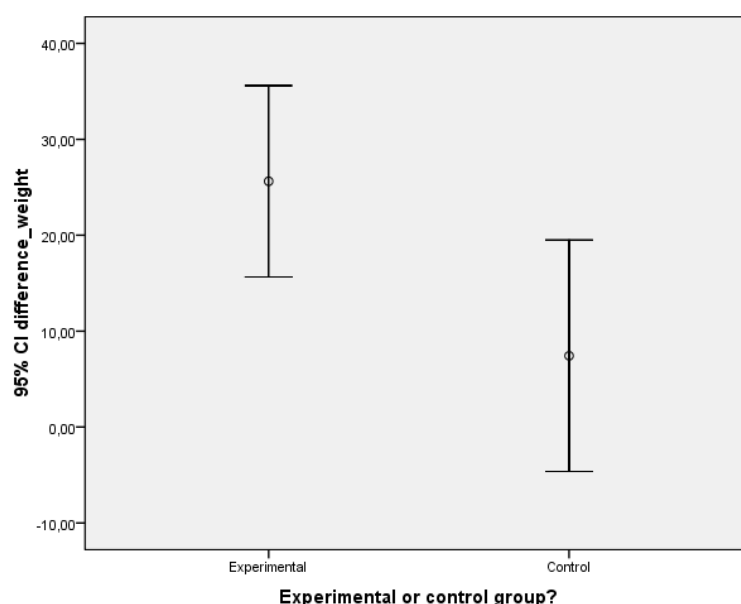


Figure 4: Differences in weight loss between the experimental and control group

Related to the change of weight also a significant decrease of heart girth between the two groups could be observed (mean difference: 3.7 cm, $p=0.030$). The experimental group showed a significant decrease of heart girth within the group (4.75 ± 3.54 cm, $p=0.007$). No significant decrease in the control group was observed (1.1 ± 1.89 cm, $p=0.176$). Besides the change of heart girth also a significant decrease of neck circumference between the two groups could be observed (mean difference: 3.81 cm, $p=0.030$). Within the experimental group a significant decrease of 3.69 ± 3.48 cm ($p=0.020$) occurred, whereas no significant decrease within the control group occurred (0.12 ± 2.40 cm, $p=0.898$).

No significant differences, but strong indications in the change of abdominal girth could be investigated between the two groups (mean difference: 5.67 cm, $p=0.056$). These strong indications are also in accordance with the significant findings within the experimental group of a decrease in abdominal girth (5.24 ± 5.45 cm, $p=0.030$) and no significant decrease within the control group (0.43 ± 4.95 cm, $p=0.826$).

No significant changes between the two groups in the change of neck thickness were evident (mean difference: 0.247 cm, $p=0.261$). However, a significant difference of thickness within the experimental group was observed (0.319 ± 0.274 cm, $p=0.013$) and none within the control group (0.071 ± 0.521 cm, $p=0.729$). No significant changes neither between nor within the experimental and control group in the body condition score ($p=0.270$, $p=0.366$ and $p=0.511$ respectively) and crest neck score ($p=0.462$, $p=0.285$, $p=1.000$) have been found.

Observations

Table 5 shows the most commonly observed symptoms and general health problems within the whole sample, being local fat accumulations, especially behind the shoulder, neck and abdominal region ($n=14$, 93.3%), easily gaining fat and losing it hardly ($n=14$, 93.3%), a hard neck ($n=13$, 86.7%), sensitive skin ($n=12$, 80%), (fat) bulbs over the whole body ($n=11$, 73.3%), being easily irritated ($n=11$, 73.3) and eczema related complaints during the previous year ($n=11$, 73.3%).

Table 5: Frequencies of the most commonly (above 70%, $n>10$) observed symptoms/ indicators of the whole sample ($n=15$) horses and the percentage of improvement in both groups.

	N	Percentage	Improvement	
			experimental	control
1. Local fat accumulations (neck, shoulder, tail head)	14	93,3%	100% (n=8)	33% (n=2)
2. Local fat accumulations at the shoulder	14	93,3%	88% (n=7)	33% (n=2)
3. Getting fat easily and loosing fat hardly	14	93,3%	88% (n=7)	17% (n=1)
4. Local fat accumulations at the neck	13	86,7%	86% (n=7)	0% (n=0)
5. Local fat accumulations at the belly	13	86,7%	100% (n=7)	33% (n=2)
6. Local fat accumulations at the tail head	12	80,0%	57% (n=4)	33% (n=2)
7. Local fat accumulations at the buttock	12	80,0%	71% (n=5)	0% (n=0)
8. Sensitive on the skin	12	80,0%	88% (n=7)	0% (n=0)
9. Eczema complaints during the previous year	11	73,3%	83% (n=5)	40% (n=2)
10. Fat bulbs over the body	11	73,3%	83% (n=5)	0% (n=0)
11. Behaviour: easily irritated	11	73,3%	83% (n=5)	20% (n=1)

Significant differences in improvement of the symptoms and health problems after the trial could be observed in sensitivity of the skin ($p=0.004$), local fat accumulations ($p=0.009$) (figure 5), (fat) bulbs over the body ($p=0.009$), getting fat easily and hardly losing it ($p=0.010$), problems with getting into heat ($p=0.014$), easily beginning to sweat a lot ($p=0.025$), shedding ($p=0.025$) and easily irritated behaviour ($p=0.045$).

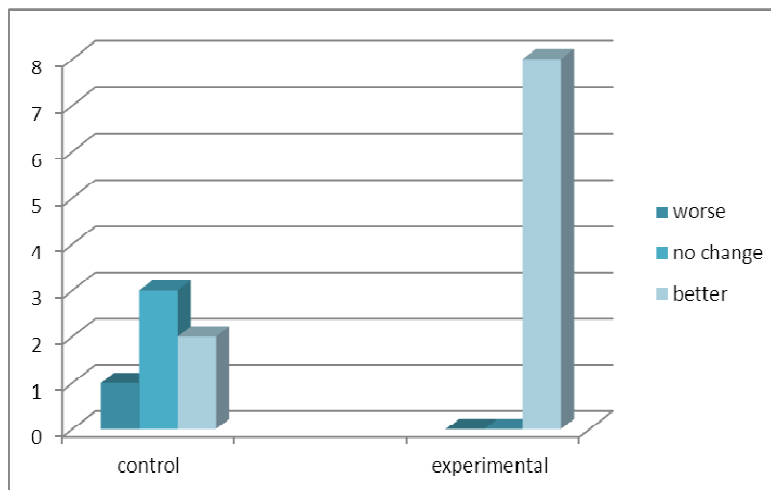


Figure 5: Improvement of local fat accumulations within the experimental and control group.

No significance, but strong indications on the improvement of stiffness ($p=0.074$) and willingness/ability to work ($p=0.074$) were observed.

No significant differences in neck consistency ($p=0.151$), hoof sensitivity ($p=0.232$), comparison of eczema complaints compared to previous year ($p=0.134$), coughing ($p=0.317$), nervous behaviour ($p=0.480$), digestive problems ($p=0.317$), lameness ($p=0.317$), dandruffs ($p=0.317$) and quickly getting out of breath ($p=0.564$) could be found. Also for slowness no significant difference between the two groups was found ($p=0.462$), although 4 out of 5 horses in the experimental group showed obvious improvement.

From the eight participating horses of the experimental group six owners reported obvious positive changes on the majority of the mentioned symptoms and indicators and on the general appearance of their horses, who became more lively and active. The experimental group showed on average an improvement of 80% ($n=107/134$) in the cases and the control group showed an improvement of 17% ($n=16/95$) in the cases with regards to the most common mentioned health problems and indicators (table 5).

From the control group, one person reported obvious changes on the majority of the mentioned indicators, which is in accordance with the percentages of improvement as can be seen in table 5. More detailed information about the statistical analysis on the impact of 'Gluco balance' on the sample can be found in appendix V.

3. DISCUSSION

In the following paragraphs the method practicability and feasibility as well as the results of the trial will be discussed.

3.1 Method Practicability and Feasibility

Method set up

The general set up of the method as a quasi-experimental, placebo controlled, double-blind clinical field study is a commonly applied research set up in researches in dietary supplementation in horses. It turned out to be a good option to be able to test this particular method and the effect of the product under real life conditions. Although not being able to control all factors, no significant differences in noted variables between the two groups were found, but other variables such as grass intake and carbohydrate contents were not able to be controlled at all. Experimental clinical studies are often regarded as the ultimate set up with regards to reliability and controllability (Petrie et al., 2006). However, the advantage of a quasi-experimental set up is in the case of this test trial that already significances and strong indications under natural conditions could be found, supporting the experience that 'Gluco balance' has a positive effect on horses living under different conditions (Petrie et al., 2006).

Also the placebo aspect turned out to be from important value. This because through a placebo controlled set up the reliability of the results could be increased. The developed placebo showed to be a proper comparable product, with high acceptance from the horses and owners. Double blindness was necessary to avoid negative influence on the results of the researcher and to be able to control the owners' perception.

Standardisation procedures

Standardisation procedures of this test trial were that all measurements were taken twice and that the measurements were taken at the same place at the same time from the same person. It showed that it is necessary that the researcher got acquainted with the measurement devices on beforehand to minimize and avoid measurement errors. No standardisation with regards to participant selection was made. It turned however out, that through non selection not all horses actually belonged to a high risk group of being insulin resistant (Treiber et al., 2005a; Carter et al., 2007). Since obesity and local fat accumulations are strongly interlinked with insulin resistance (Johnson, 2002; Hoffman et al., 2003; Frank et al., 2006b; Treiber et al., 2006; Vick et al., 2007, Carter et al. 2010) doing a more thorough selection based on predefined indicators such as diet and degree of obesity the measurements and results will increase in their consistency and reliability. It is therefore advisable to collect as much information based on the participating horses as possible on beforehand, which has not been done to such an extend in the test trial. An average body condition score of 7-8 will increase the chance of actually using insulin resistant horses in the trial considerably. Management, especially feeding and training should be standardized as much as possible; through choosing participants which have alike conditions or are willing to adapt them. It has become clear that it is important to only include horses being active in training to be able to make comparisons. For continuative research horses should show obvious obesity and a fixed number of related

problems/indicators, taking the most commonly mentioned problems from this research as a basis. With regards to breed selection it is not advisable to only include one breed, but rather choosing categories, such as robust breeds. Care needs to be taken that individual differences between the participating horses are not too large. During the test trial one mini Shetland pony participated, which eventually was not included in the data processing since the differences with regards to weight, diet and training, but also measurement practicability were too extreme. When doing quasi-experimental field studies it is important to reduce external variables as much as possible. In this trial it was not possible to standardize management conditions, this partly because the test trial covered the transition period from winter to summer management. For continuative research it is important to choose a constant period to be able to minimize such external factors. Since it has been shown that symptoms of EMS and with that insulin resistance increase several fold during summer time (Bailey et al., 2008), it is advisable to conduct research during this period. However, also in the transition period from summer to winter also many IR related health problems, such as laminitis occur and develop (Treiber et al., 2005a). Therefore ideally research should cover this phase as well. A long term study of 10-12 weeks starting in April would thus cover both periods. It than however, is important to integrate an intermediate visit at the half of the research phase. Although the results are already positive after only five weeks, to increase reliability and significances a long term study is essential.

Participation/Sample size

An objective of the test trial with regards to participation was to discover the general interest of people participating in this research. The interest was with more than 30 people larger than expected, giving an indication that for future research a larger sample size is feasible. Even though the sample size of 15 horses was rather small already significant results were obtained, strengthening the outcomes even further. The chance of deriving significant results with a small sample size is smaller than deriving significances with a larger sample size. The power in a small sample size is low and the risk for accounting type II errors, failing to reject a false Ho hypothesis, increases. If significance is accomplished with a smaller sample size it speaks for a more dramatic effect. However, finding significance in a larger sample increases the reliability that the effect could be transferred to the overall population and that the sample has not been a specific group not being representative for the overall population, which might be the case in the applied sample. The question about whether significance is stronger in a small or large sample size has been argued and is not always conclusive, probably depending on the individual method and research topic. (Royall, 1986)

Another positive outcome with regards to participation was that no participant stopped precociously, but for two horses not all data could be processed. It thus can be presumed that the commitment to such researches is rather high, especially since the owners did not need to do any extra effort, such as clinic retention and transport. It is expectable that 10-15% buffer need to be accounted, especially since the chance that more data is not usable increases in long term trials. A drawback of a prolonged duration applying a placebo controlled set up might be that participants are not willing to take the risk of supplying a placebo product for an extended period. It is therefore advisable to apply management advices, especially with regards to diet for both groups, so that all participants have the feeling of actually doing something against the current situation of their horses. To further increase reliability and reproducibility a larger sample size is necessary. However, an optimal sample

size is not always feasible in equine science; this because several factors are involved in participation, such as owners' awareness and willingness to participate and involved expenses, mainly time and money. Besides, the problem of insulin resistance is still a grey zone and many horses might unknowingly be identified as insulin resistant. To the author's knowledge it is not known at all how many horses within the Netherlands might belong to a risk group, making a proper sample size estimation being representative for the whole population very difficult.

Expanding the duration of searching participants and increasing the platforms and combining this with the interest of 32 people in this trial a feasible sample size for continuative research should be 30-40 horses. Looking at researches from the similar fields the average sample size does commonly not exceed 30 horses. (Vervuert et al. 2010 n=27; Respondek et al. 2011 n=8; Dugdale et al., 2010 n=5; Carter et al., 2010 n=12; Schell, 2010 n=6; Weyenberg et al., 2008 n=9; Gentry et al., 2004 n=24; Tinworth et al., 2011 n=6). More extensive researches applying specific measurement devices under clinical conditions commonly do not exceed 10 horses. The sample size decreases with the research effort, such as endocrinological testing. It is thus a conflict between statistical power and feasibility of such researches.

Assessment Tool/Measurement Practicability and Usability

The assessment tool itself worked well under the given circumstances. Minor adjustments on the usability have been done after the first visit. The tool forced the researcher to apply the same method and structure on all horses and with that minimizing measurement errors and differences between the horses. From the actual measurements not all turned out to be beneficial for such a short term study. Especially the neck thickness measurements and the administration of the BCS and NCS turned out to be inaccurate and with that not being reliable. However, during this trial it has been found that the scoring in the beginning can be a supportive device for determining the horse's body condition and thus the degree of belonging to a risk group. Measuring the weight, heart girth, abdominal girth and neck circumference showed high usability and reliability. The reliability got increased by taking each measurement twice. Not taking repeated measurements leaves these methods rather inaccurate and unreliable. It is thus necessary to do repeated measurements, advisably even three times instead of two. All measurements turned out being sensitive to the researchers' consistency. To reduce this dependency and with that increase reliability it is advisable to use a real scale to determine the weight. However, using such a real scale implies that the participating horses would either need to come to a clinic or high costs would be involved in transporting such a scale throughout the Netherlands. Both options are not easy to execute and therefore the option with taking a weighing tape and taking three measurements might be the most feasible under field conditions. Furthermore, a larger sample size would diminish small inaccuracies. The pictures made of the body posture and hoof condition could not be processed in a quantitative way, but rather serve as a substantiating support for the findings and owners observations. On long term studies more obvious changes could be expected and specific indicators could be assigned to the pictures to be able to make quantitative analyses.

Owners Observations

Administering the owners observations were regarded as a valuable tool, since changes in the horses' condition and behaviour were considerable. However, due to the diverse clinical presentation of IR related symptoms, it turned out to be difficult to standardize these observations. The small sample size and the distribution of observations resulted in several of the individual

observations in a very small sample. Therefore both the significant and the non-significant values need to be interpreted with care and not too much reliability should be put on them. However, observations such as the improvement of local fat accumulations are in line with the specific measurements and therefore exhibit higher reliability. Also the obvious difference in overall improvement of the complaints between the two groups is more reliable than the individual observations. It is to be expected that a long term study with a larger sample size will deliver more obvious results. Another factor which needs to be considered is that not all indicators have the same weight and severity, making it difficult to compare or categorize them. A horse showing improvement in severe stiffness is not necessarily compared with a horse showing improvement in shedding. To be able to match the groups better with each other for continuative research it would be advisable to let the participants fill in their observations on beforehand. The disadvantage from this approach is that it turned out that not all participants are necessarily aware of certain indicators or problems or they interpret them differently.

Continuative Research Feasibility

The method and with that the measurements applied in the test trial were able and suitable to detect possible IR related symptoms and measure the potential effect of 'Gluco balance' on external clinical signs. However, the method misses to directly measure the impact of 'Gluco balance' on insulin sensitivity and glucose tolerance and therefore cannot support the claims made that the product actually has a positive influence on the receptor sensitivity and with on IR directly. For continuative research it is thus necessary to include endocrinological testing to a certain degree. The application of such specific measurements would accord the method more power, reliability and acceptance in the field of veterinarian research. However, apply such methods increases complexity and costs to a considerable extend.

However, until now no tests fulfilling requirements both for accuracy and practicability are applicable for the field of equine research (Firshman et al., 2007). Since reliability is from major importance it is advisable to use the EHC as the most accurate test (Pratt et al., 2005, Firshman et al., 2007) and deciding to apply this one on a small sample size of 6-8 horses. Researches applying this method handle similar sample sizes (Kim et al., 2003; Pratt et al. 2005). The disadvantage of this method is however that it will be far more difficult to find participants being willing to let their horses retain under clinical conditions for several days. To increase the value of (clinical retention and) blood work other values related to insulin resistance, such as adipokine, especially leptin, concentrations, concentrations of pro inflammatory substances, lipid concentrations, especially FFA's and triglycerides, but also general liver values should be analysed as well. The scale of potentially related substances and their value of determining and controlling IR is ample. (Frank et al. 2010) A more complete picture of specific measurements increases reliability as well as power to the claims made for 'Gluco balance'. Promising indicative results on the use of blood pressure measurements for insulin resistant horses are present and since after familiarization with the procedure the indirect oscillometric technique is a simple method to test for changes in blood pressure. However, this method is not commonly applied in equine science yet and does not give direct results on insulin sensitivity and potential improvement of IR. (Bailey et al. 2008)

Refining the method and with that increasing further reliability and consistency primarily the sample size, duration, season, standardisation procedures, especially feeding regimes and the availability and reliability of more diverse and specific measuring methods need to be addressed.

3.2 Impact of 'Gluco balance'

Weight Loss

The most significant differences between the two groups could be observed in the change of body weight. The experimental group lost on average 18.2 Kg more than the control group. Since no significant differences between the two groups with regards to restricted and ad libitum access to roughage were evident, reliability that the weight loss indeed is accountable for the impact of the product increases. The weight loss was against expectations, this because under normal weather conditions most horses would most likely have gained weight, rather than losing weight. It has been expected that horses in the experimental group however, would gain less weight. This because it is assumed that 'Gluco balance' makes the insulin receptors more sensitive, allowing to better regulate the glucose level and to lower the insulin concentration. Since high insulin concentrations promote fat synthesis and stop fat lipolysis and thus weight loss (reviewed by Wilcox, 2005), insulin resistant horses most commonly lose weight more difficult and gain weight more easily. However, being able to lower insulin levels, energy can be converted more effectively and less energy is stored as fat. 'Gluco balance' seems to promote and increase weight loss and with that indirectly will influence insulin sensitivity (van Weyenberg et al., 2008). However, it can be assumed that the product also directly influences insulin sensitivity, but this can only be proven right with proper endocrinological testing.

Since the owners of the participating horses seemed to be more aware that their horses already belong to a risk group most of them had taken precautions with regards to energy intake. Even the horses having had access to grass 24/7 either were restricted with a grassing muzzle and/or due to weather circumstances the pasture was already eaten down very low, so that actually all participating horses could be considered having been on a certain rationed diet. The combination of diet and supplementation of 'Gluco balance' seems to be an effective way of increasing weight loss, without too heavily restricting horses in dry matter intake, as this is often the consequence of weight reduction purely based on diet (Van Weyenberg et al., 2008; Dugdale et al. 2010).

Factors which need to be considered with regards to the change of weight, is that not the direct weight has been measured, but with a help of a measuring tape. In fact only girth circumference has been measured and has been translated into weight loss. It thus can be that a certain percentage of the decrease in heart girth can also be attributed to change, loss and distribution of oedemas and/or gases. Furthermore, the measurements have taken place during the shedding period. For this also a certain percentage might be attributable to loss of hair. Nevertheless, these changes are accountable for both groups, possibly changing the mean difference, but the overall significant difference between the two groups stays the same. To avoid uncertainty, real scaling would be beneficial, but more difficult to apply under field conditions. Another option is to measure the change of subcutaneous fat, with the help ultrasonic measurements as it has been done by Gentry et al. in 2004. Both options decrease uncertainty and deliver more reliable data. A strong indication of indeed actual weight loss having been measured is that the observations also clearly state a considerable decrease of fat accumulations. The same reasoning can be attributed to the decrease of abdominal girth and neck circumference.

The test trial thus supports the claim and experience that 'Gluco balance' indeed can help horses with symptoms of insulin resistance. It is suggested that 'Gluco balance' significantly can increase weight loss in horses, which is in accordance with the experiences made by practitioners and veterinarians. Since the feeding factor has not been controlled through the researcher, but all participating horses received a kind of a rationed diet, it cannot be reliably stated that 'Gluco balance' induces weight loss. It rather increases and supports the effect of dietary management regimes and/or is the needed factor to induce improvement. 'Gluco balance' will most likely not show such significant results when no further management adjustments, to avoid exacerbation, are introduced. To gain more confidence in this field, research would be necessary observing and measuring the participating horses for a certain period before supplying them with the product; this to be able to see how the individual horse behaved compared before and after the supplementation with 'Gluco balance'.

Further Measurement Outcomes

It was unexpected that the neck thickness did not decrease significantly in line with the other measurements. However, neck thickness was also the least inaccurate measurement and the thick crest can not only be attributed to pure fat mass, but also to oedema's and general swelling through low graded inflammation of adipose tissue (Das, 2001). In one pony even a slight increase in neck thickness could be observed although generally the pony lost weight significantly. It can be assumed that no decrease or even an increase in neck thickness may be attributable due to beginning irritation and thus swelling through eczema related discomfort. It is expectable that also the neck thickness measurement will show more significant results on long term studies. Achieving a considerable decrease of neck thickness will most likely take several weeks or months to be noticeable with such measurements. The measurement also misses to differentiate between hardness of the neck. This observation is left to the owners' observation.

No significant difference in the change of abdominal circumference between the groups could be observed, however it is highly indicative. A larger sample size and longer duration are very likely to show significant differences. Also the BCS and the NCS showed no significant differences. As already stated above, Dugdale et al. (2010) did already indicate that general condition scores are not highly appropriate for determining early weight loss. However, the scores were useful to determine risk groups based on a BCS >6 and a NCS ≥ 3 (Treiber et al., 2005a; Carter et al., 2007).

Observational Outcomes

The observational outcomes showed an obvious overall improvement of complaints within the experimental group with 80% and the control group with only 17%. 6 out of 8 horses from the experimental group were reported to show considerable overall improvement, whereas from the other two the observational outcomes were not highly usable. The obvious differences in the overall improvement between the two groups is also in accordance with that only one horse from the control group was reported to show considerable improvement. It can be assumed that in longer trials and larger samples even more obvious results and significant differences in the individual observations will be derived. It was surprisingly that no significant differences between both groups in stiffness and unwillingness to work could be observed. However, the strong indications are in accordance with the owners reports and the non-significance is probably attributable to the small sample size. The pictures made were mostly non conclusive, because a weight loss of on average 25

Kg from the experimental group, especially at obese horses is likely not to be discovered with the eye. Also since the trial took place during the shedding phase, pictures might give a wrong impression. The hoof pictures were made, expecting in some cases developing growth rings, as commonly observed when drastic metabolic changes occur. It is likely that the period of five weeks was just too short for such an interpretation. It is to be expected that on the long term pictures might turn out to be a valuable supportive tool, with however rather little scientific value, but rather applicable for illustrative purposes.

With the method and measurements applied, the test trial does not support the statements that 'Gluco balance' has a positive impact on insulin resistant horses, but on horses showing symptoms of insulin resistance and belonging to a risk group. This since no evidence was present on beforehand that all participating horses were affected, neither has there been specific evidence that the insulin resistance improved during/after the trial.

4. CONCLUSIONS

Insulin resistance in horses is a serious concern with long ranging consequences, laminitis being one of the most serious effects. However, the field of IR in horses is rather new, making the diagnosis and effective treatment still challenging and controversial.

The applied method in this trial, especially with regards to the actual external measurements resulted in significant outcomes in changes of body weight, heart girth, neck circumference and overall improvement of the observed complaints. With this the method can be regarded as suitable and feasible for determining changes of external indicators with the major focus on obesity, though not all applied measurements were highly accurate and with that feasible for determining changes throughout the trial.

The measurements applied in the test trial are non-specific methods and only indirectly can provide insight on the effect 'Gluco balance' has on horses belonging to a risk group of being insulin resistant. The test trail supports the claims that 'Gluco balance' has a positive impact on symptoms and complaints related to IR such as obesity, muscle tension, hormonal imbalances and general fitness. However, it does not provide any support that it improves insulin cell receptor sensitivity and thus improves insulin resistance. To be able to provide evidence that 'Gluco balance' has a specific effect of insulin sensitivity, endocrinological testing is indispensable. Also the test trial cannot support the statements that 'Gluco balance' has a positive impact on insulin resistant horses, but on horses showing symptoms of insulin resistance and belonging to a risk group. This since no evidence was present on beforehand that all participating horses had IR, neither has there been specific evidence that the insulin resistance improved during/after the trial. Furthermore, it can only be supported that 'Gluco balance' rather increases and supports the effect of dietary management regimes and/or is the needed factor to induce improvement. 'Gluco balance' will most likely not show such significant results when no further management adjustments, to avoid exacerbation, are introduced.

Factors having affected the method set up and with that the outcomes have mainly been the duration, the sample size, standardisation and pre selection procedures and the availability of more specific measurement methods. For continuative research it is thus important to put more attention on above named factors to increase reliability and acceptance in the veterinarian field.

Extensive continuative research seems to be feasible and even necessary when wanting to directly proof the impact of 'Gluco balance' on insulin sensitivity and with that on improvement of Insulin resistance. However, continuative research will be cost and time effective and challenging. A plain clinical research will not be feasible. The expenses related to the regulated conditions most likely do not stand in relation to the benefit for the company. The most feasible options are to improve and repeat the applied method in practicability and reliability and including endocrinological testing in a certain percentage of the participants, or to develop another pilot method for a research focusing exclusively on endocrinological measurement.

Concluding, both the results on the method practicability and the indicative results on the impact of 'Gluco balance' give the company Phytonics insight in the potential of scientifically research the effect of 'Gluco balance' and to decide whether and how to apply this information in the field. To be able to effectively support IR in horses the trial suggests that 'Gluco balance' indeed has beneficial

impacts, but more specific testing is required for more precise statements about the impact of 'Gluco balance' on (obese and/or) insulin resistant horses.

5. RECOMMENDATIONS

The recommendations presented in this chapter are based on extensive literature review in the field of insulin resistance in horses and on the outcomes of the test trial. Together with the provision and discussion of the applied method the company Phytonics gets insight in the potential, practicability and feasibility on conducting a continuative research.

Although the test trial already delivered promising outcomes, it is recommendable to continue with this type of research, especially enlarging the duration and sample size and refining and specifying the measurement techniques. To be able to proof the direct positive effect of 'Gluco balance' on insulin resistant horses endocrinological testing is necessary. For this another pilot study with a small sample size of 6-8 horses should be developed and executed to collect valuable data. In later stages both the methods as well as outcomes of the trials with nonspecific and specific measurements could be combined, increasing reliability and trustworthiness in the equine industry.

Possible adjustments and refinements when wanting to continue the applied research method should be:

- Extending period to 10-12 weeks, including one intermediate visit
- Enlarging sample size to 30-40 horses, applying endocrinological tests on 20-25% of the sample
- Refining measurement techniques, using more specific tools
- Refining standardisation procedures, better pre selection and constant conditions

Both the outcomes as well as the actual developed assessment tool and the collected literature should be used for educational and marketing related purposes. The research report, with focus on the literature review and the outcomes with regards to the impact of 'Gluco balance', should be transformed in a more understandable and briefer version. Such a version should than be published on (internet) platforms such as company websites, forums, newsletters and magazines and specifically sending it to the participants to increase the awareness of insulin resistance, its diagnosis and possible management. Through this, horse owners will be stimulated to have a more detailed look at the horses' condition. Such a version, including hands on tips on how to administer the horses' condition gives horse owners the chance to make comparable measurements before and after a potential treatment. A booklet alike version could be added to the 'Gluco balance' or relating websites. Increasing awareness plus the positive results of the test trial are most likely to stimulate recognition and sales of 'Gluco balance'.

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APPENDICES

Appendix I: 'Gluco balance' Product Description

'Gluco balance' paard

Bij insulineresistentie.

Geschikt voor: Paard, Pony

Samenstelling: Agnus castus, Berberis vulgaris, Brassica oleracea, Brassica oleracea italica, Carduus marianus, Cinnamomum ceylanicum, Corylus avellana, Cynara scolymus, Trigonella foenum-graecum, Foeniculum vulgare, Glycyrrhiza glabra, Herba polygoni, Hibiscus sabdariffa, Petroselinum crispum, Rosmarinus officinalis, Salvia officinalis, Spinacia oleracea, Urtica urens, Vitis vinifera, Zingiber. Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis, Fructo Oligo Sacchariden.

Bioflavonoiden, Hesperidine. 4,66 mg Choline, 0,8 mg L-Lysine. 3,21 mg Calciumcitraat, 49,29 µg Chroompicolinaat, 6,43 µg Foliumzuur, 1,21 mg Fosfor, 0,32 mg IJzer, 12,22 mg Inositol, 6,43 µg Jodium, 1,61 mg Kalium, 1,61 µg Koper, 18,54 mg Magnesiumcitraat, 0,06 mg Mangaan, 6,11 µg Selenium, 0,8 mg Silicium (bamboe extract), 11,9 mg Zinkcitraat. Vitamine A 643,2 IE, B1 1,61 mg, B2 1,61 mg, B3 6,43 mg, B5 1,61 mg, B6 3,54 mg, B12 41,81 µg, C 6,43 µg, D 64,43 IE, E 3,59 mg, Biotine 1,29 µg, PABA 1,61 mg, Himalayazout, Montmorilloniet.

Indicatie(s):

Bij insulineresistentie. Werkt tevens ondersteunend bij hypoglycaemie. Bij verminderd functioneren van de pancreas. 'Gluco balance' werkt ondersteunend bij de gevolgen van de ziekte van Cushing.

- Bij hoefbevangenheid
- Bij tying-up
- Bij staart- en maneneceem
- Bij spierstijfheid bij –onder andere- oudere dieren



Eigenschappen per ingrediënt:

'Gluco balance' bevordert een evenwichtige bloedsuikerspiegel.

Bij insulineresistentie zijn de insulinerceptoren die verantwoordelijk zijn voor het transport van glucose de cel in, minder gevoelig voor insuline. De glucose blijft dan in de bloedbaan ondanks een verhoogde concentratie aan insuline in het bloed. Hierdoor raakt de energiehuishouding verstoord.

'Gluco balance' zorgt ervoor dat de receptoren gevoeliger worden voor insuline waardoor de glucose-opname verbeterd wordt. Ook werkt het regulerend op de lever, dit orgaan speelt een belangrijke rol in de (glucose)stofwisseling. Bij diabetes mellitus is het immuunsysteem verstoord, wat zich vaak uit in een verhoogde vatbaarheid voor infecties en een abnormale wondgenezing. Ook schade aan bloedvaten is een complicatie bij een verstoorde glucosehuishouding, omdat een te hoog glucosegehalte het aantal reactieve zuurstofverbindingen in het bloed sterk toenemen. 'Gluco balance' bevat dan ook anti-oxidanten en immuunstimulerende bestanddelen.

Dosering en toepassing:

Dosering, tenzij arts/therapeut anders voorschrijft:

Eerste week 2 x daags :

Paard: 1 maatschep

Pony : $\frac{3}{4}$ maatschep

Verlaag de dosering na de eerste week naar 1 x daags.

1 maatschep bevat ongeveer 8 gram.

Toedienen: kan over het voer gegeven worden. Bevochtig het poeder zodat het niet uit de voerbak geblazen wordt.

Waarschuwing

Gebruik tijdens dracht en lactatieperiode in overleg met arts/ therapeut. Wanneer er vermagering optreedt de dosering halveren of eventueel stoppen.

*Ervaring uit de praktijk leert dat in sommige gevallen kan worden volstaan met een lagere dosering dan zoals aangegeven op de bijsluiter. Ook wanneer dieren (te) heftig reageren kan worden begonnen met de helft van de aanbevolen dosering.

Inhoud en Adviesprijs voor consumenten:

500 gram - € 59,95

Producent: Laboratorium Bonusan BV, Numansdorp

Toelichting: De producten van Phytonics worden uitsluitend verkocht via de dierenarts of gediplomeerd therapeut.

Eigenschappen van de bestanddelen:

- Agnus castus** (Monnikspeper) heeft een regulerende invloed op de hypofyse.
- Berberis vulgaris** (Zuurbes) ondersteunt de reinigende functie van de uitscheidingsorganen.
- Brassica oleracea italica** (Broccoli) bevat sulforafaan, dit heeft een beschermend effect op de De sulforafaan induceert ontgiftingsenzymen, welke de bloedvatwand herstellen en beschermen.
- Carduus marianus** (Mariadistel) werkt leveropbouwend, ondersteunt de reinigende werking van de lever en zorgt voor een goede galproductie en -transport.
- Cinnamomum ceylanicum** (Kaneel) bevat wateroplosbare polyfenol type-A polymeren welke de glucosestofwisseling positief ondersteunen. Kaneel bevat biologisch actieve substanties met een insulineachtige werking waardoor de glucoseopname door de cel wordt bevorderd.
- Corylus avellana** (Hazelaarsblad) Stimuleert de leverfunctie. Bevordert de vorming van nieuwe erythrocyten en granulocyten en reguleert de bloedstolling.
- Cynara scolymus** (Artisjok) draagt zorg voor opbouw van het leverweefsel, heeft choleretische eigenschappen, beschermt de lever tegen beschadigingen en heeft een ontstekingsremmende werking.
- Foeniculum vulgare** (Venkel) bevordert de maagdarm-motiliteit. Werkt spasmolytisch.
- Glycyrrhiza glabra** (Zoethout) Werkt ontstekingsremmend en pijstillend door inhibitie van COX. Bevat coumarine en werkt hierdoor bloedverdunnend. Heeft een gunstige invloed op de spijsvertering, wordt ingezet bij maagzweren en heeft een spasmolytische werking.

- Herba polygoni** (Ye Jiao Teng) is een chinees kruid met positieve invloed op de lever en pancreas.
- Hibiscus sabdariffa** (Chinese roos) verbetert de vetstofwisseling, stimuleert vaatverwijding, bevordert de diurese, verbetert de doorstroming van het bloed en is een krachtige antioxidant.
- Petroselinum crispum** (Peterselie) zorgt voor afdrijving van gas uit het maagdarmkanaal. Werkt diuretisch en verlaagt de bloeddruk.
- Rosmarinus officinalis** (Rozemarijn) is een hepatorenaal drainagemiddel. Door de choloretische en spasmolytische eigenschappen, wordt ook de galblaas beïnvloed. Rosmarinus officinalis werkt tevens immuunversterkend en is een goede antioxidant.
- Salvia officinalis** (Salie) heeft een samentrekkende, antibacteriële en ontstekingsremmende werking op ontstoken slijmvliezen. Salvia reguleert en stimuleert de bloed- en lymfecirculatie en kan de bloedsuikerspiegel verlagen.
- Spinacia oleracea** (Spinazie) bevat plantaardige steroidverbindingen, de ecdysteroiden. Deze hebben een anabole werking. Ecdysteroiden verlagen de cholesterol- en de suikerspiegel en stimuleren het immuunsysteem.
- Trigonella foenum-graecum** (Fenegriek) Verbetert de insulinegevoeligheid, de glucoserespons en vergroot het aantal insulinerceptoren. Fenegriekzaden bevatten een uniek aminozuur 4-hydroxyisoleucine (4-OH-Ile) dat de insulinesecretie (na glucosetoediening) door de bètacellen van de pancreas verhoogt. Ook bevatten de zaden veel vezels waardoor de bloedsuikerspiegel daalt.
- Urtica urens** (kleine Brandnetel) werkt ontstekingsremmend, weerstandsverhogend, antiviraal, antibacterieel, fungicide, en diuretisch. Het wordt ingezet bij maag- en darmzweren en ter ondersteuning van de behandeling bij suikerziekte.
- Vitis vinifera** (Rode Druifstok) werkt ontstekingsremmend en weerstandsverhogend. Het reguleert de aanmaak van leukocyten en lymfocyten.
- Zingiber** (Gember) beschermt het maagdarmslijmvlies en verbetert de absorptie van vitaminen en mineralen, stimuleert de galsecretie en beschermt de levercellen tegen chemische toxinen. Gember verlaagt de bloedspiegels van glucose, cholesterol en triacylglycerol. Door remming van de bloedplaatjesaggregatie werkt het bloedverdunnend, heeft tevens een beschermende werking op de bloedvatwand. Door remming van de prostaglandinesynthese werkt het ontstekingsremmend en pijnstillend.
- **Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis:** De goede darmbacteriën maken azijnzuur dat de groei van schimmels, gistcellen en bepaalde ongunstige bacteriën remt. De verlaging van de zuurgraad in de darmen zorgt voor een goede balans tussen gunstige en ongunstige bacteriën. De darmbacteriën ondersteunen het immuunsysteem door de aanmaak van antistoffen. Door de productie van korte vetzuurketens bevorderen ze het herstel en onderhoud van de darmwand. Tevens spelen de darmbacteriën een rol bij de productie van bepaalde B vitaminen en Vitamine K.
- De aanwezige **prebiotica (Fructo-Oligo-Sacchariden, FOS)** vormen een matrix die ondersteunend werkt voor de groei van de verschillende bacteriestammen.
- Bioflavonoïden (90% Hesperidine)** zijn in water oplosbare, plantaardige pigmenten en hebben een anti-oxidatieve werking. verbeteren de insulinesecretie en remmen de ophoping van sorbitol in de cel. Ze versterken de werking van vitamine C, hierdoor wordt het immuunsysteem gestimuleerd. Daarnaast is er een positief effect op capillairen, bindweefsel en bloedvatwanden.
- Choline** is van belang voor de gezondheid van de lever en nieren. Choline kan de bloed-hersenbarrière passeren en is een belangrijk onderdeel van de neurotransmitter acetylcholine welke

van belang is voor de chemische prikkeloverdracht in zenuwweefsel.

-L-Lysine is een aminozuur wat van belang is voor de productie van elementaire lichaamseiwitten, zoals enzymen, hormonen en antilichamen. Het is nodig voor de groei en herstel van weefsel vanwege de collageenvormende eigenschappen. Het bevordert de absorptie van calcium uit het maagdarmkanaal en stimuleert de secretie van maagsappen.

-**Calciumcitraat** een calciumtekort draagt mogelijk bij aan het ontstaan van diabetes.

-**Chroompicolinaat**: chroom is van belang voor de opbouw van chromoduline, een complex van aan maximaal vier chroomionen, glycine, glutaminezuur, cysteïne en asparaginezuur. Dit complex bindt aan (door insuline) geactiveerde insulinerectoren en zet daar second messengersystemen (tyrosine-kinase) in gang. Het kan daarmee de werking van insuline versterken. Chromoduline kan vier chroomionen binden en naarmate meer chroom aanwezig is, vindt een sterkere activatie van de insulinerector plaats. Onvoldoende activatie van de insulinerector door chroomdeficiëntie is mogelijk een oorzaak van insulineresistentie. Ook remt chromoduline fosfatases, die de werking van de insulinerector verminderen. Er zijn aanwijzingen dat chroom ook het aantal insulinerectoren en de gevoeligheid van bètacellen in de pancreas vermeerderd.

-**Foliumzuur**: is betrokken bij de gluconeogenese als cofactor van diverse leverenzymen.

-**Fosfor**: bevindt zich in elke lichaamscel, het is een belangrijk bestanddeel van biochemische verbindingen en speelt een essentiële rol in een aantal stofwisselingsprocessen.

-**IJzer**: Is een belangrijke bouwsteen van rode bloedcellen en dan met name het zuurstoftransporterende deel. Is essentieel voor bepaalde enzymsystemen.

-**Inositol**: regelt de omzetting van vetten en cholesterol in het lichaam. Het verbetert de stressbeheersing.

-**Jodium**: is nodig voor de vorming van schildklierhormonen die belangrijk zijn voor de stofwisseling.

- **Kalium**: door hoge insulinespiegels in het bloed is er een groot verlies van kalium via de urine. Kaliumsuppletie verlaagt de insulineresistentie en verbetert de uitscheiding van insuline.

- **Koper**: een gebrek aan koper remt de glucosetolerantie en verhoogt de weefselconcentraties van sorbitol bij een hoge koolhydraatconsumptie. Sorbitolophoping in weefsels kan uiteindelijk aanleiding geven tot allerlei diabetescomplicaties als retinopathie, cataract en neuropathie.

-**Magnesiumcitraat**: op verschillende plaatsen in de glucosestofwisseling speelt magnesium een rol. Magnesiumgebrek wordt in verband gebracht met atrofie van de bètacellen in de pancreas.

-**Mangaan** als antioxidant speelt een belangrijke rol in de koolhydraatstofwisseling. Het is een cofactor van verteringsenzymen. Mangaan als cofactor, is nodig voor de activatie van het enzym pyruvaatcarboxylase. Dit enzym speelt een cruciale rol in de glucosestofwisseling.

Mangaandeficiëntie gaat, onder andere, gepaard met een verminderde glucosetolerantie, evenals met aan diabetes verwante afwijkingen in de koolhydraat- en vetstofwisseling.

- **Selenium**: speelt een belangrijke rol in de schildklierstofwisseling. Het enzym dat het schildklierhormoon T4 omzet in het actieve T3 is seleniumafhankelijk. Ook maakt het deel uit van de vier enzymen van het glutathionperoxidase enzymstelsel, dit is een belangrijke component van de enzymatische afweer tegen oxidatieve stress. Ook werkt het ontstekingsremmend en is het van belang voor het immuunsysteem en dan met name voor het functioneren van de macrofagen. Tevens is selenium belangrijk bij de detoxificatie, een voldoende hoge spiegel is in staat zware metalen als cadmium, zilver, kwik en lood te binden en de toxiciteit ervan te verminderen. Ook is selenium essentieel voor het cytochroom P450 enzymstelsel, dat verantwoordelijk is voor de ontgiftiging door de lever.

-**Silicium** (uit bamboe extract) is van belang voor de conditie van bindweefsel en de vaatwand.

- **Zinkcitraat** Zink speelt een belangrijke rol bij de insulinstofwisseling. Het stabiliseert de bloedsuikerwaarden, activeert de -resterende- insulineproductie en beschermt de bètacellen tegen beschadiging. Als gevolg van de bij diabetes voorkomende proteïnurie is de zinkuitscheiding twee tot driemaal zo hoog. Dit kan leiden tot een zinktekort, met als gevolg een verminderde glucosetolerantie en insulinegevoeligheid en een verhoogde insulineresistentie.
- **Vitamine A** heeft een belangrijke functie in de celdeling van epitheelcellen.
- **Vitamine B1 (Thiamine HCl)** is van belang voor de glucosestofwisseling.
- **Vitamine B2 (Riboflavine)** heeft een belangrijke functie in de energiestofwisseling.
- **Vitamine B3 (Nicotinamide)** doet bètacellen beter functioneren door ze te beschermen tegen beschadiging door het immuunsysteem.
- **Vitamine B5 (Calciumpantothenaat)** verbetert de koolhydraatstofwisseling.
- **Vitamine B6 (Pyridoxine HCl)** remt de glycosylatie en biedt bescherming tegen diabetische neuropathieën.
- **Vitamine B12 (Cyanocobalamine):** er is een relatie tussen een verstoorde vitamine B12-stofwisseling en suikerziekte.
In sommige gevallen kan vitamine B12 diabetische neuropathie terugdringen.
- **Vitamine C:** gaat glycosylatie tegen en is in staat sorbitolophoping in de erythrocyten tegen te gaan. Insuline is van belang voor de opname van Vitamine C in de cel.
- **Vitamine D:** is nodig voor een normale insulinesecretie en glucosetolerantie.
- **Vitamine E** verbetert de glucosetolerantie. Hoge doses vitamine E verbeteren de werking van insuline en verlagen zo de glucose- en insulineconcentraties. Het heeft een antioxidatieve werking, beschermt onverzadigde vetzuren tegen vrije radicalen. Vitamine E heeft een positieve werking op de bloedvaten, gaat vorming van bloedstolsels tegen, verbetert de bloedcirculatie en versterkt de capillairwanden.
- **Biotine:** reguleert de expressie van genen die nodig zijn voor de synthese van enzymen in de glucosestofwisseling. Biotine reguleert daarnaast de expressie van genen voor insuline en insulinerceptoren, hierdoor leidt biotine-suppletie tot een verbeterde insulinegevoeligheid en glucosetolerantie.
- **PABA (para-aminobenzoëzuur)** is van belang voor de gezondheid van het darmstelsel en de huid. Het is ook betrokken bij de werking van afweercellen en rode bloedcellen. PABA is onderdeel van het co-enzym tetrahydrofoliumzuur en speelt een belangrijke rol in het verlagen van een hoog homocysteïnegehalte. Verder is het een krachtige bestrijder van het singlet-zuurstof radicaal.
- **Montmorilloniet:** katalyseert en versterkt de fytotherapeutische ingrediënten.
- **Himalayazout:** bevat mineralen- en sporenelementen en bio-energetische frequenties in het Terahertz gebied welke speciaal zijn gekozen bij de genoemde indicaties van dit product.

Appendix II: Research Instruction Paper

Geachte.....,

Hartelijk dank voor uw interesse aan dit vooronderzoek, en insulineresistentie in het algemeen. In dit vooronderzoek gaat het om de ontwikkeling en evaluatie van een betrouwbare methode om het effect van Phytonics 'Gluco balance' op insulineresistente paarden wetenschappelijk aan te kunnen tonen.

'Gluco balance' is een natuurlijk gezondheidsproduct en is nu –maart 2011- ruim vier jaar, met groot en groeiend succes, op de markt.

Phytonics wil de fantastische ervaringen uit de praktijk ook graag degelijk onderbouwen, hiervoor is onderzoek nodig, zodat het effect ook wetenschappelijk aangetoond kan worden en zo meer paarden van dit supplement kunnen profiteren.

'Gluco balance':

'Gluco balance' is een natuurlijk gezondheidsproduct (op basis van planten/kruiden en belangrijke voedingsstoffen) en bevordert een evenwichtige bloedsuikerspiegel. Het zorgt ervoor dat de receptoren gevoeliger worden voor insuline waardoor de glucose-opname verbeterd wordt. Ook werkt het regulerend op de lever, dit orgaan speelt een belangrijke rol in de (glucose)stofwisseling. Bij diabetes mellitus is het immuunsysteem verstoord, wat zich vaak uit in een verhoogde vatbaarheid voor infecties en een abnormale wondgenezing. Ook schade aan bloedvaten is een complicatie bij een verstoorde glucosehuishouding, omdat een te hoog glucosegehalte het aantal reactieve zuurstofverbindingen in het bloed sterk doet toenemen. 'Gluco balance' bevat dan ook anti-oxidanten en immuunstimulerende bestanddelen.

'Gluco balance' kan ook positieve uitwerking hebben op aandoeningen die in relatie staan tot insulineresistentie, zoals:

- Hoefbevangenheid
- Zomereczeem
- Spierbevangenheid
- Overmatige hengstigheid
- Stijfheid
- Hoesten
- Kreupelheid
- Gedragsproblemen

Bijwerkingen van dit product zijn tot nu toe niet waargenomen. Als u hierover vragen heeft neemt u dan contact op met de holistische dierenpraktijk Den Hoek in De Bilt (tel: 030-2961462).

Opzet van het onderzoek:

Het onderzoek is een vooronderzoek om te testen of de ontwikkelde methode werkt en hoe praktisch deze is. Het eigenlijke testen van het effect van 'Gluco balance' staat bij dit onderzoek nog niet centraal, maar wordt wel als indicatie meegenomen. Het is dus belangrijk om te realiseren, dat wij in eerste instantie de methode gaan testen om te kijken hoe wij in een latere fase een echt betrouwbaar onderzoek op kunnen zetten. Vaak wordt deze ontwikkelingsfase onderschat waardoor in het eigenlijke onderzoek onbetrouwbare resultaten verkregen kunnen worden.

Het eigenlijke veldonderzoek is gerandomiseerd en dubbelblind gecontroleerd. Dit betekent dat de deelnemende groep willekeurig in twee groepen wordt gesplitst. Eén groep krijgt het echte product ('Gluco balance') en de andere groep krijgt een onschuldig product zonder effect (placebo). Noch wij als onderzoekers noch u als deelnemers weten wie welk product krijgt. Hiermee voorkomen wij subjectieve beïnvloeding op de uitkomsten.

Beide producten worden tijdens de eerste week twee keer per dag en tijdens de erop volgende vier weken een keer per dag toegediend. Het poeder kan door het voer gemengd worden.

Dosering: eerste week 2 x daags :

Paard: 1 maatschep

Pony : $\frac{3}{4}$ maatschep

Verlaag de dosering na de eerste week naar 1 x daags.

Het onderzoek duurt 5 weken (van 03.04. tot 11.05.2011). Ieder paard wordt voor en na het veldonderzoek met behulp van een diagnostisch protocol beoordeeld, zodat na afloop van het onderzoek de resultaten vergeleken kunnen worden. Het protocol bestaat uit meerdere vragen rondom het management van uw paard. Naast deze vragen worden onder andere de buikomvang, het gewicht, de halsomvang, de hoof- en vachtconditie en de algemene body condition score vastgesteld. Dit met behulp van verschillende meetmethodes. In dit onderzoek wordt **niet** met bloedmonsters gewerkt, maar vooral met bepaling en beoordeling van uiterlijke kenmerken en uw eigen waarneming. Het invullen van het protocol duurt ongeveer een uur.

Wat vragen wij van u en uw paard?

- uw paard is al insuline resistent getest of
- uw paard is (te) dik (en heeft/had andere klachten zoals hoofbevangenheid, zomereczeem, gevoelige hoeven, spierbevangenheid, stijfheid etc.).
- u bent bereid om tijdens de vijf weken geen andere behandeling te ondergaan (afgezien van niet vermijdbare behandelingen).
- u bent zich ervan bewust dat het zijn kan dat uw paard tijdens het onderzoek een onschuldig placebo zonder negatieve effecten toegediend zou kunnen krijgen.
- u kunt op twee van de onderaan genoemde dagen ongeveer een uur vrijmaken.
- het paard is bij aankomst geborsteld en heeft schone hoeven (dit omdat foto's worden genomen).
- het management (bv. voer) blijft tijdens het onderzoek zo constant als mogelijk

Wat kunt u van ons verwachten?

- vergelijkende gezondheidsinspectie en beoordeling van de conditie van uw paard (uiterlijke kenmerken).
- professionele begeleiding tijdens en na afloop van het onderzoek.
- na afloop van het onderzoek ontvangt u een gratis pot 'Gluco balance' (500 gram) voor ongeveer een maand.

Om te kunnen garanderen dat het onderzoek voor iedere deelnemer net zo lang duurt is het belangrijk om al van te voren te besluiten wanneer wij u en uw paard kunnen bezoeken. In principe geldt dus dat als u bijvoorbeeld uw keuze voor 03.04.2011 heeft gemaakt wij proberen om dan ook na precies vijf weken weer terug te komen, dus op 08.05.2011.

Graag in de onderstaande tabel aangeven wanneer het bij u het beste uitkomt. U kunt drie keuzes maken, graag aangeven met 1,2 en 3. Wij zullen proberen u voor een van de keuzes in te roosteren. In het geval dat u op alle vier mogelijke dagen, op alle vier mogelijke tijdstippen niet beschikbaar bent, kunt u ook een ander tijdstip (het moet echter wel nog licht buiten zijn) invullen en zullen wij proberen hier rekening mee te houden.

	03.04. (08.05.)	04.04. (09.05.)	05.04. (10.05.)	06.04. (11.05.)
9.00-10.00				
10.45-11.45				
12.30-13.30				
14.15-15.15				
Ander tijdstip				

Adres gegevens:

Straat:

Plaats, postcode:

Onderaan hebben wij enkele regeltjes voor een korte omschrijving van de gezondheidsconditie van uw paard vrijgelaten. Dit om zeker te stellen dat uw paard voor dit onderzoek in aanmerking komt. Het ingevulde document graag terug sturen naar ironderzoek@gmail.com.

Voor nadere informatie over insulineresistentie en Phytonics 'Gluco balance':
www.nlmhealth.com of www.phytonicsmed.com

Heeft u nog vragen over het opzet en de afloop van dit onderzoek?

Graag contact opnemen met Lennart Denkhaus:

E-mail: ironderzoek@gmail.com

T: 06-34318483

Appendix III: Subli Lucerne Ingredient List

Voedermiddelen

- Gedroogde Franse luzerne
- Plantaardig vet

Gehalten

- | | |
|---------------|--------|
| -Ruw as | 10% |
| -Ruw eiwit | 14% |
| -Ruw vet | 5% |
| -Ruwe celstof | 28% |
| -Calcium | 2,3% |
| -Fosfor | 0,25% |
| -Ewpa | 0,65 e |
| -Vrep | 10 g |

Appendix IV: Assessment Tool

-Assessment tool voor paarden met (vermoeden naar) insulineresistentie-

Datum, Plaats: _____ Product nummer: _____

Naam Eigenaar: _____

Naam Paard: _____

Leeftijd: _____

Geslacht: H M R

Ras: _____

Huisvestiging (bij begin van het onderzoek):

Aantal uren per dag: op stal _____ op paddock _____ op de wei _____

Solitair In gezelschap met soortgenoten Allebei

Voer (bij begin van het onderzoek):

Ruwvoer: Gras Hooi Kuil Voordroog Stro Mix namelijk:..... Anders

Hoeveelheid (kg): _____

Maaltijden per dag: _____

Krachtvoer: Brokken Granen Muesli Mix namelijk:..... Geen Anders

Merk: _____

Hoeveelheid (kg): _____

Maaltijden per dag: _____

(Voedings)supplementen:

Wat: _____

Waarvoor: _____

Bent u bekend met 'Gluco balance'? Ja Nee Als ja waardoor: _____

Training (bij begin van het onderzoek):

Discipline: Dressuur Springen Western Recreatie Anders

Niveau: _____

Trainingsintensiteit per dag (minuten): _____

Trainingsintensiteit per week (dagen): _____

Anamnese:

Ziekte(s): _____

Wanneer & hoe lang: _____

Reden voor ziekte(s) (als bekend): _____

Behandeling: _____

Management veranderingen tijdens het onderzoek (in te vullen na afloop van het onderzoek):

Ja Nee

Huisvestiging:

Aantal uren per dag: op stal _____ op paddock _____ op de wei _____

Solitair In gezelschap met soortgenoten Allebei

Voer:

Ruwvoer: Gras Hooi Kuil Voordroog Stro Mix namelijk:..... Anders

Hoeveelheid (kg): _____

Maaltijden per dag: _____

Krachtvoer: Brokken Granen Muesli Mix namelijk:..... Geen Anders

Merk: _____

Hoeveelheid (kg): _____

Maaltijden per dag: _____

(Voedings)supplementen:

Wat: _____

Waarvoor: _____

Training:

Discipline: Dressuur Springen Western Recreatie Anders

Niveau: _____

Trainingsintensiteit per dag (minuten): _____

Trainingsintensiteit per week (dagen): _____

Anamnese:

Ziekte(s): _____

Wanneer & hoe lang: _____

Reden voor ziekte(s) (als bekend): _____

Behandeling: _____

Voor het onderzoek (Datum _____ Product nr. _____)

1. **Gewicht (kg):** _____ = _____
2. **Omvang (heart girth)(cm):** _____ = _____
3. **Buikomvang (cm):** _____ = _____ (2/3 van schouder tot heup: _____ cm)
4. **Body condition score (1-9):**
 - a. Voor: _____
 - b. Midden: _____
 - c. Achter: _____
5. **Halsomvang (cm)*:** 0,25: _____ 0,5: _____ 0,75: _____
*0,25= _____ cm 0,50= _____ cm 0,75= _____ cm, totale lengte _____ cm
6. **Halsdikte (cm):** _____ = _____
7. **Crest neck score (0-5):** _____
8. **(Vacht)conditie:** foto's _____ (mind. 4 stuks)
9. **Hoefconditie:** foto's _____ (mind. 6 stuks)

10. Observaties:

- | | | | | | |
|------------------------|-----------------------------|------------------------------|-------------------------|-----------------------------|------------------------------|
| Lokale vetophopingen: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Vetbulten: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Harde nek: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Bult boven ogen: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Snek dik worden: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Moeilijk afvallen: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Hoefbevangen: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Gevoelige hoeven: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Verbredend witte lijn: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Kreupel: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Stijf: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Onwillig om te werken: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Veel en snel zweten: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Snel buiten adem: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Sloom: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Hoesten: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Haarwisselproblemen: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Eczeem/schuren: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Gevoelige Huid: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Onregelmatig hengstig: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Rugklachten: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Spijverteringsklachten: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |
| Nervus: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> | Agressief: | Ja <input type="checkbox"/> | Nee <input type="checkbox"/> |

Andere Observaties: _____

Na het onderzoek (Datum _____ Product nr. _____)

1. Gewicht (kg): _____ = _____
2. Omvang (heart girth)(cm): _____ = _____
3. Buikomvang (cm): _____ = _____ (2/3 van schouder tot heup: _____ cm)
4. Body condition score (1-9):
 - a. Voor: _____
 - b. Midden: _____
 - c. Achter: _____
5. Halsomvang (cm)*: 0,25: _____ 0,5: _____ 0,75: _____
 *0,25= _____ cm 0,50= _____ cm 0,75= _____ cm, totale lengte _____ cm
6. Halsdikte (cm): _____ = _____
7. Crest neck score (0-5): _____
8. (Vacht)conditie: foto's _____ (mind. 4 stuks)
9. Hoefconditie: foto's _____ (mind. 6 stuks)
10. Observaties:

_____:	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Begin onderzoek
	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Afgelopen jaar
_____:	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Begin onderzoek
	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Afgelopen jaar
_____:	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Begin onderzoek
	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Afgelopen jaar
_____:	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Begin onderzoek
	slechter <input type="checkbox"/>	gelijk <input type="checkbox"/>	beter <input type="checkbox"/>	Afgelopen jaar

Andere observaties/aanmerkingen:

Appendix V: Statistical Analysis

Group statistics of the experimental and control group before the trial

	Experimental or control group?	N	Mean	Std. Deviation	Std. Error Mean
What is the horses' age?	Experimental	8	12,9375	5,41451	1,91432
	Control	7	11,8571	3,33809	1,26168
For how many hours per day is the horse standing in the stable?	Experimental	8	5,0000	6,94879	2,45677
	Control	7	8,4286	8,32380	3,14610
For how many hours per day is the horse standing in the paddock?	Experimental	8	5,6250	8,81456	3,11642
	Control	7	5,7143	9,19627	3,47586
For how many hours per day is the horse standing in the pasture?	Experimental	8	13,3750	11,69783	4,13581
	Control	7	9,8571	10,77696	4,07331
How much roughage does the horse get on a daily basis?	Experimental	8	1,3875E10	3,49712E10	1,23642E10
	Control	7	2,8571E9	4,87950E9	1,84428E9
How much concentrates does the horse get on a daily basis?	Experimental	8	200,0000	220,38927	77,91937
	Control	7	535,7143	887,27781	335,35949
What is the average trainings intensity per day (minutes)?	Experimental	8	55,6250	34,37581	12,15368
	Control	7	47,8571	8,59125	3,24719
What is the average trainings intensity per week (days)?	Experimental	8	3,7500	2,25198	,79620
	Control	7	4,4286	1,13389	,42857
What is the horses' heart girth (cm)?	Experimental	8	177,5625	12,52520	4,42833
	Control	7	181,4500	22,52833	8,51491
What is the horses' abdominal girth (cm)?	Experimental	8	200,0125	14,63220	5,17326
	Control	7	203,3929	24,24485	9,16369
What is the horses' body condition score (front region)?	Experimental	8	7,0375	1,22350	,43257
	Control	7	7,3571	,89974	,34007
What is the horses' body condition score (mid region)?	Experimental	8	7,3750	,95431	,33740
	Control	7	7,5714	,67259	,25422
What is the horses' body condition score (hind region)?	Experimental	8	7,0625	1,11604	,39458
	Control	7	7,3571	,98802	,37344
What is the horses' body condition score (mean)?	Experimental	8	7,1500	1,01559	,35907
	Control	7	7,4143	,78831	,29795
What is the horses' neck circumference?	Experimental	8	97,8750	8,84401	3,12683
	Control	7	99,1000	13,58194	5,13349

How thick is the horses' neck?	Experimental	8	6,8125	1,46963	,51959
	Control	7	6,6143	1,13788	,43008
What is the horses' crest neck score?	Experimental	8	2,1250	,69437	,24550
	Control	7	2,5000	1,25831	,47559
How much does the horse weigh (kg)?	Experimental	8	455,9375	94,82745	33,52657
	Control	7	500,2143	154,63313	58,44583

Frequencies of the observed symptoms/ indicators of the whole sample and the percentage of improvement in both the experimental and control group.

	N	Percentage	Improvement experimental	Improvement control
1. Problems with shedding	6	40,0%	100% (n=4)	0% (n=0)
2. Problems with getting into heat	7	46,7%	100% (n=5)	0% (n=0)
3. Local fat accumulations (neck, shoulder, tail head)	14	93,3%	100% (n=8)	33% (n=2)
4. Local fat accumulations at the neck	13	86,7%	86% (n=7)	0% (n=0)
5. Local fat accumulations at the shoulder	14	93,3%	88% (n=7)	33% (n=2)
6. Local fat accumulations at the belly	13	86,7%	100% (n=7)	33% (n=2)
7. Local fat accumulations at the tail head	12	80,0%	57% (n=4)	33% (n=2)
8. Local fat accumulations at the buttock	12	80,0%	71% (n=5)	0% (n=0)
9. Fat bulbs over the body	11	73,3%	83% (n=5)	0% (n=0)
10. Hard neck	13	86,7%	57% (n=4)	17% (n=1)
11. Getting fat easily and loosing fat hardly	14	93,3%	88% (n=7)	17% (n=1)
12. Sensitive hooves	10	66,7%	60% (n=3)	0% (n=0)
13. Stiffness	7	46,7%	100% (n=4)	33% (n=1)
14. Itchy/eczema	3	20,0%	50% (n=5)	0% (n=0)
15. Change of eczema compared to the previous year	11	73,3%	83% (n=5)	40% (n=2)
16. Coughing	4	26,7%	67% 9 (n=2)	0% (n=0)
17. Sensitive on the skin	12	80,0%	88% (n=7)	0% (n=0)
18. Behaviour: easily irritated	11	73,3%	83% (n=5)	20% (n=1)
19. Behaviour: nervous	3	20,0%	50% (n=1)	0% (n=0)
20. Unwilling/unable to work	7	46,7%	100% (n=4)	33% (n=1)
21. Slow/dull	7	46,7%	80% (n=4)	50% (n=1)
22. Back problems	3	20,0%	/	33% (n=1)
23. Beginning to sweat quickly and a lot	6	40,0%	100% (n=4)	0% (n=0)
24. Fat accumulation above the eyes	3	20,0%	0% (n=0)	/
25. Getting out of breath quickly	4	26,7%	67% (n=2)	100% (n=1)

Weight

Paired sampled t-testing for the difference in weight before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	How much does the horse weigh (kg)? - How much does the horse weigh (kg)?	25,62500	11,93659	4,22022	15,64576	35,60424	6,072	7	,001

Paired sampled t-testing for the difference in weight before and after the trial within the control group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	How much does the horse weigh (kg)? - How much does the horse weigh (kg)?	7,42857	13,04616	4,93099	-4,63712	19,49426	1,507	6	,183

Independent sampled t-testing for the difference in the change of the weight between both groups

	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
difference_weight	Equal variances assumed	,024	,879	2,822	13	,014	18,19643	6,44917	4,26384	32,12902
	Equal variances not assumed			2,804	12,336	,016	18,19643	6,49037	4,09772	32,29514

Heart girth

Paired sampled t-testing for the difference in heart girth before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' heart girth (cm)? - What is the horses' heart girth (cm)?	4,75000	3,53897	1,25121	1,79135	7,70865	3,796	7	,007

Paired sampled t-testing for the difference in heart girth before and after the trial within the control group

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 What is the horses' heart girth (cm)? - What is the horses' heart girth (cm)?	1,09286	1,88468	,71234	-,65018	2,83590	1,534	6	,176

Independent sampled t-testing for the difference in the change of the heart girth between both groups

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
heart_girth_change	1,051	,324	2,441	13	,030	3,65714	1,49850	,41982	6,89446
			2,540	10,933	,028	3,65714	1,43978	,48584	6,82844

Abdominal girth

Paired sampled t-testing for the difference in abdominal girth before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' abdominal girth (cm)? - What is the horses' abdominal girth (cm)?	5,23750	5,45172	1,92747	,67975	9,79525	2,717	7	,030

Paired sampled t-testing for the difference in abdominal girth before and after the trial within the control group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' abdominal girth (cm)? - What is the horses' abdominal girth (cm)?	-,42857	4,94527	1,86914	-5,00219	4,14504	-,229	6	,826

Independent sampled t-testing for the difference in the change of the abdominal girth between both groups

	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
abdominal_girth_change	Equal variances assumed	,093	,765	2,096	13	,056	5,66607	2,70372	-,17496	11,50710
	Equal variances not assumed			2,110	12,972	,055	5,66607	2,68493	-,13563	11,46777

Body condition score

Paired sampled t-testing for the difference in BCS before and after the trial within the experimental group

	Paired Differences					t	df	Sig. (2-tailed)	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	What is the horses' body condition score (mean)? - What is the horses' body condition score (mean)?	,10000	,29277	,10351	-,14476	,34476	,966	7	,366

Paired sampled t-testing for the difference in BCS before and after the trial within the control group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' body condition score (mean)? - What is the horses' body condition score (mean)?	-,10000	,37859	,14310	-,45014	,25014	-,699	6	,511

Independent sampled t-testing for the difference in the change of the BCS between both groups

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
BCS_change	Equal variances assumed	,370	,554	1,153	13	,270	,20000	,17344	-,17470	,57470
	Equal variances not assumed			1,132	11,276	,281	,20000	,17661	-,18756	,58756

Neck circumference

Paired sampled t-testing for the difference in neck circumference before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' neck circumference? - What is the horses' neck circumference?	3,68750	3,47365	1,22812	,78346	6,59154	3,003	7	,020

Paired sampled t-testing for the difference in neck circumference before and after the trial within the control group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' neck circumference? - What is the horses' neck circumference?	-,12143	2,40275	,90815	-2,34360	2,10075	-,134	6	,898

Independent sampled t-testing for the difference in the change of the neck circumference between both groups

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
neckcircumference_change	Equal variances assumed	1,252	,283	2,431	13	,030	3,80893	1,56654	,42463	7,19323
	Equal variances not assumed			2,494	12,417	,028	3,80893	1,52742	,49331	7,12455

Neck thickness

Paired sampled t-testing for the difference in neck thickness before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	How thick is the horses' neck? - How thick is the horses' neck?	,31875	,27378	,09680	,08986	,54764	3,293	7	,013

Paired sampled t-testing for the difference in neck thickness before and after the trial within the control group

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 How thick is the horses' neck? - How thick is the horses' neck?	,07143	,52110	,19696	-,41051	,55337	,363	6	,729

Independent sampled t-testing for the difference in the change of the neck thickness between both groups

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
neckthickness_change	3,636	,079	1,174	13	,261	,24732	,21067	-,20780	,70244
			1,127	8,808	,290	,24732	,21946	-,25078	,74543

Crest neck score

Paired sampled t-testing for the difference in CNS before and after the trial within the experimental group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' crest neck score? - What is the horses' crest neck score?	,18750	,45806	,16195	-,19545	,57045	1,158	7	,285

Paired sampled t-testing for the difference in CNS before and after the trial within the control group

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	What is the horses' crest neck score? - What is the horses' crest neck score?	,00000	,50000	,18898	-,46242	,46242	,000	6	1,000

Independent sampled t-testing for the difference in the change of the CNS between both groups

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
CNS_change									
Equal variances assumed	,193	,668	,758	13	,462	,18750	,24732	-,34681	,72181
Equal variances not assumed			,753	12,343	,465	,18750	,24888	-,35310	,72810

Mann-Whitney testing for differences of observed problems and whether they became better or not

	Problems with getting into heat	Local fat accumulations (neck, shoulder, tailhead)	Local fat accumulations at the neck	Local fat accumulations at the shoulder	Local fat accumulations at the belly	Local fat accumulations at the tailhead	Local fat accumulations at the buttock	Fat bulbs over the body
Mann-Whitney U	,000	8,000	2,500	10,500	7,000	7,500	5,000	2,000
Wilcoxon W	3,000	29,000	23,500	31,500	28,000	22,500	20,000	17,000
Z	-2,449	-2,605	-2,941	-2,062	-2,463	-1,982	-2,369	-2,624
Asymp. Sig. (2-tailed)	,014	,009	,003	,039	,014	,047	,018	,009
Exact Sig. [2*(1-tailed Sig.)]	,095 ^a	,043 ^a	,005 ^a	,081 ^a	,051 ^a	,106 ^a	,048 ^a	,017 ^a

	hard neck	Getting fat easily and loosing fat hardly	Sensitive hooves	Stiffness	Itchy/eczema	Change of eczema compared to the previous year	Coughing	Sensitive on the skin
Mann-Whitney U	12,500	6,500	7,500	2,000	,500	8,000	,500	1,000
Wilcoxon W	33,500	27,500	22,500	8,000	1,500	23,000	1,500	11,000
Z	-1,437	-2,572	-1,195	-1,789	-,707	-1,499	-1,000	-2,872
Asymp. Sig. (2-tailed)	,151	,010	,232	,074	,480	,134	,317	,004
Exact Sig. [2*(1-tailed Sig.)]	,234 ^a	,020 ^a	,310 ^a	,229 ^a	,667 ^a	,247 ^a	,500 ^a	,008 ^a

	Behaviour: easily irritated	Behaviour: nervous	Unwilling/unabl e to work-slow	slow/dull	Beginning to sweat quickly and a lot	Problems with digestion	Lameness	Dandruffs	Getting out of breath quickly
Mann-Whitney U	5,500	,500	2,000	3,500	,000	,000	,000	,000	1,000
Wilcoxon W	20,500	1,500	8,000	6,500	3,000	1,000	1,000	1,000	7,000
Z	-2,003	-,707	-1,789	-,735	-2,236	-1,000	-1,000	-1,000	-,577
Asymp. Sig. (2-tailed)	,045	,480	,074	,462	,025	,317	,317	,317	,564
Exact Sig. [2*(1-tailed Sig.)]	,082 ^a	,667 ^a	,229 ^a	,571 ^a	,133 ^a	1,000 ^a	1,000 ^a	1,000 ^a	1,000 ^a

a. Not corrected for ties.

b. Grouping Variable: Experimental or control group?

