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# INFLUENCE OF NUTRITION AT YOUNG AGE ON CANINE HIP DYSPLASIA IN GERMAN SHEPHERD DOGS

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Tutkimuksen tavoitteena oli selvittää pentuajan ruokinnan vaukutusta lonkkakuvaustulokseen saksanpaimenkoirilla. Työ liittyi laajempaan lonkkatutkimusprojektiin, jonka tarkoituksena oli paikantaa lonkkadysplasiaan vaikuttavia geenejä eri roduissa, sekä selvittää lonkkadysplasian kehittymiseen ja siihen liittyvään kliiniseen oireiluun vaikuttavia ympäristötekijöitä. Kirjallisuuskatsauksessa on tietoa lonkkadysplasiasta ja sairauden syntyyn vaikuttavista ruokinnallisista tekijöistä. Tutkimusosa suoritettiin epidemiologisena eksploratiivisena tapaus-verrokki tutkimuksena. Hypoteesina tässä tutkimuksessa oli aikaisemmin tehdyn pilottikyselyn pohjalta, että raakaruokinta suojaisi koiraa lonkkadysplasian kehitykseltä.				
Lonkkadysplasia on yksi yleisimmistä ortopedisista sairauksista suurilla koiraroduilla. Se on perinnöllinen sairaus, jonka esiintymiseen myös ympäristötekijöillä on vaikutusta. Ruokinnan ja etenkin ylipainon vaikutusta lonkkadysplasian kehittymiseen on tutkittu paljon suurilla koiraroduilla, ja ylipainon on todettu olevan yksi merkittävimmistä lonkkadysplasian kehitykseen vaikutavista ympäristötekijöistä. Yleisimmin suurten rotujen pentujen ruokintaan suositellaan suurille roduille tarkoitettua teollista pentuajan kuivamuonaa. Muiden ruokintavaihtoehtojen kuten kotiruuan ja raakaruuan vaikutuksesta nivelten kehitykseen on hyvin rajoitetusti tietoa saatavilla. Silti monet omistajista valitsevat tänä päivänä ruokkia koiransa esimerkiksi raakaruualla tai antaa koiralleen raakaruokaa muun ravinnon lisänä.				
Tutkimusaineisto on peräisin DOGRISK kyselytutkimuksesta, jossa kysyttiin koiran ruokinnasta eri elämänvaiheissa. Tämän tutkimuksen kiinnostuksen kohteena oli ruokinta 2-6kk ja 6-18kk iässä. Tutkimuksen alussa kyselyyn oli tullut vastauksia 2-6 kuukauden ikää koskeviin kysymyksiin 157 ja 6-18 kuukauden ikää koskeviin kysymyksiin 130, koskien saksanpaimenkoiria, joilla kaikilla oli virallinen lonkkakuvaustulos. Tulokset käsiteltiin tilastollisesti ristiintaulukoimalla, Mann-Whitney U -testillä ja pääkomponenttianalyysillä.				
Tulosten perusteella BARF ruokinta tai raa'an lihan, raakojen sisäelinten, raakojen luiden ja rustojen, raa'an kalan, raa'an kananmunan ja raa'an naudan mahan syöttäminen pennulle osana muuta ruokavaliota suojaa saksanpaimenkoiria lonkkadysplasialta. Toisaalta kypsennetyn lihan sekä kypsennettyjen luiden ja rustojen syöttäminen tutkimuksen mukaan vaikuttaa altistavan lonkkadysplasialle. Tulokset olivat tilastollisesti merkitseviä. Teollisen kuivamuonan syöttäminen oli yleistä sekä tapaus-, että verokkiryhmissä, eikä kuivamuonan syöttämisellä tässä tutkimuksessa havaittu olevan yhteyttä lonkkadysplasiaan. Omistajia oli myös pyydetty arvioimaan, kuinka suuri osa koiran ravinnosta on kuivamuonaa, muuta teollista ruokaa, raakaruokaa ja kotiruokaa. Tulosten perusteella tervelonkkaiset koirat saivat enemmän raakaravintoa kuin lonkkadysplasiaa sairastavat koirat. Pääkomponenttianalyysissä raakaruuista koostunut komponentti oli ainoa komponenteista, joka korreloi merkitsevästi lonkkadysplasian kanssa. Korrelaatio oli negatiivinen tarkoittaen tässä tutkimuksessa suojaavaa vaikutusta. Tulosten varmistamiseksi tarvitaan vielä jatkoanalyysejä ja kliinisiä jatkotutkimuksia.				
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The aim of this study was to see if there is an association between nutrition at young age and the dog's hip screening results of either severe canine hip dysplasia (CHD) or healthy hips at the age of 18 months. This study was part of a wider CHD study in Finland which aim is to locate genes affecting development of CHD in different breeds and to find environmental factors influencing the development and the clinical sings of CHD. The literature review consists of a general overview on canine hip dysplasia and an overview on nutritional substances that have or might have an influence on the development of CHD. The clinical study part was conducted as an epidemiological explorative case-control study. The hypothesis in this study was that feeding raw food could protect large-breed dogs from CHD. This hypothesis was based on results from a pilot questionnaire that was done earlier.

Canine hip dysplasia is one of the most common orthopaedic problems seen in small animal practice. It is an inherited, developmental condition leading to osteoarthritis. Additionally to genetic factors, there is also evidence that several environmental factors such as nutrition are contributing to the development of the disease. Especially overfeeding has been shown to increase the risk for CHD. In general, the feeding of commercial food for growing large-breed puppies is advised, but there is only a minimal amount of information available about the influence of other feeding methods on developmental orthopaedic diseases, even though it nowadays is more common among dog owners to choose to feed their dogs with more unconventional diets such as the bone and raw food (BARF) and home prepared diets.

The DOGRISK questionnaire database was used and all German Shepherd Dogs with official hip screening results and adequate reported diet data were eligible for the statistical analyses. The time windows of interest in this study were the feeding at the age of 2-6 and >6-18 months. Results were analyzed by cross tabulating using Pearson Chi-square test, Mann-Whitney U-test and the Principal component analysis.

This study suggest that feeding a bone and raw food diet (BARF) or raw meat, raw offal, raw bone and raw cartilage, raw fish, raw egg and raw tripe as a supplementation to other diets or as a part of the BARF diet showed protective effect vis a vis CHD. The study also suggests that feeding cooked meat, bone and cartilage might increase the risk of CHD. Feeding of dry commercial food was common in both the case and control groups and did not show any association to CHD in this study. The proportion of BARF food fed in puppyhood, on the contrary, showed a significant difference between hip dysplastic and non-dysplastic dogs in both age groups, indicating that even if only a part of the dog's diet is raw food, it could already help protect puppies from CHD. Further analyses as well as clinical trials should be done next to test these results.

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# **1** INTRODUCTION

Canine hip dysplasia (CHD) is one of the most common orthopedic diseases in fast growing large-breed dogs<sup>1</sup>. It is an inherited, developmental condition leading to osteoarthritis<sup>2</sup>. CHD is diagnosed radiographically and it involves a lack of conformity between the head of the femur and the acetabulum <sup>2</sup>. In Finland the prevalence of CHD is over 30 % in many large-breed dogs and despite the selective breeding that has been done based on radiographic screening, no clear progress has been reported in screening results<sup>3</sup>. Additionally to genetic factors, there is also evidence that several environmental factors such as nutrition are contributing to the development of the disease <sup>4</sup>. The aim of this study was to see if there is an association between nutrition at young age and the dog's hip screening results of either severe CHD or healthy hips at the age of 18 months. The data was collected between December 2009 and October 2012 using an online questionnaire (appendix 1). The nutritional questions were asked as widely and accurately as possible to get precise information about how puppies are fed in Finland and to be able to study the possible influence of specific food items on CHD. In this study we evaluated only one breed to reduce bias. The German Shepherd dog was chosen as this work was part of a larger German Shepherd study, as it is a common breed in Finland, and as the breed has a high prevalence of CHD <sup>5-8</sup>. The hypothesis was that feeding raw food could protect large-breed dogs from CHD. This hypothesis was based on results from a pilot questionnaire that was done earlier. The bone and raw food (BARF) diet also contains high quality nutrients in their natural form, and in right proportion, and it is also closer to the diet that ancestral canines consumed in the wild. Additionally raw bones and cartilages contain lots of compounds that are already defined as chondroprotective substances and used commonly as supplementations in prevention and treatment of osteoarthritis due to CHD. This study was part of a wider CHD study in Finland which aim is to locate genes affecting development of CHD in different breeds and to find environmental factors influencing the development and the clinical sings of CHD. The literature review consists of a general overview on canine hip dysplasia and an overview on nutritional substances that have or might have an influence on the development of CHD. The clinical study part was conducted as an epidemiological explorative casecontrol study.

# 2 LITERATURE REVIEW

#### 2.1 Synovial joints

#### 2.1.1 Structure of the synovial joint

The synovial joint is formed by the articulating bony parts, a joint capsule and supporting ligaments. The articulating bone parts are coated with articular cartilage <sup>9</sup>. The joint capsule is surrounding the joint cavity filled with synovial fluid. The synovial fluid acts as a lubricant and together with the articular cartilage it reduces friction between the articulating bony ends, whereas the ligaments and muscle tendons function in balancing and stabilizing the joint <sup>10</sup>. The outer part of the joint capsule is fibrous and the inner surface is covered by a thin synovial membrane. Synovial joints provide movement and shock-absorption <sup>9</sup>.

## 2.1.2 Articular cartilage

The articular cartilage maximizes the contact area of the joint and it is thicker at the weight bearing sites. Under load the cartilage functions as a bearing substance when mechanical forces focus on the joint surfaces <sup>10</sup>. Right under the cartilage there is subchondral bone which acts as a supporting material <sup>9</sup>. Appearance of the cartilage is normally whitish or mild grey-white, uniform and smooth <sup>11</sup>. There are no blood vessels, nerves or lymphatic tissue in the articular cartilage, which means that it is dependent on diffusion from the synovial fluid for nutrition <sup>2</sup>. Only in the deepest layers, the capillaries derived from bone marrow sinusoids are nourishing the cartilage <sup>9</sup>. The lack of own blood supply also limits the maximum thickness of the cartilage <sup>2</sup>.

Approximately 70% to 80% of the weight of the articular cartilage is water which enables equal distribution of nutrients around the cartilage. In addition to water there are two main components in articular cartilage: chondrocytes and matrix <sup>9</sup>. Despite the lack of an own blood supply, cartilage is a metabolically active tissue and there are ongoing processes of

remodeling and reorganizing of matrix by chondrocytes <sup>12</sup>. Chondrocytes are mainly responsible for production, maintenance and turnover of intercellular substances found in the matrix. The matrix consists of collagen fibers, noncollagenous proteins, and proteoglycan aggregates <sup>9</sup>.

The largest proteoglycan aggregate in cartilage is aggrecan, and it is responsible for the high osmotic swelling pressure of the cartilage <sup>9</sup>. Aggrecan is composed of core proteins that are linked to a long nonpolysulfated glyconaminoglycan (GAG), hyaluronan. Additionally there are smaller, negatively charged and polysulfated glycosaminoglycans, chondroitin sulfate and keratin sulfate, attached to the core proteins. <sup>9</sup>. The amino sugar glucosamine is a precursor for GAGs <sup>13, 14</sup>. The structure of articular cartilage is shown in the Figure 1.

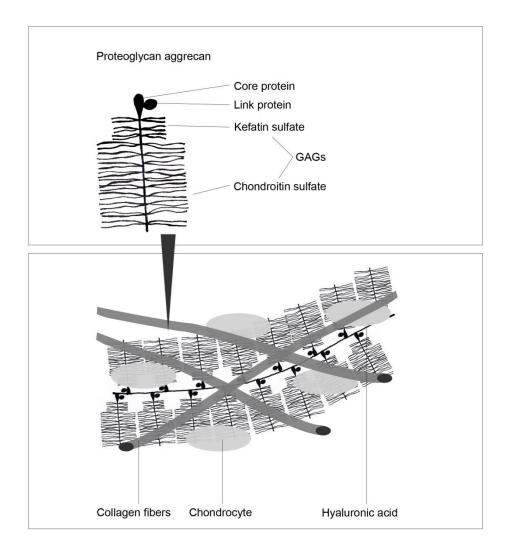


Figure 1. Structure of articular cartilage. Modified from Richardson et al 2010<sup>13</sup>.

#### 2.1.3 Joint capsule and the synovial fluid

The normal joint capsule is supplied with blood vessels, lymphatic tissue and nerves and it is formed by two layers: the peripheral fibrous layer and the thin inner layer; the synovial membrane <sup>9</sup>. The peripheral fibrous layer continues from the periosteum and consists of loose fibrous connective tissue and some adipose tissue <sup>2</sup>. It contributes to the joint stability <sup>9</sup>. The synovial membrane surrounds the entire joint cavity from the inside, except the articular surfaces. The inner layer of the synovial membrane consists of synoviocytes type A and B. Type A synoviocytes are magrophages responsible for removal of microbes and repairing the capsule after injuries, whereas type B synoviocytes produces synovial fluid <sup>9</sup>.

The synovial fluid contains hyaluronic acid, glycoprotein lubricin, proteinases and collagenase. It functions as a lubricant and takes care of the nutrition and metabolism of the chondrocytes of the articular cartilage <sup>9</sup>.

#### 2.1.4 Structure of the canine hip joint

The canine hip joint (articulation coxae, coxofemoral joint) is formed by the acetabulum and the head of the femur. The acetabulum is formed by the three bones of the pelvis: os ilium, os pubis, and os ischium. In the center of the canine acetabular cavity there is an additional bone, the small acetabular bone (os acetabula). The spheroidal shape of the joint is deepened by a fibrocartilagious band, the acetabular lip (labrum acetabulare), which is attached to the acetabular rim. The head of the femur is round-shaped and it is supposed to be congruent with the acetabulum. In addition to the bony parts, there is a joint capsule and ligaments, forming the functional hip joint <sup>15</sup>.

One of the participating ligaments is the intraarticular ligament of the head of the femur (ligamentum capitis ossis femoris, ligamentum teres), which extends from the head of the femur to the acetabular fossa. The other ligament, the transverse acetabular ligament (ligamentum transversum acetabuli), bridges the acetabular notch. The joint cavity is surrounded by a joint capsule, which is attached to the acetabular lip, to the acetabular rim and the femur. The ligament of the head of the femur is also covered by the joint capsule <sup>15</sup>. The hind legs are primary responsible for propulsion. In normal quadruped animals the hind legs bears approximately 20% of the body weight each <sup>16</sup>. The anatomic relationships of the bony components as well as the integrity of the ligaments, tendons and muscles are crucial for the motion and the weight bearing capability of the hip joint <sup>17</sup>. The schematic presentation of the canine hip joint is shown in the Figure 2.

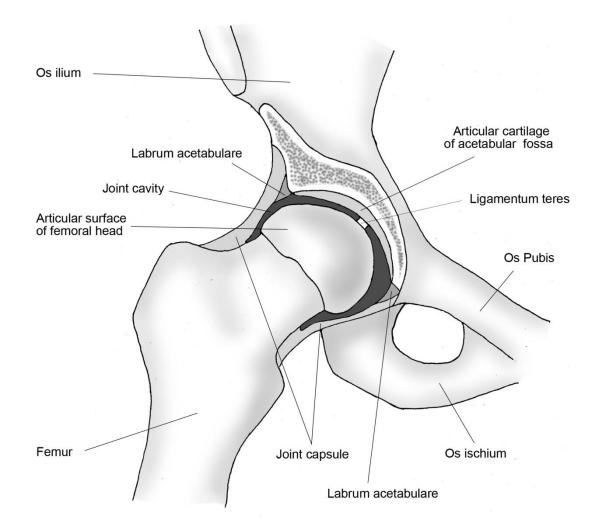


Figure 2. Schematic anatomy of the canine hip joint

In large- and giant breed dogs the longitudinal skeletal growth is fast and takes place during the first 18 months of life. It is important that the there is no disturbances during this growth phase; then congruity between the articulating bone parts of the joints can be accomplished <sup>12</sup>.

In adult dogs there is a calcified disk of cartilage called the metaphyseal growth plate, between the shaft (diaphysis) and the ends (epiphysis) of the long bones, persisting from the embryonic cartilaginous scaffold. In puppies these metaphyseal growth plates are still cartilaginous and are primarily responsible for the lengthening of bones. The growth plates are divided in three zones: resting zone, proliferative zone and hypertrophic zone. In these zones the differentiation, proliferation, maturation and hypertrophy of the chondrocytes and the mineralization of the matrix takes place in a process called endochondral ossification. In the metaphysis there is also an ongoing process of modeling and remodeling of new formed bone, accomplished by osteoblast and osteoclast <sup>9, 10</sup>.

The longitudinal skeletal growth at the metaphyseal growth plates contributes to both the length and the shape of the bone ends. The growth plates are thickest when the growth is fastest and towards maturity the growth plates become thin and are replaced by bone when no ossification longer takes place. Also at the site of the articular cartilage, the endochondral ossification stops, even though the cartilage remains and does not calcify <sup>9</sup>, <sup>10</sup>.

Both the physeal growth which elongates the bone and the modeling and remodeling of the metaphyseal bone are strictly regulated. Substances that are involved in regulating the longitudinal bone growth are hormones and growth factors like growth hormone (GH), parathyroid hormone (PTH), calcitonin, vitamin D and insulin-like growth factors (IGFs). In the growth plate GH stimulates chondrocyte differentiation, whereas PTH, vitamin D, and calcitonin, have an influence on skeletal mineralization. Maintaining the calcium homeostasis is important as calcium is a main component of the mineralized osteoid produced by the osteoblasts and chondroblasts <sup>12</sup>.

#### 2.3 Canine hip dysplasia

## 2.3.1 General information

Canine hip dysplasia (CHD) is a common orthopedic disease in large- and giant-breed dogs and has been reported as a hereditary malformance of the hip joint developing during the rapid growth period of puppyhood <sup>1, 18</sup>. It is considered a polygenic multifactorial disease with quantitative trait inheritance leading to osteoarthritis <sup>2, 8</sup>. LaFond et al. (2002) found that especially large-breed dogs such as German Shepherd Dogs, Golden retrievers, Labrador retrievers and Rottweilers have an increased risk of developing CHD <sup>19</sup>. When the inheritance of CHD was studied in four Finnish dog populations: German Shepherd Dogs, Rottweilers, Labrador retrievers and Golden retrievers, they were able to prove that the mode of inheritance of CHD was quantitative also in Finnish dog populations <sup>7</sup>. Additionally, it was suggested that a major gene with multiple minor genes affected the trait.

CHD is diagnosed radiographically and it involves a lack of congruity between the head of the femur and the acetabulum <sup>2</sup>. Despite the selective breeding that has been conducted based on radiographic evaluation, CHD is still a common orthopedic disease especially in fast-growing large-breed dogs <sup>2, 19</sup>.

#### 2.3.2 Etiology

The hereditary estimates of CHD in German Shepherd dogs varies between 0.19 and 0.27 in Germany  $^{20}$  and 0.306 to 0.354 in Finland <sup>6</sup>. The hereditability of CHD has been considered to be high enough to breed the trait according to phenotype  $^{21}$ .

In a study of Guo et al (2011) it was suggested that prediction of CHD could be done from genomic data before maturity, which would be advantageous to the breeding programs and then more effective in the prevention of CHD. However, the genetic prediction is not yet cost effective and therefore not available for public use; it is still awaiting to become a routine part of the breeding selection <sup>22</sup>. At the moment the so called

BLUP-values (Best Linear Unbiased Prediction) are used when breeding German Shepherd dogs in Finland, and also some other large breed dogs. The idea of the BLUP-values is that not only the individual phenotype of the dog, but also the phenotypes of the relatives are taken into account when choosing a suitable dog for breeding <sup>23</sup>.

Additionally to genetic factors, it is known that nutrition through balanced energy, calcium, phosphorus and vitamin intake, food electrolyte content as well as gut microbiota and immune system interaction, all play a key role in the development of joints <sup>4, 12, 24, 25</sup>. Also increased body weight, obesity or a higher body condition score might contribute to development of CHD <sup>26</sup>. Smith et al (2006) found that during growth, restricted-fed Labrador retrievers had lower prevalence and later onset of hip joint osteoarthritis, compared to ad libitum fed Labrador retrievers: median age of onset of hip joint osteoarthritis was significantly lower in the ad libitum fed group (6 years) than in restricted-fed group (12 years) <sup>27</sup>. Also, the symptoms of osteoarthritis have been noticed to decline after weight reduction <sup>28, 29</sup>.

In a study of Boxer dogs', risk factors for developing clinical signs related to CHD included aging, high birth weight, slippery pre-weaning floor cover, and neutering <sup>30</sup>. Also, dogs born in spring or summer have shown to have better hip screening results compared to puppies born in fall and winter <sup>5</sup>. In the study of Krontveit et al. (2012) puppies at the age of 12 months that had the opportunity to exercise off-leash compared to puppies with no chance to exercise off-leash were less likely to develop signs of CHD early in life <sup>31</sup>. It is also suggested that abnormalities in pelvic muscle mass are associated with development of CHD <sup>32</sup>.

#### 2.3.3 Pathogenesis

At birth, dogs with CHD have normally developed hips, but with time they develop sings of hip join laxity, which is the main structural risk factor for development of CHD <sup>33</sup>. The lax hip joint leads to subluxation and uneven distribution of weight in the joint, later leading to abnormal development of the head of the femur and the acetabulum <sup>33</sup>. When the hips are evaluated later by radiographs, the acetabulum is characteristically flattened

and there is incongruity between the articular surfaces <sup>34</sup>. Other joints might be affected as well <sup>35</sup>.

Fast growth rate of large- and giant-breed dogs is considered to cause extra biomechanical stress to the joints, leading to dysplastic changes <sup>25</sup>. Compared to small breed dogs the articular cartilage of large rapidly growing dogs is less well supported by solid bone plates and the giant-breeds dogs also have less dense physeal spongiosa which is therefore assumed to be weaker <sup>13, 25</sup>. However, it is still unclear whether the biomechanical stress itself causes the lesions to the articular cartilage <sup>36</sup> or if small local cartilaginous lesions occur first, leading to disability to protect the joint surfaces later from weight bear and muscle pull <sup>25</sup>. Determining the precise pathogenesis behind CHD is difficult, because CHD leads to degenerative joint disease and osteoarthritis, which are common also in other joint diseases <sup>37</sup>. Even though the complete sequence of events in the development of CHD is unclear, it is known that instability in the joint leads to degenerative joint disease <sup>2, 11</sup>.

The dogs with a genetic predisposition to CHD develop increased hip joint laxity as early as in the age of 0.5 to 2 months of age <sup>33</sup>. Subsequently to hip joint laxity, proliferative synovitis starts to occur, causing extra secretion of synovial fluid and stretching of ligamentum teres, still increasing the laxity, which then predispose the joint to subluxation <sup>33</sup>. Before CHD causes any clinical sings, the sequel leading to cartilage damage, starts slowly from the weight bearing areas <sup>2, 33</sup>. The early stage changes include changes in the homogeneity of the articular cartilage <sup>2</sup>. There are fissures and roughening of the surface of the femoral head and acetabulum, which are later causing most of the clinical signs in young growing dogs, together with the stretching of the ligamentum teres <sup>2, 34</sup>. When CHD is progressing, there is marked loss of articular cartilage and exposure of the subchondral bone <sup>2</sup>. Also, the colour of cartilage turns from whitish to red or redbrown. In severe lesions there might also be sclerosis of the subchondral bone, osteophyte formation and manifestation of new periosteal bone <sup>2</sup>. Quite severe changes may occur in the articular cartilage before it causes any pain perception, because articular cartilage itself lacks nerve tissue and blood vessels <sup>38</sup>.

These changes are characteristic of osteoarthritis, which is described as a progressive disease of synovial joints, with degeneration of articular cartilage and new bone formation at the joint margins, irritating the joint <sup>34</sup>. Osteoarthritic changes can develop as early as

in the age of 4 to 6 months, but the onset and progression of the disease may vary between individual dogs  $^{33}$ . As well as the articular cartilage, also the joint capsule undergoes a variety of changes <sup>2</sup>. Gross changes of the joint capsule may include thickening of the capsule and proliferation of the fibrous connective tissue, which extends over the articular cartilage surface. Sometimes synovial villous hypertrophy can also be seen, but it is not often seen on CHD <sup>2</sup>.

On the cellular level, there is localized loss of matrix proteoglycans, glycosaminoglycans, and collagen, which result in free radical formation. Free radicals together with cartilage break down products provoke a cytokine response by synovial cells, leading to inflammation. Additionally to the release of cytokines, the damage to the synovial cell membranes itself can induce the synthesis of arachidonic acid, which furthermore can lead to production of many mediators of inflammation such as prostaglandins and leukotrienes, both capable of maintaining inflammation and causing pain perception. This makes CHD an extremely painful disease, which may lead to immobility and disability and loss of ability to cope with normal life <sup>34, 39</sup>.

#### 2.3.4 Symptoms

Clinical signs of CHD vary depending on the degree of joint destruction, the severity of the disease and the age of the dog. Young, less than 1 year old dogs have a tendency to have acute episodes of unilateral or bilateral lameness in hind legs whereas older dogs usually have intermittent or continuous hind leg lameness with a tendency to put more weight to the front legs <sup>40</sup>. Subsequent to degenerative joint disease, also other symptoms than lameness are common in more severe cases. Dogs might have problems in rising, exercise intolerance, lameness after exercise, atrophy of the pelvic musculature, and/or wobbliness in gait due to an abnormal movement of the hind legs <sup>34</sup>. General soreness of hind legs is also common and it is a disease of undulating symptoms. Older CHD dogs can sometimes be spotted as having well developed musculature in the fore legs compared to hind legs due to weight transfer <sup>40</sup>.

#### 2.3.5 Diagnosis of CHD

Diagnosing of CHD is most commonly done by radiographs. Many organizations such as The Orthopedic Foundation for Animals (OFA), Fédération Cynologique Internationale (FCI) and the British Veterinary Assosiation/ Kennel Club (BVA/KC) have their own scoring systems to evaluate CHD. Additionally, specific programs have been developed to evaluate CHD: e.g. the Pennsylvania Hip Improvement Program (PennHIP) and the Dorsolateral Subluxation Score (DSL)<sup>41</sup>.

The OFA, FCI and BVA/KC use standard ventrodorsal hip-extended radiographs taken in sedation. The hips are scored by descriptive grading methods. Radiographic evaluation criteria focus on signs of incongruence, degenerative joint disease and hip joint laxity. Differences between the programs are in evaluation methods and grading. Additional tests such as the Norberg angle measurement (NA) in the FCI can be added. The NA represents the angle of the line between the femoral head centers and the line from that center to the cranial lateral margin of the acetabulum. There are some differences in screening age recommendations, and they vary from 1 to 2 years <sup>41</sup>.

In Finland the official scoring system is based on the FCI program, where the hips are scored by a 5-point grading system from A to E. Grades A-B are considered nondysplastic and grades C-E are considered dysplastic <sup>42</sup>. Recommended screening age is over 12 months or even over 18 months in large and giant breeds <sup>41</sup>. Currently 71 breeds in Finland are using the hip-dysplasia-control program under the Finnish Kennel Club. The effectiveness of control programs has been widely discussed and the slow progress of the program in reducing the prevalence of hip dysplasia has been criticized <sup>3</sup>.

#### 2.3.6 Prevalence of CHD in Finland

According to Leppänen and Saloniemi (1999) the screening prevalence for CHD in Finland varies between 18 - 64 % depending of the breed <sup>3</sup>. In their study they found great differences in CHD prevalence between breeds: all from 2 % in smooth collies to 80 % in long-haired Saint Bernard <sup>3</sup>. Significant changes in CHD prevalence were

detected in nine dog breeds when they compared the years 1988 - 1995 to the situation before year 1988. The prevalence increased in four breeds – boxer, Doberman, German Shepherd dog and rough collie, whereas the prevalence decreased in five breeds – English cocker spaniel, flat-coated retriever, golden retriever, Labrador retriever and Rottweiler <sup>3</sup>. They also stated that the prevalence of CHD in Finland between the years 1988 – 1995 was over 30 % in 10 breeds including the German Shepher dog having a CHD prevalence of 46 % <sup>3</sup>. The prevalence of severe CHD (hip score D or E) in all breeds was 17-27 % between the years 1988 – 1995 <sup>43</sup>.

According to the Finnish Kennel Club's database the overall situation is now slightly better for the German Shepherd dog. The prevalence of CHD has been 32-37% between the years 2007-2010 and the prevalence of severe CHD (hip score D or E) has been 9 - 14 % in the German Shepherd Dogs. <sup>43</sup>.

#### 2.4 Influence of nutrition on CHD

#### 2.4.1 Energy intake

#### 2.4.1.1 General about energy and over feeding

One of the nutritional theories behind developmental orthopedic diseases is that a high calorie diet, regardless of the calorie source, leads to an excessive growth rate, excessive body weight, potentially hip joint laxity and finally to hip dysplastic changes <sup>4, 25</sup>.

It is stated that overfeeding is one of the most important nutritional risk factors for development of CHD, and a balanced overall energy intake during growth is crucial in preventing developmental orthopedic diseases <sup>25, 27, 44-46</sup>. Obesity increases forces affecting the joints and increase the risk of developmental orthopedic diseases in young growing animals <sup>13</sup>. Respectively, it has been shown that limited food consumption during growth reduced significantly prevalence of CHD <sup>47</sup>. The high energy content of food has a direct effect on growth rate due to increased nutrient supply and an indirect

effect by affecting concentrations of growth hormone, IGF-1, triiodothyroine ( $T_3$ ), thyroxine ( $T_4$ ) and insulin <sup>13</sup>.

The excess energy intake due to free choice feeding might lead to incongruence between bone growth and body growth resulting in inadequate diaphyseal shaft cross-sectional area compared to body weight and also a less dense epiphyseal cancellous bone <sup>13</sup>. Dämmrich (1991) studied the influence of ad libitum feeding on fast growing large- and giant-breed dogs. In the study unrestrictedly fed dogs were predisposed to disturbances in skeletal growth. Overnutrition was found to overstimulate skeletal growth and cancellous bone remodeling in breeds that have an inherited tendency for rapid skeletal growth. Furthermore breeds with high BMI (Body Mass Index) are known to have a high prevalence of CHD <sup>25, 48</sup>.

So is there any evidence on any energy source being superior to another? Different sources of energy are discussed in the following chapters, and listed in Table 1 together with the NRC recommendations for growing large-breed puppies <sup>49</sup>, and the approximate nutritional content of ancestral diet of canines, according to Brown (2010) <sup>50</sup>.

Nutrient	Effect on bone and joint development	NRC (% from dry mater)	Ancestral canine diet (% of calories from carbohydrates, proteins, and fats)**
Carbohydrates	Diets containing large amounts of carbohydrates might influence negatively on absorption of other nutrients.	NA* (42-68)	6
Proteins	It is important that carnivors get sufficient amounts of high quality proteins. Diet high in protein might result in increased lean body mass and later onset of osteoarthritis.	22-32	49
Fats	The high energy content of food has a direct effect on growth velocity due to increased nutrient supply, and weight gain.	10-25	44

Table 1. Summary of energy sources and their effect on skeletal growth

\*NA= NRC has no recommendation on this, but as they recommend a min-max of proteins and fats, it will be about 42-68%.

\*\*All values have been rounded to the nearest whole number and therefore the total number may not equal 100%.

NRC recommendations <sup>49</sup>. Ancestral canine diet according to Brown (2010) <sup>50</sup>.

#### 2.4.1.2 Carbohydrates

Carbohydrates are considered to have only minimal influence on CHD, except when dealing with overall reduction of food consumption, but when fed excessively they might influence negatively on absorption of other nutrients like Cu and Zn<sup>4, 13</sup>. The diet of an ancestral canine was low in carbohydrates and it is estimated that only 6 % of the energy content of the diet was carbohydrates <sup>50</sup>. Dogs are very adaptive, and as a result of living with humans, they have adapted to higher carbohydrate level in their diet. It is still a matter of discussion, how much carbohydrates should be in canine diet. According to Axelsson et al. 2013, only few genes that are involved in starch digestion and glucose uptake were under selection pressure during domestication, which indicates that the dog's digesting physiology is close to ancestral dogs and wolves, but the dogs have adapted to ingest more starch <sup>51</sup>.

## 2.4.1.3 Proteins

Proteins are the main structural components of the body <sup>52</sup>. It has been estimated that 49 % of the energy content of the ancestral canine diet was protein, and because the dog is a carnivore it is important that they get sufficient amounts of high quality proteins <sup>50</sup>. Higher protein intake has been studied as a potential preventative factor for CHD, but feeding diets with different protein levels, e.g. a low protein diet (14.6 %) and a high protein diet (31.6 % protein of dry mater), did not demonstrate differences in calcium metabolism or skeletal development <sup>53</sup>. Based on this, it is currently thought that high protein intake is not so important for normal development of the joints if all the essential amino acids are found in the diet <sup>4, 13</sup>. However, excess protein intake has not been shown to affect skeletal development negatively <sup>53</sup>.

There are, however, some specific proteins that are found to be important for the normal functioning of the cartilage tissue. Matrix GLA proteins and osteocalcin are both calciumbinding proteins that take part in the mineralization and differentiation of chondrocytes <sup>54</sup>. Both of these proteins bind calcium ions and hydroxyapatite via their glutamic acid residues (GLA –residues) in a reaction that requires vitamin K <sup>54</sup>. Studies done in mice suggest that matrix GLA protein is an important inhibitor of cartilage matrix mineralization and that is why it is taught to be important in regulation of matrix mineralization <sup>55</sup>.

# 2.4.1.4 Fats, Omega-3 and Omega-6 fatty acids

Lipids serve as an energy source for the body and are the most concentrated form of energy in pet foods. Lipids are fundamental in stabilizing all cell membranes and for hormonal synthesis. They also take part in the absorption of fat soluble vitamins. Members of the omega-3 family, such as a-linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are required for brain and retinal function. The omega-6 fatty acid family includes linoleic acid, y-linoleic acid and arachidonic acid <sup>52</sup>.

Omega-3 fatty acids are naturally found both in fish and krill as well as in some plants <sup>13</sup>, <sup>14</sup>. Omega-3 fatty acids are desaturated in the body to form EPA and DHA, which are analogs to the arachidonic acid that is an endproduct of the omega-6 fatty acids <sup>13</sup>. EPA and arachidonic acid are precursors for the eicosanoids that are the immunoregulatory molecules in the body; such as prostaglandins, thromboxanes and leukotriens. Eicosanoids formed from EPA are less vasoactive and less proinflammatory than eicosanoids formed from arachonoid acid <sup>13, 56</sup>.

Omega-3 fatty acid supplementation has already been used in the prevention and management of osteoarthritis in dogs with promising results <sup>14, 57</sup>. The help for dogs suffering from degenerative joint disease is theoretically achieved by decreasing inflammation and reducing the occurrence of microthrombi <sup>14</sup>. Some of the omega-3 fatty acids have also been found to alter the gene expression in the genes that take part in the progression of degenerative changes in the cartilage <sup>13</sup>. The ratio between omega-6 and omega-3 fatty acids in the diet is recommended to be between 10:1 and 5:1 whereas it is recommended to be 1:1 for osteoarthritic dogs <sup>13, 14</sup>. To achieve this ratio the literature recommends special diets rather than providing a supplementation <sup>13</sup>.

#### 2.4.2 Calcium and phosphorus

Another nutritional theory behind developmental orthopedic diseases is imbalanced calcium and/or phosphorus intake <sup>13</sup>. Calcium is a main component of mineralized osteoid and chondroitin-sulfate produced by the osteoblasts and chondroblasts and it is important in blood clotting, muscle function, nerve transmission and sustaining cell membrane permeability <sup>12, 13</sup>. After calcium, phosphorus is the second largest constituent of bone. Additionally phosphorus is an important factor in muscle formation, it is a structural component of nucleic acids (RNA, DNA), high-energy phosphate compounds like ATP, and cell membranes <sup>58</sup>.

Plasma calcium concentration is tightly regulated by three hormonal factors: parathyroid hormone (PTH), calcitonin and calcitriol (1,25 dihydroxycholecalciferol, active vitamin D). These hormonal factors have an important role in balancing new bone formation and bone remodeling <sup>4</sup>. PTH is a peptide hormone synthesized in the parathyroid glands and its secretion is stimulated primarily by a reduction in plasma calcium concentration. PTH mobilizes calcium from bone to maintain adequate plasma calcium concentrations <sup>12, 13</sup>. Calcitonin is produced by the thyroid and the parathyroid gland C-cells. Calcitonin is also released to maintain adequate plasma calcium concentration, but it is secreted in case of hypercalcaemia to increase mineralization of bone <sup>13</sup>. Calcitonin and PTH have an antagonistic action in bone resorption, but they both decrease the renal tubular reabsorption of phosphorus and stimulate the calcitriol synthesis <sup>12</sup>.

Vitamin  $D_3$  is metabolized to calcitriol (active vitamin  $D_3$ ) in the kidneys when there is an increase in plasma PTH, decrease in  $Ca^{2+}$  or decrease in phosphate. In bone tissue calcitriol induce osteoclastic bone resorption and in the intestines calcitriol stimulates active intestinal absorption of calcium and phosphorus. Calcitriol formation is inhibited by hypercalcemia and hyperphosphatemia <sup>12,59</sup>.

During growth phase, most of the calcium is absorbed passively from the gastrointestinal tract <sup>13</sup>. Also both active and facilitated absorption takes place and the main hormonal stimulating agent in calcium absorption is calcitriol <sup>13</sup>. As most of the calcium is absorbed passively from the gastrointestinal tract at young age, it is thought that young dogs might be unable to control excess calcium intake from the food <sup>36</sup>.

Calcium influences developmental orthopedic diseases directly by competing with other minerals, and has an indirect influence through stimulating hormonal effect (PTH or calcitonin) or acid-base balance. If large-breed dog puppies are fed with food high in calcium or high in calcium and phosphorus during growth, it leads to disturbed endochondral ossification and delayed skeletal maturation and growth of bone length <sup>12, 13</sup>.

In puppies of large-breed dogs chronic excess of calcium intake has led to hypercalcemia, resulting in retarded bone maturation and remodeling, a higher percentage of total bone volume, decrease in bone resorption cells, and retarded maturation of cartilage with disturbances in endochondral ossification <sup>60</sup>. According to earlier studies the amount of calcium in the diet rather than the imbalance in the calcium phosphorus ratio, is thought to be the main factor behind growth problems in large-breed dogs <sup>60-62</sup>.

However, an insufficient or excessive phosphorus intake affects calcium homeostasis. Chronic inadequate phosphorus intake may lead to increased calcitriol production stimulating calcium and phosphorus resorption from bone and absorption from the gastrointestinal tract, whereas excess phosphorus intake together with insufficient calcium intake may lead to nutritional secondary hyperparathyroidism. Then calcium is mobilized from bone, because excess phosphorus in serum leads to decreased ionized calcium concentration in serum due to mass action equilibrium. Thus both excess and insufficient phosphorus intake may lead to excessive osteoclasia and pathologic fractures of growing bone, and it is why the calcium-phosphorus ratio of the food is recommended to be kept between 1.1:1 to 2:1. To allow normal structural and physiologic functions it is apparent that adequate but not excessive amounts of calcium and phosphorus are required in the diet of growing young dogs <sup>13, 63</sup>. The influence of calcium and phosphorus for growing large-breed puppies <sup>49</sup>, and the approximate nutritional content of the ancestral diet of canines, according to Brown (2010) <sup>50</sup>.

Nutrient	Effect on bone and joint development	NRC	Ancestral canine diet
Calcium	Calcium influence directly developmental orthopedic diseases by competing with other minerals, and has an indirect influence through stimulating hormonal effect (PTH or calcitonin) or acid-base balance.	3g/ 1000kcal	5.7g/ 1000kcal
Phosphorus	Both excess and inadequate phosphorus intake can alter the calcium homeostasis and may lead to excessive osteoclasia and pathologic fractures of growing bone.	2.5g/ 1000kcal	3.3g/ 1000kcal
Ca:P ratio		1.2:1	1.7:1

Table 2. Summary of macrominerals and their effect on joint growth

NRC recommendations <sup>49</sup>. Ancestral canine diet according to Brown (2010) <sup>50</sup>.

#### 2.4.3 Cation-anion balance

The synovial fluid of hip dysplastic joint has a higher osmolarity and there is an increase in volume of the synovial fluid compared to disease-free hip joints <sup>13</sup>. The dietary cationanion balance in foods fed to large-breed puppies during growth has been noticed to influence the development of canine hip dysplasia <sup>4</sup>. Dietary cation-anion balance is calculated by the formula: Na<sup>+</sup> + K<sup>+</sup> - Cl<sup>-</sup>. When the combination of dietary electrolytes was kept below 23 mEq/100g dry mater, it was associated with less severe hip joint laxity <sup>13</sup>. The mechanism behind the preventative influence of a low cation-anion balance in foods fed during growth is unclear, but when the cation-anion balance is increased the net physiologic effect is alkalization whereas when the balance is lower the net effect is acidification leading to calcium loss via urine. This results in intensified osteoclasia and bone remodeling in young dogs <sup>13</sup>. This might be related to regulation of acid-base balance, calcium homeostasis and/or the osmolarity of synovial fluid <sup>4, 13</sup>.

#### 2.4.4 Other important nutritional factors in bone and joint development

#### 2.4.4.1 Hormonal functions

Other substances that are involved in regulating the longitudinal bone growth are hormones and growth factors like growth hormone (GH), and insulin-like growth factors (IGFs). GH is a peptide hormone secreted from the anterior pituitary gland. Secretion of GH is pulsatile and the main stimuli of GH secretion include physical activity, stress, fasting, catecholamines, hypoglycaemia, high protein intake and certain amino acids. The main effect of GH on the growth plate is stimulating chondrocyte differentiation. Disturbances in GH metabolism can lead to different kinds of diseases depending on the age of the animal and secreted GH values <sup>12</sup>.

The effect of GH on growth is mainly mediated by IGFs (IGF-I and IGF-II). IGF-I is produced in the liver under the influence of GH, but it has also a paracrine effect and can be produced locally in the tissues like growth plate cartilage. IGF-I effect on chondrocytes results in increased longitudinal bone growth. The most important regulators of plasma IGF-I concentrations are GH and nutrition. Energy and protein are the main stimulators of plasma IGF-I concentrations, whereas fasting is decreasing plasma IGF-I concentrations. The role of IGF-II in dogs longitudinal bone growth is still unclear <sup>12</sup>.

Thyroid hormones  $T_4$  and  $T_3$  take part in skeletal growth by influencing normal maturation of growth cartilage, penetration of capillaries, and mineralization of new formed bone. Thyroid hormones increase the metabolic rate in many tissues including bone that results in increased remodeling of bone. In the absence of thyroid hormones the long bones remain short and there are disturbances of ossification and mineralization of new formed bone <sup>13</sup>.

Sex hormones have been studied as contributors to development of CHD. Hip dysplasia can be induced by estradiol injections to pups, but also if the bitch receives estradiol injections, it has a long term negative effect on the development of the hip joints of their offsprings <sup>64</sup>. In an old study of Kasström et al. (1975) it was found that the plasma estradiol concentrations were actually lower in hip dysplastic dogs compared to dogs with

healthy hips, which does not support the idea that high estradiol concentrations could cause hip dysplasia <sup>64</sup>.

#### 2.4.4.2 Microminerals

Zinc takes part in over 200 enzyme functions and is essential for the body's normal metabolism <sup>58</sup>. Zinc is an important cofactor in connective tissue metabolism and is of practical relevance mainly with regard to skin diseases. During growth, an inadequate zinc supply can cause growth depression and skeletal disorders <sup>13</sup>. There are also some inborn or genetically defined diseases described in Alaskan malamutes and bull terriers that cause zinc deficiency leading to skeletal abnormalities and malformations together with severe skin problems <sup>13</sup>. A marginal zinc supply has been shown to result in a decrease in the zinc concentration of metaphyseal bone, but it is still unclear how such a marginal zinc supply contributes to developmental orthopedic disease <sup>13</sup>. Zinc absorption from intestines might be impaired if there is high dietary intake of phytic acid, calcium, copper, or poorly digestible carbohydrates <sup>65</sup>.

Copper is also a component in several enzymes. Because of copper's enzymatic activity, copper deficiency disturbs hematopoiesis and the maintaining of the connective tissues integrity <sup>58</sup>. In cartilage and bone tissue copper is essential for collagen and elastin cross-linking <sup>13, 66</sup>. Copper deficiency may lead to severe skeletal disease and in dogs the inadequate copper intake during growth is related to severe deformities like hyperextension of the forelegs <sup>67</sup>. To assure adequate copper intake from food, the absorption from the gastro-intestinal tract should also be considered <sup>13</sup>. It is known that high dietary calcium and zinc may cause impaired copper absorption <sup>13, 68</sup>. Also poorly digestible carbohydrates and fibers may reduce the copper absorption <sup>65, 69</sup>.

Manganese deficiency is not very commonly seen in dogs <sup>58</sup>. Like copper, manganese is essential for collagen and elastin cross-linking in cartilage and bone tissue <sup>66</sup>. Together with selenium manganese is an important cofactor for enzymes involved in biosynthesis of glycosaminoglycans and proteoglycans <sup>69, 70</sup>. Experimental dietary manganese deficiency has led to development of disproportionate, shortened and thickened long bones <sup>13</sup>.

Boron is an ultra-trace mineral and nutritional requirement for boron has not been determined. Boron influences calcium, phosphorus, magnesium, and cholecalciferol metabolism by indirectly influencing PTH activity <sup>58</sup>. In osteoarthritis boron might play a role in supporting and maintaining the structural and functional integrity of subchondral bone <sup>69</sup>. Helliwell et al (1996) found that the bone tissue of the femoral head tends to be less mineralized in osteoarthrosis than in healthy bone tissue with significantly lower concentrations of boron, lead, and zinc <sup>71</sup>. This finding indicates that boron possibly has a beneficial effect on osteochondral bone <sup>69</sup>. The influence of microminerals on joint development is summarized in Table 3 together with the NRC recommendations for growing large-breed puppies <sup>49</sup>, and the approximate nutritional content of the ancestral diet of canines, according to Brown (2010) <sup>50</sup>.

Nutrient	Effect on bone and joint development	NRC	Ancestral canine diet
Zinc	Zinc is important cofactor in connective tissue metabolism and a marginal zinc supply results in a decrease in the zinc concentration of metaphyseal bone. The influence of zinc in orthopedic diseases is unclear.	25mg/ 1000kcal	24mg/ 1000kcal
Copper	In cartilage and bone tissue copper is essential for collagen and elastin cross-linking. Copper deficiency may lead to severe skeletal diseases and in dogs the inadequate copper intake during growth is related to severe growth deformities.	2.7mg/ 1000kcal	6.0mg/ 1000kcal
Manganase	Manganese is essential for collagen and elastin cross-linking in cartilage and bone tissue. Manganese is also together with selenium an important cofactor for enzymes involved in biosynthesis of glycosaminoglycans and proteoglycans.	1.4mg/ 1000kcal	3.1mg/ 1000kcal
Boron	Boron influences calcium, phosphorus, magnesium, and cholecalciferol metabolism by influencing indirectly on PTH activity. Boron might play a role in osteoarthritis by supporting and maintaining the structural and functional integrity of subchondral bone.	NA	NA

Table 3. Summary of microminerals and their effect on joint development

\*NA= Has not been established.

NRC recommendations <sup>49</sup>. Ancestral canine diet according to Brown (2010) <sup>50</sup>.

#### 2.4.4.3 Vitamins and Antioxidants

Antioxidants are commonly used in treatment of osteoarthritis to prevent damage caused by free radicals <sup>14</sup>. Antioxidants such as superoxide dismutase, bioflavonoids, glutathione, dimethyl sulfoxide, vitamin C and E are advantageous, because of their ability to reduce inflammation, and they are naturally found in raw berries, fruits, vegetables and vegetable oils <sup>13, 14</sup>.

Free radical formation is a part of the aging process of cartilage and contributes to the progression of DJD through their ability to damage cells by oxidative injury <sup>13, 14</sup>. Damage results in depolymerization of the hyalyronic acid, destruction of the collagen, and decreased production of proteoglycans <sup>72, 73</sup>. Antioxidants are used as oral supplements for growing dogs with the purpose of supporting growth. However, we were not able to find any scientific evidence of the efficacy of such preventative use of antioxidants.

Regarding joints, vitamins are essential in normal enzyme functions and take part in regulation of oxidative stress <sup>58</sup>. They also support chondrocyte metabolism and the integrity of the extracellular matrix <sup>69</sup>. Oxidative stress is characteristic for diseases like osteoarthritis where the reactive oxygen species overwhelm the endogenous defense system sustained by vitamins and enzymes like glutathione peroxidase, superoxide dismutase, and catalase <sup>69</sup>. Reactive oxygen species has shown to influence also to reduction of collagen synthesis and cartilage metabolism <sup>74</sup>.

Vitamins are determined as organic compounds that are not fats, carbohydrates or protein. They are essential for normal physiologic function and will cause some deficiency symptoms if not provided sufficiently in food. Vitamins are classified in two groups: fatsoluble and water-soluble vitamins. Fat-soluble vitamins are vitamins A, D, E and K, whereas water-soluble vitamins are Thiamin, Riboflavin, Niacin, Pyridoxine, Pantothenic acid, Folic acid, Biotin, Vitamin B12, Choline and Vitamin C<sup>58</sup>.

Vitamin A is a group of compounds that are fat-soluble and are necessary for normal vision, growth, reproduction, immune function and maintenance of healthy epithelial tissue. Good sources of vitamin A include fish liver oil, liver, egg, and dairy products. <sup>58</sup>. Vitamin A is essential in bone metabolism, especially for osteoclastic activity. Both deficiency and oversupplementation during growth may lead to severe metabolic bone

disease in growing dogs. Excess vitamin A consumption may lead to narrowing of long bone epiphyseal cartilage, ankylosis, new bone formation without osteolysis, and thin bone cortices <sup>13</sup>.

The vitamin B group is not known to effect joint development, and is there for not discussed here.

Vitamin C in an antioxidant, and it also functions as a coenzyme in hydroxylation of proline and lysine during synthesis of collagen fibrils in cartilage <sup>4, 13</sup>. The influence of vitamin C fed during growth on CHD has been studied without any clear results <sup>4, 33</sup>. Excess vitamin C supplementation during growth is generally considered to have a minor or no effect on skeletal growth <sup>13</sup>. One old study reported that high dosages of vitamin C fed to the bitch during pregnancy and to offspring until adulthood, eliminated CHD <sup>75</sup>. In human medicine studies, a reduced risk of cartilage loss and osteoarthritis progression after oral supplementation of vitamin C, is also reported <sup>76</sup>. Knee osteoarthritis was studied in humans by analyzing their eating habits and antioxidant consumption by a questionnaire <sup>77</sup>. A higher vitamin C intake was associated with a reduced risk of bone marrow lesions and with a reduction in the tibial plateau bone area, but no significant association was found in cartilage volume or cartilage defects and antioxidant consumption <sup>77</sup>. The study suggest that both vitamin C intake and fruit consumption could be beneficial in osteoarthritis as they are associated with a reduction in bone size and the number of bone marrow lesions <sup>77</sup>. However, more well-controlled studies are needed to determine whether vitamin C supplementation is beneficial for growth and/or as a treatment for osteoarthritis. It is also important to notice that excess vitamin C consumption can perpetuate hypercalcemia, which might lead to delay of cartilage maturation and have an effect on normal bone remodeling<sup>4</sup>. However, as vitamin C is not on the list of essential nutrients for the dog, it is also very seldom added to commercial foods <sup>13</sup>. As long as there is no clear consensus about the benefits and/or disadvantages of vitamin C in regard to CHD, Richardson et al. (2010) do not recommend vitamin C supplementation for growing puppies <sup>13</sup>.

D vitamins are fat-soluble compounds that can originate from plant sources (vitamin  $D_2$ , ergocalciferol) or from animal sources (vitamin  $D_3$ , cholecalciferol) <sup>12</sup>. Cholecalciferol can be synthetized in subcutaneous skin from provitamin D following the exposure to sun light, but dogs seem to be unable to synthetize it in sufficient amounts to fulfill daily

requirements <sup>12</sup>. Good natural sources of vitamin  $D_3$  are marine fish and fish oils, but also fresh water fish and eggs are sources of vitamin  $D_3$ . Small amounts of vitamin  $D_3$  exist in liver, beef and dairy products <sup>58</sup>. Also in humans it has lately been noticed that vitamin D deficiency is more common than it was thought to be <sup>78</sup>. The D vitamins are later metabolized in the body to active vitamin  $D_3$ , which influence the body's calcium and phosphorus homeostasis discussed already in chapter 2.4.1.2. Therefore D vitamins are also important for skeletal growth <sup>13</sup>. Severe deficiency of vitamin  $D_3$  is known to lead to osteomalasia, and during the growth phase, to a disease called rickets in humans and dogs <sup>13</sup>. In an experiment with Great Dane puppies, vitamin  $D_3$  excess was shown to cause disturbances in endochondral ossification <sup>79</sup>. The active vitamin  $D_3$  has been noticed to have immunomodulatory actions, and the researchers suggest that correcting vitamin D deficiency should be an important part of the management of all human patients with joint disease <sup>80</sup>.

Vitamin E is the term for a group of compounds that have the biologic activity of alfatocopherol <sup>58</sup>. Vegetable oils, seeds and cereal grains are good sources of vitamin E <sup>58</sup>. Vitamin E functions as an antioxidant in the body and in the food. With glutathione peroxidase vitamin E works protecting cells from free radicals. Vitamin E is also important for reproduction and it takes part in cellular signaling, regulating gene transcription, modulating immune function, and inducing apoptosis <sup>58</sup>. Additionally vitamin E has been shown to enhance growth of chondrocytes in cell culture, and it reduced pain and stiffness in human studies on osteoarthritis <sup>69, 81</sup>.

The two major naturally occurring forms of vitamin K are vitamin K1 (phylloquinone) and vitamin K2 (menaquinone) <sup>58</sup>. Vitamin K1 can be synthetized in the gut in small amounts <sup>69</sup>, and natural sources of vitamin K are alfalfa meal, oilseed meals, fish meal and liver <sup>58</sup>. The most important role of vitamin K is to convert prothrombin to thrombin in normal blood clotting <sup>58</sup>. Vitamin K1 is also essential in the metabolism of glutamic acid residues which are found in GLA-proteins and osteocalcin. Both affect chondrocyte mineralization and differentiation positively, which is why vitamin K deficiency is also considered to be related to development of osteoarthritis in humans <sup>54, 82</sup>.

The influence of vitamins on joint development is summarized in Table 4 together with the NRC recommendations for growing large-breed puppies <sup>49</sup>, and the approximate nutritional content of the ancestral diet of canines, according to Brown (2010) <sup>50</sup>.

Nutrient	Effect on bone and joint development	NRC (recommendation s for puppy)	Ancestral canine diet
A	Vitamin A is essential in osteoclastic activity. Excess vitamin A consumption may lead to narrowing of long bone epiphyseal cartilage, ankylosis, new bone formation without osteolysis, and thin bone cortices.	1263 IU	15375 IU
С	Vitamin C in an antioxidant that also function as a coenzyme in hydroxylation of proline and lysine during synthesis of collagen fibrils in cartilage. There is no clear evidence on the effect of oral vitamin C supplementation on growth.	NA	NA
D	Vitamin D influences the body's calcium and phosphorus homeostasis. Severe deficiency of vitamin $D_3$ leads to osteomalasia.	136 IU	NA
Е	Vitamin E functions as an antioxidant in the body. It enhances growth of chondrocytes in cell culture.	11.3 IU	23 IU
K	Vitamin K1 is essential in the metabolism of glutamic acid residues which are found in GLA-proteins and osteocalcin that both affect positively on chondrocyte mineralization and differentiation.	NA	NA

Table 4. Summary of vitamins and their effects on joint development

 $\overline{NA} = Has not been established.$ 

NRC recommendations <sup>49</sup>. Ancestral canine diet according to Brown (2010) <sup>50</sup>.

#### 2.4.4.4 Chondroprotective substances

#### 2.4.4.1 General information on chondroprotective substances

The chondroprotective substances are defined as various compounds that have a positive effect on health and on the metabolism of chondrocytes and synoviocytes. The main effects of the chondroprotective substances are to support or enhance the metabolism of chondrocytes and synoviocytes and to inhibit catabolic enzymes within the synovial fluid and cartilage matrix. They also have an antitrombotic influence inhibiting formation of trombi in small blood vessels supplying the joints <sup>14</sup>.

Chondroprotective nutraceuticals are considered non-drug substances that are administered orally to provide compounds required for normal joint structure and function with the intent to improve health and well-being <sup>13, 14</sup>. These nutraceuticals include glucosamine, chondroitin sulfate, glycosaminoglycans (GAGs), omega-3 fatty acids, antioxidants, MSM, and sources of them like Green lipped mussel, curcuma, ginger, and uocca <sup>13,14</sup>. Chondroprotective substances have been used to treat osteoarthritis in dogs with good results, but they usually need a long treatment period <sup>14</sup>, <sup>83</sup>. Many of them are mediators of pain, but most of the mechanisms of action are still unknown or unproven <sup>14</sup>. Orally administrated GAGs have a good bioavailability in dogs, and an increase in plasma values of chondroitin sulfate can be measured after oral administration<sup>84, 85</sup>. Orally administrated chondroitin sulfate shows a tropism for cartilagineous tissues, and it is shown to have anti-inflammatory and chondroprotective actions, which further on supports the use of orally administrated GAGs <sup>86, 87,88</sup>. There are also injectable chondroprotectants that are used in the treatment of osteoarthritis, and they are also called slow-acting disease modifying anti-osteoarthritis drugs (SAMOAD): e.g. Pentosan polysulphate, Hyaluronate and Polysulfated glycosaminoglycans<sup>14</sup>.

Some of the chondroprotective substances are normal compounds of the articular cartilage, and are given as supplements for the growing puppies to support skeletal growth. Actions of chondroprotective substances are discussed in the following chapters and summarized in Table 5.

#### 2.4.4.2 Glucosamine and GAGs

Glucosamine and GAGs can be provided for the dog as supplements, but they are also available naturally in raw cartilage and in Green lipped mussel <sup>9</sup>. Glucosamine and GAGs support the regeneration of cartilage, because they are the basic components used by chondrocytes to produce the matrix of articular cartilage <sup>13</sup>.

Glucosamine is an amino sugar. It is a precursor to GAGs and normal chondrocytes are able to synthesize it <sup>13, 14</sup>. It has been shown that exogenous glucosamine stimulates proteoglycan and collagen production in chondrocytes in cell culture <sup>14</sup>. The results from human chondrocyte cell cultures support clinical observations suggesting that glucosamine may have a beneficial effect in the prevention of articular cartilage loss in treatment of osteoarthritis <sup>14, 89</sup>.

The predominant GAG in the extracellular matrix of the articular cartilage is chondroitin sulfate, but also keratin sulfate is found there in lesser amounts <sup>13, 14</sup>. Chondroitin sulfate has been shown to decrease interleukin-1 production, block complement activation, inhibit metalloproteinases, inhibit histamine-mediated inflammation and stimulate GAG and collagen synthesis <sup>14, 90, 91</sup>. Oral supplementation of GAGs has been used in treatment of osteoarthritis with promising results <sup>14</sup>. Also clinical studies in humans have shown improvement of clinical signs in osteoarthritis after receiving chondroitin sulfate supplementation <sup>92, 93</sup>.

Both glucosamine and GAGs has been shown to have chondroprotective actions, but there are no studies available of their efficacy in preventative use.

#### 2.4.4.3 Methyl-sulfonyl-methane (MSM)

MSM is derived from dimethyl sulfoxide (DMSO) and because it anecdotally has a positive effect and it contains sulfur needed for the formation of connective tissue, it has been studied as a possible chondroprotective substance <sup>14</sup>. The use of oral supplementation decreased significantly the mean pain index in humans with osteoarthritic symptoms <sup>94</sup>.

Chondroprotective substances	Explanation	Effect on joint health
GAGs	Glycosaminoglycans (GAGs) are main components of the articular cartilage matrix. The predominant GAG in the cartilage matrix is chondroitin sulfate.	Chondroitin sulfate decreases interleukin-1 production, blocks complement activation, inhibits metalloproteinases, inhibits histamine- mediated inflammation and stimulates GAG and collagen synthesis
Glucosamine	Glucosamine is an amino sugar that is a precursor to GAGs	Glucosamine stimulates proteoglycan and collagen production in chondrocytes
Omega 3 fatty acids	Omega-3 fatty acids include: a- linoleic acid and eicosapentaenoic acid (EPA) from plants, and docosahexaenoic acid (DHA) and other fatty acids from animal sources.	Decrease inflammation and reduce the occurrence of microthrombi. Some of the omega-3 fatty acids alter the gene expression in the genes that take part in the progression of degenerative changes in the cartilage.
Antioxidants	Superoxide dismutase, bioflavonoids, glutathione, dimethyl sulfoxide, vitamin C and vitamin E are compounds that protect cells from oxidative injury.	Antioxidants reduce inflammation and protect catrilage from free oxygen radicals originating from cartilage degradation.
MSM	MSM is derived from dimethyl sulfoxide (DMSO).	Can function as a sulfur donor in formation of connective tissue.

# Table 5. Effect of chondroprotective substances on joint health

#### 2.4.5 Association between puppyhood diet and orthopedic problems

Nutrition plays a key role especially in puppyhood during the first 12 months of life when the dog is growing fast and before physeal closure. This might take place even later when talking about large- and giant-breed dogs <sup>12, 13</sup>. Large- and giant-breed dogs are most susceptible to developmental orthopedic diseases because of their genetic propensity for rapid growth <sup>25</sup>. For the purpose of preventing orthopedic problems including CHD, by nutrition, it is important to understand what kind of food people are providing to their dogs and why.

When purchasing a puppy the owner nowadays has numerous sources of information about nutrition and feeding methods <sup>95</sup>. Many commercial foods, produced by various manufacturers, are nowadays prepared especially for growing large-breed dogs, and it seems that commercial foods are the most common way to take care of your dog's nutrition <sup>96</sup>. A study done in Australia and USA revealed that over 90% of the dogs were fed with commercial foods so that at least half of the intake was commercial, usually dry food <sup>96</sup>. Even though the commercial foods are dominating, people have recently been more interested in feeding their dogs more naturally and they more often chose to feed their dog unconventional diets such as the bone and raw food (BARF) diet, home prepared diets or even vegetarian diets <sup>95</sup>.

A general point of view has been that it is best for the puppies if they are fed with commercial growth foods, as there then supposedly is only a minimal risk for dietary deficiencies as the commercial foods should be so well balanced <sup>97</sup>. The problem is thought to be associated more with dietary excess than with dietary deficiencies as the high-quality growth foods are supplemented with minerals, vitamins and contain a lot of energy <sup>98</sup>. If growing dogs are fed home prepared or BARF diets, the risk of deficiencies or oversupplementations is taught to increase <sup>4</sup>.

There is only a minimal amount of evidence based information on how different diets influence the development of orthopedic disease. Generally commercial foods are considered safe to use and according to some manufacturers unpublished research that they use as announcements on the food packages, there is some evidence that dogs fed commercial foods even have a decreased risk of suffering from numerous health problems, compared to dogs that were fed home prepared foods, but there is a lack of objective studies about the issue <sup>99</sup>.

In the following chapters there is some basic information about the ancestral diet of canines and more modern feeding options.

#### 2.4.5.1 The Ancestral canine diet

Dogs (*Canis l. familiaris*) started to divergent from gray wolfs (*Canis lupus*) about 12 000 years ago <sup>100</sup>. First they scavenged kills or took wounded animals that escaped from nomadic hunter-gatherers, and later when humans became sedentary, the waste attracted proto-dogs developed, which, at some level, lead to an adaptation towards the omnivorous life style of humans <sup>101</sup>. As a result of intense breeding, there is high prevalence of different phenotypes in dogs, but these changes are located only in 4 quantitative trait loci <sup>51</sup>. Only three genes that are involved in starch digestion and glucose uptake were under selection pressure during domestication, but other metabolic traits like capacity to down-regulate animo acid catabolism and the synthesis of essential nutrients were unaffected <sup>51</sup>. These results indicate that the dog's digesting physiology is still close to the ancestral dogs and that of the wolves, but the dogs have adapted and are able to ingest more starch. In nature the carnivore diet consist of raw food items: bones, meat, and offals including partly digested content of intestines, as well as grass, vegetables and berries found in nature <sup>50</sup>.

Today the use of a raw food diet is considered as an alternative new diet option, but actually it is exactly what the ancestors of modern dogs have been eating through millennia in the wild. It is estimated that the ancestral canine diet consisted of about 85-90% meat originating primarily from whole prey together with small amounts of fish and eggs. 10-15% of the diet consisted of scavenged grasses, berries, nuts, and other vegetation. When consuming an ancestral diet like the one described, about 49% of the energy intake comes from protein, 44% from fats, and 6% from carbohydrates <sup>50</sup>. It is known that the nutrient intake of gray wolves can change markedly due to differences of prey availability; when there is prey available their intake might exceed 22 % of their body weight <sup>102</sup>. They go first after internal organs like the liver then intestine, muscles,

joints and tendons and lastly bones <sup>102</sup>. But when times are hard, they go back to the prey and ingest low-nutritious parts like the bones <sup>102</sup>. Furthermore, during these times when there is low availability of prey, wolves are required to conserve body proteins to maintain a synthesis capacity for essential nutrients. This ability to adapt to surrounding conditions also made it possible for dogs to adapt to human waste diets when prey availability was low.

In a typical BARF diet it is recommended that 60 % of the food is offered as raw meaty bones, 30 % of the food is vegetation, and 5-10% is offals, eggs etc <sup>103</sup>. From the raw meaty bones dogs get high quality proteins, which is especially important for carnivores, as their diet mainly should consist of protein and fats <sup>50</sup>. In a typical frozen commercial raw food in the states, there is 36% of protein, 59% of fat and 5% carbohydrates <sup>50</sup>. Wheras in a typical Finnish frozen raw food, there is 41% protein, 44% fat, no carbohydrates and 15% crude ash <sup>104</sup>. There are however many variations of raw food diets. Schultze's diet is based on a canine nutrition pyramid where the basis is raw meat and eggs, after them comes raw bones and raw vegetables, and in a minor role in this diet are alfalfa, fatty acids and vitamin C. Volhard's diet feeds the dog porridge consisting of grain, molasses, oil, eggs and vitamins in the morning, and in the evening a meal consisting of raw meat, fresh and dry vegetables and herbs together with wheatgerm, bran, bones, garlic, beer yeast, apple vinegar, cod liver oil and vitamin C <sup>105</sup>.

There are some concerns about feeding raw food diets to dogs. One is the possible nutritional inadequacy, similar to that of any other home prepared foods. Another concern is public health, because of the possible pathogens in raw foods. There have been claims that even the commercial raw food diets do not fill the nutrient recommendations and that there are clear health risks associated with feeding raw food diets for the dogs, because of nutritional deficiencies and nutritional excess found in some raw food diets tested <sup>105,</sup> <sup>106</sup>. Billinghurst and Brown consider feeding raw food with bones a healthy diet for dogs, whereas Freeman and Michelin state that feeding raw bones is dangerous and might cause severe health problems including intestinal obstructions, perforations, gastroenteritis and broken teeth <sup>50, 103, 105</sup>.

## 2.4.5.2 Commercial dry foods

At the moment commercial food is the most common way to provide food for dogs <sup>96</sup>. There are countless different brands and options where to choose from.

In a typical dry food there is 25% protein, 32% fats and 43% carbohydrates <sup>50</sup>. The amount of carbohydrates in commercial foods is a constant subject of discussion. However, even as the ancestral canine diet did not consist of that high amount of carbohydrates, they are not considered harmful for the dogs and there are studies showing that dogs are able to utilize them as a source of energy <sup>51</sup>.

# 2.4.5.3 Home prepared diets

Home prepared diets mean foods that are cooked for the dog at home, but it also includes all the ready meals sold for humans and dogs in grocery stores. Why choose to feed a home prepared diet for a dog? The reason can simply be that some people desire to cook food for their pets or they might question the wholesomeness of the commercial pet foods. Home prepared food might be indicated also to diagnose allergies in elimination diets when there is no suitable commercial diet available or the dog is refusing to eat the appropriate diets available <sup>106</sup>.

There are some drawbacks in feeding home prepared diets. First of all it takes more time to prepare and it might not be as cost effective as commercial pet foods. Secondly, to achieve a well-balanced diet, the owner must acquire sufficient amount of knowledge about a dog's nutritional requirements, to be able to make the food. In general it is harder to achieve a balanced nutritional content when preparing the food oneself and if a dog is fed with human meal remains it is unlikely that it will match nutritional requirements for dogs <sup>106</sup>. Most human diets have a higher P content <sup>107</sup>. This leads to a Ca:P ratio of 1:20 or even 1:100, when it should be between 1.2-2:1 <sup>107</sup>. Also, most common ingredients are rich in copper, but for instance some homemade unsupplemented foods made of rice, dairy products, fat or starch may have low copper concentrations <sup>13</sup>.

# 2.4.6 Conclusions about different feeding methods

According to Brown 2010 the modern dog foods have three weaknesses: they do not contain enough proteins, the fats are usually unbalanced or incomplete and some part of the nutrition needs to be provided fresh <sup>50</sup>. According to Billinhurst's book (1993), feeding raw food is recommended because cooking is destroying vitamins and enzymes, among these many of the antioxidants <sup>103</sup>. Cooking may also reduce the nutritional value of the proteins and make them harder to digest <sup>103</sup>. Amino acids lysine and methionine are destroyed during heating and heating can also produce completely new substances <sup>103</sup>. He also stated that when proteins, fats and carbohydrates are heated they change and new substances may form <sup>103</sup>.

However there is only little evidence based information on how different diets influence the development of orthopedic disease. In general it is thought that the diet of a growing puppy should be balanced, include high quality proteins, and all the essential nutrients. At this time, still there is no evidence of any diet being superior to another.

To this end we wanted to compare the diets of healthy dogs and dogs that were diagnosed with CHD. We hoped that we at least would find some trends, that later can be tested in clinical trials.

# 3 MATERIALS AND METHODS

## 3.1 Study design

The study was conducted as an online questionnaire and it was advertised to dog owners on the website of the University of Helsinki Veterinary Faculty, in veterinary clinics, in pet food stores, in kennel journals and in newspapers. The questionnaire was available online for all dog owners to complete. An announcement about the study was published in the journal of the Finnish German Shepherd Club (Saksanpaimenkoiralehti 4/2012) and on the website of the German Shepherd dog Club (www.spl.fi). To increase the study material a personal letter was sent to German Shepherd owners known to have a dysplastic dog (D or E hips). The link to the questionnaire was spread also through social media. The study was designed as an epidemiological explorative case-control study and the data was collected between December 2009 and October 2012.

# 3.2 The questionnaire and the data

The questionnaire (Appendix 1) questions were in Finnish. The questionnaire included questions about the dog's general descriptives, information about the dog's living environment, diseases, its mother's diseases, its mother's feeding during pregnancy, the dog's vaccinations, deworming and the dog's nutrition. Nutritional questions were distributed into four phases of life: 0-2 months, 2-6 months, 6-12 months (or 6-18 months in large-breed dogs) and adulthood. To avoid completing the same answers twice the owners could choose an option which indicated that the dog was fed like described earlier. In the present study the point of interest was the feeding during puppyhood and as a youngster, especially phases of life from 2-6 months and 6-12 or 18 months. In the questionnaire there were drop-down menus for a lot of food groups, from which the owners then were able to choose specific food items, dog foods, or human foods, as parts of the dog's diet. For each food item or nutrient there was a 5-point descriptive scale for the owners to estimate how often it was given. The options were: 1 = never, 2 = a couple of times per year, 3 = a couple of times per month, 4 = a couple of times per week and 5

= daily or always, or nearly always. The answers were later categorized into only three classes: "rarely/never (1+2), "seldom" (3), "often/always" (4+5).

In each phase of life the owners were also requested to estimate the proportion of different diets that they were providing to their dog. The options were a) bone and raw food diet (BARF), b) home prepared food, c) commercial dry food or d) other heated commercial foods. As people had a hard time adding up the proportions to a 100%, a ratio was calculated from each percentage value so that the answers above and below 100 % could be used in comparison. Percentage values were then categorized into five classes: 0 - 20, 20.01 - 40, 40.01 - 60, 60.01 - 80 and 80.01 - 100%.

It was not necessary for the owners to answer every question in the questionnaire, leading to a situation where some of the owners answered only what they were feeding to the dog and left other parts empty. If the questionnaire was otherwise completed properly, the parts left empty were completed with a number one (1), meaning rarely or never.

## 3.3 Subject selection

The study population chosen for this study was German Shepherd Dog, partly because this study was a part of a bigger GSD study, and partly because of the breed's high prevalence of the CHD <sup>3, 19</sup>. Inclusion criteria were that the owner had properly completed the feeding parts of the questionnaire and there was an official hip screening result available. If an owner had completed the feeding parts properly, but the screening result of CHD status was missing, it was verified from the Finnish Kennel Club's database, if possible. Dog's elbow dysplasia screening status and sex, if missing, were also verified from the Finnish Kennel Club's database. The case group was chosen from the questionnaire by including dogs with severe CHD indicated by a screening result A/A were included into the control group. Both the case group and the control group were chosen separately for each phase of life because not all the owners had answered all "phases of life" questions asked.

Exclusion criteria were an inadequate filling of the questionnaire or an absence of the official hip screening result. Dogs were also excluded if both the official name of the dog

and the registration ID were missing, leading to failure to verify the screening result from the Finnish Kennel Club's official database.

## 3.4 Statistical methods

Baseline bias for nonparametric variables was assessed by a Chi-Square test and cross tabulation whereas a T-test was used for parametric variables <sup>108</sup>. Associations between the nutrition of puppyhood and CHD were analyzed using cross-tabulation. Test for normality was performed using the Kolmogorov-Smirnov test. Because of the uneven distribution of the data, a Pearson Chi-Square test and the Spearman's correlation coefficients  $(r_s)$  were used to compare the questionnaire answers between the case and the control groups <sup>108</sup>. The difference in percentage of given dry, other commercial, homecooked, or bone and raw food (BARF) diets per case or control group were analyzed using the Mann-Whitney U-test., because of the uneven distribution of the data <sup>108</sup>. Principal component analysis was done to describe correlations between food items provided to the dog. In this test the rotation of the components was done by the Varimax equation. Only the nutriment groups with an eigenvalue > 1, and loading values > 0.4 were interpreted <sup>109, 110</sup>. The association of the factors formed by the principal component analysis to the CHD was studied using the T-test. Also a Cronbach's alpha test was performed for each component to interpret internal consistency. The internal consistency was good with Cronbach's alphas  $\geq 0.7$ . All tests were 2-tailed, and significance was set at P < 0.05. The statistical tests were performed by IBM SPSS Statistics, version 20.0.0 for Windows (SPSS Inc., Chicago, IL, USA).

# 4 RESULTS

## 4.1 Descriptives of the dogs

In the age group of 2-6 months the total number of subjects was 157: 54 hip dysplastic dogs and 103 nondysplastic dogs. In the age group of 6-18 months the total number of subjects was 130: 49 hip dysplastic dogs and 81 nondysplastic dogs (Table 6). There was no statistically significant difference between sex, body weight or number of dogs with concurrent elbow dysplastic changes in the case and control groups (Table 6). The total distribution of feeding habits in the current data is shown in the Tables 7 and 8.

Table 6. Baseline characteristics of the case and control groups where the P-value signifies the difference between the two groups.

	2-6 mon	th		6-18 mo	6-18 month			
	Case	Control	P-value	Case	Control	P-value		
Male/Female	20/34	45/58	0.496	20/29	34/47	1.000		
	33.25	32.31	0.316	33.40	32.75	0.519		
Mean bodyweight + SD, kg	$\pm 5.2$	$\pm 5.6$	0.510	$\pm 5.4$	$\pm 5.6$	0.319		
No dysplastic changes in elbows / Changes in elbows	39/13	85/18	0.292	34/13	66/15	0.270		

# 4.2 Questions about feeding at the age of 2 - 6 months

In this study a negative correlation means that the food item has a protective influence on CHD and positive correlation represents increased risk for the disease.

At the age of 2-6 months the frequency of feeding raw offals, raw fish, raw meat, raw bone and cartilage, raw tripe and raw egg to the dog were significantly different between the control group and the case group. Furthermore, these raw foods had a negative correlation with CHD indicating that raw foods could protect the dog from CHD (P-

values 0.000-0.024). Also oils and fats of animal origin showed a significant difference between the study groups and the correlation to CHD was again negative (P-value 0.022). Cooked meat and cooked bone and cartilage also had a significant difference between the control group and the case group, but on the contrary to raw food items, they had a positive correlation to CHD (P-values 0.005 and 0.037). At the age of 2-6 months 90.7% of the case group and 83.5% of the control group received dry commercial food daily, but this difference between the two groups was not significant (P-value = 0.117). Other interesting food items were cooked tripe, cooked rice, potato, and dog biscuits with P-values 0.139-0.177, but not significant. All the P-values and correlation coefficients for each food item given to the dog at age 2 - 6 months are shown in Table 7.

Table 7. Associations between food items and CHD at the age of 2 - 6 months. The P-value is the statistical difference between dysplastic and nondysplastic dogs.\* = significant on the level  $p \le 0.05$ ,\*\* =  $p \le 0.001$ .  $r_s$  = Spearman's correlation coefficient. Statistically significant results are also written in bolded italics.

	DYSPL	ASTIC D	OGS	NONDYSPLASTIC DOGS					
	Rarely/ never	Seldom	Often/ always	Rarely/ never	Seldom	Often/ always	rs	P-value	
Raw foods	(%)	(%)	(%)	(%)	(%)	(%)			
Raw offals*	90.7	3.7	5.6	69.9	18.4	11.7	-0.23	0.010	
Raw fish *	88.9	5.6	5.6	69.9	19.4	10.7	-0.21	0.024	
Raw meat *	55.6	20.4	24.1	32.0	14.6	53.4	-0.28	0.002	
Raw bone and									
cartilage **	63.0	24.1	13.0	29.1	22.3	48.5	-0.38	0.000	
Raw tripe *	77.8	13.0	9.3	55.3	20.4	24.3	-0.23	0.018	
Raw egg *	74.1	16.7	9.3	51.5	27.2	21.4	-0.22	0.022	
Raw vegetables	70.4	13.0	16.7	68.0	10.7	21.4	-0.03	0.754	
Raw fruits	77.8	13.0	9.3	71.8	16.5	11.7	-0.06	0.730	
Raw berries	79.6	14.8	5.6	71.8	14.6	13.6	-0.10	0.307	
Fresh food for									
dogs (only 50% of the items raw)	59.3	16.7	24.1	49.5	14.6	35.9	-0.11	0.349	
Cooked foods									
Cooked meat *	63.0	22.2	14.8	85.4	9.7	4.9	0.26	0.005	
Cooked offals	90.7	5.6	3.7	88.3	8.7	2.9	-0.03	0.767	
Cooked fish	77.8	18.5	3.7	87.4	11.7	1.0	0.13	0.200	
Cooked bone									
and cartilage *	75.9	18.5	5.6	89.3	5.8	4.9	0.17	0.037	
Cooked tripe	87.0	13.0	0.0	93.2	4.9	1.9	0.10	0.146	
Cooked egg	79.6	16.7	3.7	77.7	17.5	4.9	-0.02	1.000	
Sausage	50.0	31.5	18.5	51.5	22.3	26.2	-0.02	0.380	

Blood crepe	90.7	9.3	0.0	89.3	10.7	0.0	-0.02	0.794
Liver casserole	79.6	16.7	3.7	85.4	10.7	3.9	0.07	0.507
Table scaps	44.4	37.0	18.5	56.3	24.3	19.4	0.08	0.235
Cooked								
vegetables	70.4	11.1	18.5	78.6	12.6	8.7	0.11	0.237
Cooked rice	42.6	37.0	20.4	59.2	26.2	14.6	0.15	0.139
Other grain								
products	75.9	11.1	13.0	77.7	12.6	9.7	0.03	0.839
Potato	72.2	24.1	3.7	83.5	15.5	1.0	0.14	0.162
Pasta	90.7	9.3	0.0	89.3	7.8	2.9	-0.03	0.545
Dry dog food	5.6	3.7	90.7	15.5	1.0	83.5	0.11	0.117
Bread	68.5	18.5	13.0	73.8	18.4	7.8	0.07	0.601
Gluten free								
bread	98.1	1.9	0.0	97.1	2.9	0.0	-0.03	1.000
Dry dog food as			<b>.</b>			<b>a</b> ( -	0.55	
a treat	46.3	22.2	31.5	42.7	23.3	34.0	-0.03	0.908
Dog biscuits	59.3	24.1	16.7	72.8	18.4	8.7	0.15	0.177
Bones made of	25.0	21 5	10 -	26.0	21.1	22.0	0.12	0.000
skin	25.9	31.5	42.6	36.9	31.1	32.0	0.12	0.293
Tinned sausages for dogs	81.5	11.1	7.4	77.7	16.5	5.8	-0.04	0.736
Therapeutic dog	61.5	11.1	7.4	//./	10.5	5.8	-0.04	0.730
feeds	100.0	0.0	0.0	99.0	0.0	1.0	-0.06	1.000
Yrjölä's porridge	85.2	1.9	13.0	80.6	7.8	11.7	-0.05	0.338
Fermented	05.2	1.9	15.0	00.0	7.0	11.7	0.05	0.550
foods								
Fermented meats	94.4	5.6	0.0	94.2	5.8	0.0	-0.01	1.000
Fermented								
grains	98.1	1.9	0.0	99.0	0.0	1.0	0.04	0.571
Fermented								
vegetables	100.0	0.0	0.0	98.1	1.0	1.0	-0.08	1.000
Dried foods								
Dried animal		a = -	a		<b>6 0 1</b>	<b>6</b> 0 1	0.01	
parts	42.6	35.2	22.2	49.5	30.1	20.4	0.06	0.723
Dried offals	74.1	16.7	9.3	82.5	12.6	4.9	0.10	0.368
Dried fish	92.6	3.7	3.7	95.1	3.9	1.0	0.05	0.633
Milk products								
Milk	96.3	1.9	1.9	98.1	1.9	0.0	0.05	0.706
Icecream	94.4	5.6	0.0	91.3	8.7	0.0	-0.06	0.547
Milk products	24.1	25.9	50.0	26.2	21.4	52.4	-0.01	0.817
Cheese	59.3	27.8	13.0	73.8	19.4	6.8	0.15	0.160
Oils				ı			I	
Vegetable oil	55.6	16.7	27.8	41.7	18.4	39.8	-0.14	0.235
Oils, fats of	22.0	10.7	27.0		10.1	27.0	0.11	0.200
animal origin *	77.8	14.8	7.4	55.3	26.2	18.4	-0.22	0.022
6								
Oil products	87.0	3.7	9.3	79.6	4.9	15.5	-0.09	0.545

Outdoor eatables								
Woden sticks	33.3	22.2	44.4	43.7	16.5	39.8	0.08	0.424
Carcasses	96.3	1.9	1.9	93.2	3.9	2.9	-0.06	0.762
Grass	35.2	38.9	25.9	40.8	29.1	30.1	0.01	0.495
Soil	90.7	5.6	3.7	83.5	10.7	5.8	-0.10	0.505
Clay and stone	83.3	11.1	5.6	82.5	7.8	9.7	-0.02	0.576
Water from								
puddles	38.9	24.1	37.0	28.2	26.2	45.6	-0.11	0.386
Feaces	59.3	22.2	18.5	74.8	12.6	12.6	0.15	0.127

The mean proportion of BARF fed to the dog at the age of 2-6 months was significantly different between hip dysplastic dogs and non-dysplastic dogs (P-value 0.021, N=150) so that dogs in the healthy control group more often received BARF food that the dogs in the case group (Figure 3). At this age the diet of 7 puppies (n=150) consisted of more than 70 % of BARF. From these puppies 6 belonged to healthy control group and 1 in to case group. The mean BARF intake in the healthy group was 15 % of the diet whereas the mean BARF intake in the case group was 5% of the diet. There was no significant difference between the groups in the proportion of other types of foods fed to the puppy at the age of 2 - 6 months.

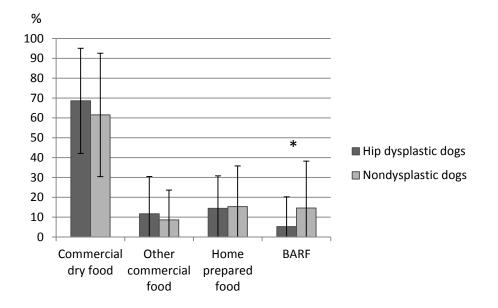


Figure 3 Mean portion of different foods fed to the dog at the age of 2-6 months and statistic deviation inside the group (hip dysplastic dogs n = 52, non-dysplastic dogs n = 98). The one marked with \* showed a statistically significant difference between study groups, where the level of significance was  $p \le 0.05$ .

# 4.3 Questions about feeding at the age of 6 - 18 months

All the P-values and correlation coefficients for each food item given to the dogs at the age of 6 - 18 months are shown in Table 8. At the age of 6 - 18 months the frequence of feeding raw offal, raw fish, raw meat, raw bone and cartilage and raw tripe to the dog were significantly different between the control group and the case group. Furthermore, these raw foods had a negative correlation to CHD. Cooked meat, cooked bone and cartilage, sausage and table scraps also showed a significant difference between the control group and the case group, but on the contrary to raw food items they had a positive correlation to CHD. As mentioned earlier, in this study a negative correlation means that the food item has a protective influence on CHD and positive correlation represents increased risk for the disease. At the age of 6-18 months 81.6% of the case group and 77.8% of the control group received dry commercial food daily, and the difference between the two groups was again not significant (P-value 0.833). However raw berries, fresh food for dogs (where 50 % was raw and 50 % heated), cooked rice, therapeutic dog feeds, and oils and fats of animal origin showed a trend towards being significant, so that raw berries, fresh food for dogs, and oils and fats of animal origin (P-values 0.074-0.096) seemed to have protective influence on CHD, whereas cooked rice and therapeutic dog feeds (P-values 0.067, 0.098) seemed to have the opposite effect. Other interesting food items in this age group were raw egg, vegetable oil (protective), cooked fish, liver casserole and potato (associated with increased risk for disease) with P-values 0.100-0.189, but again not significant.

Table 8. Associations between food items and CHD at the age of 6 - 18 months. The P-value is the statistical difference between dysplastic and non-dysplastic dogs.\* = significant on the level  $p \le 0.05$ , \*\* =  $p \le 0.001$ .  $r_s$  = Spearman's correlation coefficient. Statistically significant results are also written in bolded italics.

	DYSPL	ASTIC DO	OGS	NONDYSPLASTIC DOGS				
	Rarely/		Often/	Rarely/		Often/		
	never	Seldom	always	never	Seldom	always	rs	<b>P-value</b>
Raw foods	(%)	(%)	(%)	(%)	(%)	(%)		
Raw offals **	91.8	4.1	4.1	63.0	24.7	12.3	-0.31	0.001
Raw fish *	87.8	6.1	6.1	61.7	21.0	17.3	-0.27	0.006
Raw meat *	53.1	16.3	30.6	29.6	17.3	53.1	-0.24	0.019
Raw bone and								
cartilage **	61.2	22.4	16.3	23.5	29.6	46.9	-0.39	0.000
Raw tripe *	69.4	18.4	12.2	46.9	22.2	30.9	-0.24	0.024
Raw egg	65.3	24.5	10.2	49.4	25.9	24.7	-0.18	0.100
Raw vegetables	65.3	12.2	22.4	55.6	16.0	28.4	-0.09	0.582
Raw fruits	79.6	14.3	6.1	67.9	16.0	16.0	-0.14	0.237
Raw berries	83.7	12.2	4.1	67.9	17.3	14.8	-0.18	0.096
Fresh food for								
dogs (only 50%	~ ~	10.0	<b>9</b> 0 f	10 5	10.6		0.00	0.074
of the items raw)	61.2	10.2	28.6	40.7	13.6	45.7	-0.20	0.074
Cooked foods	< <b>7</b> 0							
Cooked meat *	67.3	16.3	16.3	87.7	9.9	2.5	0.26	0.005
Cooked offals	89.8	6.1	4.1	85.2	9.9	4.9	-0.07	0.779
Cooked fish	75.5	22.4	2.0	88.9	9.9	1.2	0.18	0.124
Cooked bone								
and cartilage *	75.5	24.5	0.0	88.9	7.4	3.7	0.16	0.007
Cooked tripe	91.8	6.1	2.0	92.6	7.4	0.0	0.02	0.563
Cooked egg	77.6	18.4	4.1	75.3	17.3	7.4	-0.03	0.849
Sausage *	49.0	36.7	14.3	53.1	17.3	29.6	-0.04	0.021
Blood crepe	85.7	14.3	0.0	88.9	9.9	1.2	0.04	0.734
Liver casserole	71.4	22.4	6.1	86.4	9.9	3.7	0.18	0.106
Table scraps *	38.8	42.9	18.4	55.6	22.2	22.2	0.10	0.044
Cooked								
vegetables	73.5	8.2	18.4	81.5	9.9	8.6	0.11	0.275
Cooked rice	42.9	38.8	18.4	61.7	23.5	14.8	0.16	0.098
Other grain								
products	73.5	20.4	6.1	79.0	12.3	8.6	0.05	0.430
Potato	71.4	22.4	6.1	85.2	12.3	2.5	0.17	0.189
Pasta	87.8	12.2	0.0	92.6	4.9	2.5	0.08	0.252
Dry dog food	16.3	2.0	81.6	21.0	1.2	77.8	0.05	0.833
Bread	63.3	24.5	12.2	76.5	16.0	7.4	0.14	0.297
Gluten free bread	98.0	2.0	0.0	98.8	1.2	0.0	0.032	1.000

Dry dog food as								
a treat	55.1	18.4	26.5	50.6	21.0	28.4	-0.04	0.913
Dog biscuits	63.3	24.5	12.2	74.1	18.5	7.4	0.12	0.429
Bones made of	05.5	24.3	12.2	/4.1	10.5	7.4	0.12	0.429
skin	28.6	38.8	32.7	40.7	33.3	25.9	0.12	0.379
Tinned sausages								
for dogs	83.7	10.2	6.1	76.5	17.3	6.2	-0.08	0.548
Therapeutic dog								
feeds	91.8	0.0	8.2	98.8	0.0	1.2	0.18	0.067
Yrjölä's porridge	91.8	2.0	6.1	91.2	3.8	5.0	-0.01	0.902
Fermented								
foods				r			r	
Fermented meats	91.8	8.2	0.0	95.1	4.9	0.0	0.07	0.709
Fermented								
grains	98.0	2.0	0.0	100.0	0.0	0.0	0.11	0.377
Fermented	100.0	0.0	0.0	00.0	0.0	1.2	0.07	1 000
vegetables	100.0	0.0	0.0	98.8	0.0	1.2	-0.07	1.000
Dried foods								
Dried animal parts	46.9	30.6	22.4	50.6	28.4	21.0	0.03	0.916
Dried offals	85.7	10.2	4.1	85.2	9.9	4.9	-0.01	1.000
Dried fish	95.9	4.1	0.0	92.6	6.2	1.2	-0.07	0.818
Milk products	05.0	4.1	0.0	00.0	1.2	0.0	0.00	0.556
Milk	95.9	4.1	0.0	98.8	1.2	0.0	0.09	0.556
Icecream	93.9	6.1	0.0	93.8	6.2	0.0	-0.00	1.000
Milk products	36.7	18.4	44.9	28.4	25.9	45.7	-0.05	0.479
Cheese	59.2	28.6	12.2	72.8	19.8	7.4	0.14	0.281
Oils				Γ			Γ	
Vegetable oil	53.1	20.4	26.5	37.0	18.5	44.4	-0.18	0.112
Oils, fats of								
animal origin	73.5	14.3	12.2	54.3	25.9	19.8	-0.18	0.092
Oil products	83.7	8.2	8.2	80.2	4.9	14.8	-0.05	0.459
Outdoor								
eatables								0.633
Wooden sticks	32.7	22.4	44.9	46.9	17.3	35.8	0.13	0.303
Carcasses	98.0	2.0	0.0	91.4	3.7	4.9	-0.14	0.219
Grass	38.8	40.8	20.4	40.7	33.3	25.9	-0.02	0.679
Soil	91.8	4.1	4.1	88.9	7.4	3.7	-0.05	0.822
Clay and stone	85.7	8.2	6.1	87.7	7.4	4.9	0.03	1.000
Water from								
puddle	38.8	30.6	30.6	28.4	30.9	40.7	-0.20	0.389
Feaces	63.3	16.3	20.4	74.1	12.3	13.6	0.12	0.439

The mean proportion of BARF food fed to the dog at the age of 6 - 18 months again was significantly different between the hip dysplastic and non-dysplastic dogs (P-value 0.006, N=102). Dogs in the control group received BARF food more often that the dogs in the

case group (Figure 4). At this age the diet of 12 puppies (n=102) consisted of more than 70 % of BARF. From these puppies 10 belonged to the healthy control group and 2 to the case group. The mean BARF intake in the healthy group was 25 % of the diet whereas the mean BARF intake in the case group was 8% of the diet. There was no significant difference between the groups in the portions of any of the other types of foods fed to the puppy at the age of 6 - 18 months.

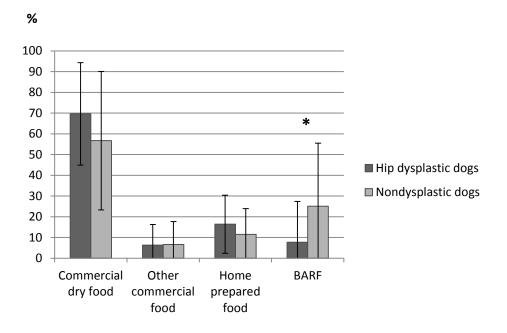


Figure 4. Mean proportions of different foods fed to the dog at the age of 6 - 18 months and statistic deviation inside the group (hip dysplastic dogs n=36, non-dysplastic dogs n=66). The one marked with \* showed statistically significant difference between study groups, \* = significant on the level  $p \le 0.05$ .

## 4.4 Principal Component Analysis

## 4.4.1 General about the Principal Component Analysis

The Principal Component Analysis (PCA) is used when there are a large number of variables and it is assumed that there might be redundancy between those variables <sup>109</sup>. It means that some of the variables correlates with one another and might actually measure

the same "thing" <sup>109</sup>. The PCA combines all the variables that have a correlation to groups called components that then can be used in further analysis <sup>109, 110</sup>. In this study, the PCA was used to analyse, if feeding of some of the food items or feeds correlated and if components could be formed from them.

#### 4.4.2 Choosing the components

In both age groups in this study the PCA suggested up to 16 components, that had an eigenvalue of > 1, because also the components from the scree plot's so called shoulder (hill like pattern commonly seen right after vertical axel) can be chosen for further examination <sup>109, 110</sup>. If all the 16 components had been chosen, the total variance explained would have been around 70 % in both age groups, but only the groups located on the vertical portion in the scree plot were chosen <sup>109, 110</sup>.

In the age group of 2-6 months all four components situating on the vertical axel of the scree plot were chosen, then explaining only 35.3% of the total variance in the study material (Figure 5). Again, in the age group of 6-18 months, all five components situating on the vertical axel of the scree plot were chosen, however, explaining only 40.9% of the total variance in the study material (Figure 6). On each component a Cronbach's  $\alpha$  test was performed.

Dry commercial food was not included in any of the components, but it had a strong negative correlation to both BARF food components shown in table 9. In the age group of 2-6 months the negative correlation of dry food was -0.441 and in the age group of 6-18 months -0.523.

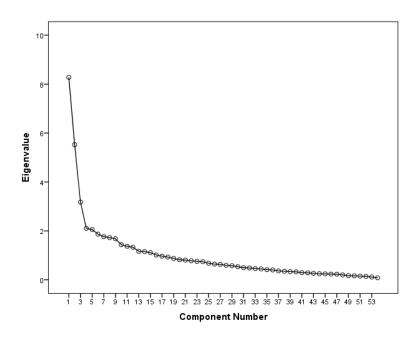


Figure 5 Scree plot of the eigenvalues of the components at the phase of life from 2-6 months. All the components from the vertical axel (4 components) were chosen to further examination.

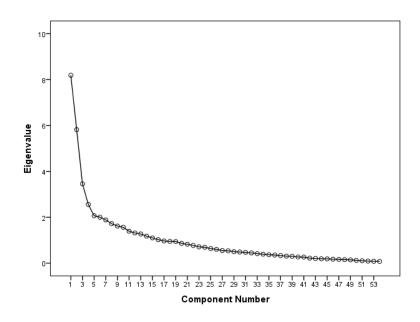


Figure 6 Scree plot of the eigenvalues of the components at the phase of life from 6-18 months. All the components from the vertical axel (5 components) were chosen to further examination.

## 4.4.3 Principal component analysis for the age group of 2-6 months

In the principal component analysis in the age group of 2-6 months the first component seemed to be a general raw food or a BARF diet component, having a strong negative association to CHD and consisting of raw offals, raw tripe, raw fish, raw egg, raw bone and cartilage, and raw meat, with good internal consistency (Table 9a). The 2<sup>nd</sup> component consisted of raw fruits, raw berries, raw vegetables and vegetable oil and its internal consistency was acceptable. The 3<sup>rd</sup> component was likely to take in the population of dogs that are at least partly fed with home prepared food and in this component there were also fermented grain, pasta, potato, and cooked fish, but the internal consistency of the component was questionable. The 4<sup>th</sup> component consisted of some of the outdoor eatables: grass, wooden sticks, water from puddles, clay and sand, and soil. The Chonbach's alpha value was acceptable for this group.

# 4.4.4 Principal component analysis for the age group of 6-18 months

The first component in this age group also seemed to be a raw food or BARF diet component, having a strong negative association to the CHD and containing raw offals, raw tripe, raw meat, raw fish, raw egg, raw bone and cartilage with a Chonbach alpha value of 0.781, indicating good internal consistency (Table 9b). The 2<sup>nd</sup> component consisted of glutein free bread, fermented grain, fermented meat, cooked offals, and dried offals with questionable internal consistency. The 3<sup>rd</sup> component consisted of different kinds of dog's treats: dry food as a treat, sausage, dog biscuit and bones made of skin and had questionable internal consistency. The 4<sup>th</sup> component in this age group resembled the 4<sup>th</sup> component in the age group of 2-6 months consisting outdoor eatables: soil, clay and stone and blood crepes with an acceptable internal consistency. The 5<sup>th</sup> component consists of cooked egg, cooked rice, and fresh food for dogs with poor internal consistency.

Table 9a and b. Food items in different components according to the principal component analysis. Cronbach's alpha values were calculated to each component to estimate internal consistency. Statistically significant Cronbach's alpha values were written in *italics* and bold.

		2-6 MONTH	IS	
		2 <sup>nd</sup>		
	1 <sup>st</sup> component	component	3 <sup>rd</sup> component	4 <sup>th</sup> component
	Raw offals	Raw fruits	Fermented grain	Grass
	Raw tripe	Raw berries	Pasta	Rods
		Raw		Water from
	Raw fish	vegetables	Potato	puddle
	Raw egg	Vegetable oil	Cooked fish	Clay and stone
	Raw bone and			
	cartilage			Soil
	Raw meat			
Cronbach'				
s alpha	0.840	0.786	0.656	0.706
Statistical association				
to CHD	0.000	0.135	0.468	0.731

Table 9a	(2-6)	months,	n =	157	).
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Table 9b (6-18 months, n = 130).

		6-18	3 MONTHS		
	1 <sup>st</sup> component	2 <sup>nd</sup> component	3 <sup>rd</sup> component	4 <sup>th</sup> component	5 <sup>th</sup> component
	Raw offals	Glutein free bread	Dry food as a treat	Soil	Cooked egg
	Raw tripe	Fermented grain	Sausage	Clay and stone	Cooked rice
	Raw fish	Fermented meat	Dog biscuit	Blood crepe	Fresh food for dogs
	Raw egg	Cooked offals	Bones made of skin		
	Raw bone and cartilage	Dried offals			
	Raw meat				
	Raw berries				
	Raw vegetables				
	Dry food (-)				
Cronbach' s alpha	0.781	0.603	0.697	0.781	0.569
Statistical association					
to CHD	0.000	0.302	0.886	0.739	0.954

# 5 DISCUSSION

#### 5.1 Results

All the analyses pointed to the same association; raw animal derived foods seemed to reduce the incidence of CHD in German Shepherd dogs in this Finnish population. It is known that nutrition plays a key role in development of joints but it is still unclear what aspect of nutrition is the most important. This is the first time that the influence of food eaten at young age (before official hip dysplasia radiographs are taken) on the development of CHD has been studied.

There might be several reasons why a negative association was seen between raw food and disease: a reason for this could be that the BARF diet contains high quality nutrients in their natural form and that the diet is closer to the diet that ancestral canines consumed in the wild. Although some recent articles of raw food diets for dogs did not recommend them due to both badly balancing and public health concerns, raw bones and cartilages contain lots of compounds that are already defined as chondroprotective substances and used commonly as supplementations in prevention and treatment of osteoarthritis due to CHD <sup>111, 112</sup>. One theory might be that the microbiota of the gut is more physiologically correct after ingestion of raw food and does not predispose for a chronic inflammatory process in the gut. This would then lead to normal permeability of the gut barrier, normal immunity, normal nutrient transport through the intestinal wall and normal nutrient production in the gut (ie.Vitamin K). Vitamin K, C and D deficiency has been associated with an inflammatory gut and with osteoarthritis in humans <sup>4, 113, 114</sup>. Also, it is known that the absorption of nutrients from the gut is impaired if the diet contains high amounts of poorly digestible carbohydrates <sup>65</sup>, which might happen when manufacturers replace animal protein by vegetal protein of low quality, to reduce costs of production and raise profits.

To avoid excess energy intake large-breed dogs are not recommended to be fed ad libitum during puppyhood. In the present study there was no dietary diary so it was not possible to calculate the energy content for each dog and therefore it is not possible to say if the dogs were over- or underfed.

To keep calcium homeostasis in balance the Ca:P ratio of the food is traditionally recommended to be kept between 1.1:1 and 2:1, and all other calcium supplementations to the food are to be avoided <sup>13, 25, 27, 45</sup>. Even though calcium excess is thought to be harmful, the practice of feeding raw bones in addition to commercial foods was shown to be beneficial for joint health in this study. In raw bone the Ca:P ratio is from 2,3:1 to 2,5:1, which could explain why they are more safe to feed than excess calcium only.

In this study all the raw food items of animal origin that were asked about in the questionnaire, fed to the growing puppy seemed to protect from CHD. The food item "fresh food for dogs" was the only exception. It was first included into the raw food section, but it cannot really be considered as raw, because some of the options were later found to be industrial cooked foods, which also explains, why it was the only raw item that did not show significant difference between the case and the control groups.

Raw food items of animal origin fed during growth together formed a component in the PCA which had good internal consistency in both age groups. It was not possible to say anything about the protective effects of individual raw food items on CHD (raw meat, raw bones, raw cartilage, raw offals, raw tripe, raw fish and raw egg), because feeding of individual raw food items had a high positive correlation together. Nevertheless, it is known that the raw cartilage itself contains glycosaminoglycans and other substances with chondroprotective actions <sup>9, 14</sup> and when combining this information and the highly significant p-values (P<0.0001) of protective influence of feeding raw bone and cartilage in both age groups, it supports the idea that raw bone and cartilage fed to the German Shepherd dogs at young age could prevent CHD. Also, together with bone and cartilage material, raw meat is usually fed. This practice is called "feeding meaty bones" and contains lots of high quality proteins that according to Brown (2010), is one of the most important factors of the canine diet <sup>50</sup>. Because the raw food items correlated together it might also be that some of these food items do not have a real protective influence on CHD, but are shown to be protective, because they are fed together with food items that protect from CHD.

Feeding of frankfurters at the age of 6-18 months also seemed to protect from CHD. This is hard to explain based on current knowledge and can of course be because of chance. But, it might also be that the sausage correlates with something else that protects from CHD and is because of that seen here as a protective food item. Frankfurter pieces are quite commonly used as training treats for dogs, so it might be that these dogs had been exercising more and that this protected them from CHD. The dietary cation-anion balance in foods fed to large-breed puppies during growth has been noticed to influence the development of canine hip dysplasia <sup>4</sup>, and keeping the combination of dietary electrolytes below 23 mEq/100g dry mater was associated with less severe hip joint laxity <sup>13</sup>. Feeding sausages that usually are high in salt could increase the dietary electrolyte balance and thus be harmful to the joint development, but the outcome was actually the opposite in this study.

Oils and fats of animal origin are usually fish oils and when fed during the age of 2-6 months of age they also had a protective influence on CHD, in this study. This might be due the fact that the fish oils contain high amounts of omega-3 fatty acids that are known to have chondroprotective actions <sup>13, 14, 56</sup>. This could maybe also be a reason why raw fish could protect from CHD in both age groups; because it is a good source of omega-3 fatty acids.

During the age of 2-6 months of age there was also a component that included raw berries, raw vegetables, raw fruits and vegetable oil. This looks like something that most likely would be a group of food items fed to dogs that are on the BARF diet. Raw berries and raw vegetables were included in the BARF diet component at the age from 6-18 months. These raw fruits, berries, and vegetables contain lots of antioxidants, that have chondroprotective actions and could be beneficial for joint development <sup>13, 14</sup>, and a weak trend (p=0.119) was seen in this study. The outdoor eatable component formed in both age groups might tell about the dogs living environment, because eating wooden sticks, grass, clay, stone, and soil material is more likely for dogs spending lots of time outdoors, or eating outdoor eatables might also tell us about gastrointestinal problems, as dogs have been noticed to eat odd things because of intestinal discomfort. As dogs still seem to look for certain things to eat, it is also possible that dogs still can crave things that they need, meaning that these dogs maybe were deficient in some minerals or trace-elements that they were looking for in this way. At the age of 2-6 months also blood crepes were included in the outdoor eatable component which is hard to explain and may be only a coincidence. It is, however, also a good source of iron. Not any of the raw berries, fruits or vegetables or any of the outdoor eatables were shown to correlate separately with CHD. On the contrary to raw food items (and frankfurters and animal oils), cooked meat and cooked bone and cartilage showed a significant positive correlation to CHD in both age groups. They were not included any of the components formed by the principal component analysis to help explain why feeding of them increase the risk for CHD. The fact that they did not seemed to protect from CHD may be explained by the harmful effect of heating to nutritional values of the food, but it is harder to explain is why they seem to increase the risk for CHD. According to Billinghurst (1993) heating is destroying vitamins and enzymes among many of the antioxidants, bringing down the nutritional value of the proteins and amino acids, and also when proteins, fats and carbohydrates are heated they change and even new substances can be formed <sup>103</sup>. Based on this information it could be thought that the harmful effect of cooked meat and bones on joint development might be related to the reduction of the quality of the proteins provided and/or loss of important vitamins and enzymes. Furthermore Brown (2010) stated that the quality of the proteins is one of the most important factors when feeding dogs by the ancestral diet <sup>50</sup>. Studies how proteins change by heating should be conducted to evaluate this.

Feeding the remains of a human meal showed a positive correlation with CHD, which could possibly be explained by that human leftovers usually are cooked or that they are high on phosphorus and very low on calcium, resulting in an unbalanced Ca:P ratio. If the dog gets a lot of leftovers, it might also correlate with overweight that is known to be harmful for joint development.

Feeding a dry commercial food was common in all the case and control groups and did not show any clear association to CHD in this study, although there was a weak trend (p=0.117) in the younger age class. The proportion of BARF food fed in puppyhood, on the contrary, showed a significant difference between hip dysplastic and non-dysplastic dogs in both age groups, indicating that even if only a part of the dog's diet is raw food, it could already protect puppies from CHD.

#### 5.2 Bias

There are lots of other environmental factors than food, influencing skeletal growth that was not possible to rule out in this study <sup>5, 19, 30, 31</sup>. The dogs taking parts in this study might also have had differences in genetic predisposition to CHD, but most probably genetic factors influenced more or less equally both case and control groups. It is also very common that breeders recommend certain type of feeding to the owners that might contribute to the distribution of genetic predisposal for CHD. Also, this study did not look if there were differences in breed lines (working line and show line) inside the German Shepherd breed, between the case and control groups. The control group was chosen to be twice as big as the case group intentionally to increase the statistical power of the study.

Limitations of the questionnaire also need to be considered: owners were able to leave some parts of the questionnaire empty, leading to a situation where not all of the answers were completed completely. This lead to a situation where some dog owners answered only what they were feeding to the dog and left other food item questions empty. Those parts left empty we assumed that meant that the owners did not feed it to the dog. This might cause some bias, if the answers that were partially filled, were not completed totally, resulting in false one-sided diets.

# 5.3 Future studies

As this preliminary study showed that there is a positive influence of feeding raw food at young age, to test the hypothesis further more studies are needed. In the future it would be interesting to test matched groups, eg. dogs from the same litters that would be fed using either raw or dry food in a randomized controlled setting. These different fed groups could also be tested for nutritional deficiencies.

# 6 CONCLUSION

This study suggest that feeding a bone and raw food diet (BARF) or raw meat, raw offals, raw bone and raw cartilage, raw fish, raw egg and raw tripe as a supplementation to other diets or as a part of the BARF diet showed protective effect towards CHD. The study also suggests that the feeding cooked meat, bone and cartilage should be avoided, because they might increase the risk of CHD. To assess the true benefit of serving dogs raw food, the pros and cons, such as zoonotic risks, should also be critically assessed.

# 7 ACKNOWLEDGEMENTS

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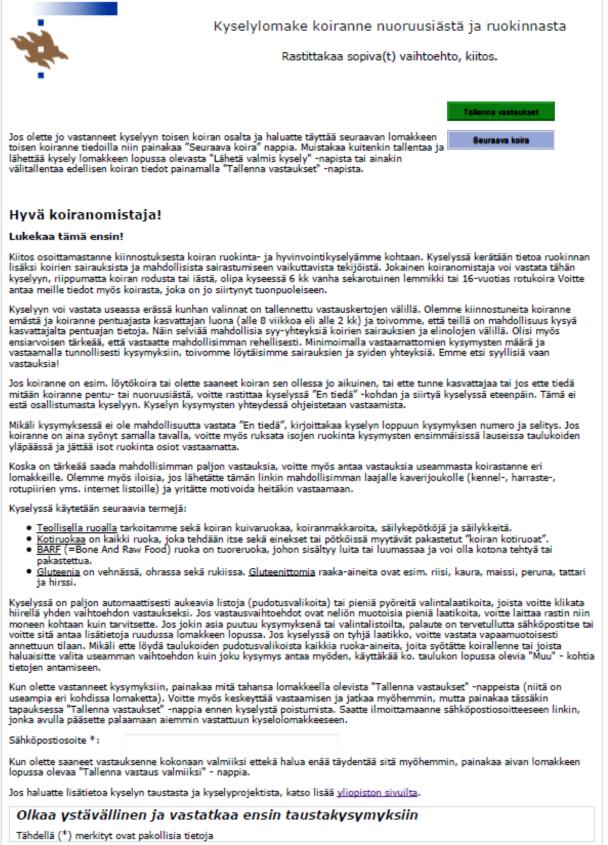
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# 9 APPENDIX

# 9.1 Appendix 1



Koiran kutsumanimi \*

Koiran virallinen nimi

Koiran rekisterinumero

1. Minkä ikäisenä koiranne tuli teille?

Noin Valitse kuukautta vanhana Noin Valitse vuotiaana Syntynyt meillä
Onko taloudessanne muita eläimiä?
Valitse kpl muita koiria Valitse kpl muita eläimiä, mitä? 2. Koiranne ikä nyt:
Pentu (0-6kk) Nuori (7-12-18kk) Aikuinen Valitse vuotta
3. Milloin koiranne syntyi?           Talvella (kk: 12-2)         Keväällä (kk: 3-5)         Kesällä (kk: 6-8)         Syksyllä (kk: 9-11)         En tiedä
4. Koiranne rotu?
Valitse Monirotuinen
Jos koiranne on vain kahden rodun sekoitus Valitse Valitse
5. Koiranne paino         Odotettu aikuispaino         Valitse         Rodun edustajan ihannepaino         Valitse
6. Koiranne sukupuoli Uros 🔍 Narttu 🔍 Kastroitu tai steriloitu 🗖
7. Koiranne väritys (meitä kiinnostaa tässä kysymyksessä vain valkoiset (=valk.) karva-alueet)?
Koko valk.(>90%)  Paljon valk. (>50%)  Vähemmän valk.  Hyvin vähän/ei ollenkaan valk.
8. Koiranne ravitsemustila normaalisti (normaali alla = kylkiluut tuntuu muttei selkäranka selästä) Hyvin hoikka ◎ Hoikka ◎ Normaali ◎ Lihava ◎ Hyvin lihava ◎
9. Koiranne luonne normaalisti
Hyvin ylivilkas ja/tai Vilkas ja /tai hieman Normaali Aika leppoisa Hyvin rauhallinen
10. Koiranne aktiivisuus normaalisti
Hyvin aktiivinen  Aktiivinen  Normaali  Hyvin "laiska"  Hyvin "laiska"
11. Koiranne pääkäyttö         Kotikoira       Metsästys         Näyttely- ja siitoskoira       Poliisikoira         Valitse
Rajakoira Valitse Huumekoira Pelastuskoira Agility
Toko Palveluskoira Valitse Avustajakoira Valitse Muuta
12. Jos koiranne metsästää         Metsästää Valitse       kk / vuosi         Ajava ©       Noutava ©       Luola ©       Jälki ©         Haukkuva ©
13. Rokotukset Pentuna normaalit sai rokotukset suositusten mukaan ○ ei saanut ○ en tiedā ○ perusrokotukset eli 2-4 rokotusta ennen vuoden ikāä:
Aikuisena: suositusten mukaan (1-3 vuoden välein harvemmin ei ollenkaan riippuen rokotteesta)
14. Madotus
Normaalit pentu-sai ei saanut en tiedä madotukset eli alle vuoden ikää 2-10 madotusta:
joka vuosi 2 kertaa tai yli 🔍 joka vuosi kerran 🔍 joka toinen vuosi 🔍
harvemmin  ei ole ikinä saanut  en tiedä
15. Tupakoiko joku/jotkut teidän talossa sisällä niin että koira on samoissa tiloissa?         Meillä poltettiin Valitae savuketta päivässä       Harvoin sisällä          Pääasiassa sisällä        Vain ulkona
Meillä poltetaan Valitse savuketta päivässä Harvoin sisällä 🔍 Pääasiassa sisällä 🔍 Vain ulkona 🔍
16. Missä koirasi asuu nyt?
Kerrostalossa   Rivitalossa  Omakotitalo, puutalo  Omakotitalo, ei puutalo
Onko teidän kotinne: erittäin puhdas © hyvin puhdas © normaalin puhdas © ei niin puhdas © ei ollenkaan puhdas ©
Koira kulkee rappusissa:       Ikerran/vikko       Ikerran/kk       Ikerran/vuosi         päivittäin monta kertaa       1 kerran/vrk       1 kerran/vikko       1 kerran/kk       1 kerran/vuosi
Koira kulkee rappusissa:       päivittäin monta kertaa       1 kerran/vrk       1 kerran/viikko       1 kerran/kk       1 kerran/vuosi
Koira kulkee rappusissa:
Koira kulkee rappusissa:       päivittäin monta kertaa       1 kerran/vrk       1 kerran/viikko       1 kerran/kk       1 kerran/vuosi         Koira on aiemmin asunut myös:

Keskusilmastointi 🔍 Ilma	-lämpö	pump	pu ©	P	ainovoi	imailm	astointi 💿		
18. Onko koirallasi piha Ei ole tarhaa/pihaa 🔲 On piha,	jossa	voi ol	la irrallaar	n = 0	)n tarha	a, jossa	a voi olla in	rallaan 🗆	
	etjussa a/vrk	ı pihal	a 🗏 Vali		)n päivi Valitse			rhassa vapaana	
19. Miten koirasi on vapaana (ilman hihnaa)									
Missä koirasi on vapaana?									
Ei ole ikinä vapaana On miltein aina vapaana On vapaana koirapuistossa									
On vapaana tarhassa / pihalla 🔍 On vapaana metsäkävelyillä									
On vapaana mökillä 🔲 On vapaana muualla, missä? 🗏									
Milloin koirasi on enemmän vap	aana?								
Lomilla Mökillä			Touko-le	okaku	un 🗉	Та	lvella 🗉		
Viikonloppuisin 🗏 🛛 Ei ole eroa l	omilla	tai vii	konloppuis	sin ym	is, 🗏				
20. Käyttääkö koirasi									
Kaulapantaa Valitse	-	ita Va						Kuonopantaa tarv	ittaessa
leveys noin Valitse cm		pi Va					I	Käytän flexiä 🔲	
materiaali Valitse	mate	naali	Valitse						
21. Onko koirallanne ollut seu	raavia	a sair	auksia?	Valits	e 1-4 ra	astia p	er rivi + alk	amisikā, jos saira	stanut
		stanut	On sairas Harvoin	tanut	Alkoi i	iässä	Sairastaa edelleen	Loppui ruokinnan vaihdoksen jälkeen	En ole huomannut, että ruokinta olisi auttanut
Sairauksia								parmeen	
Esim. sairaus	0	۰	0	۰	2	6	¥.	1	<b>V</b>
Korvatulehdusta		0	0	0	Valits	Valits		10	
Ihotulehduksia (esim. ihottumaa, hot-spot)	0	0	0	0	Valits	Valits		8	
Demodikoosia		0	0		Valits	Valits			
Varvasvälitulehduksia (= furunkuloosi)	0	0	0	0	Valits	Valits			
"Allergiaa", atopiaa, (iho-oireita)	0	0	0	0	Valits	Valits		2	
Achantosis nigrificans	0	0	0	0	Valits	Valits			
Seborrhea	0	0	0	0	Valits	Valits			
"Allergiaa", vatsan yliherkkyyttä, IBD	0	0	0	0	Valits	Valits			
Muita suolisto-ongelmia tai sairauksia		0	0	0	Valits	Valite			
Hammaskiveä						V ditte			
	0	0	0	0		Valits			
Keuhkosairauksia	·		0	0	Valits				
Keuhkosairauksia Anaalirauhastulehdusta	0	0			Valite Valite	Valits			
	0	0	0	0	Valits Valits Valits	Valite Valite		8	
Anaalirauhastulehdusta	0	0	0	0	Valits Valits Valits Valits	Valits Valits Valits			
Anaalirauhastulehdusta Rasvapatteja Syöpä-sairauksia	0	0 0 0	0	0	Valits Valits Valits Valits Valits	Valits Valits Valits Valits			
Anaalirauhastulehdusta Rasvapatteja Syöpä-sairauksia <i>Diagnoosi</i> Valitse	000000000000000000000000000000000000000	0 0 0	0	0	Valits Valits Valits Valits Valits Valits	Valits Valits Valits Valits Valits			
Anaalirauhastulehdusta Rasvapatteja Syöpä-sairauksia <i>Diagnoosi</i> Valitse Epilepsiaa	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0	0 0 0	0 0 0	Valite Valite Valite Valite Valite Valite Valite	Valite Valite Valite Valite Valite Valite			
Anaalirauhastulehdusta Rasvapatteja Syöpä-sairauksia <i>Diagnoosi</i> Valitee Epilepsiaa AIHAa	0 0 0 0		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	Valits Valits Valits Valits Valits Valits Valits Valits	Valite Valite Valite Valite Valite Valite Valite			
Anaalirauhastulehdusta Rasvapatteja Syöpä-sairauksia <i>Diagnoosi</i> Valitee Epilepsiaa AIHAa Cushingin tauti				000000000000000000000000000000000000000	Valite Valite Valite Valite Valite Valite Valite Valite Valite	Valite Valite Valite Valite Valite Valite Valite Valite			

Silmāsairautta					1.1.17	11.5	-	_	_
Diagnoosi Valitee	0	0	0	0	Valits	Valits			
Virtsatietulehduksia	0	0	0	0	Valits	Valits			
Virtsankarkailua	0	0	0	0	Valits	Valits			
Virtsakivitautia Diagnoosi Valitee	0	0	0	0	Valits	Valits			
Munuais-sairautta Diagnoosi Valitee	0	0			Valits	Valite			
Diabetestä (sokeritauti)		0			Volite	Valite			
Sydänsairauksia	0	0			Y CINC	Y dinc			
Diagnoosi Valitee	0	0			Valits	Valits			
Luusto-ongelmia - Osteokondrosis dissecans (OD)	-	-	-	-	Valle	Valite			
	0	0	0	0					
- HOD	0	0	0	0	_	Valits			
- panostitis	0	0	0	0		Valits			
- murtuma	0	0			Valits	Valits			
- Luusyöpä	0	0			Valits	Valits			
Nivelongelmia									
- Ionkkavika, -dysplasia, -nivelrikko Diseneesi, Vellise	0	0			Valits	Valits			
Diagnoosi Valltae - kyynärpään dysplasia, - nivelrikko									
Diagnoosi Valitee	•	0				Valits	8		
- ristisiteen ongelmat	0	0	0	0	- cante	Valits			
- polvinivelrikko	0	0			Valits	Valits			
- patella luksaatio	0	0			Valits	Valits			
- Löysät ranteet	0	0			Valits	Valits			
- Nivelrikko/muut nivelet	0	0			Valits	Valits			
Selkä-ongelmia									
- sillottumat eli spondyloosi	0	0			Valits	Valits			
- degeneratiivinen myelopatia	0	0			Valits	Valits			1
<ul> <li>mäyräkoirahalvaus/välilevysairaus</li> </ul>	0	0	0	0	Valits	Valits		E	
- embolus	0	0	_	_	Valits	Valits			
chibolita	0	0			T Chine	T GINC	1	See 1	
Dnko koiraltanne leikattu Ei On O vierasesine suolistosta? 22. Sairastaako koiranne jotain Sairaus Valitse	mu	Val uta k	roonist						
Alkoi Valitse sitten.			Ei sair	asta 🗉					
23. Onko koiranne jatkuvalla lä	äkit	ykse	llä, mill	ä?					
								Talenna v	stoukset
Kysymyksiä koiranne en	ias	ta:							
loudutte ehkä ottamaan koiranne kasv kiitollisia. Jos ette millään saa tietoon kysymykseen 29.	-								-
24. Rokotettiinko EMÄ tiineyder (yllä ◎ Ei ◎ En tiedā / en				ri enne	en?				

26. Muistatko/tiedätkö mitä koirasi EMÄ söi koirasi tiineyden aikana? Lähinnä kotiruokaa ® Lähinnä teollista muonaa ® Sekoitus molemmista ® En tiedä / en muista ®

Muistatko mitä

Arvioitko että se oli gluteenitonta? Kyllä 🏾 🛛 Ei 🔍 En tiedä / en muista 🔍

27. Muistatko/tiedätkö mitä koirasi EMÄ söi koirasi imetysajan aikana? Lähinnä kotiruokaa ◎ Lähinnä teollista muonaa ◎ Sekoitus molemmista ◎ En tiedä / en muista ◎

Muistatko mitä

Arvioitko että se oli gluteenitonta? Kyllä 🏾 🛛 Ei 🔍 En tiedä / en muista 🔍

	Saira: Ei		On saira Harvoin		Alkoi vv		Sairastaa edelleen	Loppui ruokinnan vaihdoksen jälkeen	En ole huomannu että ruokinta olisi auttanut
Sairauksia									
Esim. sairaus	٠	0	0	٠	2	6	4		×.
Korvatulehdusta	0	0	0	0	Valits	Valits			
Ihotulehduksia (esim. ihottumaa, hot-spot)	0	0	0	0	Valits	Valits		8	
Demodikoosia	0	0	0	0	Valits	Valits			
Varvasvälitulehduksia (= furunkuloosi)	•	0	0	0	Valits	Valits			
"Allergiaa", atopiaa, (iho-oireita)	0	0	0	0	Valits	Valits			
Achantosis nigrificans	0	0	0	0	Valits	Valits			
Seborrhea		0	0	0	Valits	Valits			
"Allergiaa", vatsan yliherkkyyttä, IBD	0	0	0	0	Valits	Valits			
Muita suolisto-ongelmia tai sairauksia	•		0	n	Valits	Valits		8	
Hammaskiveä	0	0	0	0	Valits	Valits			
Keuhkosairauksia	0	0	0	0	Valits	Valits			
Anaalirauhastulehdusta	0	0	0	0	Valits	Valits			
Rasvapatteja	0	0	0	0	Valits	Valits			
Syöpä-sairauksia Diagnoosi Valitse	•	0	0	0	Valits	Valits			
Epilepsiaa	0	0	0	0	Valits	Valits			
AIHAa	0	0	0	0	Valits	Valits			
Cushingin tauti	0	0	0	0	Valits	Valits			
Addisonin tauti	0	0	0	0	Valits	Valits			
Haiman vajaatoiminta		0			Valits	Valits			
Kilpirauhasen vajaatoimintaa	0	0			Valits	Valits			
Silmäsairautta Diagnoosi Valitae		0	0	0	Valits	Valite		8	
Virtsatietulehduksia		0	0	0	Valits	Valits			
Virtsankarkailua		0	0	0	Valits	Valits		8	
Virtsakivitautia Diagnoosi Valilae	•	0	0	0	Valits	Valite			8
Munuais-sairautta Diagnoosi Valitee	•	0			Valits	Valite		6	8
Diabetestä (sokeritauti)	0	0			Valits	Valits			
Sydänsairauksia Diagnoosi Vallixe Luusto-ongelmia	•	0				Valits			

- HOD	0	0	0	0	Valits	Valits			
- panostitis	0	0	0	0	Valits	Valits		8	8
- murtuma	0	0			Valits	Valits			
- Luusyöpä	0	0			Valits	Valits		8	
Nivelongelmia									
· lonkkavika, -dysplasia, -nivelrikko Diagnoosi Valitxe	0	0			Valits	'Valits			
· kyynärpään dysplasia, - nivelrikko Diagnoosi Valike	0	0			Valits	'VaĨits			
· ristisiteen ongelmat	0	0	0	0	Valits	Valits			
polvinivelrikko	0	0			Valits	Valits			
patella luksaatio	0	0			Valits	Valits			1
· Löysät ranteet	0	0			Valits	Valits			
Nivelrikko/muut nivelet	0	0			Valits	Valits			
Selkä-ongelmia									
sillottumat eli spondyloosi	0	0			Valits	Valits			
degeneratiivinen myelopatia	0	0			Valits	Valits			
mäyräkoirahalvaus/välilevysairaus	0	0	0	0	Valits	Valits			
• embolus	0	0			Valits	Valits			
								Tallenna v	slaukset
Kysymyksiä koiranne al los osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän 29. Tiedätkö miten usein koira Monta kertaa päivässä Fiollenkaan	tä 29- ja si <b>si oli</b> päiväs	-32, ni iirtykä ulko sä ©	iin olemm ä kysymyl na PIKK	ie jo ki kseen 3 10 PEN	iitollisia 33. NTUNA	. Jos ett	uovutusi		
os osaatte vastata osaan kysymyksis a ette itse tiedā, rastittakaa tähān 29. Tiedätkö miten usein koira Monta kertaa päivässä O Kerran p ä ollenkaan O En tie kivioi pennun aika auringonvalossa ( 80. Millainen koirasi alusta oli akattu puu, liukas muovi, kivi	stā 29- ja si si oli päivās edā ulkon PIKK Jaatt	-32, ni iirtykä sä a, ei la (U PEI a)	iin olemm ä kysymyl na PIKK Muu asin läpi), NTUNA (	e jo ki kseen : U PEN utaman Valits (alle I	iitollisia 33. NTUNA kerran ie tunti	a. Jos ett A (alle I Viikossa ia / päivä usikäin	uovutusi a M ä en 2 kk)	käisen 2 kk)? uutaman kerran ? (liukas= esii	kuukaudessa © m. parketti,
os osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän 29. Tiedätkö miten usein koira Monta kertaa päivässä OKerran p ä ollenkaan OEn tie Arvioi pennun aika auringonvalossa ( 80. Millainen koirasi alusta oli akattu puu, liukas muovi, kivi ääasiassa liukas lattia OPääas	stā 29- ja si si oli pāivās adā o ulkon PIKK Jaatta	-32, ni iirtykä sä a, ei la (U PE a) ei-liul	iin olemm ä kysymyl <b>na PIKK</b> Muu asin läpi),	e jo ki kseen i U PEN utaman Valts (alle I	iitollisia 33. NTUNA h kerran ke tunti luovut	a. Jos ett A (alle I Viikossa ia / päivä usikäin	uovutusi a M ä ien 2 kk) kas jää M	käisen 2 kk)? uutaman kerran ? (liukas= esii	kuukaudessa © m. parketti,
los osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän 29. Tiedätkö miten usein koira Monta kertaa päivässä OKerran p Ei ollenkaan OKERTA Arvioi pennun aika auringonvalossa ( 30. Millainen koirasi alusta oli lakattu puu, liukas muovi, kivi Pääasiassa liukas lattia OPääas	stā 29- ja si si oli pāivās edā ulkon PIKK Jaatta siassa jeā alu si lep	-32, ni iirtykä sä a, ei la a, ei la (U PE a) ei-liul usta, n päsi P	iin olemm ä kysymyl na PIKK Muu asin läpi), NTUNA ( kas lattia nattoja © PIKKU PI	valts (alle I (tuntia	iitollisia 33. NTUNA h kerran ke tunti luovut Ul Er NA (al	A. Jos ett A (alle I viikossa a / päivä usikäin kona liu n tiedä ( le luov okausi:	uovutusi a M i i en 2 kk) kas jää utusikäis	<b>käisen 2 kk)?</b> luutaman kerran ? ( <i>liukas= esii</i> Maalattia/nu <b>maalattia</b> /nu <b>men 2 kk)?</b> En tie	kuukaudessa © m <b>. parketti</b> , ırmikko ©
los osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän 29. Tiedätkö miten usein koiraa Monta kertaa päivässä Kerran p Ei ollenkaan En tie Arvioi pennun aika auringonvalossa ( 80. Millainen koirasi alusta oli akattu puu, liukas muovi, kivi Pääasiassa liukas lattia Pääas Sanomapaperia Pehm 81. Tiedätkö miten usein koira Arvioi pennun lepoaika (sisältää sekä Millainen pentu hän oli: nyvin pullea pullea 82. Muistatko/voitko selvittää emän maidon lisäksi)?	tä 29- ja si oli si oli päiväs edä 0 ulkon PIKK Jaatta siassa neä alu si lep päivä mitä	-32, ni iirtykä ulko sä a, ei li a, ei li a, ei li a, ei li a, ei li a a, ei li a a, ei li a a, ei li a a a, ei li a a a, ei li a a a, ei li a a a, ei li a a a, ei li a a a, ei li a a a a a a a a a a a a a a a a a a a	iin olemm ä kysymyl na PIKK Muu asin läpi), NTUNA ( kas lattia nattoja PIKKU PE yõunen), normaa asi söi e	valts (alle I tuntia	iitollisia 33, NTUNA h kerran e tunti luovut Ul Er NA (al y vuon mäiset	A Jos ett Vikossa a / päivä usikäin kona liu n tiedä le luova okausi: hoik	uovutusi a M i i een 2 kk) kas jää w tusikäis Valitse kautta el	<b>käisen 2 kk)?</b> luutaman kerran ? ( <i>liukas= esii</i> Maalattia/nu <b>sen 2 kk)?</b> En tie hyvin	kuukaudessa m. parketti, urmikko dä hoikka <u>se tuli teille</u>
os osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän P9. Tiedätkö miten usein koira Ionta kertaa päivässä Kerran p ä ollenkaan En tie Invioi pennun aika auringonvalossa ( 20. Millainen koirasi alusta oli akattu puu, liukas muovi, kivi ääasiassa liukas lattia Pääas Sanomapaperia Pehm P1. Tiedätkö miten usein koira Invioi pennun lepoaika (sisältää sekä Millainen pentu hän oli: Iyvin pullea pullea 22. Muistatko/voitko selvittää emän maidon lisäksi)?	tä 29- ja si si oli päiväs edä ulkon ulkon p <b>IKK</b> Jaatta siassa si lep päivä si lep päivä	-32, ni iirtykä. ulko ssä a, ei l: a, ei l: (U PEI a) ei-liuk ssta, n bäsi P bäsi P ä- että	iin olemm ä kysymyl na PIKK Muu asin läpi), NTUNA ( kas lattia nattoja PIKKU PI yõunen), normaa asi söi <u>e</u> aa	i Sekoit	iitollisia 33. NTUNA h kerran luovut Ul Er NA (al Vuon mäiset aus mok	a. Jos ett (alle I viikossa ia / päivä usikäin kona liu h tiedä le luova okausi: hoik t 2 kuu	uovutusi a M i i een 2 kk) kas jää w tusikäis Valitse kautta el	käisen 2 kk)? luutaman kerran ? (liukas= esii Maalattia/nu maalattia/nu En tie hyvin li ennen kuin s	kuukaudessa ( m. parketti, urmikko () dä () hoikka () <u>se tuli teille</u>
os osaatte vastata osaan kysymyksis a ette itse tiedä, rastittakaa tähän 19. Tiedätkö miten usein koira Ionta kertaa päivässä Kerran p i ollenkaan En tie avioi pennun aika auringonvalossa ( 20. Millainen koirasi alusta oli akattu puu, liukas muovi, kivi ääasiassa liukas lattia Pääas anomapaperia Pehm 21. Tiedätkö miten usein koira avioi pennun lepoaika (sisältää sekä fillainen pentu hän oli: yvin pullea pullea 22. Muistatko/voitko selvittää emän maidon lisäksi)? ähinnä kotiruokaa Lähinnä teo fuistatko mitä	tä 29 ja si si oli päiväs edä ulkon pIKK Jaatta siassa eä alu si lep i päivä si lep i päivä si lep	-32, ni iirtykä ulko ssä a, ei l: a, ei l: upäsi P päsi P päsi P ä- että i koir muona	iin olemm ä kysymyl na PIKK Muu asin läpi), NTUNA ( kas lattia nattoja PIKKU PI yõunen), normaa asi sõi <u>e</u> sa En	Valts (alle I tuntia di • Sekoit	iitollisia 33. NTUNA h kerran ie tunti luovut Ul Er NA (al NA (al vuon mäiset us mole / en mu	a. Jos ett A (alle I a viikossa a / päivä usikäin kona liu h tiedä le luov okausi: hoik t 2 kuu emmista	uovutusi a M i i een 2 kk) kas jää M utusikäis Valtse kautta el En	käisen 2 kk)? uutaman kerran l ? ( <i>liukas= esii</i> Maalattia/nu ien 2 kk)? En tie hyvin li ennen kuin s tiedä / en muista	kuukaudessa m. parketti, urmikko dä hoikka se tuli teille a setaukset

Koiraa ei saa liikuttaa vapaasti ennen Valitse kk:n ikää Noudatin täydellisesti 💿 Noudatin osittain 💿 En noudattanut ollenkaan 🔍 En saanut tätä ohjetta 🄍 Koiraa ei saa kävelyttää rappusissa ennen Valtse kk:n ikää Noudatin täydellisesti 🔍 Noudatin osittain 🔍 En noudattanut ollenkaan 🔍 En saanut tätä ohjetta 🔍 "Koiraa saa liikuttaa miten vain" Noudatin täydellisesti 🌼 Noudatin osittain 🔍 En noudattanut ollenkaan 🌼 En saanut tätä ohjetta 🌼 Sain tällaisen ohjeen: (kirjoita) Noudatin täydellisesti 💿 Noudatin osittain 💿 En noudattanut ollenkaan 💿 34. Miten paljon vuorokaudessa liikutitte/lenkititte pentua iässä 3-7 kk? 3 kk:n ikäisenä: Alle 30 min./ päivä 🌼 30-60 min./ päivä 🔍 60-120 min./ päivä 💿 Vli 2 tuntia päivässä 🔍 Valitse tuntia / päivä Arvioi pennun aika auringonvalossa (ulkona, ei lasin läpi), Valtse tuntia / päivä 4 kk:n ikäisenä: Alle 30 min./ päivä 🌼 30-60 min./ päivä 🔍 60-120 min./ päivä 🔍 Yli 2 tuntia päivässä 💿 Valitse tuntia / päivä Arvioi pennun aika auringonvalossa (ulkona, ei lasin läpi), Valtse tuntia / päivä 5 kk:n ikäisenä: Alle 30 min./ päivä 🌼 30-60 min./ päivä 🔍 60-120 min./ päivä 🔍 Vli 2 tuntia päivässä 🔍 Valitse tuntia / päivä Arvioi pennun aika auringonvalossa (ulkona, ei lasin läpi), Valtse tuntia / päivä 6 kk:n ikäisenä: Alle 30 min./ päivä 🌼 30-60 min./ päivä 🌻 60-120 min./ päivä 🔍 Vli 2 tuntia päivässä 🔍 Valitse tuntia / päivä Arvioi pennun aika auringonvalossa (ulkona, ei lasin läpi), Valitse tuntia / päivä 35. Millainen koirasi alusta oli ISOMPANA PENTUNA (2-6 kk)? (liukas= esim. parketti, lakattu puu, liukas muovi, kivilaatta) Pääasiassa liukas lattia 🔲 Ulkona liukas jää 🔍 Maalattia/nurmikko 🗖 Pääasiassa ei liukas lattia 📃 Pehmeä alusta, mattoja 🔲 En tiedä 🔲 Sanomapaperia 🗉 36. Tiedätkö miten usein koirasi lepäsi ISOMPANA PENTUNA (2-6 kk vanhana)? Arvioi pennun lepoaika (sisältää sekä päivä- että yöunen), tuntia / vuorokausi: Valitse En tiedä 📃 Millainen pentu hän oli: hyvin pullea 💿 pullea 💿 normaali 💿 hoikka 💿 hyvin hoikka 💿 37. Minkälaiset ohjeet saitte kasvattajalta penturuokinnan suhteen? "Koiralle ei saa antaa mitään kotiruokaa, ainoastaan tiettyä kuivamuonaa" Noudatin täydellisesti 🔍 Noudatin osittain 🔍 En noudattanut ollenkaan 🔍 En saanut tätä ohjetta 🔍 "Koiralle saa antaa vain tiettyä kuivamuonaa plus piimää" Noudatin täydellisesti 🔍 Noudatin osittain 🔍 En noudattanut ollenkaan 🔍 En saanut tätä ohjetta 🄍 Koiralle tehdään sekoitus kotiruuasta ja teollisesta ruuasta sisältäen esim. Noudatin täydellisesti 🔍 Noudatin osittain 🔍 En noudattanut ollenkaan 🔍 En saanut tätä ohjetta 🔍 Sain tällaisen ohjeen: (kirjoita)

Noudatin täydellisesti 💿

Noudatin osittain 💿

En noudattanut ollenkaan 💿

38. Mitä koiranne söi ISOMPANA PENTUNA (2-6 kk)? Lisäohjeet: Jos ette esim. syötä jotakin alla olevaa ollenkaan, niin "Valitse"-valikosta ei tarvitse valita mikään, vaan laitatte vain merkin ensimmäisen nappi-rivin 1-kohtaan. Jos taas syötätte esim, kuivamuonaa mutta ette muista mikä se oli nimeltään, tai jos syötätte juustoa mutta ette muista mitä, niin ei tarvitse valita mikään "Valitse"-valikosta mutta täytätte aina kuitenkin nappi-rivin. Kiitos.

Tarkenna, minkä ikäinen koira oli kun se söi kuten alla: noin Valitse - Valitse kuukautta vanha

En muista ollenkaan 🔍 Koira ei ollut silloin meillä 🔍

## Rastittakaa yksi sopiva vaihtoehto per rivi: 1 = ei koskaan, 2 = muutaman kerran vuodessa, 3 = muutaman kerran kuukaudessa, 4 = muutaman kerran viikossa, 5 = aina / miltei aina / päivittäin)

	1	2	3	4	5		1	2	3	4	5
Kuivamuonaa		_	-	-	_	Koirille tarkoitettua säilykeruokaa	tai koirar	nakk	araa	~	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	e
Sairauksien erikoisruuat						Koirille tarkoitettua tuoreruokaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	e
Valitse	0	0	0	0	0	Valitse	0	0	•	0	
Kypsennyttyä lihaa						Kypsentämätöntä lihaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyjä sisäelimiä						Kypsentämättömiä sisäelimiä					-
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyä kalaa						Kypsentämätöntä kalaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyjä luita tai rustoluita						Kypsentämättömiä luita tai rustoja					
sypsemetryja runa tar rustolulta	0	0	0	0	0	Rypsentamatornia fuita tal fustoja		0	0	0	
Kypsennettyä naudan mahaa	_	-	-	-	-	Naudan mahaa raakana	-		-	-	_
cypsermettya naddan manaa	0	0	0	0	0			0	0	0	
Kypsennettyä kananmunaa						Kypsentämätöntä kananmunaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Nakki, lenkkimakkara yms.						Verilätyt					
Nakki, leikkimakkara yms.	0	0	0	0	0	venacyc		0	0	0	
						Ruoantähteitä tai ihmisille tarkoite	ttua ruok	aa (	esim.	kaik	kki
Maksalaatikko			0			tähteet, eineksiä, laatikoita)					
							0	0	0	0	
Fermentoitua lihaa						Kuivattuja eläintenosia (esim sian	tai lampa	aank	orvia,	, här	ān
Valitse	0		•	•	0	häntiä, kuivattua kanaa)		8	8	8	
Maitoa						Jäätelöä	0	0	0	0	_
Maitoa			0			Jaateloa			0	0	
Maitotuotteet	-	-	-	-	-	Juustot		-	-	-	
Valitse	0	0	0	0	0	Valitse			0	0	
Valitse				0		Valitse			0		- 2
			-				5	-	-		_
Kypsennettyjä vihanneksia Valitse						Kypsentämättömiä vihanneksia Valitse					
Valitse			0	0		Valitse			0	0	1
	0	0	0	0	0			0	0	0	_
Fermentoitua viljaa		_	-	-	_	Fermentoituja kasviksia	-	-	-	~	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsentämättömiä hedelmiä						Kypsentämättömiä marjoja					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Keitettyä riisiä						Muita viljatuotteita					
-	0	0	0	0	0	-	0	0	0	0	

		0	0	0	0	0	Pastaa, couscousia
.eipää		_					Gluteenitonta leipää
		0	0	0	0	0	0 0 0 0
(uivamuonaa makupaloin		0	0	0	0		Koirankeksejä 🛛 💿 💿 💿
uivattuja sisäelimiä							Kuivattua kalaa
/alitse		0	0	0	0	0	Valitse O O O
/alitse		0	0	0	0	0	Valitse O O O
uruluita (nahasta valmist							Ulkona keppejä
Ilkona raatoja yms.		0	0	0	0	0	Ulkona ruohoa
Iultaa		0	0	0	0	0	0 0 0 0
luitaa		0	0	0	0	0	Savea, kiviä
							Ulkona ulosteita
II							Valitse 0000
lkona vettä lätäköistä yn		0	0		•	•	Valitze
							Mitä?
asvisöljyä							Eläinperäiset öljyt ja rasvat
/alitse		0	0	0	0	0	Valitse
/alitse		0	0	0	0	0	Valitse
							Vijölän puuroa, merkitse myös käytetyt viljat:
							durra 🗖 hirssi 🗖 intiaaniriisi 🗖
ljytuotteet							italianpantaheinä 🗖 kaura 🗖 ohra
/alitse		0	0	0	0	0	
/alitse		ö	ė	Ö	ē	0	riisi 🛑 ruis 🖏 speltti 🖷
							tattari 🗖 tefheinä 🗖 vehnä 🗖
							0 0 0 0
uu		0	0	0	0	0	Muu 0 0 0 0
							1
äytitkö varmasti kaikk	Mitä? ti? Tarkastatl	han	vie	lä ko	егта	n? Ki	Mitä?
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite	ai? Tarkastatl en iso osuus en iso osuus en iso osuus	2-8 2-8 2-8	kk kk kk	ikäi: ikäi: ikäi:	sen sen sen	koira koira koira	
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite 9. Jos et vastannut l oirasi pääasiassa söi	ai? Tarkastatl en iso osuus en iso osuus en iso osuus en iso osuus kysymyksee noin 2-6 kk ähinnä teollist	2-8 2-8 2-8 2-8 n i a m	kk kk kk 8. r iäss	ikäi: ikäi: ikäi: ikäi: niin sä?	sen sen sen vaik	koira koira koira koira koira	itos! nne ruuasta on kuivamuonaa? Valitse % nne ruuasta oli muuta teollista ruokaa? Valitse %
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite 9. Jos et vastannut l oirasi pääasiassa söi ihinnä kotiruokaa © L uistatko mitä	ai? Tarkastatl en iso osuus en iso osuus en iso osuus en iso osuus kysymyksee noin 2-6 kk ähinnä teollist	2-8 2-8 2-8 2-8 n i a m	kk kk kk 8. r iäss	ikäi: ikäi: ikäi: niin iä? aa	sen sen sen vaik	koira koira koira koira koira	itos! inne ruuasta on kuivamuonaa? Valitse % inne ruuasta oli muuta teollista ruokaa? Valitse % inne ruuasta oli kotiruokaa? Valitse % inne ruuasta oli BARFia? Valitse % et muista kaikkea niin muistatko/tiedätkö mitä oitus molemmista © En tiedä / en muista ©
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite 9. Jos et vastannut k oirasi pääasiassa söi shinnä kotiruokaa Luistatko mitä rvioitko että se oli glutee	an iso osuus en iso esuus en iso esuus es	2-8 2-8 2-8 2-8 3::n 3::n 3::n 3::n 1: 2-8 0 7:00	kk kk kk 8. r iäss nuon E	ikäi ikäi ikäi ikäi iä? aa i aa i aa i aa	sen sen sen vaik E	koira koira koira koira cka e Sek n tiec ( <i>pi</i>	itos! inne ruuasta on kuivamuonaa? Valitse % inne ruuasta oli muuta teollista ruokaa? Valitse % inne ruuasta oli kotiruokaa? Valitse % inne ruuasta oli BARFia? Valitse % it muista kaikkea niin muistatko/tiedätkö mitä oitus molemmista © En tiedä / en muista © lä / en muista © Talionna vastaukset eni rotu 6-12kk, iso rotu 6-18kk):
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite 9. Jos et vastannut k oirasi pääasiassa söi ihinnä kotiruokaa © L uistatko mitä vioitko että se oli glutee Vysymyksiä koira is osaatte vastata osaan	an iso osuus en iso osuus en iso osuus en iso osuus en iso osuus en iso osuus kysymyksee noin 2-6 kk ähinnä teollist enitonta? Kyllä anne nuo kysymyksistä 4	2-8 2-8 2-8 2-8 3 ::n i a m a m	kk kk kk 8. r iäss uon E USä 41, r	ikäi: ikäi: ikäi: ikä: aa ( a ajas	sen sen vaik E	koira koira koira koira koira koira koira Sek n tiec ( <i>pi</i> mejo	itos! inne ruuasta on kuivamuonaa? Valitse % inne ruuasta oli muuta teollista ruokaa? Valitse % inne ruuasta oli kotiruokaa? Valitse % inne ruuasta oli BARFia? Valitse % et muista kaikkea niin muistatko/tiedätkö mitä oitus molemmista © En tiedä / en muista © lä / en muista ©
oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite oisitteko arvioida mite 9. Jos et vastannut l oirasi pääasiassa söi ihinnä kotiruokaa Luistatko mitä vioitko että se oli glutee vioitko että se oli glutee oronyksiä koira s osaatte vastata osaan onha ja jos ette millään s	ai? Tarkastatl en iso osuus en iso osuus en iso osuus en iso osuus kysymyksee noin 2-6 kk ähinnä teollist enitonta? Kyllä anne nuo kysymyksistä aa tietoonne v noin 6-12 (-	2-8 2-8 2-8 2-8 3 3 ::n i a m 0 700 40-4 asta	kk kk kk kk 8. r iäss iuon E USZ 41, n auks ) kk	ikäi ikäi ikäi ikäi aa i aa a ja a a a a a a a a a a a a a	sen sen sen vaik E 5 <i>ta</i> olemr entuia	koira koira koira koira koira cka e Sek n tiec n tiec <u>(pi</u> me jo ästä j na?	itos! inne ruuasta on kuivamuonaa? Valitse % inne ruuasta oli muuta teollista ruokaa? Valitse % inne ruuasta oli kotiruokaa? Valitse % inne ruuasta oli BARFia? Valitse % it muista kaikkea niin muistatko/tiedätkö mitä oitus molemmista  En tiedä / en muista ä / en muista • Talonna vastaukset eni rotu 6-12kk, iso rotu 6-18kk): kiitollisia. Jos ette omistaneet koiraa silloin kun se oli 6-11 a ette itse tiedä, rastittakaa tähän  ja siirtykää sitten

## Rastittakaa sopiva vaihtoehto: 1 = ei koskaan, 2 = muutaman kerran vuodessa, 3 = muutaman kerran kuukaudessa, 4 = muutaman kerran viikossa, 5 = aina / miltei aina / päivittäin)

Kuivamuonaa	1	2	3	4	5	Koirille tarkoitettua säilykeruokaa t	1 ai koirar	2 makk	3 araa	4	5
Valitse	0	0	0	0	0	Valitse	0	0	0	0	e
Valitse	0	0	0	0	0	Valitse	0	0	0	0	e
Sairauksien erikoisruuat						Koirille tarkoitettua tuoreruokaa					
Valitse	0			0		Valitse				0	
Valitse	0	0	0	0	0	Valitse			0	0	6
											_
Kypsennyttyä lihaa	-	_	_	_	_	Kypsentämätöntä lihaa	_	_	_	_	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyjä sisäelimiä						Kypsentämättömiä sisäelimiä					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyä kalaa						Kypsentämätöntä kalaa					_
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Kypsennettyjä luita tai rustoluita						Kypsentämättömiä luita tai rustoja					
kypsennettyja ruita tai rustoiuita	0	0	0	0	0	kypsentamattornia fuita tai fustoja	0	0	0	0	e
Kypsennettyä naudan mahaa						Naudan mahaa raakana					
rypsernettya naddan manaa	0	0	0	0	0		0	0	0	0	6
Kypsennettyä kananmunaa						Kypsentämätöntä kananmunaa					
Valitse		0	0	0	0	Valitse	0	0	0	0	
Nakki, lenkkimakkara yms.						Verilätyt					
Nakki, lenkkimakkara yms.	0		0	0	0	Verhacyc		~	~	~	
	-	-	-	-	-	Ruoantähteitä tai ihmisille tarkoitet	-		ocim	kail	
Maksalaatikko						tähteet, eineksiä, laatikoita)	tua ruor	(aa (	esim	Kdir	aki
	0	0	0	0	0	,	0	0	0	0	
Fermentoitua lihaa						Kuivattuja eläintenosia (esim sian	tai lamp	aank	orvia	. här	ān
Valitse	0	0	0	0	0	häntiä, kuivattua kanaa)					
vanse	0	<u> </u>		0			0	•	0	0	
Maitoa						Jäätelöä					
	0	0	0	0	0		0	0	0	0	
Maitotuotteet						Juustot					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	6
Kypsennettyjä vihanneksia						Kypsentämättömiä vihanneksia					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Fermentoitua viljaa						Fermentoituja kasviksia					
Valitse	0	0	0	0		Valitse	0	0	0	0	
Kypsentämättömiä hedelmiä Valitse	0	0	0	0	0	Kypsentämättömiä marjoja Valitse		0	0	0	
Valitse				0		Valitse			0	0	ł
							0				
Keitettyä riisiä						Muita viljatuotteita					
	0	0	0	0	0	_	0	0	0	0	
Perunaa	_		_	_	-	Pastaa, couscousia			_	_	
	0	0	0	0	0		0	0	0	0	
Leipää	_	-	~	~	_	Gluteenitonta leipää		-	-	~	
	0	0	0	0	0		0	0	0	0	
Kuivamuonaa makupaloina	_		_	_	_	Koirankeksejä		-	_	_	
	0	0	0	0	0		0	0	0	0	_
Kuivattuja sisäelimiä						Kuivattua kalaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	
Puruluita (nahasta valmistettuja)						Ulkona keppejä					_
	0	0	0	0	0		0	0	0	0	
Ulkona raatoja yms.						Ulkona ruohoa					
	0	0	0	0	0	1	0	0	0	0	e

				0	~	0	0	~	0	0
		-	-	-	-	Ulkona ulosteita	-	-	-	-
						Valitse	0	0	0	
Ulkona vettä lätäköistä yms.						Valitse	0	0	0	
	0	0	8	8	0					
						Mitä?				
Kasvisöljyä						Eläinperäiset öljyt ja rasvat				
Valitse	0	0	0	0	0	Valitse o	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	e
						Vrjölän puuroa, merkitse myös käytetyt vilja	-+-			
						durra hirssi initiaaniriisi	0L.			
Öljytuotteet						italianpantaheinä 🔍 kaura 🔍 ohra				
Valitse	0	ē	ō	ō	ō					
Valitse	ė.	ö	ö	ö	ö	riisi 🗖 ruis 🗖 speltti 🗖				
						tattari 🗆 tefheinä 🗖 vehnä 🗖				
								0		
Muu						Muu	-	-	-	
	0	0	0	0	0		0	0	0	e
	Mitä?					Mitä?				
1illainen koirasi alusta oli 6	osuus 6-1 -18kk ikäi	L8 kl senä	k ikä i? ( <i>li</i>	iisen iuka:	n koii s= e	anne ruuasta oli kotiruokaa? Valtse % anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää				
Aillainen koirasi alusta oli 6 Vääasiassa liukas lattia Sanomapaperia Tiedätkö miten usein koirasi Invioi pennun lepoaika (sisältää H1. Jos et vastannut kohta Jähinnä kotiruokaa Lähinn	oosuus 6-1 -18kk ikäi Pääasiassa e Pehmeä alu: Iepäsi 6-1 sekä päivä- nan 40 niii	18 kl senä ei liu sta, r 18 kl - että n mi	k ikä á? (li kas l matto k ikä á yõu uista	iisen iuka: attia oja iisen iisen nen) atko	s= e aä? , tun	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää 🔲 Maalattia/nu En tiedä 🔲	urmik dä	dko		a
Millainen koirasi alusta oli 6 Pääasiassa liukas lattia Sanomapaperia Fiedätkö miten usein koirasi Arvioi pennun lepoaika (sisältää #1. Jos et vastannut kohta Lähinnä kotiruokaa Lähinn	o osuus 6-1 -18kk ikäi Pääasiassa o Pehmeä alu: Iepäsi 6-1 sekä päivä nan 40 niii ä teollista n	18 kl senä ei liu sta, r 18 kl 18 kl n mu nuon	k ikä á? (li kas l matto k ikä á yõu uista	iisen iuka: attia jja E iisen nen) <b>atko</b>	a koin s= e: hä? h, tunt Sek	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä E ia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo oitus molemmista En tiedä / en muista ä / en muista	adä a	a ko		a
Millainen koirasi alusta oli 6 Pääasiassa liukas lattia Sanomapaperia Tiedätkö miten usein koirasi Arvioi pennun lepoaika (sisältää 41. Jos et vastannut kohta Lähinnä kotiruokaa Lähinn Muistatko mitä Arvioitko että se oli gluteenitont	o osuus 6-1 -18kk ikäi: Pääasiassa o Pehmeä alu: Iepäsi 6-1 sekä päivä aekä päivä aan 40 niii ä teollista n	18 kl senä ei liu sta, r 18 kl 18 kl n mo nuon	k ikä i? (li kas l matto k ikä i yöu uista aa	iisen iuka: attia ja iisen iisen nen) atko	n koin s= e: iä? ), tunn /tie Sek	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä En tia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo bitus molemmista En tiedä / en muista	adä a	a ko		a
Millainen koirasi alusta oli 6         Vääasiassa liukas lattia         Sanomapaperia         Giedätkö miten usein koirasi         Arvioi pennun lepoaika (sisältää         41. Jos et vastannut kohta         Aihinnä kotiruokaa         Lähinnä         Aivioitko että se oli gluteenitont         Kysymyksiä aikuisen         Kos aikuinen koiranne on aikaise         Sina syönyt suunnilleen samalla         Koiranne syö nyt. Jos aikuinen koiranne on aikaise	o osuus 6-1 -18kk ikäi: Pääasiassa e Pehmeä alu: lepäsi 6-1 sekä päivä- man 40 niii ä teollista n a? Kyllä © A koiran emmin syön tavalla, täy	L8 kl senä ei liu sta, r L8 kl - että nuon E nuon E yt eri ttäkä	k ikä i? ( <i>li</i> kas l matto k ikä i yöu uista aa ( i jaill ii aill ii aill	iisen iuka: attia oja E iisen nen) atko E E Dkir a kui loin v	n koin s= e: jä? ), tuni //tie Sek n tied	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä E ia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo bitus molemmista En tiedä / en muista ä / en muista	urmik dä oren: a uiner tä te	a koo	iran rann	e c
Aillainen koirasi alusta oli 6         Vääasiassa liukas lattia         Sanomapaperia         Tiedätkö miten usein koirasi kuvioi pennun lepoaika (sisältää         41. Jos et vastannut kohta aihinnä kotiruokaa         Kustatko mitä         Muistatko mitä         Kuvioitko että se oli gluteenitont         Kustatko mitä         Kustatkoiranne on aikaise         K	osuus 6-1 -18kk ikäi: Pääasiassa o Pehmeä alu: lepäsi 6-1 sekä päivä- man 40 niii ä teollista n a? Kyllä • <i>koiran</i> emmin syön tavalla, täy oiranne on yt viimeis kun se söi	L8 kl senä ei liu sta, r L8 kl - että nuon E nuon E M e yt eri ttäkä aikai en v kuter	k ikä i? ( <i>li</i> kas l: matto k ikä i yöu uista aa <i>ruc</i> i laill isemr rucd n alla	iisen iuka: attia attia iisen iisen iisen iisen iisen iisen atko Ei okin boin min s at noi	n koin s= e: jä? ), tunt //tie Sek n tied n tied n n tied n n tied n n se : vain   syöny aikar in Va	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä ia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo bitus molemmista En tiedä / en muista ä / en muista ä / en muista ta / en muista sta ja terveydestä ( > 1 v): syö nyt, täyttäkää silloin kysymys 42. Jos aiku kysymys 46. Täyttäkää tämä osio ajatellen mii t eri lailla kun se syö nyt voitte yrittää saada ma? sitse - Valtse vuotta vanha	urmik dä oren: a uiner tä te sen	a ko keet n koin selita	ranne	e c
Millainen koirasi alusta oli 6         Pääasiassa liukas lattia         Sanomapaperia         Tiedätkö miten usein koirasi kuvioi pennun lepoaika (sisältää         #1. Jos et vastannut kohta ähinnä kotiruokaa         Auvioitko että se oli gluteenitont         Muistatko mitä         Auvioitko että se oli gluteenitont         Kysymyksiä aikuinen koiranne on aikaise sina syönyt suunnilleen samalla koiranne syö nyt. Jos aikuinen k sysymyksissä 43-44.         42. Mitä koiranne on syöny Merkitse minkä ikäinen koira oli         Koira on aina syönyt samalla tav äyttää, jos koira vielä pentu- ta	o osuus 6-1 -18kk ikäi: Pääasiassa e Pehmeä alu: lepäsi 6-1 sekä päivä- taan 40 niii ä teollista n a 40 niii ä teollista n a 70 koiran emmin syön tavalla, täy toiranne on yt viimeis- kun se söi valla kuin ky	L8 kl senä ei liu sta, r L8 kl - että nuon E nuon E yt eri ttäkä aikai aikai aikai aikai aikai	k ikä i? ( <i>li</i> kas l: matto k ikä i yöu uista aa <i>ruco</i> i laill isemr rucod n alla ykse:	iisen iuka: attia oja iisen nen) atko El okir la kun loin v min s a: noi ssä n	n koin s= e: jä? ), tunn //tiee Sek n tied n tied tied n tied n t	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä E tia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo bitus molemmista En tiedä / en muista ä / en muista En tiedä / en muista ta / en muista Talenna va sta ja terveydestä ( > 1 v): syö nyt, täyttäkää silloin kysymys 42. Jos aiku syymys 46. Täyttäkää tämä osio ajatellen mi t eri lailla kun se syö nyt voitte yrittää saada ma? iltse - Valtse vuotta vanha tai kuin kysymyksessä no. 40 . Tätä ko	urmik dä nren: a uinen tä te sen bhtaa	a koo n koin idän selite	ranne	e c
Millainen koirasi alusta oli 6         Pääasiassa liukas lattia         Sanomapaperia         Fiedätkö miten usein koirasi         Arvioi pennun lepoaika (sisältää         41. Jos et vastannut kohta         Lähinnä kotiruokaa         Lähinnä kotiruokaa         Arvioitko että se oli gluteenitont         Muistatko mitä         Arvioitko että se oli gluteenitont         Kysymyksiää aikuinen koiranne on aikaise         aina syönyt suunnilleen samalla         koiranne syö nyt. Jos aikuinen k         tysymyksissä 43-44.         42. Mitä koiranne on syöny         Koira on aina syönyt samalla tav         äyttää, jos koira vielä pentu- ta	o osuus 6-1 -18kk ikäi: Pääasiassa e Pehmeä alu: lepäsi 6-1 sekä päivä- man 40 niin ä teollista n a? Kyllä <i>koiran</i> emmin syön tavalla, täy toiranne on yt viimeis- kun se söi valla kuin ky i kasvuiässä to: 1 = ei k	L8 kl senä ei liu sta, r L8 kl - että nuon E nuon E yt en ttäkä aikai aikai aikai aikai sosk:	k ikä i? ( <i>li</i> kas l: matto k ikä i yöu uista aa ( i laill is sill is sill is sill semr vuod n alla aan,	iisen iuka: attia oja E iisen nen) atko E bkir ia kuu loin v min s ssä n 2 =	n koin s= e: jä? ), tuni //tie Sek n tied n	anne ruuasta oli BARFia? Valtse % sim. parketti, lakattu puu, liukas muovi, Ulkona liukas jää Maalattia/nu En tiedä E ia / vuorokausi: Valtse En tie dätkö mitä koirasi pääasiassa söi nuo bitus molemmista En tiedä / en muista ä / en muista In tiedä / en muista ä / en muista In tiedä / en muista sta ja terveydestä ( > 1 v): syö nyt, täyttäkää silloin kysymys 42. Jos aiki ysymys 46. Täyttäkää tämä osio ajatellen mi t eri lailla kun se syö nyt voitte yrittää saada ma? altse - Valtse vuotta vanha tai kuin kysymyksessä no. 40 . Tätä ko taman kerran vuodessa, 3 = muutaman	urmik dä nren: a uinen tä te sen bhtaa	a koo n koin idän selite	ranne	e c

Kuivamuonaa	_	_	_	_	_	Koirille tarkoitettua säilykeruokaa		makk		_	
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Sairauksien erikoisruuat						Koirille tarkoitettua tuoreruokaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsennyttyä lihaa						Kypsentämätöntä lihaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsennettyjä sisäelimiä						Kypsentämättömiä sisäelimiä					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsennettyä kalaa						Kypsentämätöntä kalaa					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsennettyjä luita tai rustoluita						Kypsentämättömiä luita tai rustoja					
Kypsennettyja ruita tai rustoiuita		0	0	0	0	Kypsentamattomia fuita tai rustoja				0	
Kypsennettyä naudan mahaa	-	-	-	-	-	Naudan mahaa raakana	-	-	-	-	-
All and a second s		0	0			regular mariad raakdiid				0	
Kypsennettyä kananmunaa	-	_	-	-	-	Kypsentämätöntä kananmunaa		_	-	-	
Valitse	0	0	0	0	0	Valitse		0	0	0	0
Nakki, lenkkimakkara yms.	_									_	
Nakki, lenkkimakkara yms.		0	0	0	0	Verilätyt			0	0	
		-	~		-	Ruoantähteitä tai ihmisille tarkoite	-			-	
Maksalaatikko						tähteet, eineksiä, laatikoita)	atua ruo	каа (	esim.	Kalk	KI
	0	0	0	0	0	,,	0	0		0	0
Farman and Share						Kuivattuja eläintenosia (esim sian	tai lamn	aank	orvia	, här	än
Fermentoitua lihaa Valitoo						häntiä, kuivattua kanaa)					
A guide			-	-		-	0	0	•	Θ	0
Maitoa						Jäätelöä					
	0	0	0	0	0		0	0	0	0	e
Maitotuotteet						Juustot					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsennettyjä vihanneksia						Kypsentämättömiä vihanneksia					
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Fermentoitua viljaa						Fermentoituja kasviksia					_
Valitse	0	0	0	0	0	Valitse	0	0	0	0	0
Kypsentämättömiä hedelmiä						Kypsentämättömiä marjoja					_
Valitse		0			0			0	0	0	0
Valitse				0		Valitse					
	-	-	-	-	-			-	-	-	
Keitettyä riisiä	0	0	0	0	0	Muita viljatuotteita	0	0	0	0	0
Demograph		0	0	0	0	Destra	0	0	0	0	0
Perunaa	0	0	0	0	0	Pastaa, couscousia	0	0	0	0	0
lainää	0	0	0	0	0	Gluteeniteetta laia ää	0		0	0	0
Leipää	0	0	0	0	0	Gluteenitonta leipää	0	0	0	0	0
	0	0	0	0	0	Koirankeksejä	0		0	0	0
Kuivamuonaa makupaloina	0	0	0	0	0	Koirankekseja	0	0	0	0	
Kuivottuio cicželimiž	0	0	0	0	0	Kuivattua kalaa	0		0	0	0
Kuivattuja sisäelimiä Valitse	0	0	0	0	0	Kuivattua kalaa Valitse	0	0	0	0	0
Valitse		0	0	0		Valitse		0	0	0	0
			-				5			-	
Puruluita (nahasta valmistettuja)	-		-	_	-	Ulkona keppejä	_	-	-	-	
	0	0	0	0	0		0	0	0	0	0
Ulkona raatoja yms.	-		-	_	_	Ulkona ruohoa	_	-	-	-	
	0	0	0	0	0	-	0	0	0	0	0
Multaa	-		_	_	_	Savea, kiviä	_			_	_
	0	0	0	0	0		0	0	0	0	0
Ulkona vettä lätäköistä yms.						Ulkona ulosteita	-			_	_
orkona vella lalakoista yms.	0	0	0	0	0	Valitse	0	0	0		0
		1.0	100	100	100	Valitse	0	0	0	0	- 0

Kasvisöljyä						N	litä?				
						Eläinperäiset öljyt ja rasvat					
Valitse			0 0	0	0	Valitse	0	0	0	0	
Valitse				0		Valitse			0	0	
Y allase											
						Vijölän puuroa, merkitse myös käy	tetyt vil	jat:			
						durra 🔍 hirssi 🔍 intiaaniriisi 🔍					
ljytuotteet						italianpantaheinä 🔍 kaura 🔍 ohra					
Valitse		5 (	5 6	0	ē						
Valitse		0 (	6 G	0	0	riisi 🔍 ruis 🔍 speltti 🗖					
- and -						tattari 🛛 tefheinä 🖉 vehnä 🗖					
						tattari 🗆 terneina 🗆 venna 🗆					
							0	0	0	0	
luu						Muu					
	(	0 (	0 0	0	0		0	0	0	0	
	Mitä?					N	litä?				
′oisitteko arvioida m	iten iso osuus a	aikui	sen	koira	nne r	uuasta on muuta teollista ruoka uuasta on kotiruokaa? Valitse % uuasta on BARFia? Valitse %	a? Vali	tse %			
ääasiassa liukas lattia						<b>rketti, lakattu puu, liukas muovi,</b> Ulkona liukas jää 🔲 Maa				-	
anomapaperia 📃	Pehmeä a	alusta	a, mat	ttoja		En tiedä 📃					
liten usein koirasi le liten paljon aikuinen ko		a/v	uorok	ausi:	Valit	se	En ti	iedä			
							vaihdo	sta.	105	ette	
alua vastata tähän insimmäisen kerran l a) Miltei tai täysin te b) Miltei tai täysin te c) Miltei tai täysin te d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruokaa	kysymykseer kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an	n,sii Din \ n Y2 k an ko	rtyk /alitse oti + tiruok	ää ky vuo Y <sub>2 tee</sub>	ysynn otta, s	ykseen 44 tai 45. silloin vaihdoin:	vaihdo	sta.	202		
alua vastata tähän insimmäisen kerran l a) Miltei tai täysin te b) Miltei tai täysin te c) Miltei tai täysin te d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruokaa	kysymykseer kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an	n,sii Din \ n Y2 k an ko	rtyk /alitse oti + tiruok	ää ky vuo Y <sub>2 tee</sub>	ysynn otta, s	ykseen 44 tai 45. silloin vaihdoin:	vaihdo	sta.	202		
alua vastata tähän nsimmäisen kerran l a) Miltei tai täysin te b) Miltei tai täysin te c) Miltei tai täysin te d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruoka h) Teollisesta teollise	kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an een ruokaan	n,sii oin ∖ 1 ¥₂k an ko BARF	rtyk: /alitse oti + tiruok iin	ää ky vuo <sup>Y</sup> ₂teo aan	ysynn otta, s	ykseen 44 tai 45. silloin vaihdoin:	vaihdo	sta.	202		
<ul> <li>Hissä iässä oletti halua vastata tähän ensimmäisen kerran li a) Miltei tai täysin tei b) Miltei tai täysin tei c) Miltei tai täysin tei c) Miltei tai täysin tei d) Kotiruuasta täysin ei Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruoka:</li> <li>h) Teollisesta teollise</li> <li>i) Y<sub>2</sub> koti + Y<sub>2</sub> teollise</li> </ul>	kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an een ruokaan	n,sii oin ∖ 1 ¥₂k an ko BARF	rtyk: /alitse oti + tiruok iin	ää ky vuo <sup>Y</sup> ₂teo aan	ysynn otta, s	ykseen 44 tai 45. silloin vaihdoin:	vaihdo	sta.	105		
alua vastata tähän insimmäisen kerran l a) Miltei tai täysin te b) Miltei tai täysin te c) Miltei tai täysin te c) Miltei tai täysin te d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruokaa h) Teollisesta teollise i) Y <sub>2</sub> koti + Y <sub>2</sub> teollise Mikä oli syy, että vaihdo inta Saata vaihtelua koiralle	kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an esta ruuasta teoll oitte dieettiä? avuus I Jon Ilmavaiva sulatuksen suhtee	h, sii bin \ 1 Y2 k an ko BARF iseer hkun at	rtyk /alitse oti + tiruok iin	ää ky vuo ½ tei kaan kaan	ysym ttta, s ollisee Haise ovaive	silloin vaihdoin: en ruokaan Sairaus Valitse eva henki Ruokahaluttomu oja nuorena Nivelrikko	Liho us nuokaa	i 🗖	105		
alua vastata tähän asimmäisen kerran l a) Miltei tai täysin tee b) Miltei tai täysin tee c) Miltei tai täysin tee c) Miltei tai täysin tee d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruoka: h) Teollisesta teollise i) ½ koti + ½ teollise ii) ½ koti + ½ teollise Mikä oli syy, että vaihdo finta Saata vaihtelua koiralle tsinyt parempaa ruuans kuuat vaihdettiin toisen Muu syy, mikä los syy oli sairaus, au	kysymykseer kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an een ruokaan esta ruuasta teoll bitte dieettiä? avuus Jor Ilmavaiva sulatuksen suhtee koiran takia	h, sii h Y <sub>2</sub> k an ko BARF iseer hkun at K an n	rtyk /alitse oti + tiruok iin 1 ruok suosi coirall	ää ky vuo ½ tei (aan (telen tuust a aina os siil	ysym ttta, s ollisee Haise ovaivv a eri r	silloin vaihdoin: silloin vaihdoin: en ruokaan sairaus Valitse eva henki Ruokahaluttomu oja nuorena Nivelrikko uuat Vaihdan ruokamerkkiä	Liho us vruokaai välillä	i -			
alua vastata tähän insimmäisen kerran l a) Miltei tai täysin teo b) Miltei tai täysin teo c) Miltei tai täysin teo d) Kotiruuasta täysin e) Kotiruuasta täysin f) Barfista teolliseen g) Barfista kotiruoka: h) Teollisesta teollise i) ½ koti + ½ teollise likä oli syy, että vaihdo finta Saata /aihtelua koiralle tsinyt parempaa ruuans tuuat vaihdettiin toisen //uu syy, mikä	kysymykseer kun koira oli no ollisesta vaihdoir ollisesta kokonaa ollisesta ruuasta teolliseen BARFiin an esta ruuasta teoll oitte dieettiä? avuus Jor Ilmavaiva sulatuksen suhtee koiran takia	h, sii h Y <sub>2</sub> k an ko BARF iseer hkun at k k an n k an n oi? V	rtyk /aitse oti + tiruok iin i ruok suosi ioirall	ää ky vuo <sup>y</sup> 2 tee caan caan ttelen Luust a aina a siil	ysym ttta, s ollisee Haise ovaivu a eri r hen? vaiht	Sairaus Valitse silloin vaihdoin: en ruokaan eva henki Ruokahaluttomu oja nuorena Nivelrikko uuat Vaihdan ruokamerkkiä Kyllä Ei En tiedä / oehdoista (a-i): Valitse	Liho us vruokaai välillä	i -			

<ul> <li>e) Kotiruuasta täysir</li> <li>f) Barfista teolliseen</li> </ul>	
/	
g) Barfista kotiruoka	
<ul> <li>h) Teollisesta teollis</li> </ul>	een ruokaan
© i) Y₂ koti + Y₂ teollis	sesta ruuasta teolliseen ruokaan
Mikä oli syy, että vaihd	oitte dieettiä?
	tavuus 🛛 Jonkun suosittelema 🖾 Sairaus Valitse 🛛 Lihoi 🗖
Vaihtelua koiralle 📃	Ilmavaivat 🔍 Haiseva henki 🔍 Ruokahaluttomuus 🔍
Etsinyt parempaa ruuan	nsulatuksen suhteen 🔍 Luustovaivoja nuorena 🔍 🛛 Nivelrikkoruokaan 🔍
Ruuat vaihdettiin toisen	n koiran takia 🔍 Koiralla aina eri ruuat 🔍 Vaihdan ruokamerkkiä välillä 🗖
Muu syy, mikä	
Jos syy oli sairaus, a	uttoiko ruokinnan muutos siihen? Kyllä 🌼 🛛 Ei 🔍 En tiedä / en muista 🔍
Jos syy oli sairaus, m	nikä vaihto auttoi? Valitse joku vaihtoehdoista (a-i): Valitse
Kolmannen kerran k	un koira oli noin Valitse vuotta, silloin vaihdoin:
a) Miltei tai täysin tr	eollisesta vaihdoin Y2 koti + Y2 teolliseenruokaan
	ollisesta kokonaan kotiruokaan
c) Miltei tai täysin te	eollisesta ruuasta BARFiin
<ul> <li>d) Kotiruuasta täysir</li> </ul>	n teolliseen
<ul> <li>e) Kotiruuasta täysir</li> </ul>	n BARFiin
<ul> <li>f) Barfista teolliseen</li> </ul>	
g) Barfista kotiruoka	
h) Teollisesta teollis	
i) ¥2 koti + ¥2 teollis	sesta ruuasta teolliseen ruokaan
Mikä oli syy, että vaihd	oitte dieettiä?
	tavuus 🕘 Jonkun suosittelema 📄 Sairaus Valitse Lihoi 🗖
Vaihtelua koiralle 🔲	Ilmavaivat Haiseva henki Ruokahaluttomuus
Etsinyt parempaa ruuar	nsulatuksen suhteen 🔍 Luustovaivoja nuorena 🔍 Nivelrikkoruokaan 🔍
	n koiran takia 🔍 Koiralla aina eri ruuat 🔍 Vaihdan ruokamerkkiä välillä 🔍
Muu syy, mikä	
Jos syy oli sairaus, a	uttoiko ruokinnan muutos siihen?Kyllä 🔍 🛛 Ei 🔍 En tiedä / en muista 🔍
	den da areas la classica de la construcción de
Jos syy oli sairaus, n	nikä vaihto auttoi? Valitse joku vaihtoehdoista (a-i): Valitse
44 5-23-41-2 -442	haimllari an maha allansia?
	<b>koirallasi on ruoka-allergia?</b> äilet että koira on yliherkkä tai allerginen (antaa iho-, korva, silmä, ja/tai tassuoireita)?
	<b>koirallasi on ruoka-allergia?</b> äilet että koira on yliherkkä tai allerginen (antaa iho-, korva, silmä, ja/tai tassuoireita)? Muu
Mille ruoka-aineelle epä Valitse	äilet että koira on yliherkkä tai allerginen (antaa iho-, korva, silmä, ja/tai tassuoireita)? Muu
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