# The effect of two diets with different carbohydrate content on glucose markers in dogs

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Effekten av två dieter med olika kolhydrathalt på glukosmarkörer hos hundar

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Tiivistelmä - Referat – Abstract

Considering that dogs originate from wolves, who are carnivores, one may speculate whether high amounts of carbohydrates are beneficial to dogs' health. The aim of this master's thesis was to compare two different type of diets regarding glucose markers in dogs. Fasting blood samples were taken before and after a diet intervention for the analysis of blood glycosylated hemoglobin (HbA1c), glucose, insulin and glucagon concentrations to compare the differences between dogs fed a high-carbohydrate diet (dry food diet) and a diet containing no dietary carbohydrates (raw food diet). Also bodyweight was evaluated before and after the trial. This master's thesis was part of a larger study that investigated associations between diet and atopic dermatitis in Staffordshire bull terrier dogs at the University of Helsinki.

The dietary intervention lasted for 50-188 days (median 136 days). The high-carbohydrate diet contained: 42% carbohydrates, 23% proteins and 34% fats of total metabolic energy dry matter. Two different low-carbohydrate diets were used. One was a pork-chicken-lamb diet, which contained: 0%: carbohydrates, 25% proteins and 75% fats of total metabolic energy dry matter, and the other was a beef-turkey-salmon, which contained: 0% carbohydrates, 30% proteins and 70% fats of total metabolic energy dry matter. Water was allowed ad libitum.

The results showed that feeding a carbohydrate-rich dry food to pet dogs for 4,5 months increased the percentage of HbA1c. In contrast, a raw food diet with low carbohydrate content did not affect the percentage of HbA1c. Both blood glucose and glucagon concentrations decreased within the raw food diet group; while they were not affected in the dry food diet group. No statistical changes in insulin concentrations were found.

Based on the results of this study it can be concluded that a high-carbohydrate diet, and a low-carbohydrate, respectively, have different effects on glucose metabolism in dogs. More research is needed to understand how this affects the dog's health.



Avainsanat - Nyckelord
HbA1c, glukos, insulin, glukagon, hög-kolhydratkost, låg-kolhydratkost, torrfoder, råfoder, hund

Keywords
HbA1c, glucose, insulin, glucagon, high-carbohydrate, low-carbohydrate, dry food, raw food, dog

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Tiivistelmä - Referat – Abstract

Med tanke på att hunden härstammar från vargen, som är köttätare, kan man spekulera om det är gynnsamt för hundens hälsa att äta en kost som består till stor del av kolhydrater. Syftet med denna magisteravhandling var att jämföra två olika typer av kost, en med hög andel kolhydrater (torrfoder) och en utan kolhydrater (råfoder), hos hundar, genom att mäta olika glukosmarkörer. Fasteblodprover togs före och efter en dietintervention för att jämföra nivåer av långtidssocker (HbA1c), glukos, insulin och glukagon mellan de två kosttyperna. Även kroppsvikten utvärderades före och efter dietinterventionen. Denna magisteravhandling utgjorde en del av ett större projekt där man undersökte sambandet mellan kost och atopisk dermatit hos Staffordshire bullterrierhundar vid Helsingfors universitet.

Dietinterventionen varade i 50–188 dagar (median 136 dagar). Hög-kolhydratkosten innehöll: 42% kolhydrater, 23% proteiner och 34% fetter av den totala metaboliska energin i torrsubstans. Två olika foder användes i gruppen som åt låg-kolhydratkost. Den ena (Gris-Kyckling-Lamm) innehöll: 0% kolhydrater, 25% proteiner och 75% fetter; den andra (Nötkött-Kalkon-Lax) innehöll: 0% kolhydrater, 30% proteiner och 70% fetter, av den totala metaboliska energin i torrsubstans. Hundarna hade fri tillgång till vatten.

Resultaten visade att utfodring av hög-kolhydratkosten under 4,5 månader höjde nivåerna av HbA1c; däremot påverkades HbA1c inte av låg-kolhydratkosten. Glukosnivån i blodet sänktes hos hundarna som åt låg-kolhydratkosten; medan den hos hundarna som åt hög-kolhydratkosten inte påverkades. Glukagonnivån sänktes hos hundarna som åt låg-kolhydratkosten; medan den inte påverkades hos hundarna som åt hög-kolhydratkosten. Varken hundarna som åt hög- eller låg-kolhydratkost uppvisade några signifikanta förändringar i insulinnivåerna.

Baserat på resultaten från denna studie kan man dra slutsatsen att hög-kolhydratkost, respektive låg-kolhydratkost, har olika inverkan på glukosmetabolismen hos hundar. Mera forskning behövs för att förstå hur detta inverkar på hundens hälsa.

Avainsanat - Nyckelord
HbA1c, glukos, insulin, glukagon, hög-kolhydratkost, låg-kolhydratkost, torrfoder, råfoder, hund

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# ABBREVIATIONS

| | |
|---|---|
| ATP | Adenosine triphosphate |
| BBL | Before baseline |
| BCS | Body condition score |
| BL | Base line |
| CAD | Canine atopic dermatitis |
| cBL | Combined baseline |
| DFD | Dry food diet |
| DM | Dry matter |
| E | End |
| HbA1c | Glycosylated hemoglobin |
| High-CHO | High carbohydrate |
| Low-CHO | Low carbohydrate |
| ME | Metabolic energy |
| RFD | Raw food diet |
| T2D | Type 2 diabetes |
| T1D | Type 1 diabetes |

# 1 INTRODUCTION

Nowadays, dry food diets (DFD), kibbles, are the most common diets fed to pets, but alternative diets, like raw unprocessed diets, are rising in popularity among pet owners (Laflamme et al. 2008). These two diet types have distinct differences in macronutrient content, since raw food diets (RFD) have little or no carbohydrates at all while DFDs have high amounts of carbohydrates (de-Oliveira et al. 2008). Considering that dogs originate from wolves, who are carnivores, one can speculate whether high amounts of carbohydrates are beneficial to dogs' health or not.

Carbohydrates are the major macronutrient that determine postprandial glucose levels (Nguyen et al. 1994). Several studies have showed that consumption of diets containing higher levels of carbohydrates resulted in increased postprandial glucose and insulin levels in cats and dogs (Elliott et al. 2012, Farrow et al. 2013, André et al. 2017). Also, other studies have shown that glycosylated hemoglobin (HbA1c) levels decreased when dietary carbohydrates were restricted, indicating improved glycemic control (Westman et al. 2008).

There has been a worldwide rise in the prevalence of obesity in dogs as well as in humans (Case 2011, Di Cesare et al. 2016). Obesity in dogs and cats frequently predispose development of glucose intolerance as well as abnormal insulin response and abnormal basal insulin concentrations (Mattheeuws et al. 1984, Feldhahn et al. 1999). According to Feinman et al. (2008), an overconsumption of carbohydrates is associated with continuously high levels of glucose and insulin, which may predispose humans to insulin resistance that further leads to development of obesity and type 2 diabetes (T2D). In obese pets, it has been theorized that persistent hyperinsulinemia is an important factor that contributes to development of diabetes mellitus (Case 2011). More knowledge is needed to be able to effectively prevent and treat these diseases and therefore it is important to research the carbohydrate metabolism.

## 1.1 Typical canine diets

### 1.1.1 Dry food diet

Dry food diets consist mainly of cereal grains, by-products derived from the milling industry and by-products of animal tissues derived from meat-packing, poultry-processing and fish-canning industries (Morris and Rogers 1994). Dry food diets typically consist of 30 to 60% carbohydrates, which are mostly starches derived from cereal grains such as wheat, corn and rice (Spears and Fahey 2004, de-Oliveira et al. 2008, Case 2011). Carbohydrates are an inexpensive raw ingredient, as well as an essential ingredient in DFDs to provide a proper structure of kibbles (Hand Michael et al. 2010). Sources of protein used in DFDs are either animal- or plan-based, or a combination of these two (Case 2011). Typically grain sources of protein, used in DFDs, are gluten meal, alfalfa meal, wheat germ, flax seed meal and various forms of soy (flour, meal and grits) (Case 2011). Sources of fat used in DFDs are various types of vegetable oils and animal fats (Case 2011). Most common oils used in DFDs are corn, safflower, and soybean oil (Case 2011). Nowadays a commercially produced DFD is the most common diet consumed by dogs (Laflamme et al. 2008).

In 1860, the first commercially available pet food (a dry “dog cookie”) was created by James Spratt (Hand Michael et al. 2010). Thus, foods especially made for dogs have been manufactured for the past 150 years (Parr and Remillard 2014). Today, approximately 95% of all DFDs are manufactured by extrusion (Spears and Fahey 2004), a thermal treatment technology that was introduced in the late 1950s (Parr and Remillard 2014).

Thermal and pressure treatments improve food safety and many of the nutritive properties of DFDs (Hullár et al. 1998). Also, heating inhibits anti-nutritional components, such as trypsin, chymotrypsin and α-amylase inhibitors, in vegetable materials (Alonso et al. 2000). The shelf-life of dry food is prolonged by thermal treatments due to destruction of viable spores and bacterial contamination (van Rooijen et al. 2013). Despite many benefits of processed DFDs, there also exist

negative outcomes. Plant-based proteins, as they are cheaper, are more commonly used in DFDs than animal-based proteins. In general, animal-based proteins provide a superior amino acid composition compared to the amino acid composition supplied by plant-based proteins (Case 2011). Thermal treatments have destructive effects on proteins and thus lead to decreased digestibility of amino acids (Hickman et al. 1992, Hendriks et al. 1999, Williams et al. 2006). A lot of vitamins are also lost during thermal treatments, particularly vitamin A, vitamin E, vitamin C, thiamin ($B_1$), and folic acid ($B_9$) (Riaz et al. 2009). Cereal by-products have higher levels of mycotoxins, due to processing, compared to raw cereals (Brera et al. 2006).

### 1.1.2 Raw food diet

An alternative option to commercial DFDs are RFDs, also referred to as *bone and raw food* or *biological appropriate raw food* (BARF), *raw-meat based diets* (RMBD) or *raw animal products* (RAP) (Freeman et al. 2013, Morgan et al. 2017). These diets consist of unprocessed ingredients, such as mostly muscle meat, bones, fat and cartilage derived from other animals as well as some vegetables and fruits (Freeman et al. 2013, Gyles 2017). However, the RFDs consist of little or no carbohydrates as the raw carbohydrates are mainly used as fibers, and therefore not absorbed.

Commercial RFDs are manufactured by homogenization of the raw ingredients and then frozen into proper sizes (Freeman et al. 2013). A RFD can be homemade or commercial, of which both are intended to be nutritionally balanced and complete (Freeman et al. 2013).

Feeding dogs RFDs is continuously increasing in popularity among pet owners (Schlesinger and Joffe 2011, Parr and Remillard 2014), even though the benefits of the diet is still unclear (Freeman et al. 2013). Raw food proponents believe that the diet improves overall health and provides better skin and coat condition as well as better teeth health (Freeman et al. 2013). However risks of feeding RFDs are commonly discussed, especially the presence of enteric pathogens in raw meat, the risk of a nutritionally unbalanced diet and the hazards of internal punctures caused by bones in the diet (Freeman et al. 2013). For example, several

studies have concluded that enteric pathogens, like salmonella, are more likely to be found in a RFD than in a commercial DFD (Joffe and Schlesinger 2002, Finley et al. 2006, Ha and Pham 2006, Nemser et al. 2014).

## 1.2 Carbohydrate metabolism

### 1.2.1 The impact of carbohydrates on metabolic response

A high carbohydrate diet is considered to be inappropriate for strict carnivores, like cats, and may have negative effects on their health (Hewson-Hughes et al. 2011). De-Oliveira et al. (2008) showed that after consumption of starches, cats have a lower postprandial (after feeding) glucose and insulin response than humans and dogs, which can be explained by the metabolic peculiarities of cats causing a delayed and less pronounced effect on their blood responses. However, according to Axelsson et al. (2013) dogs have adapted genetically through domestication to have improved starch digestion.

Eating a low-carbohydrate-high-fat diet, compared to a high-carbohydrate-low-fat diet, resulted in minimal perturbations in pancreatic cell activity as well as in glucose homeostasis in a non-human primate model (Fabbrini et al. 2013). Elliott et al. (2012) showed that feed containing 25% (of metabolic energy (ME)) carbohydrates compared to 45% and 55% carbohydrates resulted in both a lower postprandial glucose peak and average glucose level in healthy dogs. Likewise, André et al. (2017) showed that a medium-carbohydrate diet (19% ME) resulted in lower postprandial insulin and plasma glucose levels compared to a high-carbohydrate diet (41% ME) in obese dogs. The authors concluded that a diet with lower carbohydrate content increased insulin sensitivity, which indicate improved control of carbohydrate metabolism (André et al. 2017).

Hill et al. (2009) concluded that a low-carbohydrate diet resulted in a higher nutrient digestibility, slower glucose release into the bloodstream and reduced carbohydrate fermentation in the large intestine compared to a high-carbohydrate diet in working dogs. A study done by Hewson-Hughes et al. (2011) on healthy dogs concluded that a diet with a higher starch content induced a greater postprandial insulin response (when compared with the pre-meal insulin concentration) than a diet with a lower starch content. However, the starch content had no

effect on plasma glucose levels (Hewson-Hughes et al. 2011). Farrow et al. (2013) also showed that a high-carbohydrate diet increased postprandial blood glucose levels in healthy cats compared to a diet high in protein or fat.

### 1.2.2 Peripheral use of carbohydrates

Carbohydrates consumed by dogs are degraded in the small intestine by enzymes into simple sugars, glucose and glucose equivalents, which are then absorbed and transported from the small intestine to the liver and further out in the body (McDonald et al. 2011).

Among other things, the body is able to balance the glucose homeostasis by regulating the rate of glucose utilization of peripheral tissues (Nordlie et al. 1999). Nguyen et al. (1994) concluded that a diet rich in starches resulted in postprandial high blood glucose and insulin levels. Elevated insulin levels increase glucose utilization of peripheral tissues (Saltiel and Kahn 2001) and stimulate glycogenesis (König et al. 2012). Glycogenesis is an anabolic process that synthesizes glycogen, which lowers blood glucose levels (Han et al. 2016). In the post-absorptive state (several hours after feeding), insulin levels decrease in response to low blood glucose levels and glucagon levels increase, which causes the liver to switch from glycogenesis to glycogenolysis (Röder et al. 2016).

### 1.2.3 Hepatic metabolism

The postprandial state, is defined as a 6 hours period that immediately follows ingestion of a meal, and the postabsorptive state corresponds to a 14-16 hours period of fasting (Poretsky 2017). The liver plays an important role in glucose production during the postabsorptive state by controlling various pathways of the glucose metabolism, such as glycogenolysis, gluconeogenesis and glycolysis (Han et al. 2016).

**Glycogenolysis** is the initial response to low blood glucose levels (Han et al. 2016). In this process, glycogen storages are broken down to maintain blood glucose levels (Han et al. 2016). The amount of carbohydrates in a diet affects glucose production mostly by modulating glycogenolysis in the post absorptive state

(Bisschop et al. 2000). A study on humans with T2D showed that a low-carbohydrate-high-fat diet improved the regulation of glucose metabolism by reducing post absorptive glycogenolysis (Allick et al. 2004). Bruijne et al. (1983) concluded that the rate of glycogenolysis under starvation was much slower in dogs than in humans and rats. Clore et al. (1995) found that hepatic glucose production increased due to five days of carbohydrate overfeeding in non-diabetic humans, despite an increase in glucose cycle activity and insulin secretion. The suppression of gluconeogenesis indicated that glucose was derived from glycogen storages in the liver by glycogenolysis (Clore et al. 1995).

During the postabsorptive phase, 80% of the glucose in the blood is released from the liver, from which 50% is due to glycogenolysis and the reminder from gluconeogenesis (Poretsky 2017). The activity of **gluconeogenesis** increases continuously with the duration of fasting, after 24 hours, as the glycogen storages become depleted, gluconeogenesis accounts for approximately 70% of all the glucose production (Poretsky 2017). Gluconeogenesis accounts over 90 % of the glucose production after 42 hours of fasting (Landau et al. 1996, Poretsky 2017). Therefore, when prolonged hypoglycemia occurs, in case of starvation, gluconeogenesis will become the primary process to sustain glucose production (Frizzell et al. 1988). Gluconeogenesis uses amino acids, glycerol and lactic acid as substrate for glucose production (Han et al. 2016). A study showed that the rate of gluconeogenesis was not affected by a high-carbohydrate diet in the post absorptive state, but increased after consumption of a very low-carbohydrate diet (Bisschop et al. 2000).

**Glycolysis** can be either a non-oxidative (producing lactate for gluconeogenesis) or oxidative, where the glucose is down to produce carbon dioxide and water (used as an energy source) (Poretsky 2017). Glycolysis is a major pathway in eliciting adenosine triphosphate (ATP) and an important catabolic process that converts glucose units into pyruvate (Han et al. 2016). Glucose is oxidized via glycolysis in the liver to supply ATP to mammalian cells (Han et al. 2016), which depend on a constant supply of glucose to meet their energy requirements (Nordlie et al. 1999).

**Other energy metabolisms** also exist in mammals. Animals that rely more on proteins as their main construction material and fats as their main energy source, such as in the RFD, use ketone bodies as their main energy supply (Manninen 2004, Paoli et al. 2013). In this thesis we will focus mainly on the glucose metabolism and their markers.

## 1.3 Hormonal regulation and indicators for glucose metabolism in blood

### 1.3.1 Glucose

Glucose is a simple sugar unit derived from carbohydrates. It is a source of energy for mammalian cells but can also cause problems at high blood glucose levels (König et al. 2012). The blood glucose level is tightly regulated, despite periods of feeding and fasting, to ensure a constant supply of energy and at the same time avoid damages associated with high blood glucose levels (Saltiel and Kahn 2001, König et al. 2012). The balance of glucose homeostasis is mainly achieved by two counter-regulatory hormones, insulin and glucagon (Saltiel and Kahn 2001).

Boden et al. (2005) concluded that consumption of a low-carbohydrate diet for two weeks in obese humans with T2D resulted in improved 24-hour glucose profiles. Another study also showed that a low-carbohydrate diet as well as a low-glycemic diet led to improvements in fasting glucose levels in humans (Westman et al. 2008). In addition, Shai et al. (2008) showed that diabetic humans that consumed a Mediterranean diet (rich in fiber and with a high ratio of monounsaturated to saturated fat), compared to a low-fat and a low-carbohydrate diet, had decreased fasting glucose levels while healthy humans showed no significant change in fasting glucose levels. Interestingly, insulin levels decreased significantly in all the diet groups including both healthy and diabetic humans (Shai et al. 2008).

### 1.3.2 Glycosylated hemoglobin

Glycosylated hemoglobin (HbA1c) can be used to evaluate average blood glucose levels over 2-3 months prior to sampling in both humans and dogs (Mortensen and Christophersen 1983, Marca and Loste 2001). HbA1c is formed through an insulin-independent, non-enzymatic and irreversible process where hemoglobin is exposed to plasma glucose (Oikonomidis et al. 2018), which then binds to the N-terminal amino groups of the beta chain of the hemoglobin (Bunn et al. 1976).

In mammals, non-enzymatic glycosylation of hemoglobin is primarily determined by red cell life span, red cell glucose permeability and the average plasma glucose level (Higgins et al. 1982). HbA1c levels accumulate continuously and slowly during the life span of erythrocytes (Bunn et al. 1976). However, red cell glucose permeability is lower in dogs compared to humans, thus lower HbA1c values are expected (Higgins et al. 1982). The dry spot method has not yet been validated in dogs. However, according to Goemans et al. (2017), measurements of HbA1c in dogs were proven reliable at least when using an immunoturbidimetric assay (Goemans et al. 2017).

A study, comparing a low-carbohydrate and a low-fat diet in humans with T2D, concluded that the HbA1c levels decreased significantly after 6 months within the low-carbohydrate diet group. Although, afterwards the HbA1c levels gradually increased and returned to baseline levels at 24 months into the trial (Guldbrand et al. 2012). In another study, done by Shai et al. (2008), it was showed that a low-carbohydrate diet consumed by humans with T2D had a significant decrease in HbA1c levels compared to a Mediterranean and a low-fat diet. Likewise, Westman et al. (2008) concluded that a low-carbohydrate diet consumed by humans with T2D (for 24 weeks) had a greater reduction of HbA1c levels compared to a low-glycemic diet. A study on humans with type 1 diabetes (T1D) showed that, compared to a standard carbohydrate diet, consumption of a low carbohydrate diet resulted in a significant decrease of HbA1c levels as well as a reduced requirement of insulin use (Krebs et al. 2016). Boden et al. (2005) found that consumption of a low-carbohydrate diet for two weeks in obese humans with T2D resulted in decreased HbA1c levels. Furthermore, Tay et al. (2015) concluded

that both a low-carbohydrate diet and a high-carbohydrate diet resulted in substantial weight loss as well as reduced HbA1c and fasting glucose levels in humans with T2D. The authors also found that the low-carbohydrate diet resulted in greater improvements in the blood glucose stability, and reduced the requirement of diabetes medication (Tay et al. 2015).

### 1.3.3 Hormonal regulation by insulin and glucagon

Insulin, a peptide hormone secreted from the beta cells of the pancreas during hyperglycemia, is the sole hormone that lowers blood glucose levels, while there are multiple glucose increasing hormones, of which glucagon is the major counter-regulating hormone to insulin (König et al. 2012). Glucagon is a peptide hormone and is secreted from the alpha cells of the pancreas during hypoglycemia (McDonald et al. 2011).

Insulin promotes storage and synthesis of carbohydrates, proteins and lipids by stimulating the uptake of glucose, amino acids and fatty acids into cells, as well as inhibits the degradation of these macronutrients (Figure 1) (Saltiel and Kahn 2001, Feinman and Volek 2008). In the liver, during hyperglycemia, insulin increases the activity of glucose utilizing pathways and decreases the activity of glucose producing pathways, whereas glucagon has opposite effects during hypoglycemia (Figure 2) (König et al. 2012). Glucagon is shown to enhance the energy expenditure and reduce food intake effects the food intake (Manninen 2004).

Therefore during hyperglycemia, insulin increases the requirement of glucose (GLUT4) receptors and promotes storage of hepatic glycogen as well as inhibits gluconeogenesis and glycogenolysis (Feinman and Volek 2008). The state of insulin resistance, however, leads to disruptions in these processes and causes persistent gluconeogenesis and increased lipolysis resulting in hyperglycemia and increased unoxidized plasma fatty acids (Feinman and Volek 2008).

Insulin resistance causes reduced effectiveness of insulin signaling and therefore impaired glucose uptake into the cells in humans (Reaven 1988, Feinman and Volek 2008), hence elevated postprandial glucose levels (Saltiel and Kahn 2001)

and fasting plasma insulin levels (Kolterman et al. 1980). The human body responds to insulin resistance by compensatory overproduction of insulin and thus develops hyperinsulinemia until the pancreas is no longer able to produce enough insulin to overcome the resistance in the peripheral tissues in (Reaven 1988, Feinman and Volek 2008). This state often leads to development of T2D in humans (Cahová et al. 2007). Moreover, Schulze et al. (2004) concluded that a diet containing high amounts of rapidly absorbed carbohydrates and a low amount of cereal fiber was associated with an increased risk of T2D in humans.

Westman et al. (2008) showed that in humans, a low-carbohydrate diet as well as a low-glycemic diet led to improvements in fasting insulin levels. In another study it was concluded that consumption of a low-carbohydrate diet for two weeks in obese humans with T2D resulted in improved insulin sensitivity (Boden et al. 2005). A study done in 2004, where dogs were overfed to develop obesity and insulin resistance, showed that an increase in plasma insulin levels was associated with development of obesity (Gayet et al. 2004). Obesity often predisposes dogs to multiple health disorders, such as cardiovascular, articular and metabolic disorders (Gayet et al. 2004). Lawler et al. (2008) concluded that a fat mass deposition over 25% was associated with increased insulin resistance in dogs. Increased insulin resistance are considered to affect the lifespan negatively and increase risk of chronic diseases (Lawler et al. 2008). Moreover, Volek et al. (2009) concluded that low-carbohydrate diets were an effective approach to improve features of metabolic syndrome and risk of cardiovascular disease in humans.

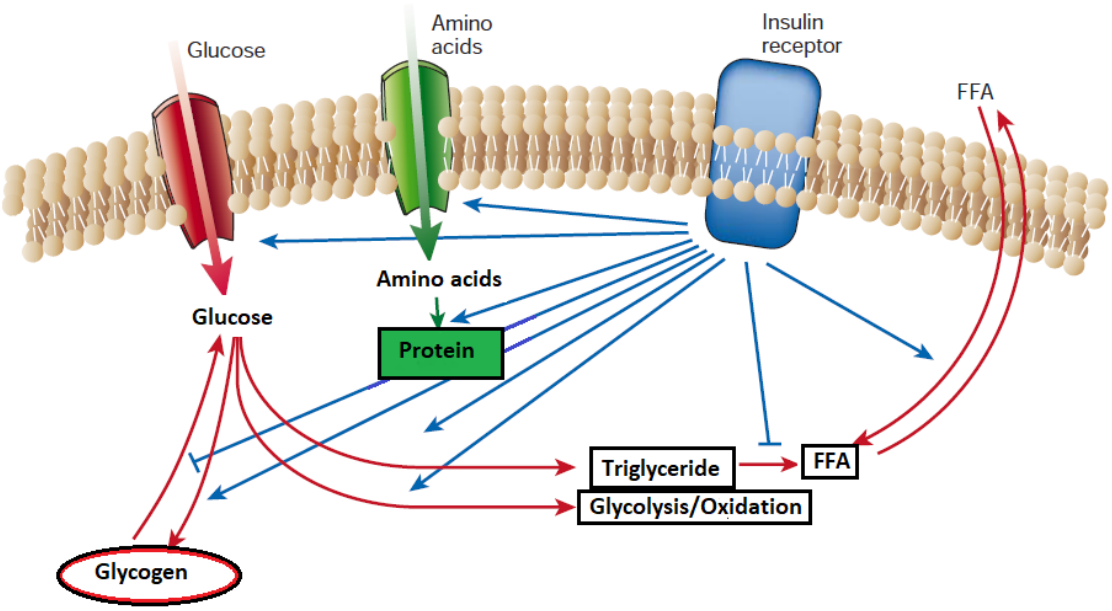


***Figure 1: The regulation of metabolism by insulin on a cellular level.*** *Insulin is an anabolic hormone and thus promotes the uptake of glucose, amino acids and fatty acids into the cells. Insulin stimulates the synthesis of glycogen, lipids and proteins, while inhibiting their degradation. Thus, insulin inhibits the degradation of glycogen into glucose and triglycerides into free fatty acids. Figure edited from Saltiel et al.* (2001)

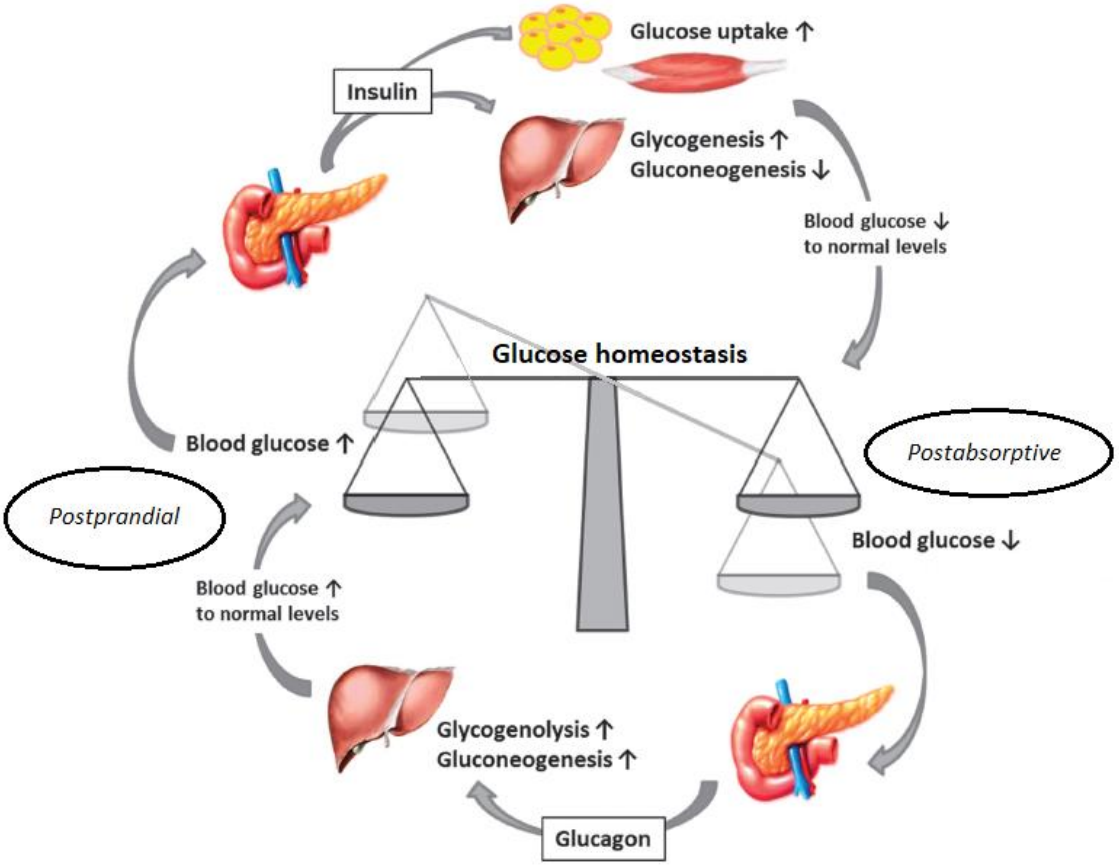


***Figure 2: Glucose homeostasis by insulin and glucagon.*** *In the postprandial state, blood glucose levels are high, which stimulates secretion of insulin. Insulin activates the process of glycogenesis and promotes glucose uptake into cells, and therefore lowers the blood glucose levels to normal. In the post-absorptive state, blood glucose levels are low which stimulate secretion of glucagon. Glucagon activates the process of glycogenolysis that increases blood glucose levels to normal levels. Figure edited from Röder et al.* (2016).

# 2 AIM OF THE STUDY

The aim of this study was to investigate glucose markers in dogs fed two different type of diets that are both commonly fed to dogs in Finland at this time: Firstly, a dry kibble type of diet being a highly processed high-carbohydrate (high-CHO) diet and secondly, a minced, mixed and frozen diet, being a minimally processed, low-carbohydrate (low-CHO) diet. In the second diet there was, however, carbohydrates in the form of fiber.

Glucose markers (glucose, HbA1c, insulin and glucagon) as well as body weight were measured before and after the dietary intervention to compare the differences between the high-CHO and the low-CHO diets.

The hypothesis was that dogs fed a high-CHO diet would show increased levels of glucose, glycated hemoglobin (HbA1c), and insulin; while dogs fed a low-CHO diet would show decreased levels of these glucose markers. We also investigated whether different CHO-levels affect blood glucagon concentrations.

# 3 MATERIALS AND METHODS

## 3.1 Study design

This study was performed as part of a larger dietary intervention study where associations between diet and atopic dermatitis in privately owned Staffordshire bullterrier dogs were studied at the University of Helsinki. Atopic and healthy dogs participated in a diet intervention trial with the intention to compare a commercial high-CHO and low-CHO diet. The results of the larger study have been published by Anturaniemi (2018)

A total of 68 dogs were registered into the study via an electric form. The owners were then contacted by phone. After the phone interview, 58 dogs were considered suitable to participate in the study. Four dog owners did not show up or were not suitable to participate in the study and were therefore excluded. Thus, the total number of animals in the beginning of the dietary intervention was 54. The dogs that entered the study were randomly divided into either a low-CHO or high-CHO diet group and stratified for previous diet, health status, and disease severity using a computerized randomisation list. At first there were 28 dogs assigned to the low-CHO diet group and 26 dogs to the high-CHO diet group. Three dogs, all from the high-CHO diet group, refused to eat their dry food diet and the owners were allowed to change into the raw food group. This was done as the diets varied at baseline anyway and as we had their baseline samples and data and did not want to lose more study subjects. Resulting in 31 dogs that participated in the low-CHO diet group and 23 dogs in the high-CHO diet group. Eight dogs discon-

tinued the diet intervention trial; Five owners chose not to continue the diet intervention trial because the diet was unsuitable for their dog (low-CHO n=2, high-CHO, n=3), one was diagnosed with immune mediated haemolytic anaemia (high-CHO diet group), one was euthanized (low-CHO diet group), and finally one owner was unreachable at the time of end visit (low-CHO diet group). The final dataset comprised a total of 46 dogs that completed the dietary intervention (Figure 3).

The study had two or three visits, depending on if an elimination diet trial was needed for the dog to be included into the study. This first inclusion visit is here called “Before baseline” (BBL) and was when some of the dogs started their elimination diet to help in diagnosing, and this was done pre-study. The baseline (BL) and end (E) visits were before and after the real diet intervention and were compulsory for all, and as explained in the text above and in the flowchart below, only dogs that came both to the BL and E visits were used in the analyses. Moreover, as these glucose markers were the last analyses to do, we lacked a lot of samples that we had just run out of. To help the situation a bit, we used both BBL and BL samples where no BL samples were left. Therefore, regarding the analyses of HbA1c, BBL and BL samples were combined (cBL) and we used 12 BBL samples. In the analysis of HbA1c one more dog was excluded, due to a lack of sample volume collected at the E visit. In the analysis of glucose eight dogs were excluded, one due to lack of samples and seven dogs who forgot to fast prior sampling. In the analysis of insulin 19 dogs were excluded, due to a lack of samples. In the analysis of glucagon 12 dogs were excluded, due to lack of samples. In the analysis of body weight measurements nine dogs were excluded, due to lack of information of bodyweight at either the BL or E visit. This left us with 45 dogs in the statistical analysis of HbA1c, 38 dogs in the statistical analysis of glucose, 27 in the statistical analysis of insulin, 34 dogs in the statistical analysis of glucagon and, 37 dogs were used in the statistical analysis of body weight measurements.

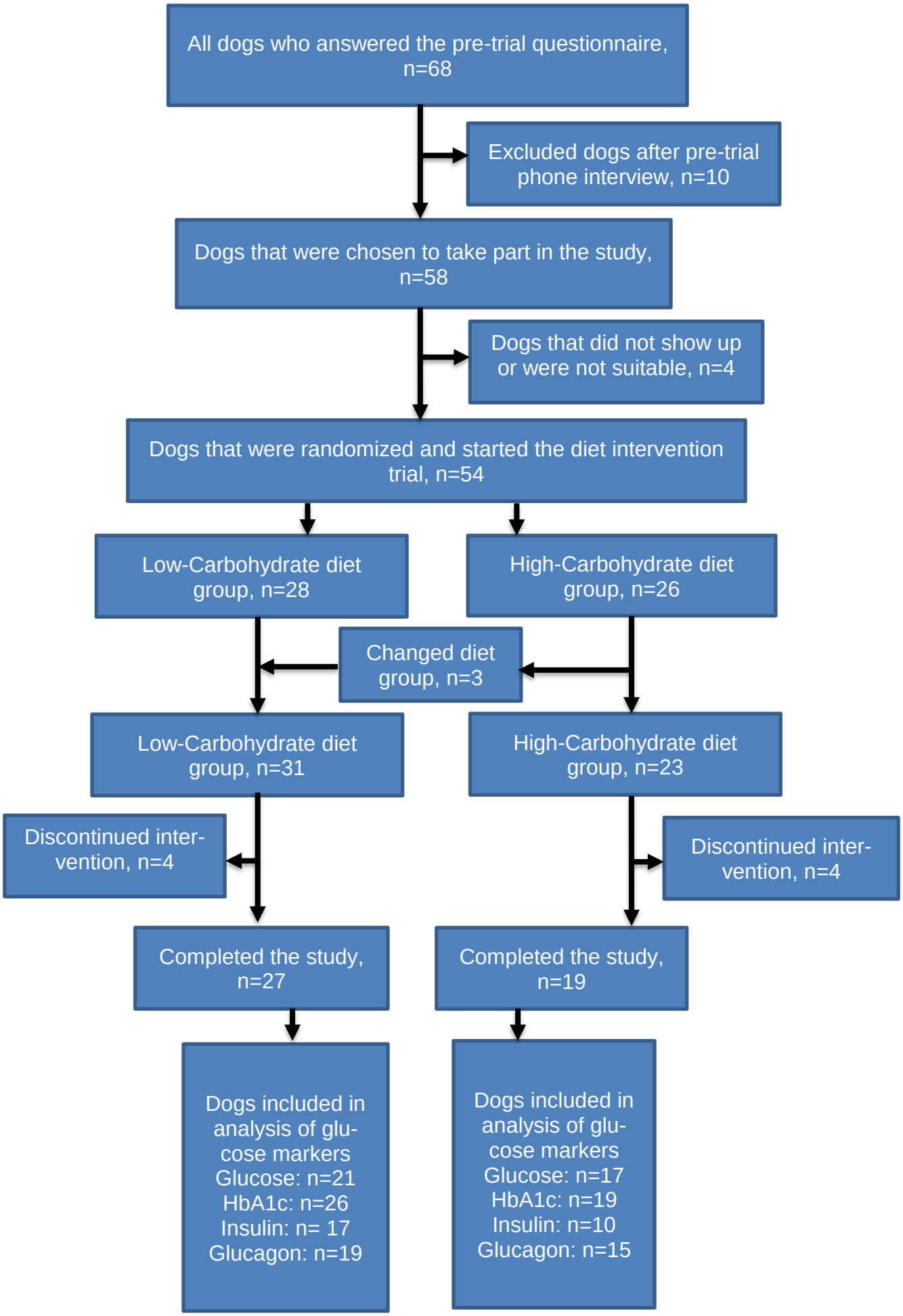


***Figure 3: Flow chart of the included and excluded dogs.***

## 3.2 Experimental diets

Three different diets were used; one commercial high-CHO diet and two different commercial low-CHO diets. Hill's Science PlanTM Canine Adult Sensitive Skin with Chicken represented the high-CHO diet. MUSH Vaisto® Pork-Chicken-Lamb and MUSH Vaisto® Beef-Turkey-Salmon represented the low-CHO diet (detailed compositions according to manufacturer shown in supplementary tables). All diets have been stated as balanced and complete by the manufacturers.

The high-carbohydrate diet contained: 42% carbohydrates, 23% proteins and 34% fats of total metabolic energy (ME) dry matter (DM). Two different low-carbohydrate diets were used. One was a pork-chicken-lamb diet, which contained: 0%: carbohydrates, 25% proteins and 75% fats of total, and the other was a beef-turkey-salmon, which contained: 0% carbohydrates, 30% proteins and 70% fats of total ME DM. Water was allowed ad libitum.

Dog owners were advised to feed the dog the proper amount according to bodyweight recommended by the manufacturer. All dogs were fed different diets before the diet intervention trial started.

## 3.3 Measurement of body weight and body condition score

The dogs were weighed on two consecutive days at the Department of Equine and Small Animal Medicine (Helsinki, Finland). The body weight of the dogs was measured using an electronic veterinary use platform balance (Model Kern EOS 150K100NXL, Kern & Sohn Gmbh, Germany) which measures with a measurement accuracy of 0,1 kg over a measurement range from 3 kg to 150 kg. The body condition score (BCS) was assessed during the BL visit. The scale (1-5) used to evaluate BCS in this study follows Hill's classifications of body scoring (Hill's 2019).

## 3.4 Animals

All dogs that participated in this study were privately owned Staffordshire bullterriers that were recruited into the trial in the Breed Club newsletter, on Facebook

and by contacting respondents of the DOGRISK questionnaire (www.ruokin-takysely.fi). Both atopic and healthy dogs participated in this study. The dogs were diagnosed as canine atopic dermatitis (CAD) according to validated scale and explained in more details in Anturaniemi (2018). The health status of the dogs is not addressed further in this study.

At BL, the average bodyweight (kg) of the dogs (n=41) was 17,79 +/- 3,31 (mean +/- S.D.) and the average age (years) of the dogs (n=45) was 5,18 +/- 2,64 (mean +/- S.D.). The average BCS of the dogs (n=44) was 2,98 +/- 0,403 (mean +/- S.D.). In the low-CHO diet group 20 dogs were neutered and 6 dogs were intact. Likewise, in the high-CHO diet group 15 dogs were neutered and 4 dogs were intact. Characteristics of the dogs, when assigned to their diet group, are shown in table 1.

***Table 1:*** *Characteristics of the dogs (n=40)*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | n | Mean | SD |
| Duration of diet intervention trial (days) | 24 | 131,96 | 32,46 | 16 | 139,31 | 18,18 |
| Age (years) | 24 | 4,98 | 2,65 | 16 | 5,72 | 2,94 |
| Weight (kg) | 24 | 17,62 | 3,30 | 16 | 18,14 | 3,50 |
| Body condition score (scale 1-5) | 24 | 3,00 | 0,42 | 16 | 3,06 | 0,25 |
| Sex (female/male) | (12/12) | | | (9/7) | | |

## 3.5 Blood metabolites and hormone concentrations

The blood samples used in this study were collected at three different occasions; At an evaluation visit BBL, BL and E of the trial. The first samples were collected between 11.4.2013 and 22.8.2013 (BBL), the second between 6.9.2013 and 8.11.2013 (BL) and the third between 25.2.2014 and 17.4.2014 (E) (Figure 3). All

blood samples were collected from the jugular vein into Vacuette® 6-10 mL plain serum tubes by a closed method (Vacutainer® Safety-Lok™ Blood collection sets, Becton Dickinson, Meylan, France). For serum samples plastic vials without a coagulant were used and for the plasma samples we used vials including Lithium heparin (Li-hep) as a coagulant. For whole blood samples we used both EDTA and Li-hep tubes. The collected serum blood was allowed to clot for a minimum of 30 min. and then centrifuged (2100 x g, 15 min.). All samples were supposed to be fasting samples but as explained before, some owners have forgotten. All samples were stored at -80 degrees in a freezer until analyzed.

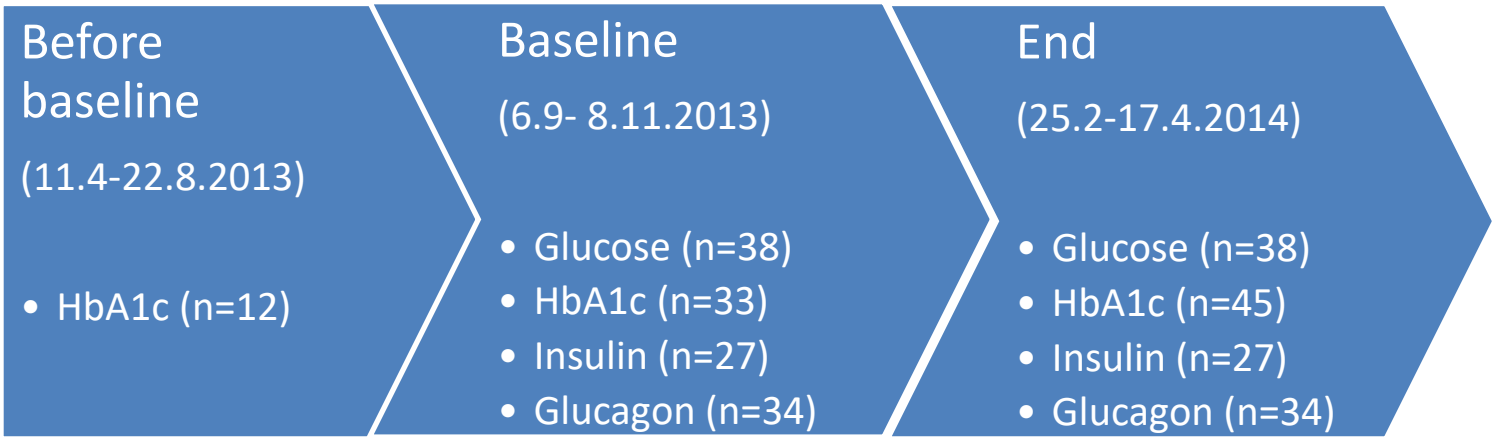


***Figure 3: Timeline of the blood samples.*** *A total of 38 samples from baseline (BL) and 38 samples from end (E) were used in the statistical analyses of glucose. The results of 12 samples from BBL (before baseline) and 33 samples from BL, which form the combined baseline, and 45 samples from E were used in the statistical analyses of HbA1c. A total of 27 samples from BL and 27 samples from E were used in the statistical analyses of Insulin. Furthermore, a total of 34 samples from BL and 34 samples from E were used in the statistical analyses of glucagon.*

### 3.5.1 Glucose

Serum was used for the analysis of glucose. Measurements were performed using Konelab 30i (ThermoFisher Scientific, Vantaa, Finland). The fotometric method used employs glucose oxidase (GOD) and a modified Trinder colour reaction, catalyzed by the enzyme peroxidase (POD). Glucose is oxidized to D-gluconate by glucose oxidase with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase, 4-aminoantipyrine and phenol

are oxidatively coupled by hydrogen peroxide to form a quinoneiminedye, coloured in red. The intensity of colour in the reaction is measured at 510 nm and it is proportional to the glucose concentration in the sample.

### 3.5.2 Glycosylated hemoglobin

Glycosylated was analyzed using a dry blood spot test. Whole blood was used for the analysis of HbA1c. For the analysis of HbA1c, the frozen samples were thawed in the refrigerator overnight and then transferred to the dry blood spot test forms after a short vortex treatment, using a pipette. When all dry blood spot test forms were filled, they were dried overnight and then sent to Baycom Diagnostics (United States) by mail. Measurements were performed using a Molecular Devices SpectraMax iD5 Multi-Mode Microplate Reader (Baycom Diagnostics, Florida, USA). The samples were analyzed in 4 replicates with a mean intra-assay CV of 2%. The Inter-assay CV was 6%. As all samples had normal range hemoglobin levels no results needed any adjustments. We did not find any validation article of this method in dogs.

### 3.5.3 Insulin

Serum was used for the analysis of insulin. Insulin was analyzed at the animal diagnostic laboratory Movet Oy (Kuopio, Finland). The insulin samples were analyzed using an immunoluminometric method (Siemens Immulite 2000, Insulin REF L2KIN2, Siemens Healthcare GmbH, Erlangen, Germany) and by a solid phase two-site bovine-spesific enzyme immunoassay method (Bo-vine Insulin ELISA, Mercodia AB, Uppsala, Sweden) with an intra-assay CV of 8.2% and an inter-assay CV of 9.5% and 7.7% for low and medium concentration, respectively.

### 3.5.4 Glucagon

The samples were collected into evacuated collection tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) and placed on ice. Blood samples were centrifuged at 2,100 x g for 15 min to separate plasma, which was then stored at -20 C for analyses of glucagon. Glucagon was analyzed at the Department of Agricultural Sciences of Helsinki University (Helsinki, Finland). Measurements

were performed using a Millipore's Glucagon Radioimmunoassay (RIA) Kit, GL-32K (Millipore, St. Charles, MO, United States).

### 3.6 Calculations and statistical analysis

All statistical analyses were performed using SPSS software (version 25, IBM Corp, Armonk, NY, USA). Normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk test. To compare glucose markers and weight at BL and E between the two diet groups, independent samples T tests was used if normality assumption held. Otherwise, differences were tested using a Mann-Whitney U test. Depending on the normality, to compare changes in glucose markers and weight measurements between BL and E within the diet groups, paired-samples T tests or a Wilcoxon Signed-rank test was used. Normality assumption held in the analysis of glucose, insulin, glucagon, and body weight measurements. However, HbA1c measurements were not normally distributed. In all test statistical significance is set at $p<0.05$ and statistical tendencies are discussed when $0.05 \leq p < 0.10$.

## 4 RESULTS

The dietary intervention lasted for 50-188 days (median 136 days), with a mean of 132,23 days in the low-CHO group (n=26) and a mean of 128,79 d in the high-CHO group (n=19). There was no difference between the two groups.

### 4.1 Glucose

There were no statistical differences in glucose levels between the two diet groups, neither at BL, nor at E (table 2). There was a statistical difference (p=0,03) in mean glucose levels between BL and E within the low-CHO diet group, so that the E values had significantly decreased compared to the BL values. However, no statistical differences were found in the mean glucose levels between BL and E within the high-CHO diet group (table 3).

***Table 2.*** *Results of glucose (mmol/l) in serum at baseline and end between diet groups.*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | | |
|---|---|---|---|---|---|---|---|
| Point of time | n | Mean | SD | n | Mean | SD | p-value |
| Baseline | 21 | 5,71 | 0,55 | 17 | 5,51 | 0,59 | 0,27 |
| End | 21 | 5,40 | 0,47 | 17 | 5,32 | 0,41 | 0,60 |

***Table 3.*** *Difference of glucose (mmol/l) in serum between baseline and end within diet groups.*

| | Baseline | | | End | | | |
|---|---|---|---|---|---|---|---|
| Diet group | n | Mean | SD | n | Mean | SD | p-value |
| Low-carbohydrate diet | 21 | 5,71 | 0,55 | 21 | 5,40 | 0,47 | 0,03 |
| High-carbohydrate diet | 17 | 5,51 | 0,59 | 17 | 5,32 | 0,41 | 0,11 |

## 4.2 Glycosylated hemoglobin

There were no statistical differences in the means of the percentage of glycolysated hemoglobin C, HbA1c, between the two diet groups, neither at cBL, nor at E (table 4). There was a statistical difference (p=0,03) in in the mean percentage of HbA1c between cBL and E within the high-CHO diet group, so that the E values had significantly increased compared to the cBL values. However, no statistical differences were found in the percentage of HbA1c between cBL and E within the low-CHO diet group (table 5).

***Table 4.*** *Results of HbA1c (%) in whole blood at baseline and end between diet groups.*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | | |
|---|---|---|---|---|---|---|---|
| Point of time | n | Mean[a] | SD | n | Mean[a] | SD | p-value |
| Combined baseline | 26 | 3,62 | 0,44 | 19 | 3,44 | 0,18 | 0,29 |
| End | 26 | 3,71 | 0,34 | 19 | 3,59 | 0,25 | 0,18 |

[a]*The percentage of the hemoglobin C fraction that is glycosylated*

***Table 5.*** *Difference of HbA1c (%) in whole blood between baseline and end within diet groups.*

| | Combined baseline | | | End | | | |
|---|---|---|---|---|---|---|---|
| Diet group | n | Mean[a] | SD | n | Mean[a] | SD | p-value |
| Low-carbohydrate diet | 26 | 3,62 | 0,44 | 26 | 3,71 | 0,34 | 0,23 |
| High-carbohydrate diet | 19 | 3,44 | 0,18 | 19 | 3,59 | 0,25 | 0,03 |

[a]*The percentage of the hemoglobin C fraction that is glycosylated*

## 4.3 Insulin

The immunoluminometric method was not suitable for analyzation of insulin concentrations in dogs, therefore we used the ELISA-method to get reliable results of insulin levels.

There were no statistical differences in mean insulin levels between the two diet groups, neither at BL, nor at E (table 6). Likewise, no statistical differences were shown between BL and E within the two diet groups. (table 7).

***Table 6.*** *Results of insulin (µIu/ml) in serum at baseline and end between diet groups.*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | | |
|---|---|---|---|---|---|---|---|
| Point of time | n | Mean | SD | n | Mean | SD | p-value |
| Baseline | 17 | 11,40 | 5,55 | 10 | 10,54 | 3,71 | 0,67 |
| End | 17 | 14,82 | 8,27 | 10 | 12,77 | 5,96 | 0,50 |

***Table 7.*** *Difference of insulin (µIu/ml) in serum between baseline and end within diet groups.*

| | Baseline | | | End | | | |
|---|---|---|---|---|---|---|---|
| Diet group | n | Mean | SD | n | Mean | SD | p-value |
| Low-carbohydrate diet | 17 | 11,40 | 5,55 | 17 | 14,82 | 8,27 | 0,13 |
| High-carbohydrate diet | 10 | 10,54 | 3,71 | 10 | 12,77 | 5,96 | 0,11 |

## 4.4 Glucagon

There were no statistical differences in mean glucagon levels between the two diet groups at BL. However, there was a statistical difference (p=0,004) between the two diet groups at E (table 8). There was a statistical difference (p=0,004) in mean glucagon levels between BL and E within the low-CHO diet group, so that the E values had significantly decreased compared to the BL values. There was no statistical difference between BL and E within the high-CHO diet group (table 9).

***Table 8.*** *Results of glucagon (pg/ml) in blood at baseline and end between diet groups.*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | | |
|---|---|---|---|---|---|---|---|
| Point of time | n | Mean | SD | n | Mean | SD | p-value |
| Baseline | 19 | 56,58 | 20,41 | 15 | 50,20 | 17,38 | 0,332 |
| End | 19 | 38,67 | 14,66 | 15 | 59,90 | 24,53 | 0,004 |

***Table 9.*** *Difference of glucagon (pg/ml) in blood between baseline and end within diet groups.*

| | Baseline | | | End | | | |
|---|---|---|---|---|---|---|---|
| Diet group | n | Mean | SD | n | Mean | SD | p-value |
| Low-carbohydrate diet | 19 | 56,58 | 20,41 | 19 | 38,67 | 14,66 | 0,004 |
| High-carbohydrate diet | 15 | 50,20 | 17,38 | 15 | 59,90 | 24,53 | 0,122 |

## 4.5 Body weight

There were no statistical differences in body weight at BL and E between the two diet groups (table 10). There was a statistical difference (p=0,02) in the body weight between BL and E within the high-CHO diet group, so that the E values had significantly increased (+0,53 kg) compared to the BL values. No statistical differences were found in the bodyweight between BL and E within the low-CHO diet group (table 11).

***Table 10.*** *Results of weight (kg) at baseline and end between diet groups.*

| | Low-carbohydrate diet | | | High-carbohydrate diet | | | |
|---|---|---|---|---|---|---|---|
| Point of time | n | Mean | SD | n | Mean | SD | p-value |
| Baseline | 20 | 17,07 | 3,22 | 17 | 18,04 | 3,42 | 0,38 |
| End | 20 | 17,01 | 3,06 | 17 | 18,57 | 3,42 | 0,15 |

***Table 11.*** *Differences of weight (kg) between baseline and end within diet groups.*

| | Baseline | | | End | | | |
|---|---|---|---|---|---|---|---|
| Diet group | n | Mean | SD | n | Mean | SD | p-value |
| Low-carbohydrate diet | 20 | 17,07 | 3,22 | 20 | 17,01 | 3,06 | 0,78 |
| High-carbohydrate diet | 17 | 18,04 | 3,42 | 17 | 18,57 | 3,42 | 0,02 |

# 5 DISCUSSION

This study showed that a high-carbohydrate, dry food diet, increased blood glycosylated hemoglobin (HbA1c) and body weight, whereas a low-carbohydrate, raw food diet, decreased blood glucose and glucagon concentrations.

The findings of an increase of HbA1c percentage within the high-CHO diet group are comparable to human studies by Boden et al. (2005), Westman et al. (2008), Shai et al. (2008) and Guldbrand et al. (2012), who concluded that restriction of carbohydrates in the diet lowers HbA1c levels in humans with T2D. However, Tay et al. (2015) concluded that both a low-CHO diet and a high-CHO diet resulted in

reduced HbA1c as well as substantial weight loss in humans with T2D. This suggest that the weight loss, not the diet changes, altered the results. Weight gain is associated with affecting the glycemic control negatively and therefore having a tendency to elevate blood glucose levels (Tomlinson et al. 2008).

In this study, the body weight increased significantly between baseline and end within the high-CHO diet group. This could be explained by the constant intake of rapidly absorbed carbohydrates that high-CHO diets consist of. This cause the body to rather oxidize carbohydrates than fat which results in accumulation of fatty acids in the body and therefore predisposes obesity (Frisancho 2003, Cahová et al. 2007). A study by Gayet et al. (2004), where dogs were overfed to develop obesity and insulin resistance, showed that an increase in plasma insulin levels was associated with development of obesity. This suggest that weight gain increases insulin levels or vice versa.

Considering that the low-CHO diet consisted of 0% carbohydrates and 70% or 75% fats of total ME DM, the insulin levels were expected to increase after the dietary intervention within the high-CHO diet group. Even though the weight of the dogs increased within the high-CHO diet group, insulin levels increased numerically more in the low-CHO diet group than in the high-CHO group. However, the number of animals included in this study is too small to make any strong conclusion about the dietary effects on changes in body weight. Also, the body weight difference was only 0.5 kg, which represents a 3% change in average body weight across all animals in the high-CHO diet group. This is a very small increase and does probably not have any physiological relevance. In addition, all included dogs had a median of 3 in body condition score, and thus no obese dogs participated in this study. Therefore, the weight observations are not considered to strongly affect the results.

The findings of a decrease in glucagon levels within the low-CHO diet group are not comparable to the findings of Manninen (2004) and Gannon et al. (2004). Manninen (2004) showed that eating a low-CHO diet was associated with increased glucagon and decreased insulin levels in humans. Likewise, Gannon et al. (2004) showed that a low-carbohydrate, high-protein diet increased plasma glucagon and decreased serum insulin in humans with T2D.

Söder et al. (2016) concluded that both glucagon and insulin increased at one hour after ingesting a high-fat-diet (51% fat, 26% carbohydrate, and 23% protein of ME) in healthy intact dogs. The authors speculated that the increased glucagon levels could be explained by the high-fat diet (Söder et al. 2016). In humans, glucagon levels decreased after ingesting pure glucose (Carr et al. 2010) and increased after ingesting pure fat (Radulescu et al. 2010). In this study, lower glucagon levels were observed in the low-CHO diet group compared to the high-CHO diet group. This could be due to the fact that dogs on a low-CHO diet have lower absorption of dietary glucose, and may have used other energy sources than glucose (i.e. ketone bodies) more efficiently as an energy source (Manninen 2004, Paoli et al. 2013). Another possibility is that the higher content of protein and fat in the low-CHO diet, compared to the high-CHO diet, have lowered the release of glucagon from the pancreas. Because the body gets proteins and fats from the diet, glucagon is not needed for release of amino acids from muscle tissue or release of fatty acids from adipose tissue (Kleinert et al. 2019).

The decrease in glucose levels within the low-CHO diet group are similar to the results of Elliott et al. (2012) and André et al. (2017), who showed that a lower amount of carbohydrates in the dogs' diet resulted in lower postprandial glucose levels. The results of glucose are also comparable to Farrow et al. (2013), who showed that a high-carbohydrate diet increased post-prandial glucose levels in healthy cats compared to a diet high in protein or fat. However, Ober et al. (2016) argue that compared to a low-fat diet and to a high-protein diet, a low-protein-high-fat diet significantly increased the glucose level in dogs.

Shai et al. (2008) concluded that consumption of a diet rich in fiber and a high ratio of monounsaturated to saturated fat, compared to a low-fat and a low-carbohydrate diet, showed decreased fasting glucose levels in diabetic humans, while in healthy humans no significant change appeared. Interestingly, insulin levels decreased significantly in both healthy and diabetic humans that consumed the diet rich in fibers (Shai et al. 2008). This would suggest that, in healthy subjects, the glucose utilization is effective enough to dispose excessive glucose, but more insulin is required to be able to maintain glucose homeostasis. Diabetic

humans on the other hand, have impaired glucose utilization and therefore glucose disposal is not as effective as in healthy humans due to increased insulin resistance. Therefore, when rapidly absorbed carbohydrates are restricted, the body is able to lower the glucose levels.

There has been a worldwide rise in the prevalence of obesity and T2D in humans as well as in dogs (Guptill et al. 2003, Di Cesare et al. 2016). A study done by Singh et al. (2015) concluded that acarbose (an anti-diabetic drug used to treat T2D) did not affect the postprandial glucose concentration much over 24 hours in healthy non-obese cats, when feeding a low-carbohydrate diet. In contrast, when a high-carbohydrate diet was fed with acarbose it reduced postprandial glucose concentrations. However, the high-carbohydrate diet with acarbose still had higher mean glucose concentrations over 24 hours compared to the low-carbohydrate diet without acarbose (Singh et al. 2015). This suggests that T2D could be treated with the diet alone. This study brings out the importance of the diet when treating diseases like T2D and although our dogs did not have T2D, the results that we got are in accordance to Singh's study.

Diabetes mellitus is a condition where a defect in pancreatic beta-cell function is present (no insulin is secreted, or too little insulin is secreted) in both humans and canines (Gilor et al. 2016). In canines this condition is quite rare, approximately 1,5% of all dogs are affected (Irvine et al. 2002); whereas in felines it is more common. This is perhaps due to the fact that felines are obligate carnivores and cannot tolerate large amounts of carbohydrates in their diets (Schermerhorn 2013) whereas canines are, to some extent at least, considered facultative carnivores, as they do have enzymes to break down carbohydrates, as opposed to felines and wolves (Axelsson et al. 2013). However, Verkest (2014) argue that obese dogs appear not to develop fasting hyperglycemia and even though insulin resistance is present, progression to T2D has not been proven to exist in dogs.

The precise function of the dog's metabolic responses regarding carbohydrate metabolism is still unknown. It is therefore unclear if the outcome of insulin resistance associated with obesity is different in dogs compared to humans. However, Monti et al. (2016) concluded that the amount of starch in the diet is a main factor affecting the postprandial glucose response in non-obese, healthy dogs,

as verified for humans. Schermerhorn (2013) argue that carnivores may be a good model for humans with T2D, due to the similarities between the human diabetes pathology and the normal metabolic processes of carnivores.

It is important to notice that the two diets in our present study differed in more ways than just the macronutrient profile, where the low-CHO diet is rich in fat and the high-CHO diet is rich in carbohydrates. Besides the fact of being raw or dry, the diets also differed in protein sources. The high-CHO diet had both animal-based (chicken, turkey and egg) and plant-based (maize gluten) protein sources, while neither of the low-CHO diets had plant-based protein sources. It is also unclear how much of the proteins in the high-CHO diet are animal-based. These differences between the low-CHO and high-CHO diet could alter the results of glucose, HbA1c, insulin and glucagon in different ways, making the results of this study difficult to interpret.

There are several limitations in this study. The limited number of dogs makes the results less reliable. The results of glucose markers might also have been affected by the dogs eating a variety of diet types prior to the diet intervention. Moreover, the dogs did not live in a controlled environment and could therefore have been exposed to other foodstuff, which could have affected the results of this study. This, however, was controlled by using a food diary. An important factor was also that not only healthy dogs participated in this study but also atopic dogs. The health status of these dogs was not considered in this study, which may have affected the result of glucose markers.

Considering that age alter the energy metabolism (Fahey et al. 2008), the glucose markers may also have been affected by the age of the dogs, which varied between 1-13 years. However, there was no significant difference in the age between the two diet groups. The energy metabolism of dogs have been shown to differ between breeds (Gomez-Fernandez-Blanco et al. 2018), although this should not be a confounding factor in this study, considering that all dogs were of the same breed. In addition, a study by Goemans et al. (2017) showed that HbA1c did not differ between breeds. Another factor that could have altered the glycemic control is stress (Kahn et al. 2001), which is challenging to measure.

The blood samples used in this study were collected during 2013-2014 and have therefore been frozen for a long time, which could have altered the glucose markers. Moreover, the reliability of the dry spot method is still unclear, as the method is not validated. The duration of the dietary intervention varied between 50-188 days, which could have had an impact on the results of HbA1c, considering that the average lifespan of the dog's erythrocyte is 86-106 days (Cline and Berlin 1963) and HbA1c accumulate throughout the lifespan of the erythrocyte (Bunn et al. 1976).

In future research it would be interesting to look at the ketone bodies in the blood as well as liver values such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and alkaline phosphatase (AFOS), as there were more than 70% of the ME from fat in the low-CHO diets. The findings in this study are difficult to interpret since the two diets differ in more ways than just the carbohydrate content. Therefore, more research is needed to be able to find out the exact reasons behind these findings.

# 6 CONCLUSIONS

This master's thesis presents information about the effects of a high-carbohydrate (dry food diet) and a low-carbohydrate (raw food diet) diet on glucose markers in dogs. The results showed that feeding a carbohydrate-rich dry food to pet dogs for 4,5 months increased the percentage of HbA1c. In contrast, a raw food diet with low carbohydrate content did not affect the percentage of HbA1c. Both blood glucose and glucagon concentrations decreased within the raw food diet group; while they were not affected in the dry food diet group. No statistical changes in insulin concentrations were found. Based on the results of this study it can be concluded that a high-carbohydrate diet, and a low-carbohydrate, respectively, have different effects on glucose metabolism in dogs. More research is needed to understand how this affects the dog's health.

# 7 ACKNOWLEDGEMENTS

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# SUPPLEMENTARY TABLES

**Table 1.** Composition and analytical constituent of food Hill's Science PlanTM Canine adult sensitive skin with chicken.

**Composition**: chicken (minimum chicken 23%, chicken and turkey combined 31%), ground rice, ground maize, chicken and turkey meal, maize gluten meal, dried whole egg, vegetable oil, flaxseed, digest, animal fat, potassium chloride, DL-methionine, salt, L-lysine hydrochloride, L-tryptophan, vitamins and trace elements. Naturally preserved with mixed tocopherols, citric acid and rosemary extract.

| **Analytical Constituent** | **In food** | **In dry matter** |
|---|---|---|
| Protein (%) | 25.3 | 27.5 |
| Fat (%) | 16 | 17.4 |
| Carbohydrate (NFE) (%) | 44.5 | 48.4 |
| Fiber (crude) (%) | 1.3 | 1.4 |
| Ash (%) | 4.9 | 5.3 |
| Moisture (%) | 8 | - |
| Calcium (%) | 0.66 | 0.72 |
| Phosphorus (%) | 0.58 | 0.63 |
| Calcium : Phosphorus | 1.1 | 1.1 |
| Sodium (%) | 0.35 | 0.38 |
| Potassium (%) | 0.64 | 0.7 |
| Magnesium (%) | 0.07 | 0.08 |
| Omega-3 fatty acids (%) | 1.2 | 1.3 |
| Omega-6 fatty acids (%) | 4.8 | 5.2 |
| ADDED per kg: | | |
| Vitamin A (IU) | 9600 | 10435 |
| Vitamin D (IU) | 480 | 522 |
| Vitamin E (mg) | 600 | 652 |

| | | |
|---|---|---|
| Vitamin C (mg) | 70 | 76 |
| Iron (mg) | 53.7 | 58.4 |
| Iodine (mg) | 0.9 | 1.0 |
| Copper (mg) | 5.3 | 5.8 |
| Manganese (mg) | 5.6 | 6.1 |
| Zinc (mg) | 111 | 121 |
| Selenium (mg) | 0.15 | 0.16 |
| Beta-carotene (mg) | 1.5 | 1.6 |

The diet is stated as complete diet by the manufacturer.

**Table 2.** Composition and analytical constituent of MUSH BARF Vaisto® diets.

| | | |
|---|---|---|
| **Composition (pork-chicken-lamb):** Finnish pork 46% (meat, lung, cartilage, heart, liver), Finnish chicken 29% (meat, bone, gizzard, skin, heart, cartilage, liver), Finnish lamb 20% (bone, meat, lung, cartilage, liver), vegetables 5% (spinach, broccoli, lettuce, cold-pressed sunflower oil), egg < 1%. | | |
| **Composition (beef-turkey-salmon):** Finnish beef, 47% (rumen, meat, lung, heart, cartilage, liver), Finnish turkey 38% (meat, bone, cartilage), Norwegian salmon 10% (salmon including bones), vegetables 5% (broccoli, lettuce, apple, carrot, cold-pressed sunflower oil, camelina oil). | | |
| **Analytical Constituent (pork-chicken-lamb)** | **In food** | **In dry matter** |
| Protein (%) | 15.2 | 38 |
| Fat (%) | 20 | 50 |
| Carbohydrate (NFE) (%) | 0.0 | 0.0 |
| Ash (crude) (%) | 4.20 | 10.5 |
| Fiber (crude) (%) | 0.60 | 1.5 |
| Moisture (%) | 60.0 | 0.0 |
| Phosphorus (%) | 0.65 | 1.6 |
| Calcium (%) | 1.09 | 2.7 |
| Calcium : Phosphorus | 1.7 | 1.7 |
| Analyzed ingredients from different batch per kg* | | |
| Omega-3 fatty acids (%) | | 0.4 |
| Omega-6 fatty acids (%) | | 3.8 |
| Vitamin A (IU) | | 143050 |
| Vitamin D (IU) | | 698 |
| Vitamin E (mg) | | 46.6 |
| Iron (mg) | | 123 |
| Iodine (mg) | | 1.86 |
| Copper (mg) | | 24.2 |
| Manganese (mg) | | 8.8 |
| Zinc (mg) | | 119 |
| Selenium (mg) | | 0.62 |

| Analytical Constituent (beef-turkey-salmon) | In food | In dry matter |
|---|---|---|
| Protein (%) | 15.0 | 42.5 |
| Fat (%) | 15.8 | 44.8 |
| Carbohydrate (NFE) (%) | 0.0 | 0.0 |
| Ash (crude) (%) | 3.70 | 10.5 |
| Fiber (crude) (%) | 0.80 | 2.3 |
| Moisture (%) | 64.7 | 0.0 |
| Phosphorus (%) | 0.34 | 1.0 |
| Calcium (%) | 0.45 | 1.3 |
| Calcium : Phosphorus | 1.3 | 1.3 |
| Analyzed ingredients from different batch per kg* | | |
| Omega-3 fatty acids (%) | | 1.1 |
| Omega-6 fatty acids (%) | | 2.7 |
| Vitamin A (IU) | | 80890 |
| Vitamin D (IU) | | 2130 |
| Vitamin E (mg) | | 54.4 |
| Iron (mg) | | 82.1 |
| Iodine (mg) | | 1.64 |
| Copper (mg) | | 31.5 |
| Manganese (mg) | | 7.4 |
| Zinc (mg) | | 79.6 |
| Selenium (mg) | | 0.73 |

The diets have been stated as complete by the manufacturer. * Ingredients were analyzed by the manufacturer from a different food batch and provided to the researchers by MUSH Ltd.

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