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Impact of nutrition on canine behaviour

Literature review

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Abstract

Behaviour issues significantly impact the welfare of both dog and owner, it can be a public health threat and are a common reason for dogs being abandoned in shelters or euthanized. Aggression, separation anxiety, and general anxiety disorder are the most seen issues to date, where multiple factors such as genetics, breed, rearing, and environment contribute. Nutrition plays a vital role in many processes of the body, but is not focused on during the treatment of animals with behavioural issues. This literature review focuses on how nutrition impacts behaviour and what links can be drawn between them.

Összefoglaló

A viselkedési problémák egyaránt jelentős hatással vannak a kutya és a tulajdonos jólétére. Közegészségügyi jelentőséggel is bírhatnak és gyakori okai annak, hogy a kutya menhelyre kerül vagy elaltatják. A leginkább említést érdemlő problémák az agresszivitás, a szeparációs szorongás és a szorongásos zavarok, amelyek kialakulásában többféle tényező is szerepet játszhat, mint az életkor, a fajta, a nevelés és a környezet. A táplálás a szervezetben zajló számos folyamatban kulcsfontosságú szerepet tölt be, de a viselkedési rendellenességek kezelésében játszott szerepe kevésbé hangsúlyos. Az összefoglaló célja a táplálás és a viselkedés közötti összefüggések bemutatása.

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Abbreviations

AA: Arachidonic acid

AAFCO: Association of American Feed Control Officials

ADHD: Attention Deficit Hyperactivity Disorder

BW: Body weight

C-BARQ: Canine Behavioural Assessment & Research Questionnaire

CBC: Canine Behavioural Checklist

CCR: Cortisol : Creatinine Ratio

CFU: Colony forming units

CP: Crude protein

DHA: Docosahexaenoic acid

DM: Dry Matter

EDED: Evaluation of dog's emotional and cognitive disorder ME: Metabolizable energy

ELISA: Enzyme Linked Immunosorbent Assay

EPA: Eicosapentaeonic acid

FEDIAF: European Pet Food Industry Federation

fT4: Free Thyroxine

GABA: Gamma-aminobutyric acid

HDL-C: High Density Lipoprotein Cholesterol

HP+/-Trp: High protein with or without tryptophan supplement

HPA: Hypothalamic-Pituitary Adrenal axis

LDL-C: Low Density Lipoprotein Cholesterol

LNAA: Large Neutral Amino Acid

LP+/-Trp: Low protein with or without tryptophan supplement

LysoPC: Lysophosphatidylcholines

LysoPE: Lysophosphatidylethanolamine

MCT: Medium Chain Triglyceride

ME: Metabolizable energy

RIA: Radioimmunoassay

SEM: Standard Error of Mean

TAG: Triacylglycerol

TSH: Thyroid Stimulating Hormone

TT4: Total Thyroxine

UCCR: Urinary Cortisol: Creatinine Ratio

VLDL-C: Very Low Density Lipoprotein Cholesterol.

1. Introduction

1.1. The importance of nutrition

Over the last decade, there have been changes in the nutrition and different food trends for dogs. Even though the domestic dog evolved from the grey wolf around 15.000 years ago, the *Canis familiaris* diet is quite different from the *Canis lupus*, the grey wolf's nutrition consists of a large amount of meat, whereas a dog's diet in later years is dry kibble containing more carbohydrates than fresh meat [1]. Even with these changes in their nutrition, little thought has been given to their effect on behaviour with the rising behavioural issues through the years.

1.2. Nutrient requirements of dogs

From a consumer perspective, there is a battle between the different food-producing brands about what type of nutritional base is the best. Some brands are exclusively using dry kibble with a main base of grains and animal byproducts that have been processed into dry meat meals. While on the other end, there are the raw food companies. They are meat-based, containing low amounts of fruit or vegetables as their carbohydrate, and therefore low in grains. A trend amongst consumers are dry kibble products with a higher percentages of fresh meat, which are either grain-free or low in grains. In the middle we have wet food, which often have a high percentage of meat from animal or fish derivates, wet food is high in moisture and are hermetically sealed [2]. Vegetarian and vegan kibble has also hit the market, created without meat by using a selection of grains, legumes, vegetables, fruit, and additionally, eggs concerning the vegetarian kibble amongst other ingredients [3].

Nutrition of dogs are widely discussed in many platforms by many people of varying knowledge with different meanings and strong opinions used by many regular people. Therefore keeping a set standard for the nutrient requirements would be important to ensure dietary needs are met by commercial or homemade diets.

Energy

Dogs need to be fed according to their body and age to have enough energy to sustain their body's needs throughout the day. The majority of energy originates from carbohydrates, protein and fats. Ideally, we want to keep a body condition score between 4 and 5 on the 9-point scale. According to the European Pet Food Industry Federation (FEDIAF), the

maintenance energy requirement varies with age, but for an adult dog, the requirement is between 95 – 130 kcal ME/kg $^{0.75}$ [4].

Protein and amino acids

Proteins and amino acids are needed for survival, especially the essential amino acids that are received from nutrition that dogs can't synthesize for themselves, which are crucial for several physiological functions [5]. The quality of protein used in dog food is significant as low-quality proteins have one or numerous limiting amino acids, whereas the best quality has none and is, therefore, more utilizable to the body. The quality of proteins is defined by the amino acids' efficiency in converting into tissues [6]. The quality of proteins can be measured by biological value and is defined as the percentage of absorbed protein that is retained, and therefore assesses the efficiency of converting these dietary amino acids in the body [6]. Eggs, turkey thigh and fish meal are protein sources with high biological value, while feather meal and various cereals are lower due to several limiting amino acids [7]. Plant proteins may be less sufficient in regards to levels of cysteine, lysine, methionine and taurine. In comparison to proteins of animal origin, plant proteins have a lower biological value due to a more deficient amino acid profile, in addition to a possible lower digestibility [8] Feeding dogs a diet of proteins with high biological value therefore aids in achieving a balanced intake of amino acids [9]. According to the FEDIAF and Association of American Feed Control Officials (AAFCO), adult dog food should contain a minimum of 18% crude protein on a dry matter basis. A maximum percentage of crude protein has not been set for adults by the FEDIAF. [4] FEDIAF recommends a minimum level of 25 grams of protein per 100 grams DM for puppies under 14 weeks and 20 grams of protein per 100 grams DM for puppies over 14 weeks. FEDIAF has only stated a maximum recommended nutritional amount of protein in regards to Lysine in puppies, which is 2,8 grams per 100 grams DM [4].

Fats and fatty acids

Bauer (2006) states fats can be categorized into facilitative and functional types of fat, the facilitative fats, including saturated and trans fats enhance palatability and assists the absorption of fat-soluble vitamins. Functional fats, including polyunsaturated fats like docosahexaenoic acid (DHA) and arachidonic acid (AA), are essential for the skin, keeping down inflammation and aids neurological development. [10] Essential fatty acids are an important nutritional need, and it is therefore important to meet the required levels of fatty

acids, where Omega-3 and -6 are especially important. Good sources of omega-3 are fish and seafood which are rich in DHA and EPA, such as salmon, herring, and mackerel. In regards to omega-6, linoleic acid are often sourced from sunflower, corn, and their oils [11]. Although, a high intake of omega-6 is found to be pro-inflammatory due to an increase of their metabolic derivatives leading to higher enzyme production of cyclooxygenase and lipoxygenase from eicosanoids derived from amino acids, causing inflammation and pro-inflammatory cytokine production [12] [13]. According to FEDIAF, the percentage of fat in a dog's diet should be a minimum of 5.5 grams per 100 grams DM, the recommended minimum for puppies is 8.5 grams per 100 grams DM. FEDIAF has not set a maximum limit for adult dogs, but linoleic acid has a nutritionally recommended maximum of 6.5 grams per 100 grams DM for puppies in early growth [4].

Carbohydrate

In the dog food industry, the carbohydrate constituents around 30-60% of the dry matter from kibble. [14] Even though carbohydrates are a macronutrient, there are no specific requirement level set for dog food. It can be divided into digestible carbohydrates from sugars and starches, and indigestible carbohydrate from fibre. Carbohydrates are an important source of energy, as well as a source of fibre [15, 16].

Vitamins

Vitamins are organic compounds that are vital for several metabolic processes in the body regarding health, sustaining growth and survival. Consequently, a small quantity of them are a dietary requirement for dogs and need to be appropriately balanced in their diet. Frequently, the precursor is added in the diet so the vitamin can be synthesized in the body from it. We can divide them into fat-soluble and water-soluble vitamins, where the water-soluble category includes the B complex and C vitamins, and the fat-soluble vitamins contain A, D, E, and K. The body does not store significant quantities of water-soluble vitamins, whereas the fat-soluble vitamins are absorbed and stored in the body, due to this there are possibilities for hypervitaminosis and even toxicity due to over-supplementation [5].

Minerals

Minerals are inorganic substances needed in the body and constitute less than 1% of the body weight (BW). Despite their small constitution in the body, they are still vital for many functional processes, including proper growth. They are categorized into Macro- and

Microminerals after the body´s need for the mineral. The macrominerals, including calcium, chlorine, magnesium, phosphorus, potassium and sodium, play a significant role in sustaining the acid-base balance, osmotic pressure and other various processes [5]. FEDIAF states diets need calcium, in a minimum of 0.50 grams in adults based on maintenance energy requirement of 110 kcal/kg$^{0.75}$ [4]. In the micromineral group there are copper, iodine, iron, manganese, selenium, and zinc aid in numerous processes and in regards to creation of many components and enzymes [5].

Dietary fibre

Dietary fibre consists of plant-originating carbohydrates that cannot be broken down by the body´s enzymes and therefore, serve as a source of nutrition for bacteria living in the digestive system. The primary constituents of dietary fibres include cellulose, pectin, hemicellulose, mucilage, gums, and lignin. Even though it cannot be absorbed, the fibre still impacts gut health, gut motility, transport time, and the gut microbiota. Dietary fibre's polysaccharide chains are connected by beta bonds, which makes them undigestible [5]. Dry kibble contains an estimate of between 2.5 – 4.5% of crude fibre, it should not exceed 10% of a dog's diet [17].

1.3. The most common behaviour problems

There are various common behaviour problems and severities within a behavioural problem, some are more prominent than others while some have a lower threshold to get different behavioural issues based on their genetics, breed, upbringing, and environment. A behavioural problem states any behaviours of a companion animal that is unacceptable to the owner [18]. The most common behaviour problems are aggression, separation anxiety, general anxiety, excessive barking, reactivity, resource guarding, and phobias.

Quite commonly, behavioural problems might stem from underlying conditions such as pain, neurological disorders and endocrine diseases, where pain might cause fearful or aggressive tendencies, several endocrine imbalances can cause behavioural changes and neurological disorders or trauma can cause various behaviour changes as for example; aggression due to tumours in the brain [19]. Pain is speculated to be the highest cause of behavioural change and there are many clinical cases where pain can be the single reason for behaviour change due to for example; the risk and vulnerability it brings or the fear of being touched at painful areas of the body and therefore feels the need to use defence as the best way out [20].

Therefore, a thorough examination and blood profiling among other diagnostics, should be the first step if behavioural changes are seen.

Behaviour problems are often related to stress where the dog is inept to cope, stress triggers a change in homeostasis, caused by a physical or emotional stressor which initiates the stress mechanism [21]. The process quickly activates and the sympathetic-adrenal medulla axis (SAM) releases catecholamines in the bloodstream which causes vasoconstriction leading to increased cardiac output and heightened blood pressure to prepare the body for fight or flight, meanwhile the hypothalamic-pituitary-adrenal axis (HPA) secretes corticotropin-releasing hormone and adrenocorticotropic hormone release, which cause the release of cortisol [22]. The HPA axis can be stimulated continuously in the case of chronic stress, leading to an increased cortisol level and causing depression of the catecholamine system, risking stress-related diseases emerging and alteration of the immune system [21].

Aggression is the most common type of behavioural problem and most commonly stems from fear, dominance or territorial aggressions [23]. Fear-based aggressive dogs display their tail down and ears back, barking at their fear-based stressor. These stimuli can be noise from the environment, unknown objects, strangers or animals. Fear-based aggressive dogs will often retreat when possible, but are triggered to growling and snapping in situations they deem uncomfortable [23].

Dominance aggression is one of the most common types of aggressivity, this form of aggressivity happens towards both other dogs and people, where they compete for resources, such as food, sleep areas and toys. Consequently, the victor claims a more dominant position in the hierarchy [24]. A dominance aggressive dog tends to have a tense body language, positions themselves on top of the other dog or person, intense direct eye contact, raised tail and ears, growls, snapping and can bite [23].

Possessive aggression occurs when the dog highly values food, toys or chews, and the owner attempts to take it from them or in some cases just approaches. Often caused or worsened by the owners not leaving the dog in peace while they are enjoying their highly valued object. Body language is often tense, licking their lips, trying to escape with their object. If not taken seriously, small signs quickly lead to growling and even biting [23]. Protective aggression is found when a dog behaves aggressively to guard their home, person or areas from external

environmental triggers [23]. To achieve this they commonly bark or growl while running around the perimeter [25].

Anxiety disorders can cause a variety of behaviours and occur when the anxiety is continual while no environmental stressors are justifying it, leading to the dog being unable to cope normally. Generalized anxiety disorder, separation anxiety, phobias and fear-related aggression are the most common behaviour issues related to anxiety.

Separation anxiety is quite common and is defined as distress while being left home alone or separated from their owner. Separation anxiety can develop when the dog is constantly together with their owner for long periods if the owner is working from home or unemployed. Examples of other common causes can be substantial changes in their routines, moving houses, or being in a kennel [25]. Separation anxiety behaviours can often escalate and the dog might feel the need to self-soothe with behaviours such as destruction, panting, restlessness, elimination and vocalization when left alone. Dogs with separation anxiety might also try to escape and can cause harm to themselves in the process due to the destruction while breaking out [26].

General anxiety disorder in dogs occurs when the dog experiences anxiety during most of their time and exhibits signs of fear as well as regularly exhibiting tensed or frightened posture, which might be regardless of any situation or non-existent stressor [27]. Commonly the dog is restless, insecure, shivering, nervous, continuously alert, struggling to properly relax or fall asleep [25].

Noise fears are one of the most common behavioural problems where dogs are typically afraid of sudden and loud noises, such as fireworks, thunder and gun shots as the most common causes. The dog exhibits tensed and frightened posture, often shaking and might go to hide under furniture or in other quieter rooms while panting and showing stressed behaviour. Treatment have previously been anti-anxiety medications such as Sileo®, thundershirts, pheromones, herbal additives and training the dog slowly upwards in noise level [28].

1.4. The importance of behavioural problems

There is a plethora of dogs being rehomed, stuck in shelters, or euthanized due to behavioural issues. Aggression has a big impact on human public health due to economic expenses, as well as physical and psychological damages from bite-related aggressions [29]. According to the British Veterinary Association, around half of veterinarians in the UK have seen a rise in aggressivity, especially following the puppies born during the pandemic. Probably due to first-time owners being more likely the majority to acquire puppies during the pandemic on impulse and were likely not critical to the breeder in regard to health testing and their temperament as well [30]. Additionally, many veterinarians does not incorporate behavioural medicine into the clinical practice, this is also something that should be shed a light on as professional help is equally important for the dogs welfare [31]. Many factors were linked to higher likelihood like lower socioeconomic status and owners spending more time away from home, and males were found to have a higher likelihood of behavioural problems [29]. Genetics, the dog breed and their instincts will also play a part in regard to behaviour, several dog breeds are more likely to get behaviour problems like aggression in cocker spaniels. For example; high-risk factors of dominance aggression include male dogs, first-time owners, no obedience schooling, a higher level of education, small-sized dogs, impulsively bought or bought for guard purposes, younger owners or elderly [32]. Nutrition today gives the dog the nutrients needed, but few commercial diets focus on how diets can be altered to possibly improve different behavioural struggles, perhaps this can be avoided or at least reduced with proper nutrition and special dietary measures according to the extra needs that might be necessary in these cases.

2. Objectives

For this literature review, I want to look into the correlation of nutrition to canine behaviour, discover what studies are done on this topic, what are their limitations and investigate what should be studied further after gaining knowledge on the topic. This literature review focuses on how nutrition impacts nutrition and what links can be drawn between them.

3. Method

While researching for literature, I utilized mostly online sources from Google Scholar, Springer, PubMed and ScienceDirect. Mostly research papers were used, but review articles, some books and papers were used for general knowledge or wherever relevant. I would

search for keywords related to “dog, canine, behaviour, behavioural problems, aggression, anxiety, nutrition, amino acids, fatty acid, phospholipid, nutraceutical, microbiome” and used together with “or” or “and” to combine keywords. During the search, the newest articles were prioritized whenever all criteria fit. The studies used were focused on different nutrients in relation to behaviour, some were focused on finding what substances were related to different behavioural issues and others focused on studying if supplementation of these would improve their linked behavioural issue. Most of the studies used control or placebo groups, while a few others had several groups go through a control diet and thereafter use the diet or supplementation in relation to the study. In addition, the studies where animals were kept in other housing facilities than the privately owned homes, all had the approval of the ethical committee in their homeland where the study took place. Most of the dogs were recruited in veterinary clinics and the owners of the privately owned dogs signed documents letting the dogs be used in the studies. This review summarizes the relevant literature on the impact of nutrition on canine behaviour.

4. Literature review

4.1. The effects of dietary amino acids and protein content on behaviour

4.1.1. Tryptophan

Bosch *et al.* (2007) states behaviour is regulated by hormones and neurotransmitters; therefore, the respective hormones and neurotransmitters can be affected by a varying or low levels of precursors. Serotonin is an important central nervous system neurotransmitter regulating mood, sleep, appetite, learning, sexual behaviour and other important functions. Tryptophan (Trp) is the precursor of serotonin and is the initial limiting factor of synthesizing serotonin, therefore increased Trp quantities would lead to an increase of serotonin in the brain [33].

A study explored by Cakiroglu *et al*. (2007) studied 23 aggressive dogs and a control group of 18 dogs with normal behaviour of all genders and a compilation of different breeds. They were assessed from an aggression chart and sampled 5 ml of blood from the jugular vein after fasting for 12 hours to measure chemically derived serotonin by a serotonin ELISA kit for quantitative measuring. An autoanalyzer was used to measure the lipids, as well as spectrophotometry [34]. During the study they examined the serum serotonin levels in all 41 dogs to determine if serotonin had a correlation to aggression, resulting in the revelation that

aggressive dogs had a significantly lower average serum concentration of serotonin, but no significant difference in the serum lipids [Table 1].

Table 1: "Mean concentrations of serotonin and serum lipids" [34].

Serum constituent	**Aggressive dogs**	**Non-aggressive dogs**	χ^2
Serotonin (ng/ml)	12.0	32.5	29.6 (P<0.01)
Total cholesterol (mg/dl)	19.7	22.6	0.60
Triglycerides (mg/dl)	20.4	21.7	0.11
HDL-C (mg/dl)	19.8	22.6	0.56
LDL-C (mg/dl)	20.8	21.3	0.01
VLDL-C	20.5	21.6	0.08

Contradictory to Cakiroglu *et al*. (2007), Jacobs *et al*. (2007) completed an experiment to measure neurons expressing the serotonin-1B receptors in the basolateral nuclear group in the amygdala. In this study, they confirmed aggressive dogs have an increased amount of serotonin-1B receptors expressing neurons compared to the normally behaved dogs. A potential interpretation could be that aggressive dogs may have reduced serotonergic activity due to activation of pre-synaptic serotonin-1B receptors, which there are an increased amount of and therefore lead to a decrease in serotonin released [35].

Bosch et al. (2007) reviewed the effects of Trp supplementation in a dogs diet and how it affected aggression. Since Trp is the precursor to serotonin and studies show aggressive dogs have lower levels, this study investigated if tryptophan could affect the serotonin levels due to being the rate-limiting substance for hydroxylation. Tryptophan hydroxylase is usually not fully saturated with Trp, with dietary supplementation this could increase the synthesis leading to higher serotonergic neurotransmission. Trp is in lower concentrations in food compared to other amino acids even though being found in most foods, therefore Trp competes for the same carriers to cross the blood-brain barriers into the brain as other amino acids which are found in higher amounts [33].

A hypothesis about lowered serotonin levels due to high carrier competition to cross the blood-brain barrier in a high protein diet causing less Trp taken up to synthesize the serotonin [33]. DeNapoli *et al.* (2000) studied the effects of diet with high and low protein content, including Trp supplementation versus without in 33 dogs. The dogs were divided into 3 study groups based on their aggressivity type, the groups were divided into dominance aggressive dogs, territorial aggressive dogs and hyperactive dogs [36].

The diets were 1) High protein content with tryptophan supplementation were given a diet with 315 g protein per kg and Trp supplement of 3.7 g, leading to a Trp:LNAA ratio of 0.06:1. 2) High protein content with no Trp supplementation, consisting of 308 g protein per kg and 2.4 g per kg Trp, giving a Trp:LNAA ratio of 0.04:1. 3) Low protein content diet with tryptophan supplement containing 188 g protein per kg and 3.0 g per kg of Trp supplement, leading to a Trp:LNAA ratio of 0.07:1. 4) Low protein content with no supplementation contained 186 g protein per kg and 1.8 g per kg Trp, giving a Trp:LNAA ratio of 0.04:1 [36].

Each diet was used for one week in randomized order, with a 3-day transitioning time between each diet and the dog participated in this study for a minimum of 40 days. The owner evaluated the dog daily with behaviour scores in regard to dominance aggression, territorial aggression, hyperactivity and fear for analyzation. Serum Trp and serotonin were obtained by blood samples before the end of each diet shortly after a meal. High-performance liquid chromatography was used and concentrations of the Trp and serotonin were calculated from the peaks and analysed by ANOVA, additionally the behavioural measurements were statistically analysed [36].

DeNapoli *et al.* (2000) did not find significant behavioural changes during the study, although, territorial aggression scores were significantly higher in the dogs fed the low protein with no tryptophan supplement in comparison to the low protein diet with Trp supplementation. Dogs fed on high protein and no Trp supplementation showed significantly higher dominance aggression scores compared to the other diets. Fearfulness and hyperactivity were not significantly different through any of the diets [36]. DeNapoli *et al.* (2000)´s study found lowered dominance and territorial aggression in both diets where Trp were supplemented, leading to the conclusion of Trp being an important factor due to both

the low and high protein diets with tryptophan supplement had a higher ratio of Trp to LNAA [36].

Contradictory to other studies mentioned, Bosch *et al.* (2009)'s study on mildly anxious dogs supplemented with Trp found increased level of plasma Trp but no significant effect were seen on behaviour of the dogs in Trp supplementation group compared to the control group on a control diet from both owner assessment and behavioral tests [37]. The findings might implicate limiting effect in healthy dogs due to the significantly difference found in other studies on Trp regarding aggression, or perhaps Trp is not the main substance to aid in anxiety.

A case study by Suñol *et al.* (2020) on a male, rescued mixed breed dog with increasing behaviour change and aggressivity were successful in dietary treatment. The dogs were examined by several veterinarians and specialists, the dogs were widely tested and examined to exclude other causes of the behaviour change as well. The aggressivity were increasing, as well as noise sensitive anxiety to usual sounds as the washing machine [38]. After physical examinations, normal haematology and biochemistry, normal endocrinological analysis were normal, as well as nothing found by magnetic resonance imaging and cerebrospinal fluid analysis. Dietary changes were trialled after the verification of the study from DeNapoli *et al.* (2000) and the microbiome communication and modulating via the gut-brain axis. Purina Pro Plan HA Hypoallergenic® was used due to the usage of gluten-free hydrolysed protein. Already a week later, a change was seen in calmer emotion and less aggressivity, another two weeks later and the owners claimed the dog were no longer aggressive [38]. Suñol *et al.* (2020) suspected a protein-losing enteropathy in relation to gluten-free hypersensitivity due to faecal alpha1-proteinase inhibitor elevation, but due to the owner not being willing to analyse further, it would only be speculation. The dog relapsed quickly to aggressivity on 2 occasions on the same day as the diet change, and diet change are found as the common denominator [38].

4.1.2. L – Theanine usage in noise- and storm-sensitive dogs

L-theanine is an amino acid which has been shown to affect brain functions. The study by Pike *et al.* (2015) was created to study if the Virbac Anxitane® product containing L-theanine were effective in aiding storm-sensitive anxious dogs. Anxitane® is a 50 mg or 100 mg tablet for dogs divided into small and medium/larger dogs, containing 99.95% active l-

theanine. Dogs were given this twice daily for 4 weeks or during a minimum of 5 thunderstorms. The dogs participating were picked out for being in accordance with the inclusion criteria, while excluded if they met exclusion criteria, such as chronic medical problems, having another behaviour disorder or already pharmacologically treated [39]. The dogs went through a physical examination and did a laboratory analysis to make sure the dogs were otherwise healthy. Twenty-six dogs were enrolled, but 2 dropped out of the study and 3 owners did not complete the paperwork, additional 3 owners were excluded due to not having enough storms. In total 18 dogs completed the study, of them both genders and various breeds were included and the dogs age ranged between 2 years and 8 years old [39]. Questionnaires were used for the owners to self evaluate the dog's behaviour during each storm and scored with a 0-5 Likert scale and evaluate how long their dog used to return to their normal behavioural state after each storm. If the dog had an improvement in the behaviour score after the 5th storm in comparison to their baseline, that were considered successful [39]. After completing the exit questionnaire, the 18 remaining dogs all showed a significant decrease in their anxiety. The time the dogs required to return to normal behaviour after a storm were also significantly shorter. All 18 owners saw improvements in their dog and were pleased with supplementing Anxitane®. The study supports the benefit of L-theanine in dogs experiencing storm-sensitive anxiety [39].

Another study by Sechi *et al.* (2017) studying nutraceutical diets effect on oxidative stress and neuroendocrine parameters including Theanine and tryptophan from green tea extract, in addition to high PUFA content concluded with increased serotonin, dopamine and beta-endorphins, while decreasing the stress markers; cortisol and noradrenalin, which further supports the nutritional influence on balancing neuroendocrinology and their potential for stress and anxiety relief [40].

4.2. The effects of dietary lipids on behaviour

Lipids are found in the highest concentration in adipose tissue and in the central nervous system. Lipids have several important roles in the body, including usage as an energy source, fatty tissue storage, precursors of chemical messengers and cellular membrane constituents which could affect behaviour.

4.2.1. Phospholipids

Puurunen *et al*. (2016)'s study examines canine Attention Deficit Hyperactivity Disorder (ADHD) like behaviour in order to find their metabolic markers, and therefore could be used as a model for ADHD in humans to aid further research, diagnosis and treatment. Questionnaire to be filled out by the owners were modelled after Tiira and Lohi (2014)´s questionnaire on canine anxiety [41]. Owners answered questions in regards to daily routine, exercise, diet, the dogs behaviour and a set of specific questions in regards to activity, inattention, impulsivity and similar ADHD-like behaviours where higher scores linked to higher ADHD-like behaviour [42] (Table 2). Twenty-two German shepherds exhibiting ADHD-like behaviour were subjected to the study, consisting of six males and sixteen females, with an age range between 16 to 91 months old. The dogs were fed the same commercially available diet in the 2 weeks before sampling to control dietary variations, then blood samples were taken from all the 22 dogs to centrifuge into plasma to use in the examination based on liquid chromatography with mass spectrometry and analysed by several software's for metabolite profiling. A questionnaire was used and scored after impulsivity, inattention and total [43].

Puurunen *et al*. (2016) found 649 out of 7058 molecular features were found in relation to ADHD-like behaviour whereas 22 metabolites correlated with ADHD-like behaviour. In relation to Trp metabolism, 3-indolepropionic acid, kynurenic acid and indoleacetic acid were found linked to ADHD-like scores. Higher scores from the questionnaire were associated with lower levels of 3-indolepropionic acid and indoleacetic acid, while higher levels were found in relation to kynurenic acid. Puurunen *et al*. (2016) found most of the metabolites to be phospholipids, where sn-1 LysoPC (18:3), sn-1 LysoPE (18:2) and phosphatidylcholine (18:3/18:2) which had the most negative correlation. Fatty acids as arachidonic acid and oleic acid (C18:1) where found in higher amounts in relation to the ADHD-like dogs [43]. Puurunen *et al*. (2016) hypothesizes oxidative stress as a factor in ADHD-like behaviours due to oxidative stress markers as fatty acid arachidonic acid were positively correlating, whereas 3-indolepropionic acid where negatively correlating. The study by Puurunen *et al*. (2016) gives valuable insights, but are limited due to small sample size and dependence on owners [43].

Table 2: “Owner completed behaviour questionnaire used by Puurunen et al. (2016)” [43]

1. My dog has a difficult time learning, because it is careless or other things can easily attract its attention.
2. It’s easy to attract its attention, but loses its interest soon.
3. It’s difficult for it to concentrate on a task or play.
4. It leaves from its place when it should stay.
5. It cannot be quiet, it cannot be easily calmed.
6. It fidgets all the time.
7. It seems that it doesn’t listen even if it knows that someone is speaking to it.
8. It is excessive, difficult to control, and if it lunges it is hard to hold back.
9. It would always play and run.
10. It solves simple tasks easily, but it often has difficulties with complicated tasks, even if it knows them and has practiced them often.
11. It is likely to react hastily and that’s why it is failing tasks.
12. It’s attention can be easily distracted
13. It cannot wait as it has no self control.

4.2.2. α - linolenic acid & docosahexaenoic acid

A pilot study by Re *et al.* (2008) explores the biochemical basis of canine aggression and takes its precedence on humans with behaviour changes and aggressivity are found linked to altered omega-3 plasma polyunsaturated fatty acids and raised ratio of omega 6:omega-3. Re *et al.* (2008) studied 18 aggressive and 18 control dogs, all included in the study were privately owned male adults of the breed German shepherd. The aggressive group was picked out based on aggressivity and was diagnosed with dominance aggression by a senior veterinary behaviourist, all dogs in this group had a record of several aggressive incidents and bite incidents without warning. Neither dogs of the aggressive or control group had received polyunsaturated fatty acid supplements or pharmaceutical treatment in relation to the aggression prior to the study [44].

Blood samples were collected after fasting around 09:00-10:00 from all 36 dogs and promptly cooled on ice before going in the centrifuge, subsequently the plasma was kept in a freezer until analysis by gas chromatography. Arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid and omega 6:omega3 ratio were measured by gas chromatography [44]. Re *et al.* (2008)’s results show no significant difference in age or BW between the groups. The aggressive dog group exhibited a significant decrease in both bilirubin and total cholesterol levels (Table 3). There were also significant changes between the two groups concerning plasma fatty acid status, aggressive dogs had lower concentrations of docosahexaenoic acid and a higher omega-6:omega-3 ratio (Table 4). Observations of this

study support the hypothesis that aggressive behaviour may be linked to the increased omega-6:omega-3 ratio or deficiency of omega-3.

Table 3: "Biochemical analysis and plasma concentrations in both study groups [1] " [44].

Parameter – Biochemical analysis	**Aggressive dogs (*n*=18)**	**Normal dogs (*n*=18)**
Arachidonic acid (20:4 n-6)	98,9±6.4	94.2±8.0
Creatinine (mg/dL)	0.9±0.1	0.9±0.2
Blood Urea Nitrogen (mg/dL)	16.4±3.1	16.8±2.7
Total protein (g/dL)	6.5±0.4	6.6±0.7
Total bilirubin (mg/dL)	0.3±0.1[2]	±0,6±0.2
Cholesterol (mg/dL)	155±7.7[3]	184±9.4

[1]Data expressed as mean ± SEM,[2] Significant difference in value between aggressive and normal dogs: $p<0.05$.[3] Significant difference in value indicated by: $p<0.01$.

Table 4: "Plasma concentrations of fatty acids in both study groups" [1] [44].

Parameter – Plasma concentrations	**Aggressive dogs (*n*=18)**	**Normal dogs (*n*=18)**
Arachidonic acid (20:4 n-6)	22.9±0.9	23.1±0.8
Eicosapentaenoic acid (20:5 n-3)	0.3±0.3	0.3±0.2
Docosahexaenoic acid (22:6 n-3)	0.4±0.1[2]	0.8±0.2
Omega-6/omega-3 ratio	24.4±2.2[3]	17.3±1.9

[1] Data expressed as the mean ± SEM, [2] Significant difference in value between the groups: $p<0.05$.
[3] Significant difference in value between groups are indicated by: $p<0.01$.

In support of Re *et al.* (2008), another study investigated the link between behaviour and supplementation of omega-3 fatty acids, zinc and magnesium where they found an improvement after 6 weeks of supplementation in dogs that were destructive, fearful or inappropriately eliminating. However, the aggressive dogs were found with no significant change and no placebo group were used, so fatty acids might be a dietary factor, but perhaps on other behavioural problems than aggression. [45].

4.2.3. Triglyceride

Triglycerides are essential lipid molecules playing a vital role in the body and are significant for storing and transporting fatty acids in cells and the bloodstream.[46] They are composed of glycerol backbone with three fatty acids linked to it. [47] Triglycerides are the main dietary fats and are broken down in the gastrointestinal tract with the help of lipases,

ultimately turning into fatty acids and monoglycerides. Additionally, the liver and adipose tissue synthesize triglycerides in low amounts. [46]

Manteca X, (2011) states as dogs age, some can develop a neurodegenerative condition with gradual decline in cognitive function, otherwise called Canine cognitive dysfunction syndrome. [48] Canine cognitive dysfunction can manifest as disorientation, changes interactions, sleep-wake cycle disruptions, unusual changes in activity, suddenly starting to urinate or defecate in the house, apathy and anxiety. [49]

Pan *et al. (2010)* created a study to evaluate if supplementation of medium-chain triglycerides (MCT) could increase cognitive function in senior dogs. [50] Reduced energy metabolism is a typical occurrence when aging and are one of the several processes associated with cognitive deterioration advancing with age [51]. The study conducted by London *et al.* (1983) of beagles showed a significant reduction in glucose metabolism in the brain in the dogs aged six compared to the one-year-old dogs [52]. Studies based on humans has also concluded that there are a decline in the glucose metabolism in the brain throughout the aging process [53]. The results of Alexander *et al.* (2002) also supports glucose metabolism after finding it considerably lower in old age with Alzheimer's disease [54]. Additionally, Drzezga *et al.* (2003) tracked the progression of cognitive deterioration in individuals exhibiting mild cognitive impairment and found that the clinical manifestations of Alzheimer's disease were linked to additional lowering in cerebral glucose metabolism leading to several studies pointing to reduced cerebral glucose metabolism that are commonly age-related [55].

Henderson *et al* (2004) hypothesized that dietary supplementation of MCT could offer a way to address deficiencies in cerebral metabolism by elevating ketone levels within the brain [56]. Glucose metabolism is the primary energy source for the brain, but ketone metabolism generated in the liver and to a lesser extent by the astrocytes in the brain could serve as an alternative energy source. [56]

In the study by Pan *et al*. (2010), twenty-four Beagles between 7 and 11 years old were studied, all the dogs had a minimum 6 months of cognitive test experience and were given stable housing and environmental factors during the experiment. The housing included enrichment in the form of toys, beds and the possibility to play outside. The test diets were

produced by Purina, the control food was a commercially available premium product and the test diets formulation changed to replace 5.5% tallow by 5.5% MCT. Pan *et al*. (2010) used both the control and test diet in their study, whereas both were isotonic and constituting of equal amounts of protein, fat and carbohydrates. [50]

Baseline blood samples were taken before the start of the study as well as after 4 and 8 months to measure clinical chemistry, blood beta-hydroxybutyrate and complete blood count. For the experiment, the apparatus used for cognitive testing in Pan *et al* (2010)´s study were a wooden box created as a modified version of Wisconsin General Test Apparatus which were used for testing in monkeys, but have been modified to many species. The apparatus used a middle and two food wells on each side or four food wells with equal spacing depending on the test at hand. [50] For the baseline testing, size discrimination learning, size discrimination reversal task [57] and Pan *et al.* (2010) cites Chan *et al.*(2002) on using the delayed-non-matching-to-position task. These results were used to divide the beagles into two groups based on similar cognition, whereas group one was fed the control diet and group two were fed on the reformulated diet with 5.5% MCT. Subsequently, after acclimating for a week, all were assessed by the landmark protocol until day 92, at 100 days they started in an egocentric protocol, at day 190 the variable object oddity tasks were started and finished after 35 days. The beagles were evaluated in one task each day [50]

Pan *et a*l (2010)´s landmark protocol involved three tasks: land-0, land-1 and land-2. Firstly, were land-0, in this task the beagles learned to choose between two objects based on their proximity to the landmark. They were trained using a yellow peg as landmark and white coasters, whereas the peg was attached on a coaster with a food reward. To avoid relying on their olfactory senses, there were food by the other coaster as well but inaccessible. Next were the land-1 task, where the landmark was shifted 1cm medially and diagonally away from the coasters edge, the beagles passing land-1 got to the next task, land-2. Land-2 were another 1cm away diagonally from the edge of previous position and 2 cm away from the coaster. Egocentric protocols were tested next, including preference phase, acquisition phase and reversal phase to assess spatial learning. The preference phase contained ten trials where food rewards were found on both sides and the side chosen most frequently were their preferred side. For the acquisition phase, the last phases preferred side were used as the positive side, a stimulus covering a reward in the preferred side and a stimulus without reward on the other side. Lastly, the dogs were tested on two reversal tests, where the

rewarded side were switched. [50] Pan *et al.* (2010) cites Christie *et al.* (2010) on variable object oddity task, which were developed assessing attentional processes. Pan *et al.* (2010) further states this task comprises of the acquisition phase, "same distractor" phase and "different distractor" phase, where the acquisition phase examined the dogs' ability to learn a two-choice discrimination task requiring them to learn how to selectively respond to a certain object, which would reward them with food. The beagles had to reach a criterion for accuracy at two stages to proceed and would not be tested further if the dogs failed with even after additional training. Nex up were the distractor phases where the beagles preformed tasks with varying number of objects, including the object trained during acquisition phase where the other objects were purely a distraction to measure accuracy and speed of the dog used as an indication of attentional processes. [50]

The results from the study of Pan *et al.* (2010) presented with no significant difference in the groups in the baseline cognitive test (Figure 1). Results from the landmark test measured by errors on land-0 and land-1 indicate the group with MCT completed with fewest errors compared to the control group, in total 22 beagles finished the land-2 test where the MCT-supplemented dogs had the greatest precision (Figure 2). Pan *et al.* (2010)´s results from the egocentric test showed more error in the reversal learning phase compared to the starting discrimination learning. The dogs were more accurate in their second reversal task compared to their first. The dogs on the MCT diet were quicker to learn in all their tasks (Figure 3). On the variable object oddity test, there were no significant differences during the acquisition phase and with only one single distractor object, all dogs were quite accurate. There were first found significant differences during 2, 3 and 4 distractors where the group supplemented with MCTs performed significantly better than the control group.

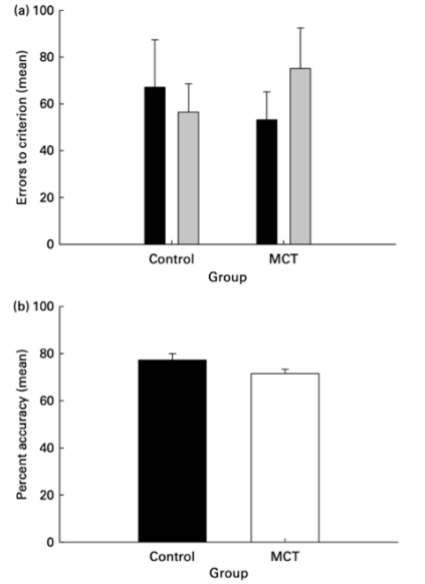

Fig. 1. Performance of dogs fed two diets in baseline cognitive tests. (a) Mean errors to criterion on the size discrimination and reversal learning tasks. (b) Percentage accuracy on the delayed-non-matching-to-position task. The data are means with their standard errors, *n* 12. There were no statistically significant differences. MCT, medium-chain TAG; ■, size; ■, size reversal.

Figure 1: Evaluation of baseline tests on control and MCT supplemented diets. A) Average number of errors on criteria collected from size discrimination and reversal learning. B) Percentage of correct responses from the task on delayed-non-matching-to-position. [50]

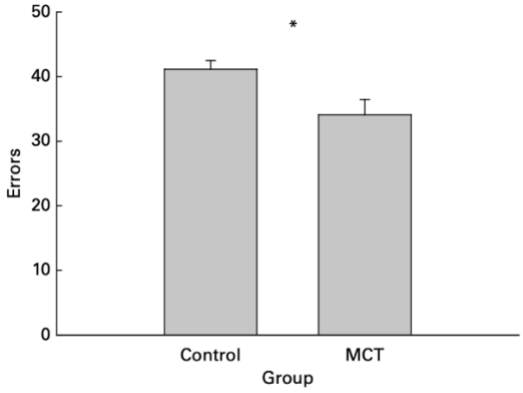

Fig. 3. Effects of dietary medium-chain TAG (MCT) supplementation on dogs' performance in complex landmark discrimination task (land-2). The data are means with their standard errors, *n* 11. The performance was expressed as total number of errors over ten sessions. * Mean values were significantly different ($P<0{\cdot}05$).

Figure 2: Effects of dietary supplementation of medium-chain TAG on a dog's performance in complex landmark discrimination task. Data are means with standard errors, n11 over ten sessions where mean values were significantly different [50]

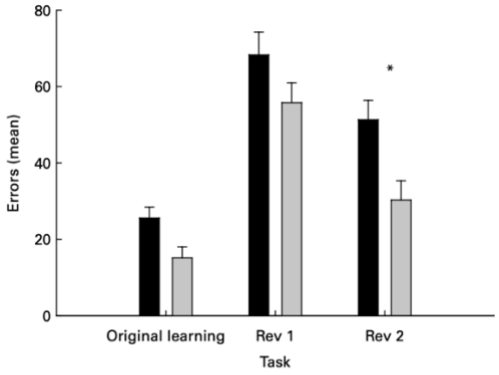

Fig. 4. Effects of dietary medium-chain TAG (MCT) supplementation on dogs' performance in the egocentric discrimination and two subsequent reversal learning tasks. The performance was expressed as errors to criterion. The data are means with their standard errors, *n* 12. * Mean values were significantly different ($P<0{\cdot}05$). Rev 1, reversal 1; Rev 2, reversal 2; ■, Controls; ■, MCT.

Figure 3: The effect of medium-chain TAG supplement on a dogs performance in the tasks of egocentric discrimination and reversal learning (rev 1 & 2) expressed as means with standard errors to the criteria [50].

Another study protocol is on a 6 month randomized trial where oil of MCT and olive oil is compared in epileptic dogs, aiming to assess changes in seizures, behaviour and change in metabolic responses, MCT is therefore getting more widely studied for their effect in other fields as well [58]. This study later completed by Berk *et al.* (2019) shows the wide variety of how MCT can be used as a dietary treatment, their findings showed some improvements in seizure control and had reduced adverse effects, but only a modest reduction in frequency of seizures [59]. Suggesting MCT can aid in more than cognitive function, but perhaps also epilepsy after further studies.

Contraindicatory to the study by Pan *et al.* (2010), another research article found no effects of either cognitive function or behaviour on senior dogs from lifelong training and an enriched diet of antioxidants, Trp, DHA and phosphatidylserine. A total of 119 pets over 6 years old were used and the training were collected by questionnaires, the dogs were tested by the Modified Vienna Canine Cognitive Battery both before the trial and after a year of the diet, the dogs on the enriched diet had no positive effect on their tests on problem-solving, boldness, dependency and trainability [60] The enriched diet might be ineffective or other factors might play a role, either way, further studies are needed under more diverse conditions to properly understand if and how diet and training can combat aging and the decline of cognitive function.

4.3. Supplements and behavioural nutraceuticals

Veterinary nutraceutical was defined by the North American Veterinary Nutraceutical council as " a non-drug substance which is produced in a purified or extracted form and administered orally to a patient to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals" [61]. The term is combined from nutrition and pharmaceutical, and is used for any substance that is food or a part of food that provides health benefits, including the prevention and treatment of a disease. [62] Nutraceuticals have been more widely used over the last years and are used in a lot of veterinary care diets given to cats and dogs.

The process and funds to apply to the European Medicines Agency can be long and costly, while there are little to no regulations regarding nutraceuticals making it easier to use in the dog food industry. Possemiers *et al.* (2010) state nutraceuticals are made from many different substances, including vitamins, minerals, essential fatty acids, amino acids, and botanicals [62]. Additionally, nutraceuticals normally have fewer side effects and usage is not contraindicated in relation to medicines or in regards to diseases [63].

4.3.1 Stress response evaluation in anxious dogs by diet.

One study by Kato *et al.* (2012) was created to evaluate the stress response of anxious dogs after using Royal Canine's veterinary Calm Canine diet®, which were supplemented with alpha-casozepine, L-tryptophan, DHA and EPA [64]. The Royal Canin Calm Canine diet had a higher ratio of Trp:LNAA of 0.048 compared to the control diet, Royal Canin's Vet Plan: Select Skin Care®, containing 0.043. The nutrient composition of the diets are equal in dry matter, but differs in amount of supplementation, amino acids, fatty acids, fat and protein (Table 5).

Table 5: Nutrient composition of trial diets

Nutrients (g/kg dry matter)	Study diet[1]	Control diet[2]
Dry matter (g/kg)	920.0	920.0
Ash	78.26	56.52
Calculated nitrogen-free extract	459.78	541.30
Protein	271.74	239.13
Fat	173.91	152.17
Crude fiber	16.3	10.87
Linoleic acic	35.65	41.30
Arachidonic acid	0.76	0.54
Isoleucine	9.78	10.11
Leucine	18.7	17.5
Phenylalanine	10.43	8.26
Tyrosine	8.37	9.02
Valine	12.07	12.5
Tryptophan	3.04	2.83
Alpha-casozepin	1.35	0

[1] Study diet: Royal Canin's Calm Canine®

[2] Control diet: Royal Canin's Vets Plan Select Skin Care®

The study was a single-blind crossover trial and the dogs were recruited in veterinary clinics and answered a standardized questionnaire which helped them to select their trial dogs that fit the behavioural qualifications. The study group was a mix of both genders and ages, containing of 12 Chihuahuas, 2 Toy poodles, 2 Shiba Inus, a Jack Russel Terrier, 2 Miniature Bull Terriers, 3 Miniature Dachshunds, a Maltese, 2 Welsh Corgi Pembrokes and 3 mixed breed dogs.

The questionnaire used was created by Hsu and Serpell (2003), The Canine Behavioural Assessment & Research Questionnaire (C-BARQ) which were used on all occasions. [65] There were 28 qualified dogs finishing the study by cooperation with their owners. The subjects started on the control kibble for 8 weeks, subsequently using a week to transition to the Calm Canine® kibble, then completing another 8 weeks on the study diet. After the 7th

week of each diet, the questionnaire was answered by the owners and urine was collected in relation to visits to the veterinary clinic for a nail trim. On each occasion, two urine samples were collected, whereas one was collected 2 hours after the clinic visit and the other was taken at home without any stressors. The samples were used to check the cortisol-to-creatinine ratio (UCCR) and measure the changes between samples at home versus after the stress at the clinic to be able to see if the nutraceuticals in the diet had an effect [64]. The results were established from behaviour and the UCCR, where the UCCR ranged between 12.84 to 75.04 x 10^{-6} and UCCR were higher after the induced stressor than the basal UCCR in both diets. Behavioural scores were lowered in favour of the Royal Canin's Calm Canine® diet, but no significant changes were found in the basal Cortisol : Creatinine Ratio (CCR) levels. Still, the UCCR based on stressor samples was higher in the urine sample taken on the control diet, leading to the confirmation of lower stress cortisol levels during stressful situations [64]. The study proves some possible efficacy of the nutraceuticals in Calm Canine, but additional studies would be key to providing insight into which nutraceutical the effect is from or if it is the combination of both Trp and alpha-casozepine as a whole.

4.3.2. Effects of DiRelax® on anxiety

Behavioural problems are negatively affecting both dogs and owners, where anxiety is increasingly more diagnosed. Anxiety is induced when an environmental stimulus is perceived improperly dangerous or threatening, it becomes pathological when it continues occurring without justified environmental conditions. The most common types of anxiety are separation anxiety and generalized anxiety which can be seen in the form of excessive vocalization, destruction, restlessness, inappropriate soiling and several other symptoms. Dogs living with only one person had a higher risk than a dog living with a family to get separation anxiety, which was observed by Flannigan and Dodman [66]. Treatment for anxiety ranges from behavioural therapy to pharmacological medicines, where for example benzodiazepine is commonly used.

Scandurra *et al.* (2021)'s study was based on 21 privately owned dogs with an anxiety diagnosis which were assessed by the C-BARQ questionnaire from Hsu and Serpell (2003) [65] and examined with in-depth blood analysis, thyroid profiling, clinical- and neurological examination to exclude other causes of the behaviour [63]. DiRelax® are mixture made by Dynamopet, originating in Italy. DiRelax® contains 24 grams 3a700 vitamin E, 100 mg/kg of vitamin B6, 3% krill oil and substances with anxiolytic properties such as 8 mg/kg

Passiflora and 55.2 mg/kg each of *Eschscholzia californica* (California poppy) hops and *Withania somnifera* (Ashawagandha). Whithania has also been studied separately by Kaur *et al.* (2022) and found it to be well tolerated for canines with no found adverse effects and the supplementation over 4 weeks resulted in reduced stress and fear [67].

The dogs were thereafter housed in suitable facilities during the duration of the study. The 21 dogs were randomly selected into two groups and were of diverse breeds, ages and genders, whereas group 1 received the supplement for 30 days, group 2 only received a placebo as a control group. Blood sampling was taken after twelve hours of fasting both before and after the supplementation period, complete blood count, blood smears and blood chemistry were all analysed, additionally TT4, fT4 and TSH were checked [68]. The experimental conditions took place in an empty room with a food container with the lid fixed onto the wooden platform, in the solvable task the food container could be manipulated to open to achieve the food reward, while in the unsolvable task, the food container was locked by the fixated lid. There were three solvable trials followed by an unsolvable trial, at all times the owner and one unknown person where on each side of the apparatus. The dogs were submitted to the tests both before and after the supplementation period (Figure 4).

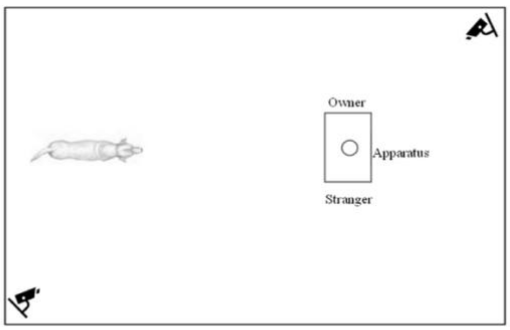

Figure 1. The figure shows the experimental room equipped with two cameras (in the corners) and the apparatus (in front of the dog). The owner and a stranger were placed on the two sides of the apparatus.

Figure 7: "Effects of a Nutritional Supplement (DiRelax®) on anxiety in dogs" [63]

The tasks were video recorded and for the solvable trials the time used where collected, while in the unsolvable task the behaviour of the dog was collected, including yawning, licking, vocalization and so on. All were statistically analysed using several software and taking variable factors into account. Scandurra *et al.* (2021) found a significant change in 6 out of 42 questions from the Q-BARQ questionnaire as listed in Table 2, whereas no significant changes were found from blood analysis. The supplemented group showed a

significant decrease in time used for solving tasks compared to no significant change in placebo group, but in the unsolvable task, no difference was seen in behaviour from both groups (Table 6) [63]. In conclusion, the study suggests that DiRelax® can effectively reduce anxiety in dogs as indicated by the improved questionnaire and without any found side effects.

Table 6: Improvements results from C-BARQ. [68]

Improved behaviour	**P**
When his/her food is taken away by a household member	0.004
When approached while eating by another (familiar) household dog	0.050
When barked, growled or lunged at by an unfamiliar dog	0.023
When groomed or bathed by a household member	0.010
Tends to follow you (or other family members) around the house	0.024
Urinates when left alone at night or during the daytime	0.00

4.3.3. The impact of Cannabidiol on noise-induced fear

Cannabidiol is derived from hemp and is a non-psychotropic phytocannabinoid [69], cannabidiol is popularly used for its anxiolytic abilities, as well as its analgesic, anti-inflammatory properties and therapeutic epileptic benefits [70]. Cannabidiol regulates neurotransmitter release by being a modulator for the G protein-coupled receptor CB1 and aids in regulating cytokine release in the immune system by being an inverse agonist for the CB2 receptor [71] [72]. More studies related to Cannabidiol usage in canines have been done the last decade, including the tolerability of Cannabidiol given orally to canines. One study by Alvarenga *et al.* (2024) found healthy dogs to tolerate both 5 and 10 mg/kg body weight a day over 36 weeks, even though 5 mg/kg were more tolerable due to soft stool in the 10 mg/kg bodyweight dosage, both caused an increase of alkaline phosphatase activity and therefore long term use are suggested to monitor liver functions [73]. Another study on daily long term tolerance showed where healthy dogs were given 5 mg/kg did not find any overall adverse effects, except for the alkaline phosphatase elevation which long term were transient and after a four week washout period the value returned to normal [74].

Morris *et al.* (2021) studied the possible alterations of the metabolome during Cannabidiol supplementation and revealed amino acid, glucose, vitamin and nucleotide metabolism were altered and therefore suggesting Cannabidiol exerts their effect through these pathways [75]. Even though more studies are done on the topic, the pharmacokinetics are not yet understood, but Cannabidiol shows promise in relation to osteoarthritis, epilepsy,

behavioural disorders and skin conditions [76]. Although several of these have either good or mixed results, more study would be needed to conclude their efficacy.

Morris *et al.* (2020) studied the impact of feeding Cannabidiol treats to alleviate noise-induced fear. Noise phobia is a common fear in dogs and their response to the noise is variable and one dog's reaction can be completely opposite of another dog's reaction. Cannabidiol has been used for people to treat anxiety and after a rise in the use of cannabidiol, many are interested in the usage of this in dogs [77]. Twenty-four adult dogs of both genders, various age and breeds were subject to the study from a rescue shelter. All dogs had complete blood profiling and had a physical examination, ruling out other causes of the noise anxiety. All dogs were given a diet of Purina Pro Plan Gastroenteric® dry food. Each period lasted 7 days with the firework test on the last day, during 1st period dogs were given placebo treats, during the 2nd period dogs received 1.4 mg cannabidiol per kg BW each day. In the 3rd period Trazodone, an antidepressant medication and no cannabidiol was given. During the 4th period dogs were given 1.4 mg cannabidiol per kg BW per day in addition to Trazodone. The cannabidiol were from industrial hemp extract from AgTech Scientific and the dogs got two treats a day with half of the full dose in each treat. The treats were otherwise made from the same ingredients, including fish and chicken, and were given at 07:00 and 18:00. Trazodone were given the evening and morning before a test at 18:30 and 10:00 [77].

The tests occurred in an isolated room with cameras and the dogs were monitored for an open field test the first day to get a baseline of this test where they could get acclimated to the room, the first firework test was done the next day and were the baseline test. The dogs were in this test room for 6 minutes every day to avoid them associating the room with the stressor and changing behaviour due to the environment instead of sound. Every 7-day period, the dogs received a new open field test and firework test. Blood samples were taken on every day of firework tests, one hour before the test and the last sample right after the stressor for the stressor cortisol and cannabidiol values. This test was also 6 minutes, where the first 3 were silent to keep the dog from anticipating the test [77].

Results found the blood cortisol to be similar in all periods, but were reduced in period 1. The blood cortisol when given trazodone were reduced, but the cortisol was unaffected by the cannabidiol. Additionally, the heart rate in period 4 were lower in comparison to the other periods, and therefore the mean beat-to-beat interval were higher as well. The periods

were cannabidiol were given, increased heart rate were found [77]. All dogs were affected by the noise with increased stressors compared to the 3 minutes before the test. The dogs on cannabidiol were less likely to use their time fixating on something in the room. During the firework test no dog showed especially fearful responses and behaved with what can be called normal to sudden sound louder sound stimulus. Therefore the test might have been not fearful enough to elicit these fear based behaviours [77].

Another study on cannabidiol in relation to stress during car travel and owner separation by Hunt *et al*. (2023) found canines receiving Cannabidiol to have lower cortisol levels and a lower heart rate during these situations, as well as reducing stress behaviours during their study compared to the placebo group. Car travel still induced a higher stress level compared to separation, but still calmed stress indicators while given 4 mg/kg body weight of Cannabidiol [78]. This study supports the theory that Cannabidiol does alleviate anxious behaviour in this case.

4.4. Gut microbiome and behaviour

Microbes are found worldwide and are considered the smallest form of life, while still having a big impact in their host's body. The gastrointestinal tract serve as a major microbial habitat, but can be found in other areas as well [79]. Possemieres *et al*. (2011) states the gastrointestinal microbiome is now acknowledged as an organ and refers to microbiome as both the bacterial cells and also their genetic material with great metabolic abilities [62]. The microbiota impacts digestion, the production of metabolites and also aids in the regulation of the immune system, but diet, immune system, environment and genetics influence the microbiome as well. Wernimont *et al.* (2020) cites Tizard and Jones (2017) stating microbiota functions to release nutrients and metabolites for the body to use, while the microbiota receives infrastructure and a proper environment for the microbiota to thrive. A barrier between the microbes and host is comprised of an epithelial barrier with mucus lining, which assists in host-microbiome homeostasis [80].

The Gut-Brain Axis is the way of communication between the gut microbiome and the host central nervous system by the autonomic nervous system. This a complex network that can affect the neural, endocrine, metabolic and immune-mediated signal pathway and thereby regulate behaviour and cognitive function. The intestinal wall are innervated by the vagal nerve, which communicates with the central nervous system when detecting metabolites

[81]. Serotonin, GABA, dopamine and norepinephrine are neurotransmitters the gut microbiota can influence and cause changes to mental state, behaviour, stress and anxiety [82]. The gut microbiota can affect serotonin synthesis due to the production in the intestine and the effect can vary due to different bacteria in the microbiota. The gut microbiota can affect the HPA axis and certain bacteria might cause an overactivity, which releases cortisol. Therefore, high stress can cause the activation of HPA and influence the gut microbiota as well. In these cases, this affects the intestinal barrier and permeability, leading to an inflammatory response and further activation of HPA by the released prostaglandins and cytokines. Cytokines and chemokines are regulated by the immune-mediated pathway where the gut microbiota and the immune system influence each other, as gut microbiota can stimulate a proinflammatory state if unbalanced [81]. Several bacteria can be found in the microbiota, including *Firmicutes*, B*acteroides, Proteobacteria, Fusobacteria* and *Actinobacteria* [79].

4.4.1. Microbiome structure in dogs with aggressive or phobic behaviour

Mondo *et al*. (2020) studied the gut microbiome and adrenocortical activity in aggressive and phobic dogs. 23 male and 19 female dogs of various breeds, ages and BWs were used in the study, they were located in three animal shelters with mixed diets. The dogs were evaluated by a veterinary behaviourist and were put in categories, where 11 were deemed aggressive, 13 were phobic and 18 were without any behavioural problems [83]. Faecal samples were taken immediately after defecating in the morning and were frozen until DNA sequencing, another portion used to test cortisol and testosterone by Radio Immuno-Assays (RIA). Bacterial DNA from faecal samplings were used to sequence V3-V4 region of the 16S rRNA.

Mondo *et al*. (2020) found that gut microbiome differed between the behavioural groups, where the aggressive group had a higher number of operational taxonomic units and the structure were distinctively different with an abundance of subdominant bacteria as *Dorea, Blautia, Collinsella, Slackia*, *Catenibacterium* and *Megamonas*. Analysis showed *Catenibacterium* and *Megamonas* as aggressive-discriminatory genera. The phobic groups had a higher amount of *Lactobacillus*, where Mondo *et al*. (2020) cites Lyte (2011) on *Lactobacillus* genus contain GABA producers which are able to regulate emotional behaviour via the vagus nerve in mice. In the healthy dogs, *Faecalibacterium*, *Bacteroide*s, *Phasscolarctobacterium*, *Fusobacterium*, *Prevotella* were found. Cortisol and testosterone

made evidently no significant change between the groups, most probably due to faecal sampling [83]. Mondo *et al*. (2020)'s findings indicate that when dogs experience prolonged stress, it might lead to imbalances in gut microbiome, leading to release of microbial by-products with potential effects on the local gut environment. Dogs with aggression had microbiomes with high diversity and a bigger quantity of less common bacteria compared to the common bacteria found in the microbiome.

4.4.2. *Lactiplantibacillus plantarum* PS128 on aggression and separation anxiety

The study by Yeh *et al*. (2022) researches the effect of PS128 in relation to aggressivity and separation anxiety [84]. Similar studies were done in mice, where decreased stress was found, but very little has been researched in the canine [85]. Forty-five dogs were used in the study and all dogs went through a physical examination, blood analysis, as well as analysis of endocrinology, urinalysis and imaging. Only healthy dogs with no treatment plan were included, 19 male dogs and 26 female dogs of various breeds, sizes and ages were enrolled to the study. Eight dogs were dog aggressive and 14 dogs were aggressive regarding humans, 15 dogs displaying symptoms of separation anxiety, 7 dogs enrolled with compulsive disorder as licking and chasing their tail, additionally there were one dog with excessive barking tendencies. The dogs were separated into the experimental or control group and after being seen by a veterinary behaviourist [84].

To reach a concentration of 5 x 10^{10} CFU/g PS128, fermented mustard greens were used for isolation and were made into a lyophilized powder with alpha-cellulose. The study group were given 0.02 g/kg after the dogs breakfast every morning for 2 weeks, the control group were only given alpha-cellulose to ensure the owners were unaware of which group their dog were placed in. The final interview with the behaviourist were on the last day of week 2 for both groups and used behavioural questionnaires and the interview to evaluate the dogs, as well as another blood sampling [84]. Significantly used behavioural questionnaire was the Evaluation of dog's emotional and cognitive disorder (EDED) scale and the Canine Behavioural Checklist (CBC) to score the dogs before and after the study period. Serotonin and 5-hydroxyindoleacetic acid were measured in blood plasma before and after supplementation, measurements were done by high-performance liquid chromatography with an electrochemical detection system and the serotonin turnover ratio were calculated.

The EDED scores on emotion were calculated from both occasions to obtain the results and the study groups were compared. The control group had a median EDED score of 16.27 before and 15.45 after the study period. While in the supplemented group, the EDED score went from median of 15.83 to 11.43. Both groups showed a significant decrease, but the supplement group showed the most significant change [84]. The CBC scores the behavioural problem severity and were measured to 33.91 and later 33.14 in the control group, the PS128 supplemented group had a median value of 33.85 and then 21.67 at the end of the period. The aggressive dogs in the control group had a median of 10.09 and 10.18, while the supplemented aggressive dogs had a median of 10.08 and later 4.09. The group with separation anxiety dogs had a median of first 13.14 and later 12.14 in the control group, the supplemented group started with a median of 14.0 and ended with 10.0. A significant decrease was therefore found in all groups [84]. Additionally, the plasma concentration of serotonin and 5-hydroxyindoleacetic acid were measured, but they found no significant changes in the plasma concentrations. Although, the separation anxious dogs had a significant reduction in the turnover ratio of serotonin were reduced after treatment. Results still suggest that supplementation of PS128 were significantly able to aid in calming behaviour problem severity and aid in regulating their emotions [84].

3. Results

The field of nutrition relating to behavioural problems are getting more knowledgeable as more studies are conducted on the field and the science progresses. There has been a growth of knowledge from new experiments and studies on the topic the last decades and canines are being more researched based on the knowledge already there of humans as well. The increase of information available and the knowledge of how to use this information can influence canine health and lower the rate of canines struggling with behavioural problems.

Bosch *et al.* (2007) highlighted the influence of hormones and neurotransmitters on canine aggressivity with emphasis on the role of serotonin. The precursor tryptophan were found to influence serotonin synthesis and was hypothesized to affect aggressive behavior. This review found tryptophan to be in lower concentrations in food compared to other amino acids and therefore competing for the same carriers to cross the blood-brain barrier as the other amino acids [33]. Cakiroglu *et al.* (2007) based their study on investigating if canines' behavior was modulated by serum lipids and serotonin, as previously seen in studies of

humans and other animals. Contrary to the hypothesis, the results showed insignificant serum lipid differences between the aggressive and healthy dogs [34]. Therefore, the result of this study does not fit with the theory that serum lipids, such as cholesterol are linked to aggressive behavior in canines and highlights the complexity of the pathways of aggression. Additionally, this study found a decreased serum serotonin in aggressive dogs, which supports the theory of serotonins role in aggression and the possibility of therapy based on regulating serotonin [34]. Contradictory findings appeared from Jacobs *et al.* (2007), where an increased expression of serotonin-1B receptors was found in aggressive dogs [35]. This result might insinuate a regulatory mechanism of the presynaptic serotonin receptors taking place in aggressive dogs. This study underlines how complex the underlying neurobiological pathways of aggression are and gives new insight into the mechanism related to aggression, as serotonin and tryptophan have been the major focus.

DeNapoli *et al*. (2000) evaluated the effect of high- and low-protein diets together with or without tryptophan supplementation on canines with dominance aggression, territorial aggression and hyperactivity, whereas a low-protein diet with tryptophan was hypothesized to cause a decreased aggressive behavior. In conflict with the hypothesis, no significant change in behavior was found in any of the groups during the dietary treatments [36]. The study indicates a potential association between dietary protein level and aggression due to the higher dominance aggressive scores in dogs fed high protein diet without Trp, which might cause a lower uptake of Trp compared to other LNAA. The result of the study does partially support the hypothesis due to Trp supplementation of both high- and low-protein diets led to a decreased aggression score in both dominance and territorial aggression [36]. Puurunen *et al*. (2016)'s evaluated the metabolic markers associated with ADHD-like behaviour. The study used the same commercial diet on all the dogs, which is good for achieving data without dietary variations [43]. This study gives good insights into the metabolites in ADHD-like behaviour, but due to the smaller sample size, further experiments with a larger size could be preferred.

Re *et al.* (2008) studied the biochemical basis of canine aggression on the basis of previous human studies where behaviour changes were linked to altered omega-3 polyunsaturated fatty acids and higher ratio of omega 6:omega 3 [44]. A senior veterinary behaviourist diagnosed the dogs and all had a history of aggressive incidents, which might help keep the objectivity due to a professional diagnosis, which were unaware of the dogs polyunsaturated

fatty acid status. Pan *et al. (2010)'s* study found supplementation of MCTs in senior dogs increased cognitive function compared to the control group, shown through several tasks and blood analysis [50].

Cannabidiol has been studied in relation to noise-induced fear, car travel and separation anxiety. In the noise-induced fear study, the dogs given Cannabidiol had increased heart rate and were less likely to fixate on objects during tests, but otherwise no significant change in cortisol levels [77]. The results of stress during car travel and separation from owner showed lower heartrate, reduced stressful behaviours and lower cortisol levels in the dogs supplemented with Cannabidiol [78].

Kato *et al*. (2012)'s study on Royal Canin's Calm Canine® diet resulted in positive findings in regard to lower stress response in the dogs on the diet. The diet Calm Canine had nutraceuticals as alpha-casozepine, L-tryptophan, DHA and EPA. Both behaviour scores were lowered during the Calm Canine® diet and UCCR samples were also lower, but not significantly [64]. Scandurra *et al.* (2021)'s study on the effect of DiRelax® also gave positive results for the nutraceutical during the owner evaluations from C-BARQ questionnaire and showed quicker resolution to the solvable tests, while the result of the blood analysis showed no significant changes [68]. The study by Mondo *et al*. (2020) found dogs with aggression have higher diversity and less common bacteria compared to a normal healthy dog's microbiome [83]. Findings support that prolonged stress leads to imbalances in the gut microbiome, as previously supported by other studies as well. *Lactiplantibacillus plantarum* PS128 were found to reduce aggression, separation anxiety and compulsive disorder by assessment of behaviour scales [84].

Canine behaviour problems have complex pathways and are regulated by hormones and neurotransmitters which can be affected by the levels of these or their precursors, in which several can be affected by nutrition. These complex pathways need even more information to be able to understand the processes completely, but as of now we know Trp and serotonin are found linked to aggressivity and can be affected by feeding dietary Trp to increase the Trp:LNAA ratio [33–38]. Dietary L-Theanine were found to improve canine storm-sensitive anxiety, while phospholipids were linked to ADHD-like behaviour modelled after human studies [39, 43]. Observations support the link of omega-6:omega-3 ratio to aggression and MCT showed a positive effect in relation to cognitive function [44, 45, 50, 52, 54, 58, 59]. The use of Cannabidiol were found to releave anxious behaviour as in car travel and separation anxiety, and dogs were less likely to fixate on something in the room during the

noise fear study [77, 78]. The Royal Canin's Calm Canine diet ® gave lowered stress scores in their study, as well as in the study of DiRelax® [64, 68]. Additionally, the gut microbiome was found significantly different in healthy dogs compared to aggressive dogs and *Lactiplantibacillus* were found calming in relation to anxiety and aggression [83, 84].

4. Discussion and conclusion

Behavioural problems are reported as more common than previously and even with proper nutrition, many dog owners need more education in canine husbandry, training and handling as well as dietary treatment as behavioural problems are quite complicated. Continuing to gain accurate knowledge would aid veterinarians, researchers and dog owners in understanding the complex relationship between nutrition and behaviour, and therefore aid in finding dietary treatments suited for their patients and their behavioural problems.

The findings of the studies in this literature review support the premise that nutrition influences canine behaviour and the information acquired from studies and experiments gives valuable insights into the relationship between nutrition and behaviour. Nevertheless, it is vital to acknowledge the further studies warranted in this field to be able to better understand canine behaviour in the future.

For instance, further investigation is necessary to understand the complex pathways of aggressive behaviour concerning the precursor tryptophan and the serotonin synthesis. Some studies indicate a correlation between aggressive dogs and low serotonin levels, while a conflicting study implies a higher complexity of the mechanism behind aggression where both serotonin receptors and tryptophan levels are involved. Even though the study of Cakiroglu *et al.* (2007) gives valuable insight, there are limitations due to being a cross-sectional study with a small sample size and therefore all the results are based on a one-time occurrence instead of over time [34]. For further study, a recommendation would be for the study to span over a longer time period, with a larger sample size and more various ages and breeds to generalize the results. DeNapoli *et al.* (2000) gave further insights into the effect of different protein level with tryptophan by their randomized controlled trial, some limitations such as smaller sample size and owner-based assessment can give subjective results [36].

Additionally, L- Theanine were researched in relation to storm anxiety and gave valuable insights into anxiety relief, but due to 8 dogs dropping out of the study, the sample size was small [39]. Due to the trial being based at home with their owners during the storms, the study is subjected to bias as each owner are the observer and assesses the dog themselves. Further studies on the same topic could improve with a larger sample size, with an added control group over a longer period of time. To make the trial more unbiased, physiological markers could be added and the dogs can be contained in the same environment with the same conditions and are observed by an unbiased person with experience in canine behaviour.

The research on canine ADHD-like behaviour gave valuable information by finding the related phospholipid metabolites and their levels in healthy dogs compared to those with ADHD-like behaviour indicating a possible metabolic link to this behaviour in canines [43]. The study is impacted by the subjectivity of owner assessment, where using a professional behaviourist could cause objectivity. A lack of other breeds can also be noted, where only German shepherds were used in the study, and a larger sample size would be preferred to see the larger picture.

Another study related to aggressive behaviour focused on omega 3-PUFA and the omega-6:omega3 ratio gave valuable insight into the impact of omega-6 and omega-3 on behaviour [44]. Further study would be valuable with larger sample size in a longitudinal study where the effect of the intake of omega-3 and omega-6 would be studied to aid in forming a dietary treatment.

Pan *et al.* (2010) conveyed the benefits of MCT supplement for cognitive function and the study have a higher reliability due to the near identical housing, environment and enrichment of the beagle dogs compared to other studies. Objectively equal tasks were used for all test groups in the same manner and the dogs chosen had training prior to the study [50]. The study could achieve a wider generalization by using various breeds and expanding the variety of ages. The age of the dogs were between 7-11 years old, and even though this was the target due to the cognitive decline in seniors, it might be interesting to see if anything of note could be seen in younger dogs as well or even used preventatively.

Kato *et al*. (2012) gave insight into the effect of Royal Canin's Calm Canine® on anxious dogs, the study was a single-blind crossover and thereby increased in credibility even though the dogs were assessed by their owner, as they were not aware of when the trial diet or control diet were given. All dogs were privately owned, meaning there would be differences in the way they are housed, their activity, and enrichment, which might be factors to take into consideration [64]. Additional studies would be recommended due to the uncertainty about which nutraceutical in particular that caused the improvement or if the group combined were the cause.

In Scandurra *et al.* (2021)'s study — feeding DiRelax® — the measurements of the dogs are thorough with in-depth blood analysis, clinical and neurological exam as well as thyroid profiling, which excluded other causes of behaviour and therefore making the study more objective. The dogs were also housed in the same facility during the study, receiving the same care, activity and enrichment. Additionally, many breeds, ages and all genders were in the study helping to generalize the width of the study. The study was controlled by having a placebo group in addition to the DiRelax® supplemented group. Blood samplings were done twelve hours after fasting where an abundance of analysis were completed. The tests were given to all dogs to solve and were filmed for control purposes and to study the dogs behaviour during the impossible tasks [63]. Privately owned dogs were used and therefore the questionnaire can be exposed to owner subjectivity and a larger sample size to control if this is the outcome in several situations, but otherwise this is a controlled study with good results and no side effects.

New insights into other potential therapeutic remedies were studied on the effect of Cannabidiol on noise-induced anxiety, the study was a crossover trial, with controls to avoid placebo effect and used both blood profiling and behaviour assessment in the same room for all dogs, which combined raises the reliability of the results [77].

The study of Mondo *et al*. (2020) — which analysed faecal microbiota — has a good variation of genders, various breeds, age and BW [83]. The dogs were also evaluated by a veterinary behaviourist who objectively put them in groups of aggressive, phobic or in the control group. Faecal samples were taken in the morning with sterile equipment to avoid contamination and frozen until testing. Both DNA sequencing and RIA were used, which are both methods with good accuracy. The dogs were kept in different animal shelters, which

could affect differently in relation to the conditions and environment at the shelters. Mixed diets were fed, dietary variations can therefore not be excluded.

The study on *Lactiplantibacillus plantarum* PS128's effect on separation anxiety, compulsive behaviours and aggression gave an insight into the usage of this as a therapeutic supplement. The dogs used in the trial were examined methodically to ensure no medical cause of the behaviour were found, blood analysis was done before and after the trial, two different standardized questionnaires were used for all dogs during interviews by a behaviourist. [84]. It could be advantageous to prolong the study to be able to see the results of using the PS128 long-term and use a double-blind trial to be certain there are no biases.

Moving on, it would be beneficial to study the effects of these dietary supplementations with behavioural training as a factor since many dogs with behaviour problems receive help in the form of training to teach the dogs to react properly to their stimulus where they are unable to cope normally and their owner to aid the dog in these situations. Studying the impact of nutrition together with training, would provide valuable insights into how effective the dietary supplements would be standing alone compared to the effect combined with regular behavioural training. The interaction of these would be interesting to see the findings of as they together might give a synergistic effect or continue to prove the impact of nutrition by itself when the training is certainly excluded as it has not been so far.

In conclusion, these studies confirm that nutrition does have a rather big impact on behaviour, and this can be used to ultimately help dogs with behavioural issues get more balanced if studied further to use with veterinary guidance, or even released commercially if no side effects are found. The future of behavioural-based nutrition could be a good way to reduce the amount of dogs in shelters or euthanized in the long run. If we can make use of this, build new studies on what is already done for further confirmation and extract it into everyday use in our canine's diets we could influence their behaviour, mood and well-being.

5. Summary

This literature review discusses the importance of nutrition on canine behaviour and focuses on the dietary components that can be given in a dog's diet. Various studies have contributed to expanding the knowledge on the influence of neurotransmitters, various dietary substances and supplements, as well as the effect of specific diets in relation to behaviour.

Tryptophan was found to influence behaviour by increasing serotonin levels through dietary supplementation and therefore reducing aggressive behaviour. Dietary lipids, specifically medium-chain triglycerides enhance cognitive function in aging dogs, therefore potentially reducing cognitive degradation. Omega-3 fatty acids such as alpha-linolenic acid and DHA are linked to canine aggression and might help aid the reduction of aggressivity if proper supplementation can be given. Nutraceuticals as DiRelax® and Royal Canin's Calm Canine ® diet, in addition to Cannabidiol were able to aid in reducing anxiety as well as in other areas with good results. In the microbiome, there have also been found links between aggression and phobic behaviour and alterations in bacterial microbiome. *Lactiplantibacillus plantarum* (PS128) were found to aid in separation anxiety and regulation of emotion. Nutrition can significantly influence canine behaviour in many areas, from aggression to cognitive function and further research should be done to learn more about how we can use it to our advantage.

However, the studies have their own limitations such as small sample size, subjective owner assessments, control or placebo group missing, using few or only a select breed in the trials among some. Therefore, recommendations for further research include using single or doble blind studies with a longitudinal design containing larger sample sizes containing control groups, when possible take advantage of physiological markers and use a wider variety of breeds, preferably including breeds and genetics prone for behavioural problems to be able to see if they respond as well. Additionally, it would be interesting to assess the effect of nutrition and training combined and separately to be able to see if the dietary treatments can stand on their own.

Despite these limitations, the findings show the importance of the nutritional influence on behaviour and how these can be used to aid in behavioural treatments in the future.

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Thesis progress report for veterinary students

Name of student: Thea Ohr

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Name and title of the supervisor: Nikoletta Hetényi, research fellow

Department: Institute of Animal Breeding, Nutrition and Laboratory Animal Science, Department for Animal Nutrition and Clinical Dietetics

Thesis title: Impact of nutrition on canine behaviour (review of the literature)

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2021	09	15	Topic declaration, articles.	
2.	2022	03	16	Online consultation. Table of contents, articles.	
3.	2022	04	04	Online consultation, thesis progress has been made, late response.	
4.	2022	05	09	Online consultation, little progress.	
5.	2022	06	09	Online consultation, little progress.	

Grade achieved at the end of the first semester: 3 (fair)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	11	02	First draft	
2.	2024	02	21	First review	
3.	2024	03	04	Online consultation, second draft	
4.	2024	03	05	Online consultation, third draft	
5.	2024	03	07	Final approval	

Grade achieved at the end of the second semester: 3 (fair)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in.....08. March 2024.....