Facoltà di Medicina Veterinaria

Master di II Livello in

Nutrizione, Alimentazione e Dietetica Clinica del cane e del gatto

Tesi di Laurea in ALIMENTAZIONE ANIMALE

CANINE EPILEPSY, MICROBIOME AND DIET: A DEEPER LOOK INSIDE

Candidato:

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Relatore:

Prof. *Jan Suchodolski*

Anno accademico 2016-2017

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Parole chiave: CANINE EPILEPSY, MICROBIOTA, MICROBIOME, KETOGENIC DIET, MEDIUM CHAIN TRIGLYCERIDES, POLYUNSATURED FATTY ACIDS

Anno accademico 2016-2017

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Introduction

Epilepsy is one of the most common neurological disorders in dogs as well as in human beings. Although some cases recognize a specific etiopathogenesis, most cases are actually classified as "idiopathic". Novel therapeutic approaches are needed since traditional drug therapeutic options may fail to treat seizures in dogs as well as in humans (Martle, Van Ham et al. 2014). Poor owners' perception of their best friends' quality of life may also be a good reason to challenge canine idiopathic epilepsy from a new point of view (Wessmann, Volk et al. 2016).

Recent research showed how deeply gut microbiota and the brain interact with each other (Wang and Wang 2016). A bidirectional communication between the central and enteric nervous systems, together with an important role played by gut microbiota, has led to the term "Gut-Microbiota-Brain Axis" (Wang and Wang 2016). It is nowadays believed that, in addition to influencing digestive system function, symbiotic microbiota can bidirectionally and reversibly impact various extra-intestinal pathogenic processes, including those of the nervous system and behavior (Packer, Law et al. 2016, Wu, Zhang et al. 2016). Some studies suggest how gut microbiota may be an alternative therapeutic target for epilepsy in humans, via diet of pharmacological modulation (Wu, Zhang et al. 2016).

Nevertheless, while in human Medicine a clear correlation between at least some cases of idiopathic epilepsy and diet is already well established (Sampaio 2016), in Veterinary Medicine we still have no final data on it (Larsen, Owens et al. 2014, Podell, Volk et al. 2016).

This thesis has been written with the purpose of going deeper into the subject of epilepsy – microbiota – diet correlation, comparing, at the best of the Author's knowledge, existing data in both human and veterinary medicine as a base for future clinical trial on canine patients.

Canine idiopathic epilepsy

Epileptic seizures are one of the most common neurological disorders in dogs, characterized by a wide spectrum of clinical signs and consequences, with variable short- and long-term morbidity. (Rusbridge 2014, Podell, Volk et al. 2016). Kearsley-Fleet and colleagues estimated a prevalence of 0.62 percent in primary veterinary practice (Kearsley-Fleet, O'neill et al. 2013).

Epilepsy is a heterogeneous disease process, often complicated by the inability to obtain a definitive diagnosis for all patients. In fact, the unpredictability of disease progression, the gaps in scientific knowledge of disease pathophysiology and, even more, the challenges in the diagnosis process due to financial constraints often lead to a lack of treatment uniformity (Podell, Volk et al. 2016). Furthermore, as for many veterinary conditions and therapies, a relatively small database of strong evidence-based clinical studies exists (Podell, Volk et al. 2016). Antiepileptic drugs (**AED**) are designed and well-studied for human use (Podell, Volk et al. 2016). What follows is a brief summary of canine seizure definitions, pathophysiology, diagnosis, and available pharmacological therapies according to present veterinary literature.

Definitions

A **seizure** is characterized by a sudden episode of transient neurological clinical signs, such as involuntary muscle movements, sensory disturbances and/or altered consciousness caused by abnormal electrical activity in the brain (Rusbridge 2014). Seizures can be generalized, affecting both cerebral hemispheres, or partial (focal) where the electrical disturbance is limited to one or more specific areas of the brain (Rusbridge 2014). Canine idiopathic epilepsy is characterized as most common clinical signs by generalized tonic-clonic seizures, with stiffening of the limbs (the so-called tonic phase), followed by jerking of the limbs and jaw (clonic phase) (Rusbridge 2014). **Ictus** in veterinary medicine is often used as a synonym for seizure.

A seizure may be characterized by an **aura** – a subjective sensation that precedes and marks the onset of a neurological condition – and/or a **postictal phase** - the recovery phase after a seizure marked by an altered state of consciousness (Rusbridge 2014). The interictal period is defined as the time between one seizure and the following (Rusbridge 2014).

Epilepsy is defined as a brain disorder characterized by a predisposition to generate epileptic seizures. The International League Against Epilepsy (ILAE) has recently modified the definition of epilepsy to a condition characterized by:

- at least two unprovoked seizures occurring more than 24 hours apart;
- or one unprovoked seizure and a high probability of further seizures;
- or at least two seizures in a setting of reflex epilepsy, provoked e.g. by flashing light (Fisher, Acevedo et al. 2014).

Idiopathic epilepsy is defined as epilepsy of unknown cause other than possible hereditary predisposition, where seizures do not occur as a consequence of some other disease or injury (Fisher, Acevedo et al. 2014). In humans, idiopathic epilepsy is defined as epilepsy of predominately genetic or presumed genetic origin with no gross neuroanatomic or neuropathologic abnormality (Shorvon 2011). The acknowledgment that idiopathic epilepsy has a genetic etiology is important:

although in the veterinary world the term idiopathic is often used inappropriately as ‘unknown cause’, individual breed prevalence of canine genetic epilepsy has been estimated. Nevertheless, despite reports showing a higher risk of epilepsy in certain breeds, or even hair color within a certain breed, there is still a lack of epidemiological studies estimating prevalence in general dog populations (Kearsley-Fleet, O’neill et al. 2013).

Pathophysiology

As previously stated, canine idiopathic epilepsy is suspected to have a **hereditary basis**. Finding the predisposing genes, however, is not an easy scientific challenge. In rodent models and humans, the majority of known epilepsy genes encode ion channels or associated proteins that modify membrane currents controlling neuronal excitability and bursting and/or affect other cellular signaling pathways (Rusbridge 2014). It is hypothesized, therefore, that also many canine "idiopathic" epilepsies may also ultimately prove to be 'channelopathies'.

Nevertheless, some **epigenetic conditions** must also be taken into consideration. The term "Epigenetics" refers to the study of heritable changes in gene expression (active versus inactive genes), that do not directly involve changes to the underlying DNA sequence, which still lead to changes in the phenotype expression, without a change in the genotype. For instance, Short and colleagues have investigated associations between sex and neuter status with treated cases of epilepsy, although there is no concluding data (Short, Dunne et al. 2011). Another epigenetic condition which is commonly correlated with a neurological disorder, both in veterinary and human medicine, is nutrition, which is, in fact, the specific topic of this master thesis.

Besides genetic or epigenetic conditions affecting or inducing seizures properly classified as idiopathic epilepsy, Figure 1 summarizes possible canine seizures causes.

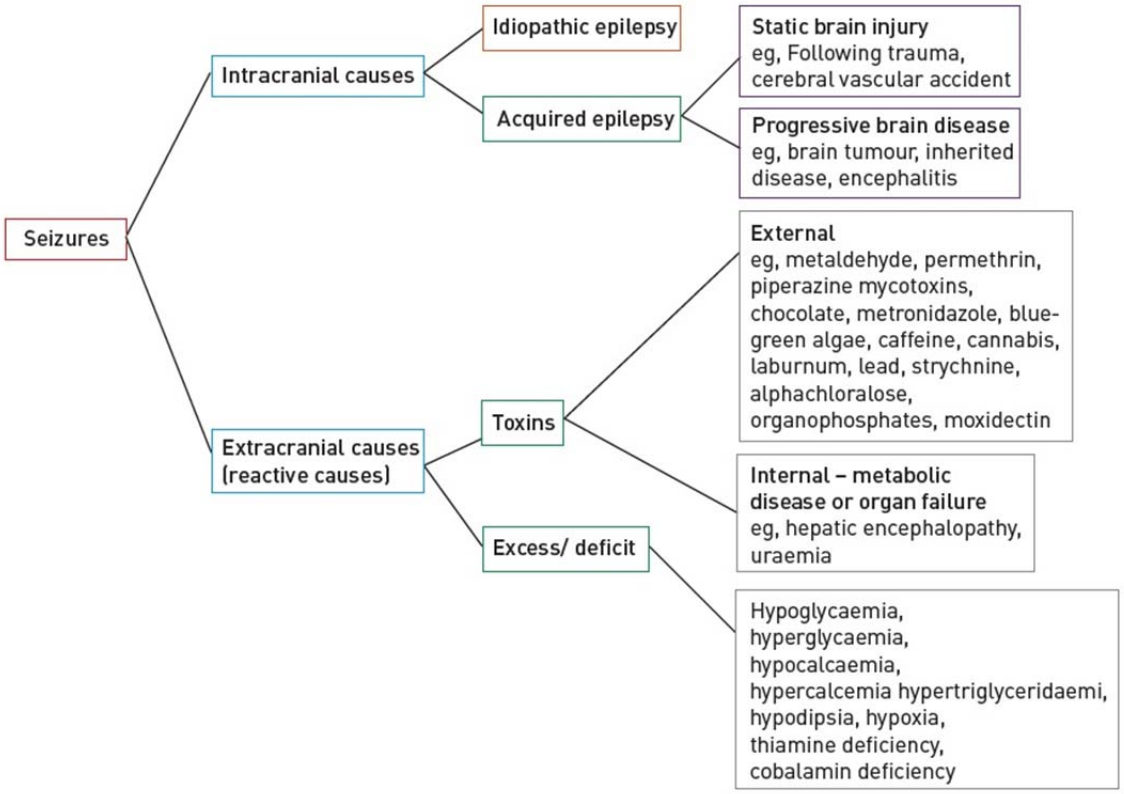

Figure 1 - Causes of seizures

Diagnosis

As shown in Figure 1, the list of possible differential diagnosis for dogs' seizures is long. For this reason, when approaching an epileptic patient, a detailed anamnesis and a systematic approach is necessary. Idiopathic epilepsy is, in fact, a diagnosis of exclusion (Rusbridge 2014).

The animal's signalment (including e.g. breed, age, and history) is very important. In fact, while for a dog aged between six months and six years, with recurrent seizures and normal interictal life, there is more than 97 percent confidence in a diagnosis of idiopathic epilepsy, brain tumors are more common in animals over six years old (Smith, Talbot et al. 2008).

While an important clinical step should be to encourage the owner to obtain a video of the event, firstly, the dog should obviously have a general clinical examination. A hematology, routine serum biochemistry, and urinalysis should also be performed to exclude or confirm other diseases that could be associated with or confused with seizures, e.g., a liver or heart disease (Rusbridge 2014).

A neurological examination should be the following step, in order to identify other signs of brain disease. Depending on the clinical history, neurological findings, and facilities available, further investigation of intracranial disease should be performed, when affordable. MRI or CT, followed by cerebrospinal fluid analysis (to rule out an inflammatory disease) are the most helpful tests to evaluate the epileptic patient. Finally, while electroencephalogram plays an important role in diagnosis and management of epilepsy in humans, it is less useful in animals (Rusbridge 2014).

Pharmacological therapy

The panel recommendations to initiate antiepileptic drugs (AED) treatment of the American College of Veterinary Internal Medicine "Consensus Statement on Seizure Management in Dogs" of 2015 are summarized as follows (Podell, Volk et al. 2016):

1. Identifiable structural lesion present or prior history of brain disease or injury;
2. Acute repetitive seizures or, status epilepticus (ictal event ≥5 minutes or ≥3 or more generalized seizures within a 24-hour period);
3. ≥2 or more seizure events within a 6-month period;
4. Prolonged, severe, or unusual postictal periods.

Treatment is aimed at reducing the frequency, duration or severity of the seizures. Selection of "the best" AED is based on seizure type, efficacy, and tolerability since there is no evidence that any single AED provides a better outcome for adults with unprovoked epilepsy. Drug selection, therefore, is often based on tolerability in both people and dogs (Podell, Volk et al. 2016). Anyway, AEDs suppress seizures but, unfortunately, not epileptogenesis and therefore they do not 'cure' the dog, but merely suppress signs of disease (Rusbridge 2014).

Figure 2 shows ACVIM recommendation of AED use, monitoring, and risk profile according to the "Consensus Statement on Seizure Management in Dogs" of 2015 cited before (Podell, Volk et al. 2016).

Drug	Monotherapy recommendation		Monitor drug levels	Risks Types				Add-on AED recommendation	
	Level	Grade		1	2	3	4	Level	Grade
Phenobarbital	I	A	Y	Y	Y	Y	N	IV	B
Bromide	I	B	Y	Y	Y	Y	N	II	B
Primidone	II	D	Y	Y	Y	Y	N	II	D
Imepitoin	I	A	N	Y	N	N	N	III	C
Levetiracetam	IV	C	N	Y	N	N	N	Ib	B
Zonisamide	III	C	Y	Y	Y	N	N	III	B

Level of study design

Level I and Ib Appropriately designed, controlled trials

1 Criteria

a I: Blinded, randomized clinical trials and drug efficacy of ≥50% for at least 6 months

b Ib: Blinded, randomized clinical trials and drug efficacy of ≥50% for less than 6 months

Level II: Case-control or cohort studies

1 Criteria:

a Nonblinded, randomized, or nonblinded and nonrandomized clinical trials with cohort size of 15 or more, drug efficacy of > or equal to 50% for > 12 weeks, or both.

Level III: Case reports or series

1 Based on individual case reports, conference proceedings, and/or other media distribution as a *potentially* effective and/or predictable outcome

2 Criteria:

a Nonblinded and nonrandomized clinical trials with cohort size of less than 15 and/or drug efficacy of ≥50% for > 12 weeks

Level IV: Expert opinion only

1 Based on any level of scientific information as an unestablished, ineffective, and/or harmful

2 Criteria: Expert opinion only without documentation of cohort studies

Grade of ACVIM panel recommendation

1 A: High recommendation and likely be effective treatment

2 B: Moderate recommendation and most likely to be effective treatment

3 C: Low recommendation and may not be effective treatment

4 D: Not recommended for treatment and may be ineffective and/or dangerous to the patient

Figure 2 - ACVIM panel recommendations of AED use, monitoring, and risk profile (Podell, Volk et al. 2016)

Since epilepsy is a progressive disorder and repeated seizures damage the brain making further seizures more likely (Sakurai, Morita et al. 2013), the number and frequency of seizures before commencing treatment are negatively correlated with prognosis. Novel therapeutic approaches are needed since classical drugs therapeutic options may fail in dogs as well as in human seizures (Martle, Van Ham et al. 2014). For example, phenobarbital monotherapy efficacy for seizure reduction was evaluated in 8 studies for a total of 311 dogs. The cumulative success rate of >50% seizure reduction to improve seizure control was 82% (258/311 dogs), with a cumulative seizure-free rate of 31% (93/311) and failure rate (no improvement) of 15% (48/311) (Podell, Volk et al. 2016). Breeds may also be an important factor leading to successful or unsuccessful therapy outcome: one study of 49 epileptic Border Collies found an apparently unsuccessful drug therapy in 71 percent of 24 dogs, even if treated with more than two AEDs (Hülsmeyer, Zimmermann et al. 2010).

Poor owners' perception of their best friends' quality of life may also be a good reason to challenge canine idiopathic epilepsy from a new point of view (Wessmann, Volk et al. 2016).

Gut-Microbiota-Brain Axis

The intestinal Microbiota

The term intestinal **microbiota** refers to the collection of the living microorganisms (bacteria, fungi, protozoa, and viruses) inhabiting the gastrointestinal (GI) tract. Thanks to novel bacterial identification approaches, we now know that several hundred different bacterial phylotypes form a highly complex ecosystem, both in humans and companion animals (Honneffer, Minamoto et al. 2014). Since sequencing studies of the 16S rRNA are performed to describe intestinal microbiota, the term "**microbiome"** is generally used to describe microbiota genes found as a synonymous of "microbiota" itself.

Apparently, a total of 10^{10}-10^{14} microbial cells live in the intestine of mammals, approximately 10 times more than the number of host cells (Honneffer, Minamoto et al. 2014). This complex ecosystem, formed by microbial cells which interplay with eukaryotic host cells, has a significant impact on host health and disease. Some of the already scientifically reported interactions are, for example:

1. Host immune system stimulation, probably due to microbial metabolites, leading to a coevolution of gastrointestinal microbiota with their host;
2. Gut microbes are a defense barrier against enteropathogens;
3. Aid in digestion of complex fiber sources;
4. Production of various short-chain fatty acids and other metabolites that provide nutritional support for enterocytes (Honneffer, Minamoto et al. 2014).

Depending on the single subject, 10 different bacterial phyla have been identified on average in the feline and canine gut, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria* being the vast majority of all gut microbes. Other minor but still abundant members of canine and feline microbiome are the phyla *Tenericutes*, *Verrucomicrobia*, *Cyanobacteria*, and *Chloroflexi*.

While *Helicobacter* are the predominant group in the stomach with more than 90% of sequencing reads, the duodenum is home to *Enterobacteriaceae*, *Clostridiales*, *Bacteroidales*, and *Lactobacillales* (Suchodolski, Camacho et al. 2008).

Anyway, growing scientific evidence shows that gut microbiota is not fixed during lifetime. Acute or chronic gastrointestinal inflammation appear in fact to modify canine and feline microbiota (Honneffer, Minamoto et al. 2014). Moreover, we also know from human studies that microbiota is changing with human development and influenced by various stress factors (Wang and Wang 2016). After receiving the initial microbiome from their mothers, individual microbiota change during times, leading to a macro balance (Wang and Wang 2016). Apparently, changes in "beneficial" bacteria, or any changes in this balance, can significantly affect the health of individuals, while some factors such as infection, drug, illness, and diet may change the microbiome (Wang and Wang 2016).

The Gut – Microbiota – Brain axis: how microbiota influences neural and behavior

Recent research showed how deeply gut microbiota and the brain interact with each other (Wang and Wang 2016). A bidirectional communication between central and enteric nervous system, together with an important role played by gut microbiota led to the coning of the term "Gut-Microbiota-Brain Axis" (GMBA) (Wang and Wang 2016). It is nowadays believed that, in addition to influencing digestive system function, symbiotic microbiota can bidirectionally and reversibly impact various extra-intestinal pathogenic processes, including those of the nervous system and behavior (Packer, Law et al. 2016, Wu, Zhang et al. 2016).

For instance, a few years ago, a study showed germ-free (GF) mice compared with conventional mice who were growing in a specific-pathogen-free (SPF) environment. Under the experimental conditions, the latter had less anxiety-like behaviors and increased 5-HT synthesis in the thalamus (Heijtz, Wang et al. 2011). If GF adult mice were moved to SPF environment, anxiety-like behavior did not increase. Nevertheless, its offspring's anxiety behavior returned to the normal state, indicating that there was a critical time window for the influence of gut microbiota on behavior development (Heijtz, Wang et al. 2011). Starting from such scientific works, a number of different projects started focusing on gut-brain interactions and on the specific role that microbiota appears to play.

Although currently the exact mechanism of communication between the gut microbiota and the brain has not yet been fully clarified, gut microbiota appear to exert effects on the brain not only through neuroanatomical pathways, but also through the endocrine system, immune system, and metabolic system (Wang and Wang 2016). The bidirectional communication between the gut and the brain is what we currently defined as **the gut-brain axis**. Looking deeply, the interaction of gut microbiota with gut-brain axis is referred to as the **gut-microbiota-brain axis**. Since gut microbiota can be considered from a clinical point of view as an independent variable and changed intentionally, more and more emphases are placed on the role of microbes in gut microbiota-brain axis (Wang and Wang 2016).

Microbiota and epilepsy

Considering what was briefly summarized in prior paragraphs, it should not sound strange if some studies suggest that gut microbiota may be an alternative therapeutic target for epilepsy at least in humans, via diet, pre-probiotic or pharmacological modulation (Wu, Zhang et al. 2016).

One hypothesis of microbiota and epilepsy correlation seems to be based on an **autoimmune pathogenesis**. In fact, according to Wu and colleagues (Wu, Zhang et al. 2016), segmented filamentous bacteria (SFB), a gut-residing species, plays a negative role through its impact on the adaptive immune system and may be correlated with epilepsy via autoimmune disorder.

Another interesting hypothesis considers **gut microbiota metabolites**. Gut bacteria, in fact, can synthesize gamma amino acid, butyric acid, 5-HT, dopamine, and Short Chain Fatty Acids (SCFAs). These substances can also exchange between cells of microorganism, especially intestinal cells in the gut can produce many 5-HT that have an effect on the brain. Bacterial enzymes can also produce neurotoxin products such as D-lactic acid and ammonia (Wang and Wang 2016). Therefore, since a lot of necessary neurotransmitters in the body are generated by the gut microbiota, exerting influence on the human body including the brain, it appears to be clear how important is a correct modulation of gut microbes.

Fecal microbiota transplantation (FMT) is a promising strategy both in human and veterinary medicine that involves reconstruction of gut microbiota through the implantation of donors' microbiota. Recently, it has been considered as a treatment for a number of gastro-enteric pathologies, including e.g. Crohn's disease (CD), as well as certain neurological diseases. A case report of 2017 documents the first case where, using FMT, remission of intestinal and neurological symptoms was achieved in a girl with CD and a 17-year history of epilepsy. FMT has proved its efficacy in preventing relapse of seizures after more than 20 months of follow-up, including the withdrawal of antiepileptic drugs. This finding highlights the role of microbiota-gut-

brain axis and may inspire novel treatments for epilepsy through remodeling gut microbiota (He, Cui et al. 2017).

Diet and epilepsy: from history through present scientific findings

Since Hippocrates (460 BC–370 BC), **fasting** has been recognized as a therapeutic treatment for epilepsy. Fasting was also documented in the Bible: Mark (9:29) relates the story of Jesus who, when his apostles asked him why they had not been able to cure a boy from epilepsy, said: "*This kind can come out by nothing but prayer and fasting*".

From fasting to the ketogenic diet

The first scientific report of the antiepileptic effect of fasting was noted almost 100 years ago (Geyelin 1921). During those years, a particular kind of dietary regime (Wilder 1921) called the **ketogenic diet** (KD) was introduced for the treatment of epilepsy in human medicine. Over the next years, the KD became a popular treatment for both children and adults with epilepsy (Martle, Van Ham et al. 2014).

Anyway, with the development of different AEDs, and also due to the difficulty of the practical management of a day-by-day diet, the popularity and use of the KD diminished (Martle, Van Ham et al. 2014).

A renewed interest and research into the efficacy of the KD was initiated during the 90s of last century, due to the successful treatment of the refractory seizures of a famous American film producer's son (Kossoff, Zupec-Kania et al. 2009).

In 2008, Neal and colleagues conducted and finally published on The Lancet, the first randomized controlled trial to assess the efficacy of the KD (Neal, Chaffe et al. 2008). One hundred and forty-five children with epilepsy who were unresponsive to two AEDs were followed by authors. Children were randomized into two groups: one received the KD immediately (KD group), and the other after three months with AEDs in a stable dose (control group). After three months, the KD group experienced a 75% reduction in seizure frequency compared to controls. Additionally, 38% of children in the KD group had > 50% seizure reduction and 7%

had > 90% reduction in seizure frequency. These results clearly showed that the KD benefits children epilepsy compared to no change in treatment. Nevertheless, some difficulties were also experienced during this study: in fact, one-fourth of the children who were on the KD reported side effects such as vomiting, lack of energy, hunger, diarrhea, abdominal pain, or taste problems. Constipation was the most reported side effect (Neal, Chaffe et al. 2008).

Also due to this study, this diet is used to the present day mainly for the treatment of refractory pediatric epilepsy in human medicine. This diet is very high in fat (80% of calories), low in carbohydrates (5%) and has adequate amounts of protein (15%), based on minimum daily requirements (Martle, Van Ham et al. 2014). The classic KD is also called the '4:1 fat: non-fat' diet (Kossoff, Zupec-Kania et al. 2009). Despite much in vitro and in vivo investigation, the precise antiepileptic mechanism of action of the KD still remains unknown (Martle, Van Ham et al. 2014).

Medium-chain triglyceride (MCT) diet

The MCT diet was basically developed to make the KD more palatable, allowing a greater proportion of carbohydrate and protein. Medium-chain triglycerides produce more ketones per kilocalorie of energy than the long-chain triglycerides used in the classic KD, requiring less fat intake to produce ketosis compared to a classic KD, because MCTs are more rapidly metabolized (Martle, Van Ham et al. 2014).

Patients consume more varieties and quantities of food, have better growth and require fewer micronutrients compared to the classic KD. There is also a positive effect on lipid levels with a significantly lower total cholesterol/high-density lipoprotein ratios compared to the classic KD. The traditional MCT diet was initially designed to deliver 60% of energy from medium-chain triglycerides (Martle, Van Ham et al. 2014, Sampaio 2016).

In this case, nevertheless, frequent side effects in children also include diarrhea, vomiting, bloating, and abdominal pain. To increase tolerability, a modified version was proposed using 30% of energy from MCTs and 30% from long-chain fatty acids with the MCT percentage needing to be increased gradually (Sampaio 2016).

The efficacy of the MCT diet is similar to that of the classic KD. A randomized controlled trial (from same authors who firstly published the KD article on The Lancet) comparing the MCT and classic versions of the KD indicated there were no significant differences between the two diets (Neal, Chaffe et al. 2009).

In 2015, a 6-month prospective, randomized, double-blinded, placebo-controlled cross-over dietary trial was designed to compare a **ketogenic medium-chain TAG diet (MCTD) with a standardized placebo diet in chronically antiepileptic drug-treated dogs with idiopathic epilepsy** (Law, Davies et al. 2015). Dogs were fed either MCTD or placebo diet for 3 months followed by a subsequent respective switch of diet for a further 3 months.

The experimental placebo and test formulae were **dry extruded kibble** (Nestle Purina PetCare) formulated to meet or exceed the nutritional guidelines established by the Association of American Feed Control Officials (AAFCO). The same ingredient characterized both formulae composition: they contain <10 % moisture, at least 28 % crude protein, at least 15 % crude fat and 50 % carbohydrates with <2 % as crude fiber (Law, Davies et al. 2015).

The only compositional exception was that zero MCT were added to the placebo formula: to ensure that the formulae had same calories, lard was used as a fat substitute (1560·6 kJ/100g (373 kcal/100 g)). The test formula on the opposite contained 5·5% MCT. MCT content was about 10 % of the total formula calories (Law, Davies et al. 2015).

The placebo diet had, therefore, a different fat composition with none or practically none of C12, C10, and C8 fatty acids. Both formulae exceeded the AAFCO minimum requirements for essential fatty acids. Twenty-one dogs were housed and fed mainly once or twice daily at home with no restrictions on water consumption. The amount of food given per day was calculated according to the weight of each dog, allowing obviously a minor deviation food consumption to account for the individual needs of each dog, taking into consideration differences in activity level and physical condition. Dogs were restricted to consumption of the study diet, and thus treats or snacks were replaced by the respective placebo or MCTD food (Law, Davies et al. 2015).

Seizure frequency, clinical and laboratory data were collected and evaluated for the twenty-one dogs completing the study. Seizure frequency was significantly lower when dogs were fed the MCTD in comparison with the placebo diet; three dogs achieved seizure freedom, seven additional dogs had ≥50 % reduction in seizure frequency, five had an overall <50 % reduction in seizures and six showed no response. Consumption of the MCTD resulted in significant elevation of blood β-hydroxybutyrate concentrations in comparison with placebo diet. No significant changes in serum concentrations of glucose, phenobarbital, potassium bromide and weight between diet groups were reported (Law, Davies et al. 2015).

Although only this study cannot represent a strong scientific evidence of the efficacy of MCT diet in canine epilepsy, reported data show some interesting antiepileptic properties associated with this commercial product designed on a ketogenic diet. This also provides some evidence for the efficacy of the MCT diet as a therapeutic option for epilepsy treatment (Law, Davies et al. 2015).

Nevertheless, according to the opinion of the Author of this thesis, it should be taken into account that, for better or for worse, a commercial product should not be taken as representative of "diet" in general. Fresh and varied food should never be compared to dry, extruded products. In veterinary medicine, the quality of food should be as important as it is in human medicine.

Comparison of different fresh diets used in human medicine

Figure 3 reports a comparison between different KD used in human medicine: ketogenic diet properly so called, Medium-chain Triglycerides Oil diet (MCT diet), Low Glycemic Index Treatment and Modified Atkins Diet (Sampaio 2016).

Questions	Ketogenic Diet	Medium-Chain Triglycerides Oil Diet	Low Glycemic Index Treatment	Modified Atkins Diet
Is medical supervision required?	Yes	Yes	Yes	Yes
Is the diet high in fat?	Yes	Yes	Yes	Yes
Is the diet low in carbohydrate?	Yes	Yes	Yes	Yes
What is the ratio of fat to carbohydrate & protein?	4:1, 3:1, 2:1, 1:1	Approximately 1:1	Approximately 1:1	Approximately 1:1
How much carbohydrate is allowed on a 1000 calorie diet?	8 g carb on a 4:1 16 g carb on a 3:1 30 g carb on a 2:1 40-60 g carb on a 1:1	40-60g	40-60g	10 g for one month 20 g afterwards Adjusted for children vs. adults
How are foods measured?	Weighed	Weighed	Fat and carbohydrate content is discriminated or estimated	Fat and carbohydrate content is discriminated or estimated
Are meal plans used?	Yes	Yes	Yes	Optional
Where is the diet initiated?	Hospital/Home	Hospital/Home	Home	Home
Are calories controlled?	Yes	Yes	Yes	No
Are vitamin and mineral supplements required?	Yes	Yes	Yes	Yes
Are liquids (fluids) restricted?	No	No	No	No
Is a pre-diet laboratory evaluation required?	Yes	Yes	Yes	Yes
Are there possible side-effects?	Yes	Yes	Yes	Yes
What is the overall difference in the design of these diets?	This is a personalized and structured diet that provides specific meal plans. Foods are weighed and meals should be consumed in their entirety for best results. The ratio of this diet can be adjusted to provide better seizure-control or better tolerance. This diet is also considered a low glycemic therapy and results in steady glucose levels.	This is a personalized and structured diet containing medium-chain triglycerides, which are highly ketogenic. This allows more carbohydrate and protein than the classic ketogenic diet. A source of essential fatty acids must be included on this diet.	This is personalized but less structured diet than the ketogenic diet. It uses exchange lists for planning meals and emphasizes complex carbohydrates. The balance of low glycemic carbohydrates in combination with fats result in steady glucose levels. It is not intended to promote ketosis.	This diet focuses on limiting the amount of carbohydrate while encouraging fat consumption. Carbohydrate may be consumed at any time during the day as long as it is within limits and should be consumed with fat. Suggested meal plans are used as a guide. Protein is not limited but excessive consumption is discouraged.

Figure 3 - Comparison of different fresh ketogenic diets used in human medicine according to (Sampaio 2016)

Diet mechanism of action

The antiepileptic effects of the diet, like the antiepileptic effects of starvation, have been attributed to an accumulation of ketones, and there are experimental data in animal models to support this hypothesis.

Some scientists, in fact, suggest that the mechanism of action should involve alterations in mitochondrial function, effects of ketone bodies on neuronal function and neurotransmitter release, antiepileptic effects of fatty acids, and/or glucose stabilization (Martle, Van Ham et al. 2014). Ketone bodies may increase membrane potential hyperpolarization and γ-aminobutyric acid synthesis, and decrease the release of glutamate, norepinephrine, or adenosine (Martle, Van Ham et al. 2014).

Recently, new data about the neuroendocrine response to the acute phase reaction (stress) have emerged, indicating the involvement of various neuropeptides, including neuropeptide Y (NPY), which is considered as an endogenous anticonvulsant (Mainardi and Albano 2008). The release of NPY is also stimulated by nutrients in the gut, particularly fats. Long-chain and, to a greater extent, medium-chain triglycerides (MCT), which are components of the ketogenic diet, stimulate NPY secretion. This effect may explain the improvement in seizure control after starvation, use of the classical ketogenic diet, especially with additional MCT in the diet (Mainardi and Albano 2008).

Essential fatty acid and epilepsy

Dietary omega-3 (Ω3) and omega-6 (Ω6) polyunsaturated fatty acids (PUFAs) are involved in many physiological processes in the brain. It is thought that Ω3 PUFAs could have anticonvulsant effects (Taha, Burnham et al. 2010). Docosahexaenoic acid (DHA) in fact plays an important structural role in the neuronal membrane, and the most commonly accepted hypothesis to explain its possible anticonvulsant mechanism of action is that it modulates ion channels, mainly voltage-dependent sodium channels (Martle, Van Ham et al. 2014).

Other hypotheses are that PUFAs might raise the seizure threshold by antagonizing neuroinflammation or by activating peroxisome proliferator-activated receptors (Taha, Burnham et al. 2010). Recent evidence from in vitro and animal studies suggests that Ω3 PUFAs could have a potential use in the treatment of epilepsy although conflicting results have been found in human clinical studies, maybe due to different products or dosages used (Taha, Burnham et al. 2010).

A **case report** about the positive effect of daily supplementation with fish oil, a rich source of PUFAs, on seizure frequency in a Great Dane with idiopathic epilepsy has been described (Scorza, Cavalheiro et al. 2009). In this particular case, it was decided to supplement patient's diet with moderate amounts of fish oil with a dosage of Ω3 polyunsaturated fatty acids of 2 g/day, after that, a dosage of 2.5 mg/kg, twice a day orally of phenobarbital for 8 weeks failed to adequately control the seizures. The frequency of the epileptic seizures markedly fell after 50 days of combination therapy with phenobarbital and Ω3 fatty acid. During the subsequent 18-month period, seizure frequency fell to one per 3 months, a reduction of about 85% (Scorza, Cavalheiro et al. 2009).

Unfortunately, while for phenobarbital the dosage was given per kg of weight, we cannot know either the exact dose of Ω3 administered, nor the exact composition of Ω3 formula (EPA, DHA and/or even ALA have been administered?), nor even the biochemical form of these PUFAs (ethyl esters, triglycerides or phospholipids?), nor whether they were declared free of

contaminants such as heavy metals. This makes the comparison with other future studies virtually impossible.

More recently, a single-blinded, placebo-controlled crossover trial investigated the effects of x-3 oil supplementation on seizure frequency and severity in dogs with idiopathic epilepsy (Matthews, Granger et al. 2012). In this study, 15 dogs were treated with triple purified Ω3 oil containing 400 mg eicosapentaenoic acid, 250 mg docosahexaenoic acid and 22 mg vitamin E per 1.5 mL at a dose of 1.5 mL/10 kg (975 total EPA + DHA/10 kg approximately equal to 175 total EPA+DHA/kg metabolic weight) once daily for 12 weeks, followed by a 12 week placebo period of supplementation with olive oil. Owners recorded seizure frequency and severity and any adverse events. In this case, no significant difference in seizure frequency and severity was found between the treatment and placebo period, but the study had low statistical power (Matthews, Granger et al. 2012, Martle, Van Ham et al. 2014).

Conclusions

Epilepsy is nowadays one of the most common chronic neurological disorders both in humans and dogs. Most dogs have idiopathic epilepsy and are managed successfully with standard antiepileptic drugs. Nevertheless, approximately 30% of cases are refractory to treatment (Martle, Van Ham et al. 2014).

Unfortunately, although a substantial number of new AEDs have become available, both in human and veterinary medicine, several long-term studies have demonstrated that few human patients became seizure free after initiating a third AED when two AEDs have failed. The same is probably true also in veterinary medicine, although we are missing such well organized and statistically significant data. Therefore, non-pharmacological treatment options are becoming increasingly important.

Diet and starvation had been used for centuries to treat human epilepsy. Ketogenic Diet (KD), with or without Medium Chain Triglycerides (MCT) and/or Polyunsaturated Fatty Acids (PUFAs) supplementation appear to have a good efficacy in human medicine, with studies published in notorious Journals such as The Lancet or other important ones (Neal, Chaffe et al. 2008, Neal, Chaffe et al. 2009).

Although in multiple studies a central role of the Gut-Microbiota-Brain axis is supposed, the exact mechanism of action of diets and starvation remains unknown.

Nevertheless, due to important scientific findings, both in vivo and clinical studies, regarding gut microbiome and its relationships with a number of neurological conditions, a deeper look should be taken also in veterinary medicine. Clinical data are needed to propose a fresh or commercial dry food to owners of dogs that are experiencing idiopathic epilepsy, also in order to decrease AEDs use and finally contribute to the quality of life of those families.

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