TERESA MORUJO DE MAGALHÃES

NUTRITIONAL SUPPLEMENTATION WITH MEDIUM CHAIN FATTY ACIDS IN DOGS WITH COGNITIVE DYSFUNCTION SYNDROME: THE TUTORS' PERSPECTIVE

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FACULTY OF VETERINARY MEDICINE

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WITH THE FOLLOWING ATTENDING JURY:

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To Polly,

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Resumo

A disfunção cognitiva canina é uma síndrome neurocomportamental na qual os animais experienciam déficits contínuos na aprendizagem, memória e orientação espacial resultando num declínio cognitivo gradual com crescente patologia cerebral. Apesar da prevalência estimada desta síndrome variar entre 22,5% e 73,5%, a maioria destes casos não é tratada desde cedo já que os tutores tendem a relacionar as alterações comportamentais dos seus animais com o processo fisiológico do envelhecimento.

O estudo apresentado de seguida, tem como objectivo, averiguar se as alterações comportamentais subsequentes de uma suplementação oral com ácidos gordos de cadeia média será perceptível pelos tutores de uma população de cães urbanos.

Os efeitos de melhoramento cognitivo dos ácidos gordos de cadeia média estão bem documentados e reportados na literatura. Contudo, quando estudando uma população de cães geriátricos com alterações comportamentais subtis, não se verificam, na literatura, estudos sobre a avaliação dos tutores do comportamento dos seus animais.

Quando se submeteram as variáveis do perído de estudo a testes de $\chi 2$, uma Taxa de resposta global ao tratamento foi criada. Os resultados indicam melhoria de sinais clínicos em 8,04% da população e agravamento dos mesmos em 5,36% dos casos. A maioria da população de estudo, 86,61%, apresentou manutenção dos sinais clínicos de início de estudo não tendo sofrido agravamento nem melhoria dos mesmos.

Palavras Chave: Disfunção cognitiva canina (DCC), comportamental, ácidos gordos de cadeia média (AGCM), suplemento, tutor

Abstract

Canine cognitive dysfunction syndrome is a neurobehavioural syndrome in which the animals experience ongoing deficits in learning, memory and spatial awareness resulting in a gradual cognitive decline with increasing brain pathology. Although the estimated prevalence of this syndrome in a population ranges between 22.5% and 73.5%, the majority of those cases is not early addressed since the tutors tend to relate the behavioural changes with the normal process of ageing.

The following study aimed to see if the behavioural changes subsequent of an oral supplementation with medium chain fatty acids would be noticeable by the tutors of a population of urban dogs.

The effects of medium chain fatty acids on cognition are well described in the literature but not when addressing a population of geriatric dogs with an early onset of behavioural changes in the tutors perspective.

When subjecting the variables throughout the trial period to $\chi 2$ tests a global response to therapy rate was created. The results indicated an improvement in 8,04% of the cases while a worsening in 5,36%. The majority of the study group, 86,61% considered that the animals had not improved nor worsened their behavioural changes.

Key words: Canine cognitive dysfunction syndrome **(**CDS), behavioural, medium chain fatty acids (MCTs), supplementation, tutors

Resumo das secções em português

Introdução

A disfunção cognitiva canina – DCC - é descrita como alguma alteração comportamental em diferentes categorias como interacção social, comportamento de eliminação, ciclo do sono e orientação (Overall, 2013). É uma síndrome neurocomportamental na qual o animal experiencia deficits na aprendizagem, memória e orientação espacial contínuas e crescentes, com exclusão de outras causas médicas para os mesmos sintomas (Landsberg, Hunthausen & Ackerman, 2013 ; Overall, 2013).

O estudo desta síndrome, nos animais, começou para dar resposta a questões sobre o Alzheimer onde, os animais estudados, eram usados como modelo para a doença de medicina humana sendo todos estudos laboratoriais. Os primeiros artigos que focavam os estudo da doença nos animais datam dos anos noventa (Cummings & Cotman, 1995). Desde então, vários estudos foram elaborados com a finalidade de estudar esta doença como um problema nos animais e, de maneira a, melhorar a sua qualidade de vida (Salvin, McGreevy, Sachdev & Valenzuela, 2010).

Nem sempre esta síndrome é tratada desde o início da sua manifestação pois os tutores tendem a relacionar as alterações comportamentais manifestadas como parte natural do processo de envelhecimento (Neilson, Hart, Cliff & Ruehl, 2001).

O envelhecimento fisiológico cerebral do cérebro inclui determinadas alterações tais como uma redução na massa cortical acompanhada com atrofia dos gânglios basais, aumento do tamanho ventricular, calcificação meningeal, diminuição do número de neurónios funcionais e aumento da sua degeneração (Borràs, Ferrer & Pumarola, 1999). Já está comprovada uma correlação positiva entre atrofia cortical e disfunção cognitiva (Rofina, Ederen, Toussaint, Secrève, van der Spek, van der Meer, et al., 2006). Existem outras alterações que podem justificar a apresentação clínica da Síndrome de Disfunção Cognitiva que incluem a insuficiência vascular cerebral por fibrose das paredes vasculares, proliferação endotelial, mineralização, microhemorragias e angiopatia amilóide das mesmas. Isto leva a uma diminuição da perfusão cerebral diminuindo o uso da glucose como fonte primária de energia (Uchida, Okuda, Yamaguchi, Tateyama, Nakayama & Goto, 1993 ; Borràs et al., 1999 ; Landsberg & Araujo, 2005).

Em relação ao que se pode apelidar de envelhecimento patológico existem três processos que acontecem: Produção de radicais livres – o que leva a alterações oxidativas, Formação de lesões cerebrais – nomeadamente a deposição de placas de β-amilóide e alterações na disponibilidade de oxigénio pelo cérebro (Overall, 2013). Estas alterações têm diversas consequências fisiológicas. Estudos indicam uma séria de alterações de neurotransmissores em animais de laboratório e humanos. Estas alterações variam desde diminuição dos níveis de dopamina e norepinefrina a alterações de metabolismo da glucose diminuindo o uso pelo cérebro. Isto leva a formação de espécies reactivas de oxigénio culminando, novamente, em danos oxidativos (Badino, Odore, Bergamasco, Barbero, Osella, D'Angelo et al., 2013 ; London, Ohata, Takei, French & Rapoport, 1983 ; Beckman & Ames, 1998). Sabe-se também que associado ao envelhecimento, para além da deplecção de catecolaminas, existe um aumento de Monoaminoxidase B – MAOB - (Milgram, Ivy, Head, Murphy, Wu, Ruehl et al., 1993).

Todavia, o seu envolvimento directo na síndrome a abordar, foi apenas provada mais tarde quando se descreveu que um declínio em dopamina, e em todas as catecolaminas, diminuição de acetilcolina e receptores muscarínicos têm, de facto, um papel patogénico (Araujo, Studzinski, & Milgram, 2005). A sua minoração está associada a declínio tanto motor como cognitivo e alterações da fase REM do sonho – fase do Movimento Rápido do Olho (Araujo, Chan, Winka, Seymour & Milgram, 2004). Estão também descritas diminuições em determinados factores neurotróficos cerebais - Fator Neurotrófico Derivado do Cérebro e Factor de Crescimento Neural em cães geriátricos. Apesar disso, ficou por provar a sua relação com a presença de DCC nestes animais (Head, McClearly, Hahn, Milgram & Cotman, 2000).

O envelhecimento patológico patológico aparece secundário a um conjunto de alterações: Aumento da produção de radicais livres por aumento da sua produção mitocondrial e diminuição dos mecanismos para a sua eliminação, diminuição da energia utilizável pelo cérebro e aumento de MAOB. É o aumento desres radicais livres que levará a dano celular irreversível que varia entre disfunção a morte celular (Landsberg & Denenberg, 2009).

Já foi provado, contudo, que existe uma correlação positiva entre a deposição de placas de β-amilóide no córtex cerebral e a presença de DCC nesses mesmos animais. Inclusivamente verificou-se que esta deposição leva a vários deficits em tarefas

discriminatórias, de aprendizagem reversa e aprendizagem espacial (Cummings et al., 1996). O tipo de depósitos que existe no cérebro canino é diferente daquele que se encontra no cérebro humano – nos primeiros apenas se encontram depósitos em forma de placas (Papaioannou, Tooten, van Ederen, Bohl, Rofina, Tsangaris et al., 2001 ; Head, Rofina & Zicker, 2008). O papel patológico destas deposições ainda não está completamente explicado mas sabe-se que esta proteína é neurotóxica e a sua deposição leva a diminuição da função neuronal o que resulta em perda de células, sinapses não funcionais e diminuição de neurotransmissores (Cummings et al., 1996 ; Head, Callahan, Muggenburg, Cotman & Milgram, 1998 ; Hauw, Crespeau, Uchihara, Akiyama, Checler et al., 2000 ; Gunn-Moore, Moffat, Christie & Head, 2007).

Outra variável que influencia a apresentação clínica da síndrome descrita, para além da quantidade de depósitos de β-amilóide, é a extensão e localização destes mesmos depósitos. A área mais afectada pela deposição é variável segundo diferentes autores sendo, no entanto, globalmente aceite que a localização de deposiação tende a alterar-se com a idade: Começam por ser encontrados depósitos no lobo frontal, passando para o parietal, sendo o lobo occipital o último a ser afectado (Colle et al., 2000 ; Head et al., 2000).

Para estudar o papel da genética na DCC há apenas um estudo onde se verificou que a extensão e localização dos depósitos desta proteína podem ser influenciados pelo genoma. Chegou-se a esta conclusão pois foi demonstrado que determinadas raças tendem a ter deposição de β-amilóide mais cedo que outras raças e que entre ninhadas de cães há de facto similaridades na extensão de depósitos (Bobik, Thompson & Russel, 1994).

Para avaliar a prevalência desta síndrome, foram elaborados diferentes estudos que permitiram concluir que segundo diferentes autores e métodos de colheita de dados obtêm-se resultados bastante diferentes. Estes resultados, dão-nos variâncias de prevalência entre 22.5% e 73.5% (Neilson et al., 2001 ; Osella, Re, Odore, Girardi, Badino, Barbero, et al., 2007 ; Azkona, García-Bellenguer, Chacón, Rosado, Léon & Palacio, 2009). Deve ser referido que alguns estudos podem sobrestimar a prevalência pois nem todas as causas médicas de doença que possam ter a mesma apresentação clínica foram excluídas enquanto outros a podem subestimar pois podem existir doenças concomitantes sem que isso exclua a presença de DCC (Landsberg & Denenberg, 2009). O ponto de coerência é o subdiagnóstico da síndrome pelos clínicos. Há um estudo que indica que numa população de cães onde 14.2% tinha DCC apenas 1.9% havia sido diagnosticado (Salvin et al., 2010).

Todos estes estudos confirmam que as alterações cognitivas que se manifestam como alterações comportamentais são facilmente ignoradas pelos tutores pois são entendidas como parte normal do envelhecimento do seu animal de companhia (Landsberg & Araujo, 2005 ; Osella et al., 2007).

Não há, até ao presente e que a autora tenha conhecimento, factores de risco associados ao desenvolvimento de DCC com coerência entre autores (Fast, Schutt, Tpft, Moller & Berendt, 2013 ; Fast et al., 2013 ; Azkona et al., 2009 ; Head et al., 2001).

Falando da apresentação clínica da síndrome é fácil entender que esta é bastante variável e sem sinais patognomnónicos. Os sinais clínicos mais comuns são: Vocalização nocturna e alterações no ciclo de sono-vigília; Alterações no comportamento social; Perda de comportamentos ou tarefas previamente aprendidos pelo animal e demonstração de ansiedade em situações nas quais previamente o animal se demonstrava confortável (Overall., 2013).

Vários testes foram elaborados com o objectivo de avaliar certas alterações comportamentais associadas com o envelhecimento. Sabe-se hoje que certas tarefas, como aprendizagem reversa ou associadas a memória são sensíveis ao avançar da idade (Tapp, Siwak, Estrada, Head, Muggenburg, Cotman et al., 2003). Os sinais mais frequentemente encontrados em animais já diagnosticados com DCC são Diminuição das interacções sociais, alterações no comportamento de eliminação seguidos de alterações no ciclo de sono-vigília (Azkona et al., 2009).

Sendo que há outras doenças que podem partilhar a mesma apresentação clínica da DCC, é de referir a extrema importância da exclusão das mesmas para que se chegue ao diagnóstico da síndrome estudada (Landsberg & Denenberg, 2009). Os diagnósticos diferenciais do clínico enquanto aborda um cão geriátrico com alterações comportamentais devem incluir, para além da SDCC, outros problemas comportamentais primários do cão geriátrico para além de outras causas orgânicas de doença. Deve também ser mencionado que a exploração minuciosa e proactiva da história médica e comportamental do animal deve ser considerada prioritária. Com isto, supõem-se verificar que não existem medicações que façam parte do plano de tratamento de doenças concomitantes do animal que justifiquem as alterações comportamentais encontradas para além de doenças que, só por si, tambéma s justifiquem (Overall, 2013).

Não deve ser esquecido que para cada comportamento há um mecanismo aprendido que o justifica. Quer isto dizer que, por exemplo, quando um animal demonstra agressividade secundária a dor, percebe que com essa demonstração o que para ele é considerado um perigo desaparece. Desta forma, é possível que o volte a demonstrar noutras situações pois aprendeu que, para este exemplo, a agressividade lhe resolveu o problema. Nestes casos são necessárias combinações de fármacos e planos de modificação comportamental para eliminar os comportamentos indesejados (Landsberg & Denenberg, 2009).

Com o objectivo de chegar a um diagnóstico de SDCC o mais precocemente possível, há que excluir outras causas que levam aos mesmos sinais clínicos através de uma anamnese cuidada e extensa aquando da consulta. Para além disto, sugere-se a realização de um exame físico, colheita de sangue para hematologia e bioquímica e endocrinologia sanguínea e preenchimento de questionários já elaborados para rastreio de alterações comportamentais em cães geriátricos. Estes questionários devem ter em conta uma avaliação de dor, perda de capacidades sensitivas, avaliação neurológica de maneira a facilitar a diferenciação entre causas médicas e comportamentais para as alterações encontradas (Landsberg & Denenberg, 2009). Sugere-se uma avaliação a cada 6 meses (Azkona et al., 2009).

Os passos descritos anteriormente dirigem o clínico a um diagnóstico e início terapêutico precoce. Desta forma, consegue atrasar-se a progressão da doença com menor risco de complicações resultando numa melhoria geral da qualidade de vida do animal e aumento da sua esperança média de vida (Landsberg & Denenberg, 2009).

Para minorar o erro e o tempo despendido pelo clínico no diagnóstico desta síndrome foram criadas várias escalas de auxílio de diagnóstico por diferentes autores. Desta forma, três escalas serão enumeradas de seguida:

A escala “Age Age-Related Cognitive and Affective Disorders” será a primeira a ser abordada. É uma escala numérica que, como o nome indica, avalia alterações comportamentais de animais geriátricos diferenciando as mesmas em alterações cognitivas tais como a tarefas aprendias e alterações emocionais onde se incluem o apetite ou o sono. Foi elaborada com o objectivo de separar as alterações encontradas nestes dois grupos para que seja mais fácil para o clínico fazer uma escolha correcta da terapêutica farmacológica a implementar no animal. Esta escala está validada sendo que resultados altos nesta escala

estão relacionados com a presença de depósitos de β-amilóide no córtex cerebral, nomeadamente no córtex temporal e hipocampo (Landsberg, Hunthausen & Ackerman, 2013 ; Colle et al., 2000).

A segunda escala a ser descrita será a Canine Cognitive Dysfunction Rating scale. Esta escala, tal como a anteriormente descrita, é uma escala numérica que foi criada pela necessidade da existência de uma escala clara e concisa para avaliação de alterações comportamentais em cães geriátricos. Foi elaborada através de um conjunto de vinte e sete comportamentos já identificados em cães com demência. Destes vinte e sete comportamentos foram escolhidos treze que quando presentes adicionam pontos ao resultado do animal. Se o resultado final do animal for superior a cinquenta, obtém-se um diagnóstico de SDCC desde que seja acompanhado de uma avaliação médico-veterinária para que se distinga entre SDCC e outros problemas comportamentais de animais geriátricos. É uma escala também validada com uma precisão diagnóstica estimada de 99.3% (Salvin et al., 2010).

A terceira e última escala a ser enumerada na presente dissertação é a escala "Disorientation, Social Interactions, Sleep-wake cycle, Housesoiling and Activity". Esta, é uma escala que agrupa as alterações comportamentais em diferentes categorias. Ao contrário das anteriores, a presente escala não é numérica e o diagnóstico de SDCC é baseado no número de sinais clínicos do animal mas pela presença de sinais clínicos de diferentes categorias o que poderá ser indicativo de doença em vez de envelhecimento fisiológico. Foi elaborada precisamente nesse sentido, auxiliando o clínico a direccionar o seu diagnóstico clínico para SDCC em vez de outros problemas comportamentais e existem diferentes versões por diversos autores (Overall, 2013 ; Landsberg, Hunthausen & Ackerman, 2013).

Para elaborar um plano de tratamento para qualquer doença comportamental e, inclusivamente, para SDCC, o clínico deverá começar por fazer um correcto diagnóstico baseado na história médica e comportamental do animal a abordar. Posteriormente, diversos exames complementares deverão ser elaborados para que se tenha um diagnóstico correcto e, consequentemente, seja elaborado um plano de tratamento escrupuloso (Landsberg, Hunthausen & Ackerman, 2013).

O primeiro passo que deve ser tomado será sempre tratar doenças concomitantes ou que possam também elas levar a alterações comportamentais. Isto é particularmente

importante se for considerado que deixando este passo para trás e ao implementado um plano de modificação comportamental este é provável que falhe. Especialmente se se tiver em conta o mecanismo aprendido do comportamento (Landsberg, Hunthausen & Ackerman, 2013).

Para que o plano de tratamento elaborado seja eficaz, deve ser focado em diversos pontos tais como a modificação comportamental, o maneio ambiental, e o reaprender de tarefas que em tempos o animal já foi capaz de elaborar. Foi aliás descrito, em diversos estudos, que este treino é essencial para a manutenção da qualidade de vida do animal a tratar pois desenvolve um papel de estimulante cognitivo (Milgram, Head, Zicker, Ikeda-Douglas, Murphey, Muggenburg et al., 2004). Em associação devem ser adicionados planos farmacológicos e nutricionais de tratamento da síndrome (Landsberg, Hunthausen & Ackerman, 2013).

O enriquecimento ambiental pode ser obtido através de brincadeira interactiva, treino e brinquedos do tipo puzzle com tarefas específicas. Sabe-se que existem várias melhorias cognitivas associadas exclusivamente ao enriquecimento ambiental (van Praag, Christie, Sejnowski & Gage, 1999 ; Milgram, Head, Zicker, Ikeda-Douglas, Murphey, Muggenburg et al., 2005) até porque é através deste, que se consegue estimular o animal resultando isto numa manutenção das funções cognitivas do mesmo e consequente atraso do no declínio cognitivo (Milgram et al., 2004). Contudo, já foi provado que os melhores resultados são obtidos quando se combinam estas técnicas com suplementos de antioxidantes pois sabe-se que quando juntas têm um efeito sinérgico (Milgram et al., 2005).

Outras formas de intervenção de igual importância são o uso único de treino através de reforço positivo, manutenção das rotinas do animal ou, quando estritamente necessário, fazer alterações progressivas e programadas para que se consiga manter uma qualidade de vida razoável ao animal a tratar (McMillan, 2003 ; Landsberg, Hunthausen & Ackerman, 2013).

O passo seguinte a tomar será sempre adaptar o plano de tratamento aos sinais clínicos demonstrados pelo animal. Devem ser ponderadas alternativas a cada ponto referido anteriormente de acordo com as capacidades não só cognitivas mas também físicas demonstradas pelo animal afectado. Para além disso, o tratamento farmacológico a escolher terá sempre por base os sinais mais graves presentes considerando sempre interacções

medicamentosas que possam existir com os planos de tratamento já elaborados para tratar doenças concomitantes (Landsberg, Hunthausen & Ackerman, 2013).

Começando por falar das opções existentes para a terapêutica farmacológica vão ser enumerados diversos princípios activos que podem auxiliar o tratamento da síndrome abordada.

A selegilina é um inibidor da Monoaminoxidase B, uma enzima que catabolisa a desaminação oxidativa de diversas aminas biogénicas, incluindo nas mesmas a dopamina. Quer isto dizer, que ao inibirmos esta enzima o resultado será um aumento da dopamina (Ruehl, Bruyette, DePaoli, Cotman, Head, Milgram et al., 1995). Outro benefício do uso deste fármaco é o aumento de produção da enzima superóxido dismutase levando a uma diminuição da quantidade de radicais livres cerebrais (Carillo, Ivy, Milgram, Head, Wu & Kitani, 1994). Por último, leva também a um aumento da feniletilamina, um percursor da dopamina com funções neuromoduladoras que facilita a trnamissão do impulso neuronal (Milgram et al., 2005).

A propentofilina é um derivado das xantinas que actua como um inibidor selectivo do transporte da adenosina e da fosdiesterase. Já tinha sido usado em cães com o objectivo de melhorar a hemodinâmica dos animais pelos seus efeitos vasodilatadores. Isto pode ser favorável em animais com SDCC pois pode aumentar a tolerância ao exercício (Siwak, Grute, Woehrlé, Muggenburg, Murphey & Milgram, 2000). Para além disto, diminui a letargia, o comportamento depressivo e influência a libertação de neurotransmissores e o impulso neuronal. Já foi referido que pode reverter deficits na aprendizagem e memória em ratos de laboratório (Fuji, Hiramatsu, Kameyama & Nabeshima 1993 ; Haragushi & Kuribara, 1991).

Tendo como base estudos de medicina humana, percebeu-se que o uso de suplementos nutricionais orais de ácidos gordos, minerais, vitaminas, antioxidantes e nutritentes levam a uma melhoria dos sinais clínicos de pessoas com doença de Alzheimer (Donini, Fellice & Canella, 2007). Então os nutracêuticos – qualquer substância considerada comida ou parte de que melhora a saúde de quem o ingere (Boothe, 2004) – surgem como solução para a neuroprotecção no cão geriátrico (Osella et al., 2008).

Alguns ácidos gordos como os ácidos docosahexaenóico e eicosapentaenóico ou mesmo a fosfatidilserina que é um fosfolípido de membrana são considerados componentes de reforço cerebral, essenciais para a optimização funcional do cérebro e tecido neuronal

(Heath et al., 2007). Para além destes benefícios a fosfatidilserina é ainda considerava chave na transmissão de sinais eléctricos nas células nervosas (Osella et al., 2008). Promove neuroprotecção de diversas formas e melhora a recuperação da memória e comportamento exploratório (Vannucchi, Casamenti & Pepeu, 1990).

Outros ácidos gordos implicados na melhoria da cognição em humanos com doença de Alzheimer são os ácidos gordos de cadeia média, onde se incluem os ácidos docosahexaenóico e eicosapentaenóico. Estes, são uma fonte de energia alternativa para um cérebro que usa ineficientemente a glucose (Reger, Henderson, Hale, Cholerton, Baker, Watson et al., 2004). Conseguem-no pois o seu consumo aumenta a concentração de ácidos gordos Ω-3 polinsaturados no cérebro por mobilização dos mesmos para o cérebro (Taha, Henderson & Burnham., 2009). Isto pode melhorar a cognição porque estes ácidos estão envolvidos na neurogénese e transmissão de sinal. Sabe-se que uma dieta com ácidos gordos Ω-3 polinsaturados normaliza os níveis de factor neurotrófico derivado do cérebro e reduz dano oxidativo levando a aumento da capacidade de aprendizagem em ratos com dano cerebral induzido (Beltz, Tlusty, Benton & Sandeman., 2007 ; Wu, Ying & Gomex-Pinilla., 2004).

O extracto de Gingko Biloba é apresentado como adjuvante da fosfatidilsierina já que estimula alguns sistemas em animais idosos, nomeadamente o colinérgico e o serotoninérgico (Lee, Chen & Wang, 2004). Outros benefícios comprovados deste extracto são Aumento de dopamina no cérebro por inibir reversivelmente a MAOA e MAOB (White, Scates & Cooper, 1996) e a protecção neuronal contra a apoptose por deposição de β – amilóide (Yao, Drieu & Papadopoulos, 2001). Pensa-se que estes benefícios sejam justificados pelas suas capacidades antioxidantes na prevenção de alterações oxidativas mitocondriais já descritas em ratos de laboratótio (Sastre, Millán, Assunción, Plá, Juan, Pallardo et al., 1998).

O ácido α – Lipóico é uma coenzima que está envolvido na utilização de hidratos de carbono necessários para a produção de adenosina trifosfato na mitocôndria (Stoll, Hartmann, Cohen & Muller, 1993). Está associada a melhorias na memória de longo prazo em ratos e a sua junção com a acetil- L- carnitina está descrita como sinérgica (Liu, Head, Charib, Yuan, Ingersoll, Hagen et al., 2002).

A piridoxina, também descrita como Vitamina B6, é de extrema importância pois existem várias enzimas dependentes do fosfato piridoxal incluindo vários

neurotransmissores tais como a dopamina, norepinefrina, serotonina, ácido y-aminobutírico e a taurina. Sabe-se quando há um declínio das concentrações desta vitamina existe, consequentemente, uma deplecção nos níveis de serotonina apesar do mesmo não se verificar em relação à dopamina. Apesar disto, o desequilíbrio entre os níveis destes dois pode levar a alterações severas no sistema neuroendócrino (Dakshinamurti, Paulose, Viswanathan, Siow & Sharma, 1990).

Já os suplementos nutricionais, são compostos que combinam vários dos componentes atrás referidos e que surgiram como resposta comercial a uma necessidade de existirem maneiras mais prácticas de administração destes nutracêuticos. Têm diferentes apresentações, desde cápsulas a comprimidos para administração enteral até rações comerciais suplementadas com diversos componentes. Cada um foi testado e os resultados comprovados.

Não há terapia específica para a SDCC e o objectivo do tratamento é atrasar a progressão da doença com manutenção da qualidade de vida do indivíduo. Para que estes objectivos sejam atingidos, há uma variedade de opções que devem ser usadas em conjugação e não como planos de tratamento individuais. As opções foram descritas anteriormente e variam entre maneio ambiental, planos de modificação comportamental, fármacos e suplementos nutricionais. A selecção de uma combinação adequada é a chave para o sucesso de tratamento e há que ter em conta que o tempo de resposta ao tratamento pode variar entre várias semanas a meses (Landsberg, Hunthausen & Ackerman, 2013).

O prognóstico é variável e o caso deve ser visto como um todo. O sucesso do plano de tratamento dependerá do cumprimento do plano por parte do tutor, da existência de doenças concomitantes, do período de diganóstico da doença e dos sinais clínicos exibidos pelo animal. Apesar da SDCC não ser por si normalmente a causa de morte de um animal, a resposta ao tratamento ao longo do tempo conjugado com a saúde geral do animal têm um papel decisivo quando se tomam decisões relativamente ao uso da eutanásia (Landsberg, Hunthausen & Ackerman, 2013).

Materiais e métodos

No presente estudo os objectivos foram responder à seguinte questão de investigação: Avaliar se, na perspectiva do tutor, eram perceptíveis alterações comportamentais nos seus animais de companhia aquando da alimentação com uma ração enriquecida com ácidos gordos de cadeia média.

Para além da questão principal surgiram dois objectivos secundários: Avaliar o papel dos ácidos gordos de cadeia média numa população de cães urbanos com alterações comportamentais e fazer uma análise descritiva de uma população de cães urbanos geriátricos.

Os critérios de selecção de indivíduos basearam-se na idade, que deveria ser igual ou superior a oito anos, e deveriam apresentar um ou dois sinais clínicos compatíveis com disfunção cognitiva. O grupo de estudo composto por dez animais que cumpriam os critérios de inclusão foi escolhido de uma amostra de sessenta animais cujos tutores responderam ao questionário para inclusão no estudo (que se encontra no anexo V) em centros de atendimento médico-veterinário do distrito de Lisboa.

Os critérios de exclusão do estudo eram animais com idade inferior a oito anos de idade, que demonstrassem três ou mais sintomas associados a SDCC ou que tivessem resultados na hematologia ou bioquímica sanguínea compatíveis com doença sistémica. Para além destes, estavam ainda excluídos animais que vivessem com o seu tutor há menos de doze meses ou que estivessem em tratamento para SDCC ou outro problema comportamental. Ficou também definido que aquando do início do estudo, todos os cães que passassem por alterações extremas no seu ambiente físico ou emocional, demonstrassem intolerância ao alimento ou fossem diagnosticados com alguma doença médica seriam excluídos do grupo de estudo.

A avaliação cognitiva dos animais que foram incluídos no grupo de estudo foi feita através do preenchimento de um questionário pelos tutores, denominado de "Assessment Tool" que pode ser encontrado nos anexos do presente trabalho.

A colheita de dados foi feita através da colaboração com cinco centros de atendimento médico-veterinário do distrito de Lisboa que disponibilizaram o questionário de inclusão no estudo durante um período de doze meses, desde Maio de 2014 até Maio de 2015. Assim se formou a amostra de sessenta cães de onde foram seleccionados dez animais

que demonstravam apenas critérios de inclusão. Assim foi criado o grupo de estudo que eram inicialmente dez animais. Dois animais foram excluídos durante o período de estudo por diagnóstico de doença médica concomitante.

O desenho de estudo foi adaptado de maneira a maximizar os resultados estatísticos que poderiam ser obtidos do grupo de estudo. Todo o grupo começou o estudo, no dia zero com uma avaliação física e colheita de sangue para hematologia e bioquímica sanguínea. Começaram também a comer uma dieta placebo – Dieta A – até ao dia trinta do estudo. A sua composição pode ser encontrada no anexo VII.

Do dia trinta ao dia noventa os animais foram alimentados com uma dieta verum – Dieta B – enriquecida com ácidos gordos de cadeia média cuja composição pode ser encontrada no anexo VIII.

Durante todo o período de estudo com intervalos de trinta dias – dia 0, dia 30, dia 60 e dia 90 – foi pedido aos tutores que preenchessem o mesmo questionário para avaliação comportamental dos seu animais de companhia, já enumerado anteriormente como "Assessment Tool". Este questionário está disponível para consulta no anexo IX.

A análise de dados foi feita através do uso de software próprio para o efeito: SPSS® 22 e Microsoft Excel® 2013. A análise estatística inicial incluiu uma análise descritiva da amostra total e do grupo de estudo como dois grupos separados. Posteriormente foram analisadas correlações entre as variáveis de cada grupo recorrendo a testes de $\chi 2$ já que todas as variáveis eram categóricas.

Resultados

A análise descritiva da amostra total populacional (n=60) foi a primeira abordagem e demonstrou que a população continha ligeiramente mais fêmeas que machos, 51.7% para 48.3%, e que 48.3% dos animais tinham entre oito e dez anos de idade, 36.7% tinham entre onze e treze anos de idade restando 15% que tinham idade superior a treze anos aquando do início do estudo. Relativamente a alterações no apetite as queixas mais reportadas foram a alteração na conclusão da refeição seguido de alterações na capacidade física de se alimentar. Quando a função de eliminação foi avaliada 73.3% dos tutores afirmaram não existirem alterações relativamente à mesma sendo que a alteração mais referida foi o aumento da frequência de necessidade de eliminação mesmo que sem eliminarem em locais inapropriados. Relativamente às interacções com humanos também 73.3% dos tutores afirmaram não existirem alterações sendo que 20% descrevem um ligeiro decréscimo de interacção sem notarem que os animais deixem de reconhecer os humanos com quem interagem. Já quando a interacção avaliada foi com outros animais de companhia apenas 66.7% dos tutores admitem não haver alterações. Já 15% admite existir um ligeiro decréscimo de interacção seguido de 13.3% de tutores que afirmam existir um decréscimo marcado de interacção com outros animais de companhia apesar de referirem sempre que o animal reconhece os outros animais com quem interage. Por último, ao ser avaliado o ciclo de sono-vigília 53.3% dos tutores referem não existirem quaisquer alterações sendo que 31.7% referem um aumento do número das horas de sono diurnas.

O passo seguinte foi a análise descritiva do grupo de estudo (n=8). Neste grupo a análise do apetite revelou que apenas 12.5% da população teria alterações no mesmo sendo que estas estariam relacionadas com a velocidade a que o animal se alimenta. Relativamente ao comportamento de eliminação não existiram quaisquer alterações reportadas pelos tutores. Passando às interacções com humanos apenas 37.5% dos tutores referem um decréscimo de interacção mantendo o animal a capacidade de reconhecer os humanos com quem interage. De seguida, foram avaliadas as interacções com outros animais. Nesta categoria, 12.5% dos tutores afirmam que existe um ligeiro decréscimo de interacção, outros 12.5% de tutores referem um decréscimo marcado de interacção com outros animais de companhia apesar de referirem sempre que o animal reconhece os outros animais com quem interage. Na mesma percentagem encontram-se animais que recusam qualquer interacção com outros animais mas mantendo a capacidade de reconhecer os animais com

quem partilham o espaço. Sobre as alterações no ciclo de sono-vigilía apenas 37.5% dos tutores referem alterações sendo estas o aumento do número de horas de sono diurnas.

Posteriormente, foram elaborados testes $\chi 2$ para avaliar correlações entre as variáveis. Foram verificadas correlações positivas entre as variáveis "idade" e "Alteração na frequência de eliminação e acidentes" ($\chi 2$ (6, N= 60) = 18,313, p =0,050), verificando um agravamento da segunda variável com um aumento da idade. Foram também verificadas correlações positivas entre as variáveis "Alteração nas interacções com os seres humanos" e "idade" ($\chi 2$ (6, N= 60) = 12,959 p =0,011) e nas variáveis "Alteração nas interacções com outros animais" e "idade" ($\chi 2$ (6, N= 60) = 15,598 p =0,016). Ambas demonstraram, tal como na anterior, um agravamento das condições da variável com um aumento da variável idade. Em último lugar foi também verificada uma correlação entre as variáveis "Alterações no ciclo de sono-vigília" e "idade" ($\chi 2$ (6, N= 60) = 17,714 p =0,023) demonstrando novamente um agravamento das condições da primeira variável aquando do aumento da idade.

Passou-se então à análise de frequências do estudo de grupo (n=8) examinando o questionário "Assessment Tool" nos diferentes momentos de resposta. Foram elaborados vários testes $\chi 2$ mas não foram encontradas quaisquer correlações entre as variáveis. Desta análise foi criada a taxa global de resposta ao tratamento. Esta taxa compara os resultados globais de um indivíduo em dois momentos de avaliação – dia 0 e 90. Obtiveram-se resultados de manutenção para a maioria do grupo: 86.61%. Já 8.04% do grupo respondeu positivamente à taxa, demonstrando uma melhoria geral de sintomas sendo que 5.36% demonstrou aumento do número de sintomas tendo sido considerado como agravamento de doença.

Discussão

Os efeitos dos ácidos gordos na cognição já foram previamente estudados e sabe-se que a mesma terá tendência a diminuir com o avançar da idade. Daí se ressalva a importância para o clínico de saber como atrasar a progressão deste declínio cognitivo de uma maneira eficiente (Heath et al., 2007 ; Reger et al., 2004 ; Taha et al., 2009).

Em relação ao estudo apresentado anteriormente, a primeira parte da análise foi uma análise descritiva da população de todos os cães que foram avaliados no estudo. A população não apresenta diferenças estatísticas quanto a grupos de idade ou género. Apenas 23,3% da população (n = 14) não tinha alterações comportamentais. Embora existam 76,7% de animais que demonstraram alterações comportamentais, estas não têm obrigatoriamente de estar relacionadas com SDCC. Portanto, a prevalência da SDCC na nossa população não vai ser estimada. Apesar disso, nenhum desses animais tinha sido referenciado para um Médico Veterinário especialista em Medicina Comportamental nem estavam as suas alterações comportamentais a ser abordadas. Um estudo anterior demonstra que um diagnóstico de SDCC apenas é concretizado em cerca de 13,3% de uma população (Salvin et al., 2010).

Mostrou-se que 50% dos tutores da nossa população afirmam que os seus animais têm alterações no apetite sendo a mais reportada a alteração da conclusão da ingestão de uma refeição (18,3%). A segunda alteração mais reportada é a deterioração na capacidade do animal para fisicamente manipular alimentos (11,7%). Ao avaliar o ciclo de sono-vigília, foram relatadas alterações em quase metade da população (46,7%). Estes resultados indicam que as alterações no apetite são os mais prevalentes seguidos pelas mudanças no ciclo de sono-vigília. Isso não significa necessariamente que estes sinais são os mais frequentes, nem o primeiro a aparecer em um cão que sofre de CDS mas pode indicar quais são os sinais mais facilmente ou frequentemente detectados pelo tutor dos animais, ou que são os sinais que mais facilmente têm impacto na vida dos tutores sendo, por isso mesmo, mais facilmente notados.

Ao avaliar o grupo de estudo (n = 8) os resultados são coerentes com a população, uma vez que a maior mudança relatada pelos tutores é a alteração no apetite dos animais (87,5%). A correlação positiva entre as variáveis " Alteração na frequência de eliminação e acidentes" sobre os comportamentos de eliminação, "Alteração nas interacções com os seres humanos" e também "Alteração nas interacções com outros animais" sobre interacções

sociais e a variável "idade" facilmente demonstram que há um agravamento dos sintomas com o aumento da idade. Estas correlações são significativas no sentido de validar os dados de estudo, uma vez que de acordo com a literatura está o um aumento da prevalência da disfunção cognitiva com o aumento da idade (Azkona et al., 2009).

Considerando uma população de sessenta cães, o facto de apenas oito animais terem sido aceites no estudo foi uma consequência dos critérios de inclusão. O objectivo foi estudar animais com comprometimento cognitivo leve e excluir os animais com comprometimento cognitivo grave. Embora esta escolha tenha sido baseada no número de sinais clínicos apresentados no momento do primeiro questionário não foi tido em conta quais os sinais apresentados e a categoria de sinais de acordo com a escala DISHA, apresentada pode também indicar a gravidade da doença (Osella et al., 2007).

Outro facto que deve ser enumerado é que, embora os animais tenham sido submetidos a testes de hematologia e bioquímica, juntamente com um exame físico, não podem ser excluídas todas as causas de doença concomitante já que não foram também elaborados todos os exames complementares de diagnóstico que permitam fazer tal afirmação. As probabilidades são baixas uma vez que apenas os animais com os resultados satisfatórios nos testes foram incluídos. No entanto, sendo a SDCC uma doença com de diagnóstico de exclusão, deve salientar-se que nenhum dos animais foi submetido a qualquer teste de imagiologia cerebral levando a que outras patologias cerebrais não possam ser excluídas.

Ao analisar a evolução dos sinais clínicos dos animais durante todo o período de estudo, a taxa global de resposta à terapêutica não foi o que seria expectável considerando que a maioria do grupo de estudo (n = 8) não apresentou melhorias nem piorias na sua cognição na perspectiva dos seus tutores - 86,61%. A quantidade de animais que apresentaram uma resposta global positiva à terapia foi de 8,04%. Ao comparar este valor com outros estudos com suplementações com ácidos gordos de cadeia média foram obtidos resultados mais baixos (Dodd et al, 2002;.. Wu et al, 2004).

Algumas razões que podem explicar o resultado anterior serão enumeradas de seguida. O facto de o estudo ter sido realizado em um cenário de "vida real", em vez de em um laboratório pode justificar grandemente os resultados. As principais diferenças estão presentes nos pontos em que os animais não estão tão estreitamente monitorados e o facto de que a avaliação comportamental foi da exclusiva responsabilidade do tutor, em vez de

um clínico, comportamentalista ou qualquer pessoal veterinário técnico. Istto pode ter levado a menor grau de eficácia no alerta para alterações comportamentais subtis. Combinando isso com o fato de que os animais escolhidos apresentavam sinais clínicos leves e do período de estudo foi curto, é compreensível que as mudanças de comportamento e, consequentemente, a resposta à terapia podem ter sido subestimadas.

Também deve ser mencionado que pode haver um viés na taxa global de resposta à terapia. Deve ser tido em conta que a manutenção dos sinais clínicos pode ser também uma resposta positiva à terapia. Esta hipótese pode ser considerada porque, tal como referido na literatura, o objectivo do tratamento da síndrome é muitas vezes para atrasar a progressão dos sinais clínicos e não eliminar sinais já instalados (Landsberg & Denenberg, 2009). Ao considerar a avaliação da desorientação pode perceber-se que não houve mudança nessa categoria porque nenhum dos animais apresentou quaisquer sinais de desorientação no início do estudo. O facto desse sinal clínico não ter aparecido durante o período de estudo pode ter sido um sinal benéfico do enriquecimento com ácidos gordos de cadeia média. Estes podem ter ajudado os cães manter a sua memória de longo prazo sobre seus ambientes familiares durante o estudo. Isso também pode ser considerado uma resposta positiva pois a suplementação pode ter estabilizado estes animais. Outra possibilidade é a de que simplesmente a região do cérebro afectada nestes animais não é a responsável por esse comportamento.

Isto também deve ser tido em conta para os outros comportamentos avaliados. Por exemplo, para avaliação das interacções sociais, a dieta suplementada estabilizou sete cães e melhorou um cão considerando interacções com seres humanos. Para as interacções com outros animais a dieta reduziu o número de cães com diminuição de interacções em cinquenta porcento. Também estabilizou os níveis de interacções em sete cães. A dieta suplementada também estabilizou sintomas noutras categorias, incluindo ciclos do ciclo sono-vigília, comportamento de eliminação e níveis de actividade.

Uma vez que o estudo teve como avaliadores os tutores, seres humanos que são emocionalmente ligados aos seus animais, o efeito placebo também devem ser considerado. Especialmente se considerado que os croquetes da ração era diferente entre as dietas. Desta forma, o tutor poderia facilmente entender que tinha havido uma mudança na dieta. Apesar de não saber qual seria a dieta verum e a dieta placebo, a mudança pode ter influenciado as respostas dos tutores aos questionários. Uma maneira possível de evitar estas possíveis

alterações seria, para além de ter croquetes iguais entre rações, ter também ter um médico ou comportamentalista a preencher a avaliação comportamental do animal, a par do tutor para o investigador para ser capaz de comparar os dois e estimar um percentual efeito placebo mais com precisão.

Para entender completamente como um alimento seco suplementado vai mudar os comportamentos e possivelmente atrasar a progressão da SDCC em cães são necessários mais estudos. Esses estudos deverão ter populações maiores e períodos experimentais mais longos. A separação da população em grupos placebo e verum como dois grupos de estudos individuais pode surgir como solução. Evita, em primeiro lugar, a mudança de dieta durante o estudo e possibilita a avaliação de flutuações comportamentais em populações de cães geriátricos com declínio cognitivo. Outra alteração sugerida, seria agrupar os cães de acordo com a categoria de sinais clínicos que mostram comprometimento em acordo com a escala DISHA para que se verifique como cada categoria responde à suplementação.

Conclusão

O estudo anterior descreve o papel de uma dieta suplementada com ácidos gordos de cadeia média numa população de cães com ligeiro declínio cognitivo quando abordado pelos tutores.

Realizando uma série de questionários, as mudanças comportamentais do grupo de estudo foram descritas ao longo de um período de alimentação placebo e, posteriormente, de alimentação verum. Ao fazer isto, pode não só avaliar-se como cada tutor entende a mudança de comportamento do seu animal de estimação, mas também notar que os sintomas são mais sensíveis à suplementação.

Durante a realização deste estudo, verificou-se que nenhum destes cães estavam a ser medicados para as suas mudanças de comportamento nem estavam a ser seguidos por um comportamentalista. Foi, desta forma, demonstrado que o diagnóstico da SDCC é facilmente subestimado, mesmo quando um animal visita regularmente o seu Médico Veterinário, tal como descrito na literatura. Isto leva a uma revisão específica da formação de veterinários de maneira a incluírem habilidades que permitam ao clínico realizar uma anamnese precisa.

Nenhuma relação estatística foi encontrada na população entre a presença de sintomas com género. Houve no entanto uma confirmação de que, quando não tratada, os sinais clínicos piorar com a idade como se poderia suspeitar. Infelizmente, embora os dados tenham sido validados, não foram encontradas correlações estatísticas entre as variáveis ou ao longo do período do estudo. Apesar disto, ao avaliar os questionários individualmente dieta suplementada ou estabilizou ou melhorou a maioria dos animais durante este estudo de curto prazo. Isto é um resultado encorajador. Para estudos futuros e, a fim de avaliar mais precisamente o cérebro da população de estudo, exames de imagiologia cerebral devem ser incluídos.

As declarações anteriores indicam que mais estudos são necessários, de maneira a analisar estatisticamente uma população de cães geriátricos com mais precisão. Desta forma conseguir-se-á também perceber como os tutores percebem as mudanças de comportamento em seus animais de companhia.

Abbreviations and symbols

CDS – Cognitive Dysfunction Syndrome

MRI – Magnetic Resonance Imaging

BDNF – Brain Derived Neurotrophic Factor

NGF – Nerve Growth Factor

MAOB – Monoamine oxidase B

DNA – Deoxyribonucleic acid

ARCAD – Age-Related Cognitive and Affective Disorders scale

CCDR – Canine Cognitive Dysfunction Rating scale

DISHA – Disorientation, Social Interactions, Sleep-wake cycle, Housesoiling and Activity scale

EE – Environmental Enrichment

PEA - 2-phenylethyalamine

SSRI – Selective Serotonin Reuptake Inhibitors

TCA – Tricyclic Antidepressant

DHA – Docosahexaenoic Acid

EPA – Eicosapentaenoic Acid

PS – Phosphatidylserine

MCT – Medium Chain Triglycerides

PUFA – Ω-3 polyunsaturated fatty acid

EGb – Gingko Biloba Extract

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1. Canine Cognitive Dysfunction

A cognitive dysfunction is termed by any changes in certain behaviours as social interaction, elimination, sleeping cycle and navigational (Overall, 2013). Besides these changes the canine cognitive dysfunction syndrome can be described as a neurobehavioural syndrome in which the animals experience deficits in learning, memory and spatial awareness (Landsberg, Hunthausen & Ackerman, 2013). The described changes are ongoing and all other medical causes for these must be ruled out (Overall, 2013). This is shown as a gradual cognitive decline with increasing brain pathology (Landsberg & Denenberg, 2009).

The study of cognitive dysfunction in animals started as a result of animals being used as models to human Alzheimer's disease. The first researches on canine cognitive dysfunction are all laboratory studies. They took place in beginning of the nineties' decade (Price, Martin, Sisodia, Wagster, Koo, Walker et al., 1991 ; Cummings, Su, Cotman, White & Russel, 1993 ; Cummings & Cotman,1995). Around the middle of the decade the first publications that were focused in studying the canine cognitive dysfunction centered on the animal itself and the veterinary medicine context can be found (Head et al., 1996).

From there and until nowadays several studies have been conducted to assess the cognitive dysfunction syndrome – CDS - and to improve animals' welfare. Once the improvements in veterinary medicine expanded companion animals life expectancy it led to a specific need to improve the treatment and management options for age-related diseases (Salvin, McGreevy, Sachdev & Valenzuela, 2010).

Although it might be said that CDS is a common syndrome in older animals not the majority of those is actually treated early on onset since the tutors tend to relate the behavioural changes with the normal process of ageing (Neilson, Hart, Cliff & Ruehl, 2001).

2. Biological Basis for Canine Cognitive Dysfunction

The forebrain holds most of the memory and behaviour pathways. In this, the hippocampus and the limbic system are included. Physiological ageing leads to several changes in the brain. Those changes include a reduction in brain mass with cerebral and basal ganglia atrophy with an increase in ventricular size, meningeal calcification, a lessening in neurons and increase in degenerative neurons and axons with demyelination and an elevation in lipofuscin apoptic bodies (Borràs, Ferrer & Pumarola, 1999).

The most common findings both in vivo imaging and in necropsies include cortical atrophy and ventricular widening of the brain (Su, Head, Brooks, Wang, Muggenburg, Adam et al., 1998). There is a confirmed correlation between cortical atrophy and cognitive dysfunction (Rofina, Ederen, Toussaint, Secrève, van der Spek, van der Meer, et al., 2006). Another suggestion to justify the clinical signs of CDS is the vascular insufficiency, fibrosis of the vascular walls, endothelial proliferation, mineralization, micro hemorrhage and amyloid angiopathy in cerebral veins that consequently decrease perfusion in the aged brain of dogs and diminishes the use of glucose as the primary source of energy (Uchida, Okuda, Yamaguchi, Tateyama, Nakayama & Goto, 1993 ; Borràs et al., 1999 ; Landsberg & Araujo, 2005).

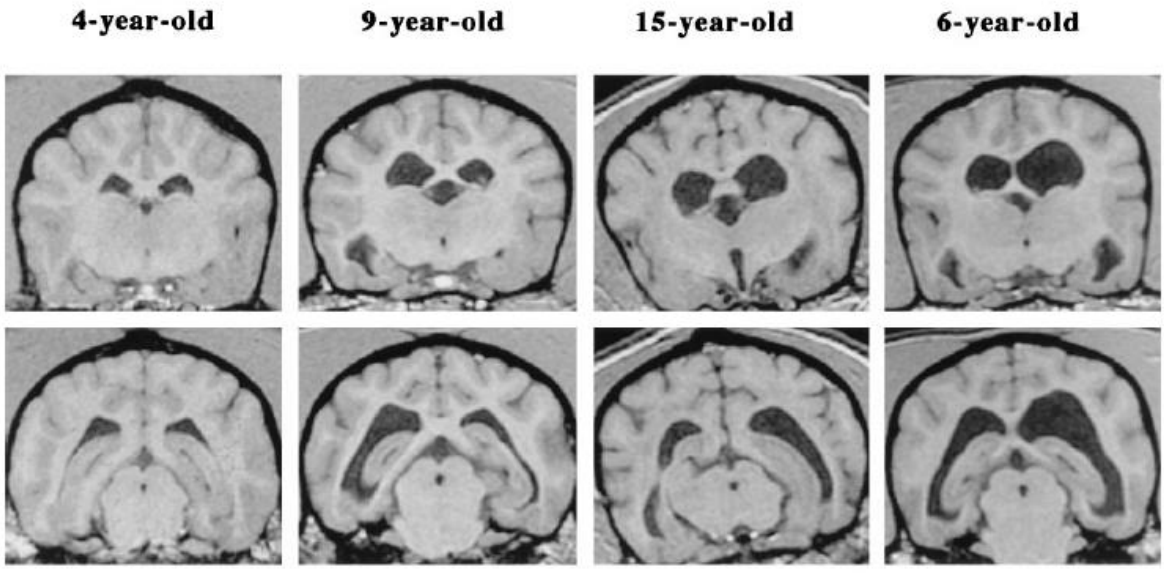


Figure 1 – Magnetic Resonance – MRI - images taken from dogs showing the thalamus and hippocampus. There is an enlargement of the ventricles and cortical atrophy with the increase of age. (Su et al., 1998).

In order for animals to go through pathological aging three main things have to happen in the brain: 1) Production of free radicals (oxidative changes); 2) Formation of lesions in the brain such as β-amyloid plaques deposition; and 3) Changes in the oxygen availability to be used by the brain (Overall, 2013).

These changes secondary to aging lead to several physiological alterations. It has been reported a series of neurotransmitter abnormalities in a variety of studies in several laboratory animals and in humans. In one study the plasma levels of several neurotransmitters were evaluated only to realize that in all aged dogs there is a severe decrease of dopamine and norepinephrine levels (Badino, Odore, Bergamasco, Barbero, Osella, D'Angelo et al., 2013). Other studies show that glucose and oxygen brain metabolism changes with age in dogs leading to an impaired used of glucose as an energy source (London, Ohata, Takei, French & Rapoport, 1983). It also results in the formation of reactive oxygen species since they are products of cellular metabolism leading to oxidative damage (Beckman & Ames, 1998).

Nevertheless, their direct involvement in cognitive dysfunction was only stated later when it was realized that a decline in dopamine, and all other catecholamines, a decrease in acetylcholine and a muscarinic receptors had an important pathogenic role (Araujo, Studzinski, & Milgram, 2005). The impairment of the cholinergic system has also been associated with both cognitive and motor decline and changes in the Rapid-Eye-Movement sleep (Araujo, Chan, Winka, Seymour & Milgram, 2004).

There are also reports that demonstrate that some neurotrophic factors such as Brain Derived Neurotrophic Factor – BDNF – and Nerve Growth Factor – NGF - are also decreased in aged dogs. Although this findings, this decline was not proven to be directly related to the existence of CDS in the dogs (Head, McClearly, Hahn, Milgram & Cotman, 2000).

Aging is also associated with a depletion of catecholamine neurotransmitters and an increase in monoamine oxidase B – MAOB (Milgram, Ivy, Head, Murphy, Wu, Ruehl et al., 1993). There is also a decrease in dopamine and dopaminergic receptors but that has not been correlated with pathological aging nor cognitive dysfunction (Rehman & Masson, 2001 ; Landsberg & Denenberg, 2009).

In the ageing process the production of free radicals increases due to an increase of mitochondrial production and a decrease in energy concurrent with an increase of MAOB

activity. The strategy to eliminate free radicals consists of enzymes - e.g. superoxidase dismutase, glutathione peroxidase - and free radical scavengers - e.g. Vitamins A, C and E. However, ageing is also responsible for a decrease in these processes. Consequently what occurs is an increase in Reactive Oxygen Species and therefore cell damage that ranges from dysfunction to mutation to cell death by reacting with deoxyribonucleic acid - DNA lipids and proteins (Landsberg & Denenberg, 2009).

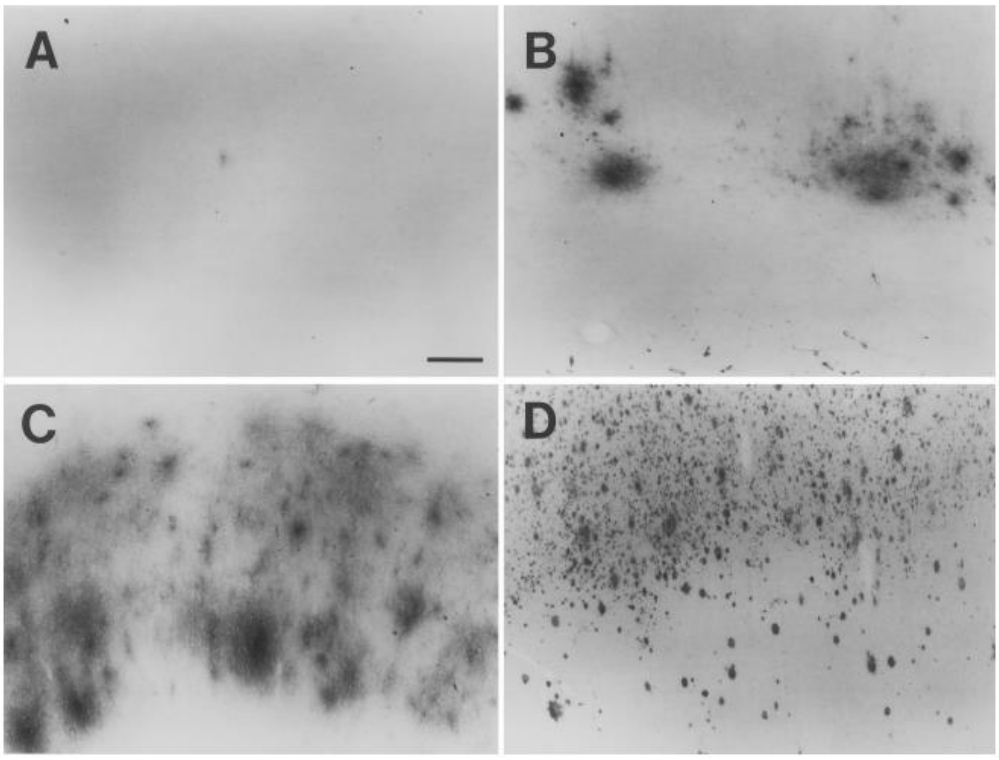


Figure 2: Photomicrographs of β-amyloid immunoreactivity in dogs at different life stages. A – Young canine; B – Old canine with mild cognitive impairment; C – Old canine with CDS; D – Severe case of Alzheimer's Disease in a human patient (Cummings, Head, Afagh, Milgram & Cotman, 1996).

In the canine ageing brain it the abnormal deposition of β-amyloid in the cerebral cortex is correlated with Canine Cognitive Dysfunction. As a matter of fact it was observed that this deposition is strongly associated with deficits in several tasks including discrimination, reversal and spatial learning (Cummings et al., 1996). In the dog's brain there are no evidences of formation of neurofibrillary tangles – contrarily to the human brain – instead they form β-amyloid plaques (Papaioannou, Tooten, van Ederen, Bohl, Rofina, Tsangaris et al. 2001 ; Head, Rofina & Zicker, 2008). The exact pathological role of β-amyloid deposition in the CDS brain is yet to be explained. What is already known is that

β-amyloid is neurotoxic and leads to an impairment in neuronal function which results in degenerate synapses, cell loss and decreased neurotransmitters as stated before (Cummings et al., 1996 ; Head, Callahan, Muggenburg, Cotman & Milgram, 1998 ; Colle, Hauw, Crespeau, Uchihara, Akiyama, Checler et al., 2000 ; Gunn-Moore, Moffat, Christie & Head, 2007).

Besides the amount of deposition, the extent and location of these plaque depositions is critical for the severity of deficits and clinical signs of this syndrome. The main location of the deposits varies according to different authors. Some say they tend to appear in the cerebral cortex, hippocampus and meningeal vessels (Head et al., 2000). Others claim that besides the prefrontal cortex the temporal lobes are also affected but exclude the brain stem or the cerebellum (Hou, White, Bobik, Marks & Russel, 1997).

Age is another variable, as it is known that the deposition sites vary according with age. The first area of the brain to accumulate β-amyloid is the frontal lobe (Colle et al., 2000 ; Head et al., 2000) which explains the early impairment in memory tasks shown by CDS dogs. It seems that the β-amyloid deposition starts in the prefrontal cortex moving into the entorhinal and parietal cortices being detected lastly in the occipital cortex (Head et al., 2000).

Nonlipidic arteriosclerosis was also identified as a result of blood vessel fibrosis, endothelial proliferation and mineralization as well as β-amyloid deposition (Colle et al., 2000).

In terms of breed specifications and the genetic role in CDS, there is one study in which it is stated that the extend of β-amyloid deposition and its distribution can be influenced by genetics. This is listed as it was established that some breeds develop this deposition at an earlier age. It was also stated that within litters there is a similar extent of β-amyloid deposition (Bobik, Thompson & Russel, 1994).

To sum up, it seems that the appearance of the signs of CDS are concordantly with the progression of the neuropathology and reflect the areas of the brain that are being affected (Head et al., 2000).

3. Prevalence and Risk Factors

Various studies determine the prevalence of cognitive impairment in dogs through interviewing the dogs' tutors. The results give us an estimated prevalence that ranges between 22.5% and 73.5% (Neilson et al., 2001 ; Osella, Re, Odore, Girardi, Badino, Barbero et al., 2007 ; Azkona, García-Bellenguer, Chacón, Rosado, León & Palacio, 2009).

It must be taken into account that in some studies the prevalence of CDS may be overestimated since not all medical causes were ruled out. On the other hand, in some studies the real prevalence may be underestimated as some medical causes are found even though CDS might be present concurrently (Landsberg & Denenberg, 2009). There is one study that correlates the percentage of dogs with canine cognitive dysfunction and the ones that had a previous diagnose of CDS. In this epidemiological approach an estimated prevalence of 14.2% was described in their sample even though only 1.9% of the dogs had been previously diagnosed (Salvin et al., 2010). Besides this, aggression and anxiety disorders are the most common cause for referral to a behavioural practice in North America (Landsberg, 1991).

All these results confirm that neurocognitive changes that conduct to behavioural manifestations are commonly ignored or perceived by the dogs' tutors as a part of the normal ageing process (Landsberg & Araujo, 2005 ; Osella et al., 2007). They must be aware that any behavioural change in a senior pet, even if it is one that is very subtle, may not be a part of the normal ageing and it should be reported to their veterinarian as it may be a sign not only of CDS but also of any medical condition (Landsberg & Denenberg, 2009).

If considering a diagnosis solely based in behavioural changes, the onset of this syndrome in dogs is eleven years or older. In contrast, if laboratory studies are added, those reports will show impairment in some memory tasks as early as six to eight years old (Studzinski, Christie, Araujo, Burnham, Head, Cotman et al., 2006).

Until the present day there is still no risk factors associated with the development of CDS in the dog (Fast, Schutt, Toft, Moller & Berendt, 2013). There are however some studies that say this syndrome has no breed or gender preference (Fast et al., 2013) and others who suggest that castrated dogs, from both genders, are significantly more affected by CDS than fertile dogs (Azkona et al., 2009 ; Hart, 2001).

4. Clinical Signs and Diagnostic Approach

In CDS clinical signs are very variable and mostly non-specific to this disease. The most common are: a)Nocturnal vocalization and changes in the sleep cycle, b)Disorientation or other changes in spatial awareness, c)Alterations in the social behavior (usually first as an extreme neediness changing into a very disengaged dog), d)Loss in previous learned behaviours commonly showed by housesoiling and also e)Anxiety in situations in which the dog was previously comfortable (Overall, 2013).

Several tests for the assessment of certain behaviour changes with ageing have been done and validated. These show that visual discrimination is not usually affected by age but memory tasks and reversal learning are sensitive to age (Tapp, Siwak, Estrada, Head, Muggenburg, Cotman et al., 2003). There is one study that showed that 37.7% of the animals previously diagnosed with CDS exhibited a decrease in social interaction, and in the same amount housesoiling, whilst sleeping cycle changes were only displayed by 20.2% of the population leaving disorientation to a 16.4% (Azkona et al., 2009).

Being CDS a neurobehavioural syndrome we have to rule out other medical causes to the same clinical signs. Especially if we take into account the fact that in senior pets behavioural manifestations might be the first or even the only signs of many medical conditions (Landsberg & Denenberg, 2009). For instance pain in an arthritic dog may lead to fewer interactions either with other pets or even humans, it can make them want to move less and in consequence housesoiling happens or their ability to get food decreases and they choose to eat less. Other normal ageing changes may lead to similar clinical signs as CDS such as the impairment in their visual or olfactory systems and this may be manifested by loss of interest in food, lower social interactions or even higher levels of reactivity (Overall, 2013).

The clinicians' differential diagnoses list should also include other primary behavioural problems of the dog. It is important to mention this, because there are several behavioural changes with no organic cause but that are not CDS related as well. Those can also worsen with the process of ageing or as a consequence of an environmental change.

It is also very important to truly explore the animals' medical history because many old pets tend to take medication for other conditions and this, with the medication secondary effects may lead to some behavioural changes - becoming ataxic, changing their

appetite or the number of hours they sleep. If so this should not be mistaken with CDS (Overall, 2013). Besides this, even when one or several medical conditions are diagnosed and treated there may be a learnt mechanism of the undesired behavior. For instance, if the dog addresses a specific behaviour such as pain related aggression, it might lead the animal to use it in future occasions even when the threat is nor present simply because at first that was the behaviour that solved its problem. Therefore, in order to resolve cases like the example given before, it is needed a combination of therapeutics using not only a medical treatment but also a behavioural modification technique (Landsberg & Denenberg, 2009).

With the purpose of ensuring the earliest detection of CDS and other medical conditions common in senior companion animals such as arthritis, periodontitis or neoplasia, these animals should be screened twice a year. The suggestion of an assessment every six months is based on studies that show that CDS is only likely to progress in terms of frequency or intensity of signs in blocks of six to twelve months (Azkona et al., 2009).

First of all a thorough medical history should be taken. In addition, this screening should include physical examination, several laboratory tests and a comprehensive questionnaire. This last tool should be left in the waiting room so that the tutor can complete it in each visit and kept in the animal's medical record for the clinician to be able to perform comparisons and assess any alteration present in the animals' behaviour (Landsberg, Hunthausen & Ackerman, 2013). It should also be focused on pain assessment sensory loss and neurological evaluation in order to ease the differentiation between medical and behavioural causes (Landsberg & Denenberg, 2009).

Being memory impairment one of the first signs of CDS, this particular sign is very difficult to be detected by tutors alone – with no laboratory testing. Only with a thorough questionnaire will the clinician be able to consider a diagnose of CDS in the early stages of disease development since the diagnosis requires a total rely on tutors' history. (Landsberg, Hunthausen & Ackerman, 2013).

The approach discussed above leads to an early diagnosis and intervention which provides the best opportunity to delay the pathology with lower complication risk and decrease the progress of several diseases. All this primes to the animals' quality of life improvement and possibly the longevity as well (Landsberg & Denenberg, 2009).

Since the clinical signs of CDS are non-specific and very variable, several scales from different authors were created in order to assess the syndrome. From those three will be enumerated.

4.1 The Age-Related Cognitive and Affective Disorders scale – ARCAD

The ARCAD designates a scoring system to evaluate Age-Related Cognitive and Affective Disorders. This scoring addresses not only cognitive parameters, such as learned tasks, but also emotional ones such as appetite or sleep.

It was designed not only to assess problems common in the elderly dog but also to discriminate emotional and cognitive disorders as described above. That would help the clinician in the pharmacological choice for the patient. (Landsberg, Hunthausen & Ackerman, 2013)

There is actually a correlation between high ARCAD scores and the presence of β-amyloid deposits in the brain particularly in the temporal cortex and hypothalamus. This was specially seen in dogs with high emotional scores (Colle et al., 2000).

4.2 The Canine Cognitive Dysfunction Rating scale - CCDR

The first step on the development of this scale was the identification of twenty-seven different behaviours that had previously been identified in dogs with dementia. It was created from the need to have a scale with clear scoring and concise assessment of behavioural changes.

In order to do so the authors isolated thirteen behaviours that when altered add points to the animal's score leading to a positive diagnose of CDS if the score is over fifty. If the final score is lower than fifty the behavioural changes are considered normal ageing or associated with other pathology, either medical our primarily behavioural. This rating scale focuses on changes in orientation, memory, activity, olfactory function and locomotion.

The definitive diagnose proposed included a score over fifty and a veterinary assessment since this allows the professional to distinguish the neurobehavioural changes related to CDS from the ones that occur in normal ageing.

The CCDR scale has a high diagnostic accuracy - 99.3% - with favorable psychometric properties and a high reliability since the retest after two months also shows a high diagnostic accuracy (Salvin et al., 2010).

4.3 The Disorientation, Social Interactions, Sleep-wake cycle, Housesoiling and Activity scale - DISHA

The DISHA acronym is a scale that designates several signs that are present in the CDS. This is not a numerical scale like the ones presented before but indicates a diagnosis in the presence of signs of each category. When using this scale, the clinician does not get a diagnosis based on a number but instead, by grouping the most common symptoms, the clinician might better differentiate between physiological and pathological ageing.

It was designed with the aim of leading the clinician to a diagnosis of CDS rather than other behavioural conditions of the senior companion animal.

There are several questionnaires that will help a diagnostic approach on the DISHA basis by several authors (Overall, 2013 ; Landsberg, Hunthausen & Ackerman, 2013).

5. Treatment Plans

When approaching behaviour problems in general, the work towards the diagnosis of those should be based on history and physical examination followed by any tests thought necessary to rule out any medical causes for that problem. From this the clinician should focus on getting a detailed behavioural history. Then, when the diagnosis is made appropriate treatment must be followed. (Landsberg, Hunthausen & Ackerman, 2013)

Once focusing on treating this syndrome there are several steps in which the clinician can focus. The first one is to treat any medical pathology concurrent with CDS or that could be leading to the behavioural signs. This must definitely be addressed otherwise the behavioural modification is likely to fail (Landsberg & Denenberg, 2009). Although, even if the medical problems can be successfully addressed, if the dog has already learned that a certain behaviour gives him a good outcome like aggression when trying to avoid contact, it will be even harder to stop that behaviour. (Landsberg, Hunthausen & Ackerman, 2013)

In order to do so, the rest of the treatment should be focused on a combination of behaviour modification, environmental management and retraining previously learnt tasks that the dog cannot perform nowadays. It has been proven that in order to maintain the dogs' quality of life mental stimulation is an essential part of the treatment (Milgram, Head, Zicker, Ikeda-Douglas, Murphey, Muggenburg et al., 2004). This must be done in combination with pharmacological and nutritional therapy (Landsberg & Denenberg, 2009 ; Landsberg, Hunthausen & Ackerman, 2013).

Environmental enrichment - EE - could be obtained through interactive playing, exercising (always considering the animals' mental and physical abilities), training and presenting novelty and puzzle type toys with specific tasks. Studies in mice reveal that running increases the dentate gyrus of the hippocampus leading to hippocampal neurogenesis and as a result memory improvement (van Praag, Christie, Sejnowski & Gage, 1999). One task that is proven to improve a lot over time from EE when comparing to a group of dogs with no enrichment, is discrimination learning task (Milgram, Head, Zicker, Ikeda-Douglas, Murphey, Muggenburg et al., 2005). These activities will be a way to stimulate the dog and help maintaining cognitive function and as well to postpone cognitive decline (Milgram et al., 2004). Combining EE with an antioxidant supplemented diet

enhances the results in the animals' cognition since when used concurrently they are more effective than any of them when used alone (Milgram et al., 2005).

A very important rule that benefits a dog with CDS is the sole use of positive reinforcement when desirable behaviours occur and ignore undesirable behaviours. The positive punishment should never be used by the tutors in any occasion. If it is used, especially in cases of aggression, it is likely to increase fear, anxiety and defensive aggression (Landsberg, Hunthausen & Ackerman, 2013). A predictable but stimulating routine should be created for the reason that older animals are less able to respond to stress and maintain homeostasis. Because of that, sudden changes are more likely to cause stress and anxiety. Besides that, the routines help the animal to improve cognition and temporal orientation summing up to a general improvement in the dogs' quality of life (McMillan, 2003). Any change that needs to be done must be done gradually and monitored. If the animal has not only a marked cognitive decline but also a severe decrease in its sensory acuity and processing, the different rooms in the animal's environment can be individually marked by an odor, a sound cue or even a tactile one to help maintain some degree of environment awareness and welfare for the dog (Landsberg & Denenberg, 2009 ; Landsberg, Hunthausen & Ackerman, 2013).

The next step is obviously adapting the treatment to the animals' signs and problems. The tutors should find alternatives considering the animals' capabilities either physical or mental. In some cases where the mental capabilities are very detrimental, or to prevent it, the clinician should consider the most adequate drugs taking into account which is the problem that causes most discomfort to the animal such as anxiety or restlessness. The drug selection also depends on how marked the signs of CDS are. It can vary from cerebral vasodilators to other drugs that will interact on metabolism, catabolism or recaptation of specific neurotransmitters. Other item that must be taken into account is the concurrent medical problems that may exist and the medication or natural supplements that are being used to treat them (Landsberg, Hunthausen & Ackerman, 2013).

Selegiline or l-Deprenyl is an inhibitor of the monoamine oxidase-B – MAOB - enzyme in the dog resulting in an increase of dopamine levels (Ruehl, Bruyette, DePaoli, Cotman, Head, Milgram et al., 1995). It also increases the production of superoxide dismutase in certain brain areas of rats (Carillo, Ivy, Milgram, Head, Wu & Kitani, 1994) decreasing the amount of free radicals in the brain. Another substance that is increased is 2-

phenylethyalamine – PEA – which by its neuromodulator function enhances dopamine and other catecholamine's functions and this enhancement may improve neuronal impulse transmission and ultimately cognition (Milgram et al., 1995). Another way this drug can improve cognition is by its metabolites: L-amphetamine and L-methamphetamine can improve cognition. However this drug should not be used concurrently with other MAOB inhibitors nor with drugs that can increase serotonin transmission such as Selective Serotonin Reuptake Inhibitors – SSRI's or tricyclic antidepressants – TCA's (Landsberg, Hunthausen & Ackerman, 2013). The time that takes before an animal responds to therapy is very variable ranging from one month (5%) to three months (15%) (Ruehl et al., 1995).

Propentofylline has previously been used in adult dogs with the aim of improving cerebral and peripheral compartments hemodynamics. This drug is a xanthine derivate that acts as a selective inhibitor of adenosine and phosphodiesterase transport. It is broadly used in human medicine both in patients with Alzheimer's disease and vascular dementia (Kapl & Rudolphi, 1998). When targeting aged dogs it is important to state that its effect in hemodynamics can be favorable to the animals since it can raise exercise tolerance. Besides that, it decreases dullness, lethargy and depressed demeanor since it acts as a vasodilator and may influence neurotransmitter release and neuronal firing (Siwak, Grute, Woehrlé, Muggenburg, Murphey & Milgram, 2000). Studies in rats and mice have shown that this drug can reverse deficits in learning and memory caused by basal forebrain damage and enhance performance of an avoidance task in mice (Fuji, Hiramatsu, Kameyama & Nabeshima 1993 ; Haragushi & Kuribara, 1991).

There are some other drugs that may also be used if the clinician thinks it is necessary to do so. Adrafinil is a stimulant used in human medicine to increase attention, vigilance, memory and mood problems when associated with age (Israel, Fondarai, Lubin, Salin & Hugonot, 1989). There is one study that stated that this drug enhanced locomotion remaining effective for as long as the duration of the treatment (Siwak et al., 2000). In the same study, no results were achieved when considering nicergoline and propentofylline. In mice, a therapy was tested using Memantine, a N-methyl-D-aspartate receptor antagonist, concluding that its use slowed the clinical signs of impaired cognition (Martinez-Coria, Green, Billings, Kitazawa, Albrecht, Rammes et al., 2010).

There is no specific treatment for CDS but the aim of the treatment is to delay the ageing process or improve the animal's quality of life. In order to achieve this, there is a range of different approaches that should be used with each other and not only individually. This range varies from medical therapy to environmental enrichment, behavioural modification therapy, cognitive drugs and drugs for specific behaviour signs. The behavioural modification is the most important part of this therapy and should be always considered. All the rest are complementary to this, but the selection of adequate combination can be the way to success. When there is a need to use drugs, the animal can take weeks to months to achieve the maximal effect of the drug and present signs of improvement (Landsberg & Denenberg, 2009).

It has to be taken into account that treating behavioural problems in senior companion animals is more difficult that doing so in the younger animals. That is so because it is harder for older companion animals to learn new tasks since they are less able to adapt to change. In addition to this, they are more likely to develop concurrent medical conditions that have an impact on their behaviour. Finally, since CDS is a progressive disease the medication that starts as effective can be less and less effective, ending up with no use and so the clinician has to be alert and ready to make changes throughout the progression of the syndrome (Landsberg & Denenberg, 2009).

The prognosis is variable and the whole case should be considered. It depends on the tutors' compliance, the existing concurrent medical problems, the diagnostic period and the clinical signs the animal exhibits. Although CDS is not usually a cause for death or euthanasia, since this syndrome is a progressive disease, the response to treatment over time and the animal general health play an important role when it comes to making a decision (Landsberg & Denenberg, 2009).

5.1 Nutritional therapy

As stated before, ageing in the dog is associated with several changes that might be physiological or become pathological (Overall, 2013). Also realizing that the brain is the organ that uses more oxygen and has a low level of antioxidant enzymes makes it particularly susceptible to oxidative degeneration (Kidd, 2005). From human studies it was also indicated that oral nutritional supplementation with not only one, but instead a combination of fatty acids, minerals, vitamins, antioxidants and trophic nutrients led to an

improvement in cognitive functions subsequently decreasing morbidity associated with Alzheimer's Disease (Donini, Felice & Cannella, 2007).

Let's consider neuroprotection – Neuron cells protection against degenerative processes. This concept has to be taken into account for animals that suffer from pathological ageing. This might be the change that promotes physiological brain ageing resulting in a better quality of life. This is one of the strategies one can use when trying to treat CDS (Osella, Re, Badino, Bergamasco & Miolo, 2008).

A nutraceutical comes from the junction of the words "Nutrition" and "Pharmaceutical" by Dr Stephen DeFelice and can be described as any substance that is either considered food or a part of it that is associated with improvements in the animals' health. They should be administered orally and must be produced in a specific way – purified or extracted (Boothe, 2004).

Nutraceuticals can arise as a solution for neuroprotection in the geriatric dog (Osella et al., 2008). It is particularly important since the study that is about to be presented recalls nutritional therapy to improve CDS signs.

Dietary supplements are known to improve antioxidants and reduce the toxic effects of free radicals (Landsberg, Hunthausen & Ackerman, 2013). However, as previously stated, the best results when approaching a multifactorial syndrome such as CDS is a combination between an enriched environment and an enriched diet (Milgram et al., 2004).

Some fatty acids such as docosahexaenoic and eicosapentaenoic acids – DHA and EPA or even phosphatidylserine – PS -, a natural phospholipid, are considered brain-strengthening components because they are mandatory for the functional optimization of brain and neural tissue (Heath et al., 2007). The last one, PS, is a cell membrane phospholipid that takes a key role in the transmission of electrical signals in nerve cells and also in the response (Osella et al., 2008). It works in very different was to promote neuroprotection – as showed in table 1 and it has be proven to improve memory retrieval and exploratory behaviour in aged rats (Vannucchi, Casamenti & Pepeu, 1990). In humans, the deficiency in these fatty acids early in life is associated with impairment in learning and exploratory behaviour (Lucas, Morley, Cole, Lister & Leeson-Payne, 1992).

Mechanisms of action	Reference
Protective effect on impairment of long-term potentiation in rats	Nolan et al., 2004
Increase of hippocampal synaptic efficacy	Borghese et al., 1993
Prevention of the age-related decrease of dendritic spine density in the hippocampus of aged rats	Nunzi et al., 1989
Increase of ACh release of the synaptosomes prepared from aged rats to the level of young rats	Suzuki et al., 2001
Increase of ACh release in cerebral cortical synaptosomes from mice	Yamatoya et al., 2000
Reversal of the age-dependent decrease in cortical ACh release in old rats	Casamenti et al., 1991
Prevention of the reduction in total evoked ACh release from cortical slices in aged rats	Vannucchi et al., 1990
Restoration of ACh release in cortical slices from aged rats	Pedata et al., 1985; Vannucchi and Pepeu, 1987
Modulation of acetylcholinesterase in the synaptosomes of the canine brain	Tsakiris and Deliconstantinos, 1984, 1985
Stimulation of exogenous GABA uptake into brain GABAergic nerve terminals in rats	Loeb et al., 1988
Enhancement of GABA uptake by rat brain synaptosomes	Chweh and Leslie, 1982
Increase of binding properties of AMPA subtypes of glutamate receptors in brain sections from adult rats	Gagne et al., 1996
Increase of AMPA binding in a dose-dependent manner in rat brain membranes	Baudry et al., 1991
Elevation of NMDA receptor density by approximately 25% in the forebrain of mice	Cohen and Müller, 1992
Reversal of the age-dependent decrease of prolactin brain receptors in hypothalamus and substantia nigra from aged rabbits	Di Carlo et al., 1988
Restoration of the reduced density of muscarinic cholinergic receptors in several regions of the aged mouse brain	Gelbmann and Muller, 1992
Restoration of the stimulated B-50/GAP-43 phosphorylation [presynaptic protein phosphorylation implicated in neuronal mechanisms related to learning and memory] in hippocampal slices from aged rats	Gianotti et al., 1993
Increase of the Na(+), K(+)-ATPase activity of the synaptosomes prepared from dog brain [implications in the physiologic processes in the CNS]	Tsakiris and Delinconstantinos, 1984
Increase of the Ca^{2+}-stimulated ATPase activity of dog brain synaptosomal plasma membranes	Tsakiris and Delinconstantinos, 1985
Increase of the Na(+), K(+)-ATPase activity of the synaptosomes prepared from aged rats to the level of young rats	Suzuki et al., 2001

AMPA, α-amino-3-hydroxy-5-methylisoxazde-4-propionic acid (artificial glutamate analog, able to activate the AMPA subtype of glutamate receptors); GABA, Gamma-aminobutyric acid (an inhibitory neurotransmitter); NMDA receptor; N-methyl-D-asparatic acid receptor (a subtype of glutamate receptor).

Table 1: Stated roles of phosphatidylserine in neuroprotection (Osella et al., 2008).

Other fatty acids known to improve cognition in humans with Alzheimer's disease are the medium chain triglycerides – MCTs - as they provide an alternate source of energy – ketone bodies from β-hydroxybutyrate – to an aged brain that uses glucose inadequately (Reger, Henderson, Hale, Cholerton, Baker, Watson et al., 2004). An alternate way in which MCTs improve cognition is because the aged brain has a depletion in Ω-3 polyunsaturated fatty acids – PUFAs – and although MCTs are not PUFAs, their consumption may increase brain PUFAs by mobilizing them from other tissues to the brain itself. In beagle dogs it has been stated that feeding a diet enriched with MCT oils will elevate the concentration of PUFAs in the parietal cortex (Taha, Henderson & Burnham., 2009). This will ultimately result in an improvement in cognitive function since PUFAs are known to be involved in neurogenesis and cell signaling (Beltz, Tlusty, Benton & Sandeman., 2007). In rats it has been proven that dietary enrichment with PUFAs will even normalize BDNF levels and reduce oxidative damage resulting in an improvement of their learning ability after brain injury (Wu, Ying & Gomex-Pinilla., 2004).

A coadjutant of PS is the Gingko Biloba Extract – EGb – for the simple reason that it also stimulates some systems in aged animals such as the cholinergic or serotoninergic one (Lee, Chen & Wang, 2004). Other stated benefits of EGb include the increase of dopamine in the brain of mice by reversible inhibiting Monoamine oxidase A and MAOB (White, Scates & Cooper, 1996) and the protection of neurons against apoptosis when that cell death is happening because of the β-amyloid protein (Yao, Drieu & Papadopoulos, 2001). It is thought that these properties are due to its antioxidant abilities in preventing oxidative changes in mice age-related mitochondrial changes (Sastre, Millán, Assunción, Plá, Juan, Pallardo et al., 1998).

The α-Lipoic acid is a coenzyme that is involved in carbohydrate utilization necessary for the production of Adenosine triphosphate in mitochondria (Stoll, Hartmann, Cohen & Muller, 1993). This coenzyme is associated to improvements in long-term memory in female mice. The association with Acetyl-L-carnitine has also been studied and proved fruitful in mice (Liu, Head, Charib, Yuan, Ingersoll, Hagen et al., 2002).

Vitamin B6 – Pyridoxine - is another studied element in the neurological/behavioural context. This vitamin has a very important role because there are several pyridoxal phosphate dependent enzymes including quite a few neurotransmitters such as dopamine, norepinephrine, serotonin, y-aminobutyric acid and taurine. In fact, when a decline in the levels of pyridoxine is induced in rats, there is a depletion in serotonin but not in dopamine in the brain. The depletion of dopamine, But, because the imbalance between these two is altered, severe alterations in the neuroendocrine system can be achieved (Dakshinamurti, Paulose, Viswanathan, Siow & Sharma, 1990).

There are several reports on this subject, but not all of them study each component individually in aged dogs. For instance, there is a successful study with a supplement with several components listed on annex I. This supplement, Aktivait®, demonstrated not only great effect in managing some CDS signs such as Social interactions and housesoiling but also in the tutor's perception of the dogs' condition culmination, obviously, in the relationship between these and their tutors (Head, 2007).

Other supplement that was studied is Senilife®, whose composition is described in annex II. In the preliminary trial the dogs treated with this supplement showed great improvement in their symptoms although they did no achieve total remission. One thing that must be stated is that the dogs enrolled for the trial did not have a baseline status of

severe CDS. Even though many had several different symptoms, they were all categorized as dogs with mild cognitive impairment (Osella et al., 2007). However, the previous study focused on client- owned dogs so a need was felt to test the supplement in a laboratory stings, testing a memory performance of Beagle Dogs. This study found improvement in dogs treated with the supplement in their short-term memory. The effects lasted for over seventy days after the discontinuation of treatment suggesting a beneficial long-term effect of oral nutritional supplementation with Senilife® (Araujo, Landsberg, Milgram & Miolo, 2008).

Another strategy presented was a study with a commercially available pet food – Hills b/d® - whose composition is enumerated in annex III. The study indicated a global improvement in cognition and behaviour in dogs by their tutors. The enhancements were in behaviours from all categories of the DISHA scale and the authors estimate a placebo effect ranging from twenty to fifty percent. (Dodd, Zicker, Jewell, Lowry, Fritsch, & Toll, 2002).

Alternatively Nestlé Purina Petcare presented a study in which beagle dogs were being fed a diet containing 5.5% of MCTs *versus* a placebo group with no MCTs. From this it was observed that the dogs fed with the supplemented diet had better results when it came to discrimination and reversal learning, allocentric spatial ability and visuospatial function. To sum up it showed that the diet improved cognitive ability and serum ketone levels (Pan, Larson, Araujo, Lau, Rivera, Santana et al., 2010).

Other supplement tested - whose formulation can be found in annex IV – with antioxidants and mitochondrial cofactors revealed that the aged dogs eating a supplemented diet had better results when tested for landmark discrimination learning and had better long-term memory maintenance. It must be stated that this was the first study that combined substances with the aim of enhancing mitochondrial function and suppression of free radicals which was assumed to work synergistically (Milgram, Head, Muggenburg, Holowachuk, Murphey, Estrada, et al., 2002). In a posterior study using the same diet it was concluded that the results were improved if combining the diet with an enrichment program that included not only cognitive enrichment but also physical activities and environmental enrichment (Milgram et al., 2004 ; Milgram et al., 2005).

6. Materials and Methods

6.1 Aim of the study

The aim of the present study was to assess if some behavioural changes were noticed by the tutors of a geriatric dog population when these were being fed a diet supplemented with medium chain fatty acids.

Besides this, the study also aimed to assess the role of a supplementation with medium chain fatty acids in a urban and geriatric dog population with mild cognitive decline – mild behavioural changes. It was also planned doing descriptive analysis of the population that was addressed.

6.2 Selection of subjects and exclusion criteria

The subjects selected to enroll the study were chosen from a pool of 60 dogs. This pool was created by leaving questionnaires in five small animal practices in the Lisbon district. After an initial approach, a group of 10 dogs was elected based on the inclusion criteria. These dogs were eight years old or older and had one or two symptoms associated with CDS. They had to be considered generally healthy. A table was elaborated to aid the identification of these symptoms, which can be found in annex V.

The exclusion criteria were dogs younger than eight years, that showed three or more symptoms associated with cognitive dysfunction, had symptoms of clinical disease at the examination or had hematology or biochemistry exams compatible with systemic concomitant disease. Furthermore, all dogs that lived with their tutors for less than 1 year or were undergoing treatment for Cognitive Dysfunction or other behavioural problems were also excluded.

Once the study began, it was defined that all dogs that went through severe changes in social or physical environment, showed intolerance to the food or were posteriorly diagnosed with any systemic disease would be withdrawn.

In order for the cognitive status to be evaluated in the tutors' perspective a questionnaire was filled by the tutors.

It has to be stated that from the initial study group of ten dogs, two were withdrawn during the study period due to presence of concurrent disease that was diagnosed during the trial period. The remaining animals (n=8) were considered the final study group.

6.3 Data collection

The questionnaire referenced above was available for tutors in five small animal veterinary practices in the district of Lisbon. The questionnaire was based on the original questionnaire present in the book "Manual of Clinical Behavioral Medicine for Dogs and Cats". - annex VI. The questionnaires were available from May 2014 to May 2015.

6.4 Experimental design

The study layout for the initial ten selected cases – that remained eight for the final study group - was designed to maximize the number of analysis that could be obtained from the sample. The whole group started the study eating a placebo dry food from day one to day thirty – Study diet A whose composition can be found in annex VII. In the next two months, from day thirty to day ninety, the animals were fed a dry enriched food – Study diet B – composition in annex VIII.

The tutors were asked to fill a numeric questionnaire specifically designed for the study – the Assessment Tool - in the day zero and at each thirty days – day 0, 30, 60 and 90. The aim of having the tutors fill the same questionnaire in four different occasions is to more accurately assess behaviour changes throughout the period trial and, consequently, throughout both diets. This questionnaire can be found in annex IX.

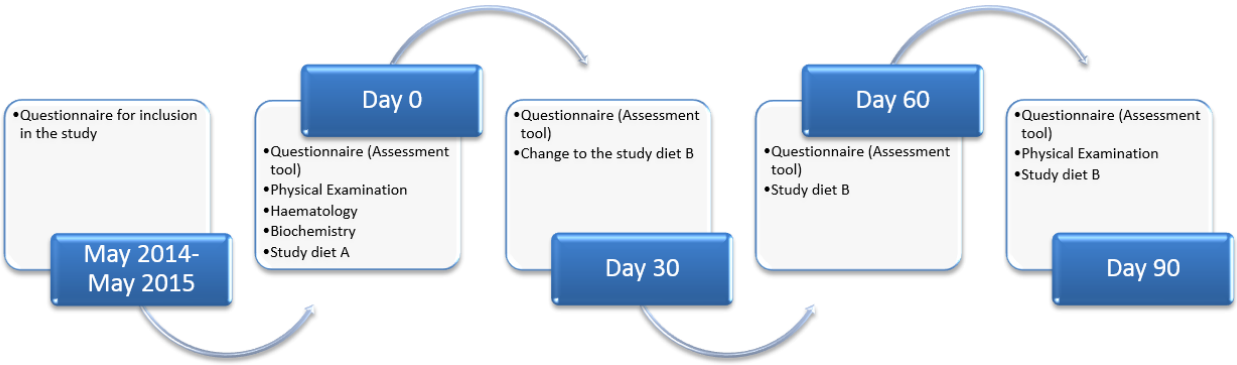


Figure 3: Study design schematic

To assess the general health of the enrolled subjects in the day 0 of the trial period a physical examination was performed and blood was collected to have hematology and biochemistry tests performed. To ensure the subjects remained healthy throughout the trial period a physical examination was performed once a month- day 0, 30, 60 and 90. If clinical signs of concurrent disease were found the animals were immediately withdrawn from the study group.

6.5 Data analysis

The collected data was treated using SPSS® 22 and Microsoft Excel® 2013.

The initial statistical analysis included a descriptive analysis of all the variables from the inclusion questionnaire (n=60). Such variables were also tested using a $\chi 2$ test, given that they were all categorical.

The smaller sample included in the study and the questionnaires' data collected at the four different moments were also subjected to a descriptive statistical analysis and the variables were tested using $\chi 2$ tests.

7. Results

7.1 Descriptive analysis – Inclusion questionnaire, IQ (n=60)

1. Appetite assessment (may tick more than one).

   a. No alterations in appetite

	Frequency	Percent
No	15	25,0
Yes	45	75,0
Total	60	100,0

Table 2: Frequency analysis of alterations in appetite of the IQ.

   b. Change in ability to physically handle food

	Frequency	Percent
No	53	88,3
Yes	7	11,7
Total	60	100,0

Table 3: Frequency analysis of ability to physically handle food of the IQ.

   c. Change in ability to retain to food (vomits or regurgitates)

	Frequency	Percent
No	59	98,3
Yes	1	1,7
Total	60	100,0

Table 4: Frequency analysis of ability to retain food of the IQ.

   d. Change in ability to find food

	Frequency	Percent
No	60	100,0
Yes	0	0,0
Total	60	100,0

Table 5: Frequency analysis of ability to find food of the IQ.

   e. Change in interest in food (may be olfactory, having to do with the ability to smell)

	Frequency	Percent
No	58	96,7
Yes	2	3,3
Total	60	100,0

Table 6: Frequency analysis of interest in food of the IQ.

f. Change in rate of eating

	Frequency	Percent
No	59	98,3
Yes	1	1,7
Total	60	100,0

Table 7: Frequency analysis of rate of eating of the IQ.

g. Change in completion of eating

	Frequency	Percent
No	49	81,7
Yes	11	18,3
Total	60	100,0

Table 8: Frequency analysis of completion of eating of the IQ.

h. Change in timing of eating

	Frequency	Percent
No	57	95,0
Yes	3	5,0
Total	60	100,0

Table 9: Frequency analysis of timing of eating of the IQ.

i. Change in preferred textures

	Frequency	Percent
No	55	91,7
Yes	5	8,3
Total	60	100,0

Table 10: Frequency analysis of preferred textures of the IQ.

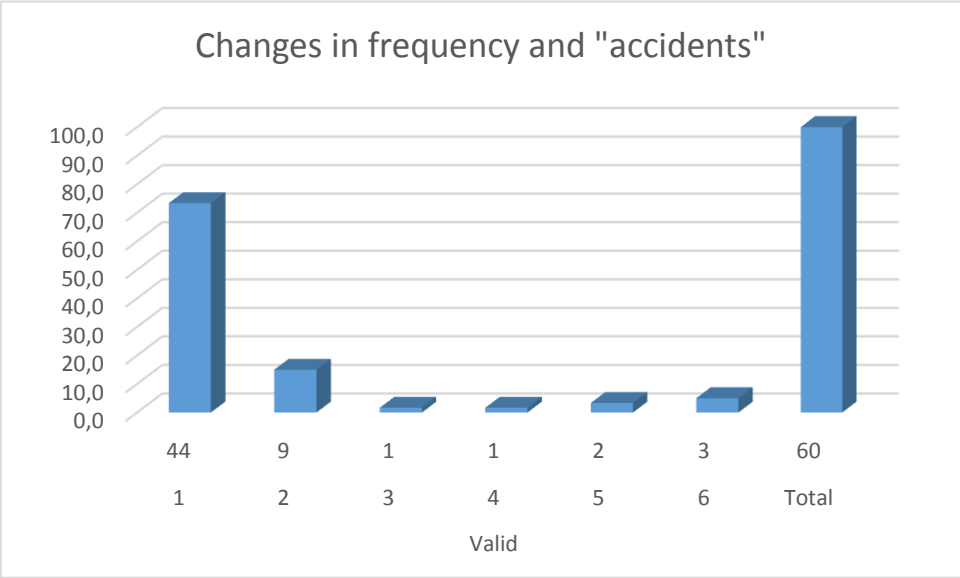
2. Assessment of elimination function

    a. Changes in frequencies and “accidents”

        1. No change in frequency and no “accidents”
        2. Increased frequency, no “accidents”
        3. Decreased frequency, no “accidents”
        4. Increased frequency with “accidents”
        5. Decreased frequency with “accidents”
        6. No change in frequency, but “accidents”

	Frequency	Percent
1	44	73,3
2	9	15,0
3	1	1,7
4	1	1,7
5	2	3,3
6	3	5,0
Total	60	100,0

Table 11: Frequency analysis of changes in frequency of elimination behaviour of the IQ.



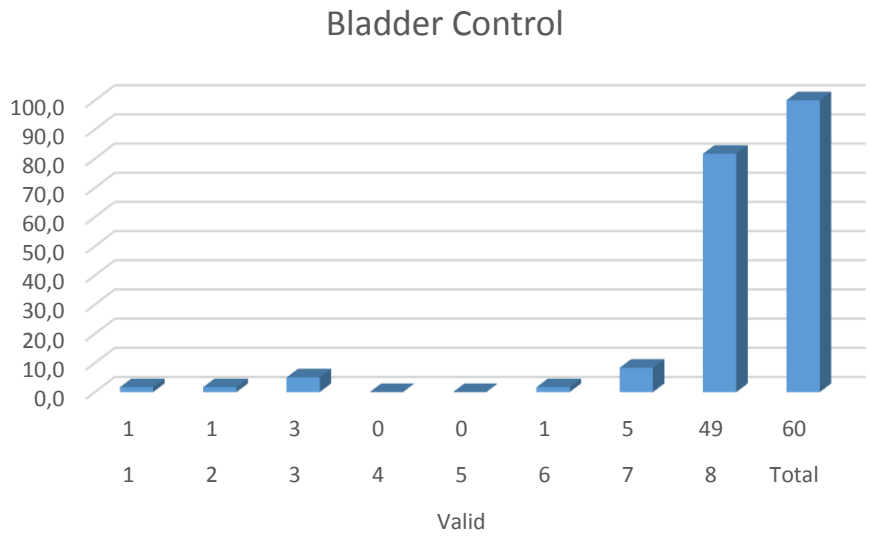
Graphic 1: Frequency analysis of changes in frequency of elimination behaviour of the IQ.

b. Bladder Control

1. Leaks urine when asleep, only
2. Leaks urine when awake, only
3. Leaks urine when awake or asleep
4. Full-stream, uncontrolled urination when asleep, only
5. Full-stream, uncontrolled urination when awake, only
6. Full-stream, uncontrolled urination when awake or asleep
7. No leakage or uncontrolled urination, all urination controlled, but in inappropriate or undesirable location
8. No change in urination control or behavior

	Frequency	Percent
1	1	1,7
2	1	1,7
3	3	5,0
4	0	0,0
5	0	0,0
6	1	1,7
7	5	8,3
8	49	81,7
Total	60	100,0

Table 12: Frequency analysis of Bladder Control of the IQ.



Graphic 2: Frequency analysis of Bladder Control of the IQ.

c. Bowel control

1. Defecates when asleep
2. Defecates without apparent awareness
3. Defecates when awake and aware of action, but in inappropriate or undesirable locations
4. No changes in bowel control

	Frequency	Percent
1	0	0,0
2	0	0,0
3	4	6,7
4	56	93,3
Total	60	100,0

Table 13: Frequency analysis of Bowel Control of the IQ.

3. Interactions with humans – which situation best describes that interaction?

a. No change in interaction with people
b. Recognizes people but slightly decreased frequency of interaction
c. Recognizes people but greatly decreased frequency of interaction
d. Withdrawal but recognizes other pets
e. Does not recognize other pets

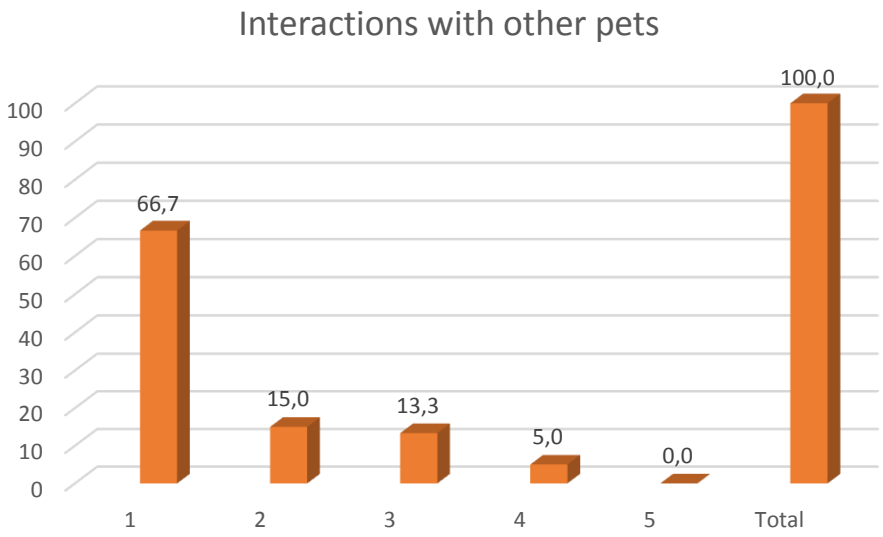
	Frequency	Percent
1	44	73,3
2	12	20,0
3	4	6,7
4	0	0,0
5	0	0,0
Total	60	100,0

Table 14: Frequency analysis of interactions with humans of the IQ.

4. Interactions with other pets – which situation best describes that interaction?
    a. No change in interaction with other pets
    b. Recognizes other pets but slightly decreased frequency of interaction
    c. Recognizes other pets but greatly decreased frequency of interaction
    d. Withdrawal but recognizes other pets
    e. Does not recognize other pets

	Frequency	Percent
1	40	66,7
2	9	15,0
3	8	13,3
4	3	5,0
5	0	0,0
Total	60	100,0

Table 15: Frequency analysis of interactions with other pets of the IQ.

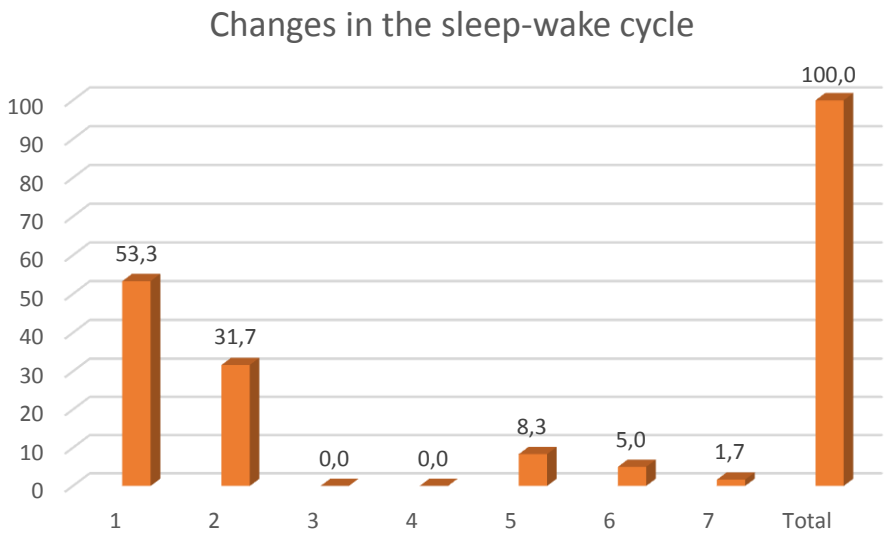


Graphic 3: Frequency analysis of interactions with other pets of the IQ.

5. Changes in sleep/wake cycle
    a. No changes in sleep patterns
    b. Sleeps more in day, only
    c. Some change – awakens at night sleeps more in day
    d. Much change – profoundly erratic nocturnal pattern and irregular daytime pattern
    e. Sleeps virtually all day, awake occasionally at night
    f. Sleeps almost around the clock

	Frequency	Percent
1	32	53,3
2	19	31,7
3	0	0,0
4	5	8,3
5	3	5,0
6	1	1,7
Total	60	100,0

Table 16: Frequency analysis of changes in the Sleep-Wake cycle of the IQ.



Graphic 4: Frequency analysis of Changes in the Sleep-Wake cycle of the IQ.

Gender

	Frequency	Percent
Male	29	48,3
Female	31	51,7
Total	60	100,0

Table 17: Frequency analysis of gender of the IQ.

Age groups

	Frequency	Percent
08 to 10 years old	29	48,3
11 to 13 years old	22	36,7
14 to 16 years old	9	15,0
Total	60	100,0

Table 18: Frequency analysis of age groups of the IQ.

7.2 Descriptive analysis – Study Group, SG (n=8)

Appetite assessment (may tick more than one).

a. No alterations in appetite

	Frequency	Percent
No	1	12,5
Yes	7	87,5
Total	60	100,0

Table 19: Frequency analysis of appetite assessment of the SG.

b. Change in ability to physically handle food

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 20: Frequency analysis of ability to handle food of the SG.

c. Change in ability to retain to food (vomits or regurgitates)

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 21: Frequency analysis of ability to retain food of the SG.

d. Change in ability to find food

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 22: Frequency analysis of ability to find food of the SG.

e. Change in interest in food (may be olfactory, having to do with the ability to smell)

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 23: Frequency analysis of interest in food of the SG.

f. Change in rate of eating

	Frequency	Percent
No	7	87,5
Yes	1	12,5
Total	8	100,0

Table 24: Frequency analysis of rate of eating of the SG.

g. Change in completion of eating

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 25: Frequency analysis of rate of eating of the SG.

h. Change in timing of eating

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 26: Frequency analysis of timing of eating of the SG.

i. Change in preferred textures

	Frequency	Percent
No	8	100,0
Yes	0	0,0
Total	8	100,0

Table 27: Frequency analysis of preferred textures of the SG.

2. Assessment of elimination function

a. Changes in frequencies and “accidents”

i. No change in frequency and no “accidents”

ii. Increased frequency, no “accidents”

iii. Decreased frequency, no “accidents”

iv. Increased frequency with “accidents”

v. Decreased frequency with “accidents”

vi. No change in frequency but “accidents”

	Frequency	Percent
1	8	100,0
2	0	0,0
3	0	0,0
4	0	0,0
5	0	0,0
6	0	0,0
Total	8	100,0

Table 28: Frequency analysis of changes in frequency of elimination behaviour of the SG.

b. Bladder Control

i. Leaks urine when asleep, only

ii. Leaks urine when awake, only

iii. Leaks urine when awake or asleep

iv. Full-stream, uncontrolled urination when asleep, only

v. Full-stream, uncontrolled urination when awake, only

vi. Full-stream, uncontrolled urination when awake or asleep

vii. No leakage or uncontrolled urination, all urination controlled, but in inappropriate or undesirable location

viii. No change in urination control or behavior

	Frequency	Percent
1	0	0,0
2	0	0,0
3	0	0,0
4	0	0,0
5	0	0,0
6	0	0,0
7	0	0,0
8	8	100,0
Total	8	100,0

Table 29: Frequency analysis of changes in frequency of bladder control of the SG.

c. Bowel control

i. Defecates when asleep

ii. Defecates without apparent awarness

iii. Defecates when awake and aware of action, but in an inapprorpiate or undesirable location

iv. No changes in bowel control

	Frequency	Percent
1	0	0,0
2	0	0,0
3	0	0,0
4	8	100,0
Total	60	100,0

Table 30: Frequency analysis of changes in bowel control of the SG.

3. Interactions with humans – which situation best describes that interaction?

a. No change in interaction with people

b. Recognizes people but slightly decreased frequency of interaction

c. Recognizes people but greatly decreased frequency of interaction

d. Withdrawal but recognizes people

e. Does not recognize people

	Frequency	Percent
1	5	62,5
2	3	37,5
3	0	0,0
4	0	0,0
5	0	0,0
Total	60	100,0

Table 31: Frequency analysis of changes in interactions with humans of the SG.

4. Interactions with other pets – which situation best describes that interaction?

    a. No change in interaction with other pets

    b. Recognizes other pets but slightly decreased frequency of interaction

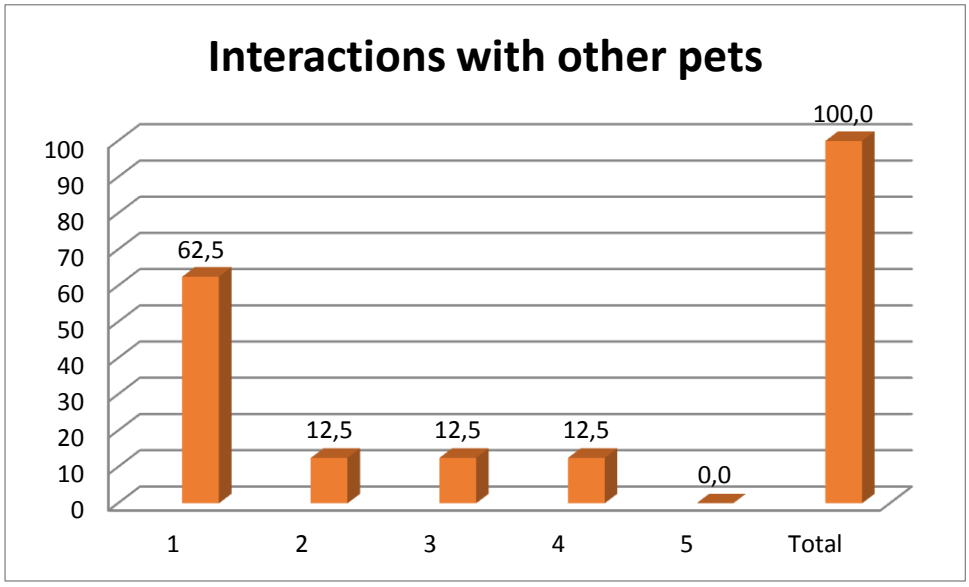
    c. Recognizes other pets but greatly decreased frequency of interaction

    d. Withdrawal but recognizes other pets

    e. Does not recognize other pets

	Frequency	Percent
1	5	62,5
2	1	12,5
3	1	12,5
4	1	12,5
5	0	0,0
Total	60	100,0

Table 32: Frequency analysis of changes in interactions with other pets of the SG.



Graphic 5: Frequency analysis of Changes in interactions with other pets of the SG.

Changes in sleep/wake cycle

a. No changes in sleep patterns
b. Sleeps more in day, only
c. Some change – awakens at night sleeps more in day
d. Much change – profoundly erratic nocturnal pattern and irregular daytime pattern
e. Sleeps virtually all day, awake occasionally at night
f. Sleeps almost around the clock

	Frequency	Percent
1	5	62,5
2	3	37,5
3	0	0,0
4	0	0,0
5	0	0,0
6	0	0,0
7	0	0,0
Total	8	100,0

Table 33: Frequency analysis of changes in the sleep-wake cycle of the SG.

The $\chi 2$ tests determined a significant relation between the variables "Changes in frequencies and "accidents"" and "age" ($\chi 2$ (6, N= 60) = 18,313, p =0,050), with a positive association between the aggravation of the conditions associated with the first variable and the age increase.

The $\chi 2$ tests also showed a significant relation between the variables "Changes in Interactions with humans" and "age" ($\chi 2$ (6, N= 60) = 12,959 p =0,011) and the variables "Changes in Interactions with other animals" and "age" ($\chi 2$ (6, N= 60) = 15,598 p =0,016). Both the relations show a positive association between the aggravation of the conditions and the age increase.

Lastly, the $\chi 2$ tests also exhibited a significant relation between the variables "Changes in the Sleep/Wake cycle" and "age" ($\chi 2$ (6, N= 60) = 17,714 p =0,023) with a positive association between the aggravation of the conditions associated with the variable and the age increase.

7.3 Frequency analysis of trial period, days 0, 30, 60 and 90

Assessing Disorientation

How frequently does your dog get lost in familiar locations? (e. g. At home they may not find their way to their bed).				
	Day 0	Day 30	Day 60	Day 90
1	8	8	8	8
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0

Table 34: Frequency analysis of assessment of disorientation 1 of the SG.

How frequently does your dog go to the wrong side of the door when leaving a room?				
	Day 0	Day 30	Day 60	Day 90
1	8	8	8	8
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0

Table 35: Frequency analysis of assessment of disorientation 2 of the SG.

How frequently does your dog wander in the house with no apparent purpose?				
	Day 0	Day 30	Day 60	Day 90
1	8	3	4	4
2	0	4	2	2
3	0	1	2	2
4	0	0	0	0
5	0	0	0	0

Table 36: Frequency analysis of assessment of disorientation 3 of the SG.

Assessing Social Interaction

Interacting with humans. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog shows interest in interacting with humans has… (e. g. playing, petting, greeting)				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	0	0	0	0
3	8	8	7	7
4	0	0	1	1
5	0	0	0	0

Table 37: Frequency analysis of assessment of social interactions 1 of the SG.

Interacting with other pets Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog shows interest in interacting with other pets has…(e. g. playing, barking, smelling)				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	3	2	2	1
3	5	6	6	7
4	0	0	0	0
5	0	0	0	0

Table 38: Frequency analysis of assessment of social interactions 2 of the SG.

Remember some tasks you taught your dog. (e.g. sitting, laying down, fetching). Nowadays the number of times your dog is capable of performing them has…				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	0	0	0	0
3	8	8	8	8
4	0	0	0	0
5	0	0	0	0

Table 39: Frequency analysis of assessment of social interactions 3 of the SG.

Assessing Sleep – Wake Cycles

Barking during the night. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog barks during the night for no apparent reason has…				
	Day 0	Day 30	Day 60	Day 90
1	3	3	2	3
2	2	2	2	2
3	3	3	4	3
4	0	0	0	0
5	0	0	0	0

Table 40: Frequency analysis of assessment of sleep-wake cycles 1 of the SG.

Sleeping during the day Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog spends most of his/her day sleeping has…				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	0	0	0	0
3	5	6	6	5
4	1	0	0	2
5	2	2	2	1

Table 41: Frequency analysis of assessment of sleep-wake cycles 2 of the SG.

Staying awake during the night. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog spends most of his/her night awake has…				
	Day 0	Day 30	Day 60	Day 90
1	2	2	2	2
2	0	1	0	0
3	4	5	5	5
4	2	0	1	1
5	0	0	0	0

Table 42: Frequency analysis of assessment of sleep-wake cycles 3 of the SG.

Assessing Housesoiling

Urinating in a “wrong” place. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog urinates in a place he is not supposed to has…				
	Day 0	Day 30	Day 60	Day 90
1	3	3	3	3
2	0	0	0	0
3	4	5	4	4
4	1	0	1	1
5	0	0	0	0

Table 43: Frequency analysis of assessment of housesoiling 1 of the SG.

Defecating in a “wrong” place. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog defecates in a place he is not supposed to has…				
	Day 0	Day 30	Day 60	Day 90
1	3	3	3	3
2	0	0	0	0
3	5	5	5	5
4	0	0	0	0
5	0	0	0	0

Table 44: Frequency analysis of assessment of housesoiling 2 of the SG.

Assessing Activity Levels

Vocalization. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog vocalizes has…				
	Day 0	Day 30	Day 60	Day 90
1	1	1	1	1
2	0	0	0	0
3	6	6	6	5
4	0	0	0	1
5	1	1	1	1

Table 45: Frequency analysis of assessment of activity levels 1 of the SG.

Appetite. Remember the typical behaviour of your dog in adult life. Nowadays your dog's appetite has… (e. g. eating more, asking your for food)				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	0	1	3	4
3	4	4	2	2
4	4	3	3	2
5	0	0	0	0

Table 46: Frequency analysis of assessment of activity levels 2 of the SG.

Greeting behaviour. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog greets you / other elements of the family has…				
	Day 0	Day 30	Day 60	Day 90
1	0	0	0	0
2	0	0	0	0
3	5	6	6	5
4	3	2	2	3
5	0	0	0	0

Table 47: Frequency analysis of assessment of activity levels 3 of the SG.

Several $\chi 2$ tests were elaborated between the variables and the day of the questionnaire but no relations, neither positive nor negative, were found between these.

7.4 Global response to therapy rate

After testing the variables an additional analysis compared the global results between the days zero and ninety. From these comparisons, a global response to therapy rate was determined.

When comparing these two moments 8.04% of the study group (n =8) had a general improvement in the lessening of their clinical signs. The majority of the study group - 86.61%, was generally considered to have no improvement nor aggravation in their clinical signs and finally 5.36% of the study group experienced a general worsening of the CDS signs.

8. Discussion

The effects of fatty acids in the cognition have been previously studied (Heath et al., 2007 ; Reger et al., 2004 ; Taha et al., 2009). Cognition may decrease with age and it is important for the clinician to know how to delay its progress in an efficient way.

Regarding the study previously presented, the first part of the analysis was a descriptive analysis of the population of all the dogs that were assessed in the study. The population had no statistical differences regarding age groups nor gender. Only 23.3% of the population (n=14) had no behavioural alterations. Although 76.7% had some described alterations not all of them have to be related to CDS. Therefore, the prevalence of CDS in our population will not be estimated. Despite of that, none of these animals had been referred to behaviouralists nor were their clinical signs being addressed. A previous study shows that a veterinary diagnosis of CDS is accomplished in 13,3% of a population (Salvin et al., 2010).

It was shown that 50% of the tutors of our population claim that their animals have alterations in appetite being the most reported one the "change in the completion of eating a meal" (18,3%) followed by an impairment in the ability of the animal to physically handle food (11,7%). When assessing sleep-wake cycles changes, these were reported in almost half of the population (46,7%). These results state that the alterations in the appetite are the most prevalent followed by the changes in the sleep-wake cycle. This does not necessarily mean that these signs are neither the most frequent nor the first to appear in a dog suffering from CDS but rather might indicate which are the signs more easily or frequently detected by the animals' tutor, or the ones that impact the most the tutors' life.

When assessing the study group (n=8) the results are coherent with the population, since the major change reported by the tutors is the alteration in the animals' appetite (87,5%). The positive association between the variables "Changes in the frequencies and accidents" regarding housesoiling, "Changes in interactions with humans" and also "Changes in interactions with other animals" regarding social interactions and the variable "age" show that there is an aggravation of the symptoms with the age increase. These significant relations validate the data since according to literature the prevalence of cognitive impairment is expected to increase with the increase of age (Azkona et al., 2009).

Considering a population of sixty dogs, the fact that only eight animals were enrolled in the study was a consequence of the inclusion criteria. The aim was to study animals with mild cognitive impairment and exclude the animals with severe cognitive impairment. Although this choice was based upon the number of clinical signs presented at the moment of the first questionnaire it was not taken into account which signs were presented. For instance none of the animals demonstrated disorientation as a clinical sign. The category of signs presented, according to the DISHA scale, might also indicate the severity of the disease (Osella et al., 2007). Other reasons include refusal from the tutor, biochemistry tests / hematology coherent with systemic disease, lack or excess of behavioural changes.

Another fact that should be nominated is that although the animals underwent hematology and biochemistry tests along with physical examination, it cannot be confirmed that the animals did not have a concomitant systemic disease. The odds are low since only animals with test results satisfying were included. But, because several diagnostic tests were left undone – such as urinalysis our fecal cytology - it cannot be stated that the animals had no concurrent disease. Since CDS is an exclusion diagnostic disease it must be stated that none of the animals had an MRI or Computerized Axial Tomography – CAT scan done so other brain pathologies cannot be excluded.

When analyzing the animals' evolution throughout the 90 days trial-period, the global response to therapy was not what was expected considering that the majority of the study group (n=8) had no improvement nor impairment in their overall cognition when addressed by the tutors – 86,61%. The amount of animals that showed a positive global response to therapy was 8,04%. When comparing this value with other studies with MCT's the previous study achieved a lower score (Dodd et al., 2002 ; Wu et al., 2004). Lastly, a smaller percentage also worsened throughout the study – 5,36%.

Some reasons that can explain the previous result are the fact that the study was conducted in a "real life" scenario rather than in a laboratory. The major differences are the fact that the animals are not so closely monitored and the fact that the behavioural evaluation was solely the tutor responsibility rather than a clinician, behaviourist or any veterinary staff. This may lead to a lower degree of efficacy on noticing subtle behavioural changes. Combining that with the fact that the animals chosen had mild clinical signs and

the short study period it is understandable that the behavioural changes and, consequently, the response to therapy may have been underestimated.

It must also be stated that there may be a *bias* in our global response to therapy rate if it is considered that the maintenance of the clinical signs might also be a positive response to therapy. This can be considered because, as stated in the literature, the goal of the treatment of this syndrome is often to slow down the progression of signs rather than eliminating them (Landsberg & Denenberg, 2009). When considering the disorientation assessment one can notice that there was no change in that category because none of the animals presented any disorientation signs to begin with. Even there was no further improvement due to the fact that they were not disoriented at the beginning, the MCTs diet may have helped the dogs maintain their long term memory about their familiar environments during the study. This might also be considered a positive response since the MCT's in the diet might have stabilized these animals or it might just be that the brain region affected in these animals is not responsible for that behaviour.

This is also true for the other behaviours assessed. For instance for social interaction assessment, MCT's diet stabilized 7 dogs and improved 1 dog in interacting with humans and reduced the number of dogs that had decreased interaction with other pets from 2 to 1, and stabilizing the interactions in other 7 dogs. The MCT diet either stabilized the symptoms or improved the symptoms in other assessments including sleep-wake cycle, housesoiling and activity levels.

Since the study dealt with humans who are emotionally attached to the animal they were evaluating, the placebo effect also must be taken into account. Specially if considered that Study diet A and Study diet B had different shaped kibbles, the tutor could easily understand that there had been a change in the diet. Although not knowing which was the *verum* and which was the *placebum* this might have influenced the tutors when answering the questionnaires. A possible way this fact could be avoided, is not only having equally shaped kibbles but also having a clinician or behaviourist completing the behaviour assessment of the animal along with the tutor for the investigator to be able to compare both and estimate a *placebum* effect percentage more accurately.

To completely understand how a supplemented dry food will change the behaviours and slow the progression of CDS in dogs further studies are needed with bigger populations and extended trial periods. Other vicissitudes that may be addressed is

separating the placebo and the verum populations as two individual group studies in order to firstly avoid the change of diet during the study and to more easily compare the fluctuations of behaviour changes in populations of geriatric dogs with cognitive impairment. Other suggested alteration would be to group the dogs accordingly to the category of clinical signs they show impairment in according to the DISHA scale to identify how they answer the supplementation.

Other possible

9. Conclusion

The previous study describes the role of a supplemented diet in a population of dogs with cognitive impairment when addressed by the tutors.

Conducting a series of questionnaires the behavioural changes of the study group was described throughout a period of non-supplemented food and a period of supplemented food. By doing this one can assess not only how does a tutor perceive the change of behaviour in his pet but also notice which symptoms are more sensitive to the supplementation.

When realizing that none of these dogs were being medicated for their behavioural changes nor were being followed by a behaviourist, it was shown that the diagnostic of CDS is easily underestimated even when an animal is regularly visiting the clinician as described in the literature. This leads to a specific revision of veterinary training and include skills that allow the clinician to perform an accurate anamnesis.

No statistical relation was found in our population between the presence of symptoms with gender. There was however a confirmation that, when untreated, the clinical signs worsen with age as one could suspect. Unfortunately, although our data was validated as a group no statistical relations were found between the variables or along the period of the study. Besides this, when assessing the questionnaires individually the MCT's diet either stabilized or improved most of the measurements alone during this short-term study which is an encouraging result. For further studies and in order to more precisely evaluate the brain MRI's or CAT scans should be included.

The previous statements indicate that further studies are needed in order to more accurately statistically analyze a population of geriatric dogs and to realize how the tutors perceive the changes of behaviour in their companion animals.

ANNEXES

Annex I

VetPlus Aktivait® Composition

Composition per capsule:	Small breeds (< 10 Kg)
DHA/EPA	35 mg
L-carnitine	20 mg
Vitamin C	20 mg
N-acetylcysteine	13.5mg
Alpha-lipoic acid	10 mg
Vitamin E	10 mg
Acetyl – carnitine	5 mg
Coenzyme Q10	1 mg
Phosphatidylserine	1 mg
Selenium (as selenometionina)	25mcg

VetPlus Aktivait®

Composition per capsule:	Medium / Large breeds (> 10 Kg)
DHA/EPA	70 mg
L-carnitine	40 mg
Vitamin C	40 mg
N-acetylcysteine	27 mg
Alpha-lipoic acid	20 mg
Vitamin E	20 mg
Acetyl - carnitine	10 mg
Coenzyme Q10	2 mg
Phosphatidylserine	2 mg
Selenium (as selenometionina)	50mcg

Annex II

Ceva Senilife® Composition

Composition per capsule:	Small breeds (< 10 Kg)
Phosphatidylserine	25 mg
Gingko Biloba extract	10 mg
Vitamin B6	20.5 mg
Vitamin E	33.5mg
Resveratrol (Grape extract)	5 mg
Other ingredients: cod liver oil, seed oil products and byproducts, gelatin, glycerin, glyceryl, monostearate, titanium dioxide, FD&C Red 40 Guaranteed Analysis: Proteins 22.5%, Oils and Fats 46%, Cellulose <1%, Moisture/Humidity 6%, Ash 6%, Vitamin E (33.5 mg), Vitamin B6 (20.5 mg)	

Ceva Senilife XL®

Composition per capsule:	Small breeds (< 10 Kg)
Phosphatidylserine	62.5 mg
Gingko Biloba extract	25 mg
Vitamin B6	51.25 mg
Vitamin E	83.75 mg
Resveratrol (Grape extract)	12.5 mg
Other ingredients: cod liver oil, seed oil products and byproducts, gelatin, glycerin, glyceryl, monostearate, titanium dioxide, FD&C Red 40 Guaranteed Analysis: Proteins 18.5%, Oils and Fats 52%, Cellulose <1%, Moisture/Humidity 5%, Ash 7%, Vitamin E (83.75 mg), Vitamin B6 (51.25 mg)	

Annex III

Hill's Prescription Diet®

b/d® Canine Healthy Aging & Alertness

Ingredients

Whole Grain Corn, Chicken By-Product Meal, Pork Fat, Brewers Rice, Soybean Mill Run, Flaxseed, Soybean Meal, Fish Meal, Lactic Acid, Chicken Liver Flavor, Dried Egg Product, Soybean Oil, Dried Carrots, Dried Spinach, Dried Grape Pomace, Dried Tomato Pomace, Dried Citrus Pulp, Potassium Chloride, Oat Fiber, Choline Chloride, vitamins (Vitamin E Supplement, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Niacin Supplement, Thiamine Mononitrate, Vitamin A Supplement, Calcium Pantothenate, Vitamin B12 Supplement, Pyridoxine Hydrochloride, Riboflavin Supplement, Vitamin D3 Supplement, Folic Acid, Biotin), L-Lysine, Iodized Salt, Calcium Carbonate, L-Tryptophan, Taurine, minerals (Ferrous Sulfate, Zinc Oxide, Copper Sulfate, Manganous Oxide, Calcium Iodate, Sodium Selenite), L-Carnitine, Mixed Tocopherols for freshness, Lipoic Acid, Phosphoric Acid, Beta-Carotene, Natural Flavors.

Annex IV

Test food ingredients and formulation

The basis of the food was formulated to meet the American Association of Feed Control Official recommendations on adult dog food.

The supplement included – on the dry matter basis - L-Carnitine (330ppm), DL-α-Lipoic acid (150ppm), DL-α tocopherol acetate (1150ppm), taurine (1095 ppm), ascorbic acid (100 ppm), and the following were included at the percentage of 1%: spinach flakes, tomato pomace, grape pomace, carrot granules and citrus pulp.

Annex V

Symptoms associated with CDS based on the DISHA scale

Category	Description	Behaviours
Disorientation	Altered spatial orientation; Failure in recognizing places, people, routines.	Gets lost in familiar locations; Does not recognize tutors, other companion animals nor familiar objects; Goes to the wrong side of the door when leaving a room; Eyes fixed on the horizon; Does not go around the obstacles that are found it its way; Agitation; Restlessness; Wandering in the house; Does not finnish a task.
Social Interactions	Altered interaction with people or other companion animals.	Decreased greeting behaviour; Decreased playing behaviour (either with tutors, other companion animals or toys); Decreased interest in petting; Decreased reactivity to stimuli; Decreased capability of performing a previously learnt task; Increased agressivness towards humans/other animals.
Sleep / Wake Cycles	Altered sleeping patterns.	Restlessness during night time; State of insomnia or hypersomnia; Alterations in the amount of sleep; Vocalizing during night; Pacing.
House Soiling	Altered urination or defecation routines.	Indoor elimination behaviour in random locations; Incontinence.
Activity	Altered activity levels, decreased purpose activities and increase in apparent aimless activities.	Decreased reactivity to stimuli; Decreased exploratory behaviour; Pacing; Wandering; Altered appetite; Increased vocalization.

(Azkona et al., 2009 ; Osella et al., 2007)

Annex VI

Questionnaire for inclusion in the study.

Please answer these questions comparing the behaviours the dog exhibits now with behaviours the dog exhibited in adult age (consider five years old).

1. Appetite assessment (may tick more than one).

    a. No alterations in appetite
    b. Change in ability to physically handle food
    c. Change in ability to retain to food (vomits or regurgitates)
    d. Change in ability to find food
    e. Change in interest in food (may be olfactory, having to do with the ability to smell)
    f. Change in rate of eating
    g. Change in completion of eating
    h. Change in timing of eating
    i. Change in preferred textures

2. Assessment of elimination function (tick only one in each section)

    a. Changes in frequencies and "accidents"
        i. No change in frequency and no "accidents"
        ii. Increased frequency, no "accidents"
        iii. Decreased frequency, no "accidents"
        iv. Increased frequency with "accidents"
        v. Decreased frequency with "accidents"
        vi. No change in frequency but "accidents"

    b. Bladder Control
        i. Leaks urine when asleep, only
        ii. Leaks urine when awake, only
        iii. Leaks urine when awake or asleep
        iv. Full-stream, uncontrolled urination when asleep, only

v. Full-stream, uncontrolled urination when awake, only

vi. Full-stream, uncontrolled urination when awake or asleep

vii. No leakage or uncontrolled urination, all urination controlled, but in inappropriate or undesirable location

viii. No change in urination control or behavior

c. Bowel control

i. Defecates when asleep

ii. Defecates without apparent awarness

iii. Defecates when awake and aware of action, but in an inappropriate or undesirable location

iv. No changes in bowel control

3. Interactions with humans – which situation best describes that interaction? (tick **only one**)

a. No change in interaction with people

b. Recognizes people but slightly decreased frequency of interaction

c. Recognizes people but greatly decreased frequency of interaction

d. Withdrawal but recognizes other pets

e. Does not recognize other pets

4. Interactions with other pets – which situation best describes that interaction? (Tick **only one**)

a. No change in interaction with other pets

b. Recognizes other pets but slightly decreased frequency of interaction

c. Recognizes other pets but greatly decreased frequency of interaction

d. Withdrawal but recognize other pets

5. Changes in sleep/wake cycle (tick **only one**)

a. No changes in sleep patterns

b. Sleeps more in day, only

c. Some change – awakens at night sleeps more in day

d. Much change – profoundly erratic nocturnal pattern and irregular daytime pattern

e. Sleeps virtually all day, awake occasionally at night

f. Sleeps almost around the clock

6. Is there anything else you think we should know?

Annex VII

Purina ProPlan Sensitive Derma

Composition: Salmon (14%) , rice (14%) , corn , dehydrated salmon protein , corn gluten meal , corn semolina , soy meal , animal fat , viscera organoleptic , dehydrated beet pulp , dehydrated egg , starch corn, mineral , fish oil, dehydrated chicory , soya oil .

NUTRIENTS	Unit	
Humidity	%	9.5
Protein	%	27.0
Fat	%	15.0
Carbohydrates	%	38.0
Fiber	%	3.0
Ash	%	7.5
MINERALS		
Calcium	%	1.40
Phosphorus	%	0.93
Sodium	%	0.50
Chlorine	%	0.88
Potassium	%	0.64
Magnesium	%	0.12
Iron	mg/kg	160
Zinc	mg/kg	205
Copper	mg/kg	19
Manganese	mg/kg	50
Iodine	mg/kg	2.7
Selenium	mg/kg	0.47
VITAMINS		
Vit A	IU/kg	24439
Vit D3	IU/kg	1900

Vit E	mg/kg	479
Vit K	mg/kg	0.17
Vit C	mg/kg	70
Vit B1	mg/kg	30
Vit B2	mg/kg	13
Vit B3	mg/kg	159
Vit B5	mg/kg	47
Vit B6	mg/kg	16
Vit B8	mg/kg	0.15
Vit B9	mg/kg	4.60
Vit B12	mg/kg	0.25
Colina	mg/kg	3540
Taurina	mg/kg	750
ESSENTIAL FATTY ACIDS		
Ómega- 6	%	2.0
Linoleic acid	%	1.7
Omega- 3	%	1.1
DHA	%	0.50
EPA + DHA	%	0.92
ENERGY		
Calculated metabolizable energy	Kcal/g	3.550

Annex VIII

Purina ProPlan Senior Original

Composition: dehydrated poultry protein , Wheat, Corn , Chicken (14%) , gluten, soy flour, fractions of vegetable oil, rice (4%) , dried beet pulp , minerals , corn semolina , animal fat , viscera organoleptic , Fish oil.

NUTRIENTS	Unit	
Humidity	%	9.5
Protein	%	29.0
Fat	%	15.0
Carbohydrates	%	37.0
Fiber	%	2.0
Ash	%	7.5
MINERALS		
Calcium	%	1.3
Phosphorus	%	0.86
Sodium	%	0.52
Chlorine	%	1.02
Potassium	%	0.86
Magnesium	%	0.12
Iron	mg/kg	201
Zinc	mg/kg	219
Copper	mg/kg	20
Manganese	mg/kg	57
Iodine	mg/kg	2.8
Selenium	mg/kg	0.4
VITAMINS		
Vit A	IU/kg	16545
Vit D3	IU/kg	713
Vit E	mg/kg	559

Vit K	mg/kg	0.10
Vit C	mg/kg	82
Vit B1	mg/kg	19
Vit B2	mg/kg	10
Vit B3	mg/kg	122
Vit B5	mg/kg	33
Vit B6	mg/kg	12
Vit B8	mg/kg	0.18
Vit B9	mg/kg	3.30
Vit B12	mg/kg	0.19
Colina	mg/kg	2025
Taurina	mg/kg	872
ESSENTIAL FATTY ACIDS		
Ómega- 6	%	2.0
Linoleic acid	%	1.9
Omega- 3	%	0.3
DHA	%	0.07
EPA + DHA	%	0.23
ENERGY		
Calculated metabolizable energy	Kcal/g	3 585

Annex IX

Questionnaire – Assessment tool

Assessing Disorientation

(1 – Never; 2 – Seldomly; 3 – Sometimes; 4 – Frequently; 5 - Always)

	0	1	2	3	4	5
1. How frequently does your dog get lost in familiar locations? (e. g. At home they may not find their way to their bed)						
2. How frequently does your dog go to the wrong side of the door when leaving a room?						
3. How frequently does your dog wanders in the house with no apparent purpose?						

Assessing Social Interaction

(1 – Almost never shows interest; 2 –Decreased; 3 – The same; 4 – More often; 5 - Always)

	0	1	2	3	4	5
1. Interacting with humans. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog shows interest in interacting with humans has… (e. g. playing, petting, greeting)						
2.Interacting with other pets Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog shows interest in interacting with other pets has…(e. g. playing, barking, smelling)						
3. Remember some tasks you taught your dog. (e.g. sitting, laying down, fetching) Nowadays the number of times your dog is capable of performing them has…						

Assessing Sleep – Wake Cycles

(1 – Almost never; 2 –Decreased; 3 – The same; 4 – More often; 5 - Always)

	0	1	2	3	4	5
1. Barking during the night. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog barks during the night for no apparent reason has…						
2. Sleeping during the day Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog spends most of his/her day sleeping has…						
3. Staying awake during the night. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog spends most of his/her night awake has…						

Assessing House Soiling

(1 – Almost never; 2 –Decreased; 3 – The same; 4 – More often; 5 - Always)

	0	1	2	3	4	5
1. Urinating in a "wrong" place. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog urinates in a place he is not supposed to has…						
2. Defecating in a "wrong" place. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog defecates in a place he is not supposed to has…						

Assessing Activity Levels

(1 – Almost never; 2 –Decreased; 3 – The same; 4 – More often; 5 - Always)

	0	1	2	3	4	5
1. Vocalization. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog vocalizes has…						
2. Appetite. Remember the typical behaviour of your dog in adult life. Nowadays your dog's appetite has… (e. g. eating more, asking your for food)						
3. Greeting behaviour. Remember the typical behaviour of your dog in adult life. Nowadays the number of times your dog greets you / other elements of the family has…						

(Azkona et al., 2009 ; Osella et al., 2007)

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