SCIENTIFIC RELEASE

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DOG AGEING:
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Victor FRAGUA, Anne LEPOUDÈRE - DIANA Pet Food

Pets' life span has been continuously increasing over the past years. In 2012, life expectancy for dogs reached 11 years, which represents an increase of 0.5 per year in comparison to 2002, while cat's life expectancy reached 12 years, a gain of 1 year for the same period (APOP, 2013). For the pet parents, the question raised is then no longer «is my pet going to live longer», but «is he or she going to get older and remain in good health».
Indeed, advanced age in dogs and cats is frequently associated with a number of changes affecting their health and welfare. Among these age-related changes, alterations of cognitive functions orchestrated by the brain such as memory, coordination of movements and learning capacity may occur. These cognitive deficits can deeply affect old dogs' and cats' behavior thus deteriorating the pet-owner relationship.
Thanks to advanced knowledge on the effect of ageing on brain, several solutions can now be proposed to preserve dog and cat cognitive performances over time. Nutritional strategies targeting cognitive health benefits are one of them.

BRAIN AGEING, WHAT'S HAPPENING?

Biological ageing is a complex set of processes leading to damages at organ and cellular levels. It results from oxidative stress characterized by a progressive accumulation of oxidative products and decreased endogenous antioxidant defense mechanisms *(Sechi 2015; Cupp 2007; Head 2008)*. It is associated with changes in body condition and composition, energy requirements, and metabolic activity, as well as impairment of organ functions and immune status.

Because the brain is a highly metabolically active organ, the oxidation damages associated with ageing have dramatic consequences on its integrity hence on its functionality *(Head 2008)*. With time, the brain undergoes various alterations ranging from brain structure modification to cellular and molecular changes (Figure 1):

- Brain main morphological changes include a loss of white matter and a reduction of cortical thickness and grey matter volume. This leads to a cerebral atrophy and a ventricular dilatation, and therefore an overall brain structure impairment.
- Age-related changes in the brain are also accompanied by a reduction in the number of neurons, as well as modifications to their morphology including an alteration of the number and length of dendrites.
- At molecular level, ageing induces biochemical changes resulting from decreased neuromediator receptor density and lowered neurotransmitter release in the synapses. It is also established that the concentrations of other synaptic markers such as neurotrophic factors are modified.
- Finally, ageing provokes a decreased sensitivity of insulin receptors, impacting brain metabolic activity and particularly brain energy metabolism.

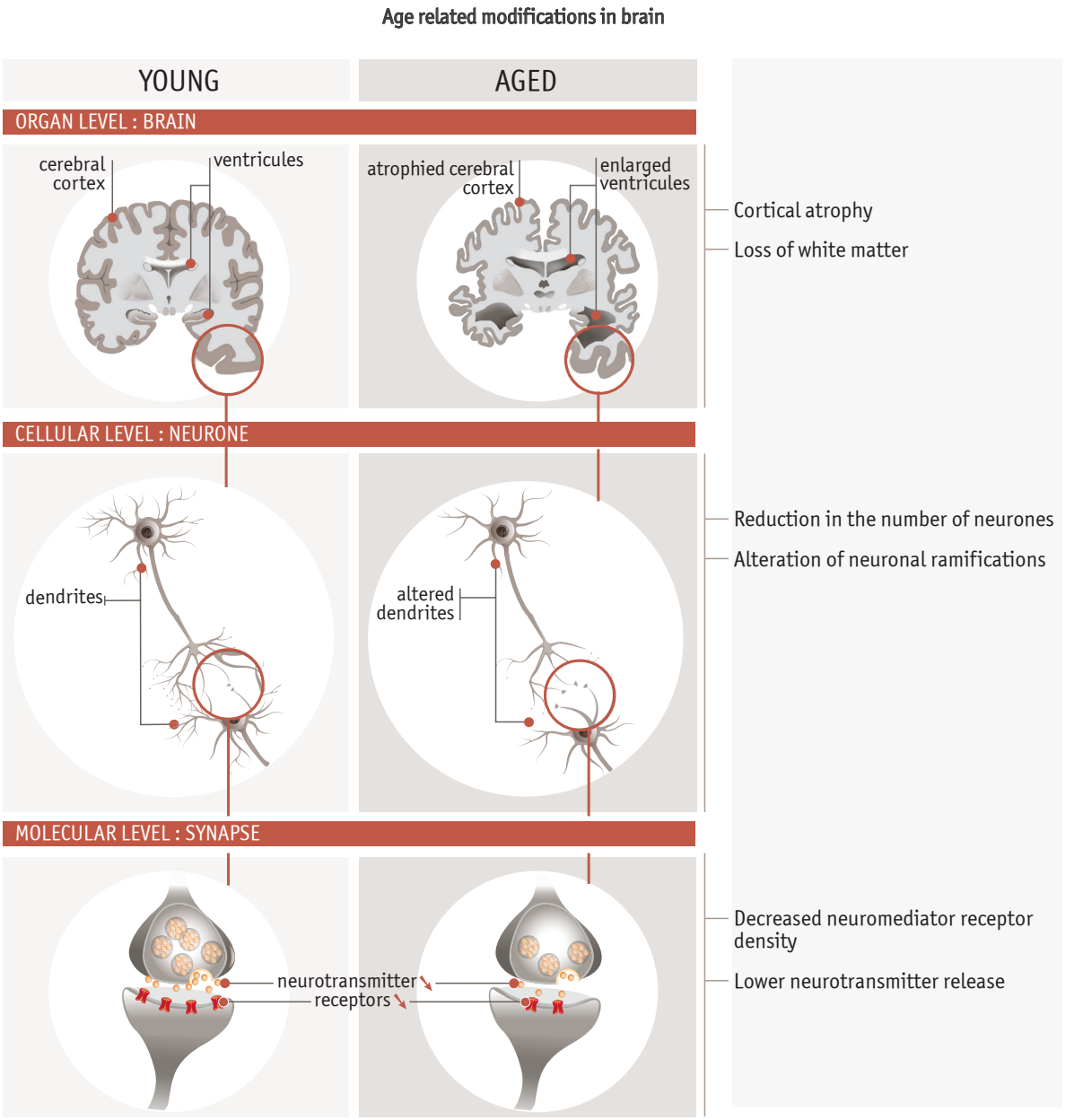


Figure 1: Main modifications occurring in dogs' and cats' brains ageing at organ, cellular and molecular levels

AGEING AND CONSEQUENCES: THE COGNITIVE DYSFUNCTION SYNDROME

Age-related impairments observed in neurons' morphological, chemical and functional properties – so called the synaptic plasticity - lead to a lower efficiency of the connection systems between neurons. They alter information transmission thus decreasing pets' cognitive performances such as memory, learning ability, attention, spatial abilities, etc. As a consequence, senior pets undergo various behavioral modifications perceived as problematic by the parents (Figure 2).

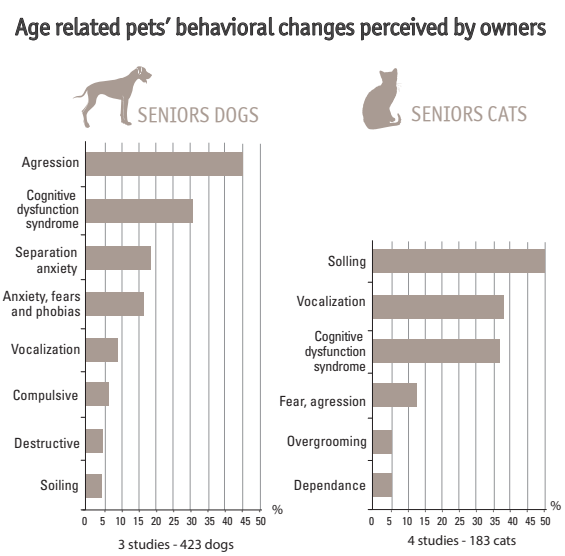


Figure 2: Prevalence of owner reported behavioral problems in senior dogs and cats *(Landsberg 2012)* – data expressed in % of pets showing each specific behavioral alteration

The behavioral and cognitive changes reported when pets get older are referred by Head and Landsberg as Cognitive Dysfunction Syndrome (CDS). In the studies conducted to evaluate the prevalence of CDS in pets, owners answer questionnaires to evaluate their animal behaviors according to the DISHA behavioral categories: Disorientation, Socio-Environmental Interaction, Sleep-wake cycles, House soiling, General Activity (Figure 3).

The frequency of CDS in dogs reported in literature range from 22.5% (age > 9 years, *Azkona 2009)* to 50% (age > 7 years, *Osella 2007*). In cats, Landsberg (2012) reported a prevalence of 28% in 11-15 year cats and 50% in cat older than 15 years.

Several risk factors are implied in CDS prevalence *(Azkona 2009; Katina 2016)*:

- Sex: higher prevalence for females in comparison to entire males
- Neutering: lower prevalence for entire dogs
- Quality of food : lower prevalence for dogs fed premium foods.
- Breed size: higher prevalence in medium/ large size older dogs (11-13 years)

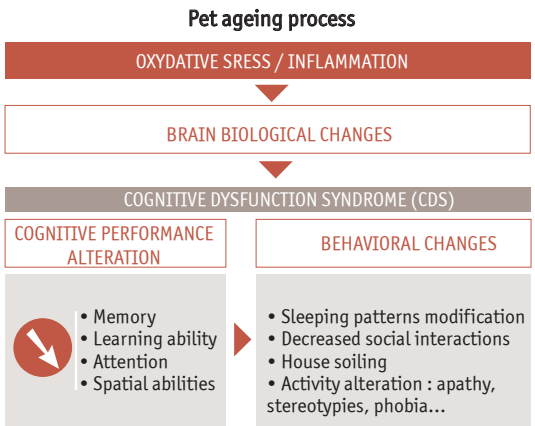


Figure 3: Impact of ageing on pet's cognitive performance and behavior

NUTRITION, A PREVENTIVE WAY TO LIMIT COGNITIVE DECLINE

Nutritional strategies to limit cognitive decline in pets mainly focus on the prevention of age-related morphological and metabolic changes in the brain. They aim at reducing neuron loss and manage brain aging by correcting early metabolic changes and minimizing risk factors such as oxidative stress. To be efficient, this nutrition must be started as early as possible.

Reducing oxidative stress and inflammation

Supplementation with antioxidants such as vitamins E and C, Selenium and polyphenols have revealed a protective effect over all pathophysiologic mechanisms involved in the development of the cognitive dysfunction syndrome *(Bosch et al., 2007; Forbes et al., 2015)*. Indeed, these molecules offer protection against oxidative and inflammation-induced damage in both brain tissue and blood vessels.

Other nutriments are known to have an effect on brain health, although their specific mechanisms of action are still unknown *(Pan et al., 2013)*:

- Docosahexaenoic acid (DHA), a fatty acid widely found in fish oil, has shown beneficial preventive properties because of its anti-inflammatory and vascular protection effect.
- Arginine, an essential amino acid for dogs and cats, has been linked to blood pressure control and cognition performance preservation.
- Vitamins of the B group limit the formation of homocysteine, a homologue of cysteine that can disturb brain metabolism.

Providing the brain with alternative sources of energy

Another well-studied strategy to counteract age-detrimental effects in dogs aims at increasing energy metabolism in the brain *(Pan et al., 2010)*. Since insulin and insulin receptors decrease in the brain with aging, the use of energy sources other than glucose can improve cognitive function in aged dogs. Several studies have shown that diet supplementation with medium-chain triglycerides, mainly found in coconut and palm kernel oil, provides the brain with a usable source of energy in the form of ketones.

POLYPHENOLS: MOLECULES OF HIGH INTEREST

What are polyphenols?

Polyphenols are phytochemical compounds, today considered as essential micronutrients. They naturally exist in plant products including fruits (e.g. grapes, apples, and various berries), vegetables (particularly broccoli, onion, cabbage), nuts, cereals, legumes (soybean), herbs, cocoa and tea. In humans, regular polyphenol consumption has been associated with a reduced risk of a number of chronic diseases, including cancer, cardiovascular disease and neurodegenerative disorders.

Over 500 different polyphenols exist and are classified based on their structure, with the phenolic hydroxyl groups as common structural feature. Figure 4 presents the four main classes of dietary polyphenols:

- Flavonoids: including flavones, flavonols, flavanols, flavanones, isoflavones and anthocyanidins; being mainly found in berries and grapes.
- Phenolic acids: the most common being caffeic acid, present in many fruits and vegetables, and ferulic acid, present in cereals.
- Stilbenes : the most studied being resveratrol, found in grapes, grape products and red wine.
- Lignans: mainly found in linseed (secoisolariciresinol).

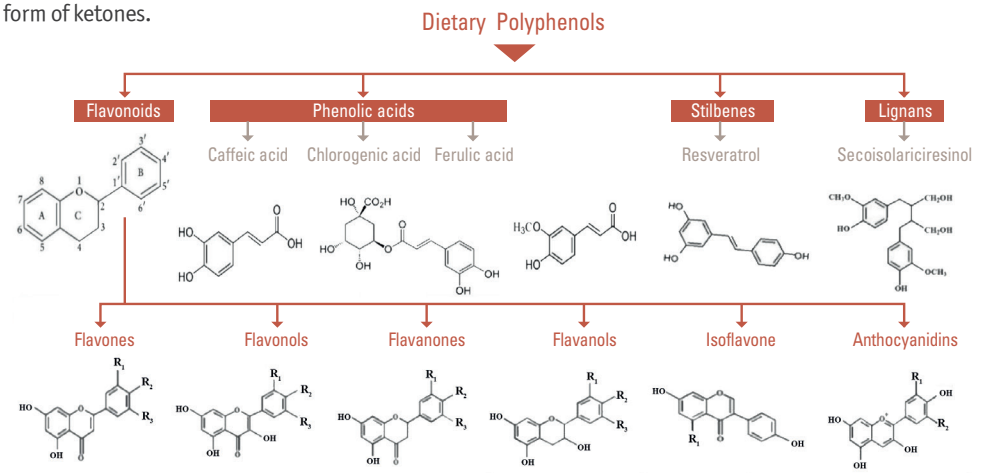


Figure 4: Dietary polyphenols - Adapted from Kim 2016

How do polyphenols work?

Polyphenols are well known for their antioxidant functions resulting from their chemical structure. However, beyond exerting direct antioxidant effects, polyphenols also interact with cellular pathways involved in chronic diseases such as cancer and neurodegenerative disorders *(Vauzour 2010)*.
Most polyphenols have to be hydrolyzed in the intestine to be absorbed. This is possible thanks to intestinal enzymes or with the support of the colic microflora. During absorption, the chemical structure of polyphenols is modified in the small intestine and later in the liver. As a consequence, polyphenols and their metabolites are found in the bloodstream *(Mata-Bilbao, 2008)*. They can then enter tissues and particularly the brain, where they are metabolized. Neuroprotective benefits have already been demonstrated in rats after resveratrol or anthocyane oral ingestion *(review by Bensalem, 2014)*.

PREVENTING DOG COGNITIVE DECLINE WITH POLYPHENOLS

Since september 2011, Diana Pet Food has been working with 10 industrial and academic partners from France and Canada to evaluate the interest of polyphenol supplementation in the prevention of cognitive decline in dogs (Neurophenols Consortium; www.neurophenols.org). In this study - Phenomind - a new patent pending ingredient mixing grape extract from *Vitis vinifera* and wild blueberry powder from *Vaccinium angustifolum* developed by Vit2be, DIANA Pet Food's activity dedicated to Pet Health and Nutrition, was evaluated.

Innocuousness

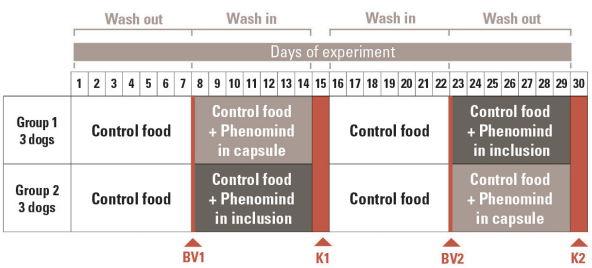
A preliminary study was first conducted at Oniris National College of Veterinary Medicine, Food Science and Engineering (Nantes, France), to confirm Phenomind safety in dogs. Twenty four Beagle dogs were fed a maintenance diet during 6 months. Among them, four groups of 6 dogs each were supplemented with capsules containing either maltodextrin (Control food) or Phenomind-rich mixtures at 4, 20 and 40 mg/kg BW/day.

Results showed that dogs supplemented with Phenomind on a long-term basis at different dosages, including up to ten times the intentional dose of the ingredient of 4 mg/kg BW/day, had no alteration in their general health status, thus showing the innocuousness of the ingredient.

Bioavailability

Phenomind bioavailability was also assessed at Oniris. The first objective of the study was to confirm that the main metabolites of polyphenol degradation were found in blood. The second objective was to compare the efficacy of two different modes of administration: capsule administration or inclusion in dry pet food before the extrusion process. Figure 5 presents the experimental design used to assess Phenomind bioavailability.

Phenomind bioavailability study Experimental design



BV : Polyphenol metabolites Basal Value
K : Polyphenol metabolites Kinetic of absorption

Figure 5: Experimental design of Phenomind bioavailability study

Six Beagle were included in a crossover design. They were divided into two groups of 3 dogs which alternated periods of 7 days wash out where they received a control food without Phenomind, and periods of 7 days wash in where they received Phenomind either in capsules (4 mg/kg BW) or included in the diet (240 ppm). After each wash out period, the dogs' basal value of plasmatic polyphenol metabolites was measured (BV1, BV2). After each wash in period, the kinetics of absorption of these metabolites were followed during 8 hours (K1, K2).

Figure 6 shows the amount of polyphenol metabolites from Stilbens, Phenolic acids, Flavanols, Flavonols and Anthocyanidins families found in the dogs' plasma after phenomind supplementation in both capsule and inclusion. Data are presented as the area under the curve, based on an extrapolation of their kinetics of absorption.

Since basal values (BV1 and BV2) were almost zero for all the polyphenol families, we may state that main polyphenols in Phenomind were extensively represented by their respective metabolites in dog plasma after each wash in period, demonstrating that polyphenols in Phenomind were bioavailable in dogs. Moreover, there was no significant effect of the mode of administration on the Phenomind metabolites dosed in the blood ($p > 0.1$).

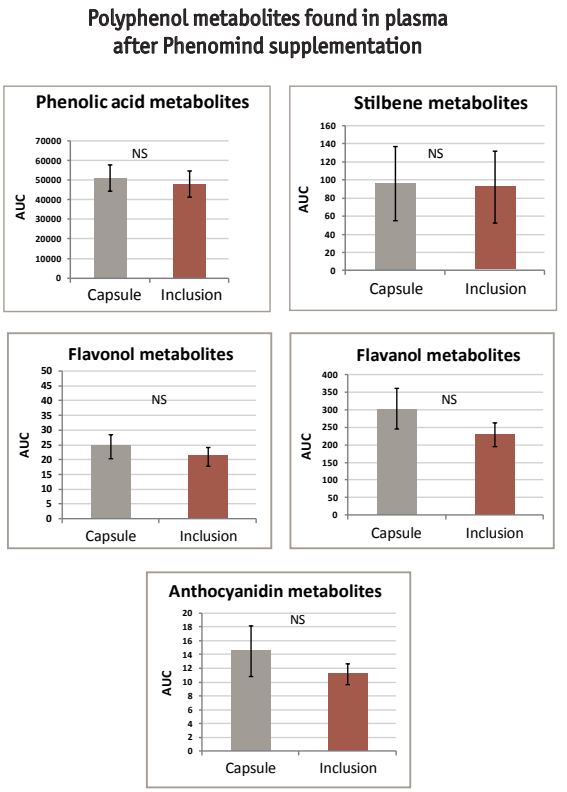


Figure 6: Plasma metabolites corresponding to the main families of polyphenols after Phenomind administration either in capsules or included in the diet - AUC

Efficacy

The efficacy of Phenomind consumption on dogs cognitive abilities, particularly on learning and working memory, was also evaluated. This trial was carried out at CanCog (Toronto, Canada). Thirty-five Beagle dogs in good general health were included in the trial. The ages of the animals ranged from 8.0 to 14.5 years at the study initiation. The dogs' cognitive abilities were evaluated based on the Delayed Non-Matching Position (DNMP) task. This test comprises two phases (Figure 7):

- Sample phase: dogs are required to displace an object placed over 1 out of 3 possible positions on a food well. The block to be displaced covers a food reward (a wet canned dog food);
- Test phase: after a delay, dogs are presented with 2 identical objects: the first object is placed at the same position as for the sample phase, the second object is placed in one of the 2 remaining positions (non matching positions). In this phase dogs must deplace the object in the new position to get the food reward.

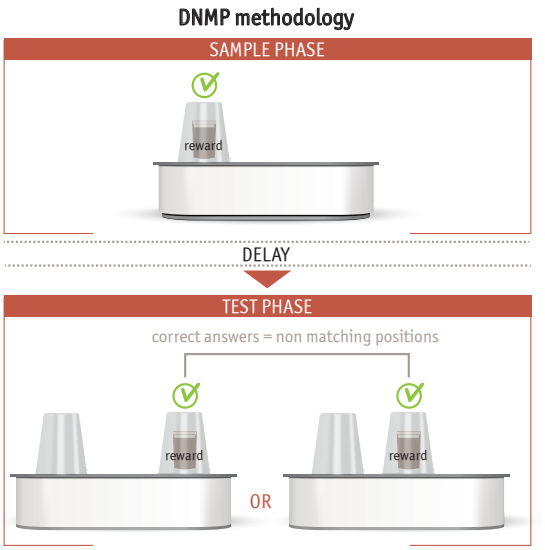


Figure 7: Dogs' cognitive abilities evaluation protocole with the Delayed Non-Matching Position (DNMP) task

As a first step, the 35 dogs performed 12 DNMP trials per day during 6 days. They were then divided into 3 groups according to their individual performance on DNMP, so as to have an homogeneous average performance between groups. The 3 groups of dogs were then fed during 58 days with experimental diets containing 0 ppm Phenomind, ("Control food", 11 dogs), 240 ppm Phenomind ("240 ppm Phenomind", 12 dogs), or 480 ppm Phenomind ("480 ppm Phenomind", 12 dogs). At the end of this period, the dogs' were tested again on DNMP performances following the same procedure as in the first step of the experiment (6 days, 12 DNMP trials per dog per day). Results shown in figure 8 indicate that the dogs ' fed Phenomind-rich diets, regardless of the dosage, showed significantly greater cognitive improvements than those which did not receive any supplementation.

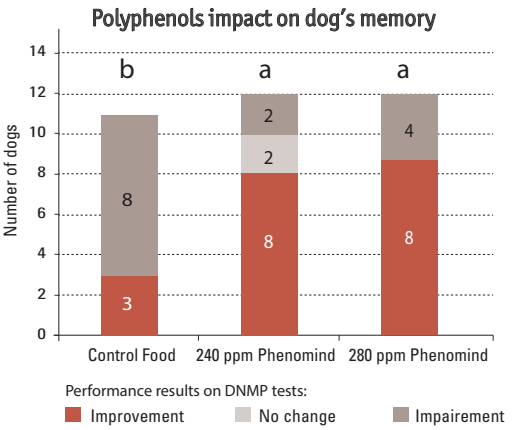


Figure 8: Change in DNMP performances prior to and after 58 days of Phenomind supplementation

CONCLUSION

The pet ageing process is associated with a high oxidative stress leading to numerous physiological changes. Brain structure and functionality are particularly sensitive to these alterations. Because of less efficient connection systems occurring between neurons, pets' cognitive performances thus significantly decrease and the related behavioral modifications ocurring when pets get older can strongly affect the owners' relation with their companions.

Food supplementation with targeted bioactive molecules is a promising strategy to counteract age related effects. By preventing early metabolic changes in the brain, specifically formulated blends combining natural sources of polyphenol are for instance able to significantly improve senior dogs' working memory and learning capabilities. Dog food manufacturers can efficiently and conveniently include this type of innovative ingredients in their products to bring valuable long term health benefits to companion animals.

REFERENCES

• APOP, 2013. Association for pet obesity prevention. National pet obesity survey results. http://www.petobesityprevention.org.

• Azkona G, García-Belenguer S, Chacón G, Rosado B, León M, Palacio J. Prevalence and risk factors of behavioural changes associated with age-related cognitive impairment in geriatric dogs. J Small Anim Pract. 2009 Feb;50(2):87-91.

• Bensalem J. PhD thesis. Effets des polyphénols de baies sur le déclin cognitive lié au vieillissement chez la souris. Université de Bordeaux, décembre 2014.

• Bosch, G., Beerda, B., Hendriks, W. H., Van der Poel, A. F. B., & Verstegen, M. W. A. (2007). Impact of nutrition on canine behaviour: current status and possible mechanisms. Nutrition research reviews, 20(02), 180-194.

• Cupp C.J., J.P. Clementine, Wendell WK, Avinash R.P., Perez-Camargo G. Effect of nutritional interventions on longevity in senior cats. Intern J Appl Res Vet Med, Vol. 5, No. 3, 2007.

• Forbes, S. C., Holroyd-Leduc, J. M., Poulin, M. J., & Hogan, D. B. (2015). Effect of nutrients, dietary supplements and vitamins on cognition: a systematic review and meta-analysis of randomized controlled trials. Canadian Geriatrics Journal, 18(4), 231.

• Head E., Rofina J., Zicker S. Oxidative Stress, Ageing and CNS disease in the Canine Model of Humain Brain Ageing. Vet Clin North Anim Pract. 2008 January; 38(1):167-vi.

• Katina S., Farbakova J., Madari A., Novak M. and Zilka N. Risk factors for canine cognitive dysfunction syndrome in Slovakia. Acta Vet Scand (2016) 58:17.

• Kim Y., Keogh J.B., Clifton P.M. Review. Polyphenols and glycemic control. Nutrients 2016, 8, 17.

• Landsberg GM, Nichol J, Araujo JA. Cognitive dysfunction syndrome: a disease of canine and feline brain aging. Veterinary Clinics of North America: Small Animal Practice. 2012 Jul 31;42(4):749-68.

• Mata-Bilbao, MD, Andrés-Lacueva C, Jáuregui O, Lamuela-Raventos RM. Determination of flavonoids in a Citrus fruit extract by LC–DAD and LC–MS. Food Chemistry. 2007 Dec 31;101(4):1742-7.

• Osella M, Re G, Odore R, Girardi C, Badino P, Barbero R, et.al. Canine cognitive dysfunction syndrome: Prevalence, clinical signs and treatment with a neuroprotective Nutraceutical. Appl Anim Behav Sci 2007; (105):297-310.

• Pan, Y., Larson, B., Araujo, J. A., Lau, W., De Rivera, C., Santana, R., ... & Milgram, N. W. (2010). Dietary supplementation with medium-chain TAG has long-lasting cognition-enhancing effects in aged dogs. British journal of nutrition, 103(12), 1746-1754.

• Pan Y., Araujo J.A., Burrows J., de Riveira C., Gore A., Bhatnagar S., Milgram N.W. Cognitive enhancement in middle-aged and old cats with dietary supplementation with a nutrient blend containing fish oil, B vitamins, antioxidant and arginin. British Journal of Nutrition (2013), 110, 40-49.

• Sechi S., Chiavolelli F., Spissu N., Di Cerbo A., Canello S., Guidetti G., Fiore F., Cocco R. Research article. An antioxidant dietary supplement improves brain-derived neurotrophic factors levels in serum of aged dogs: preliminary results. Hindawi Publishing Corporation, Journal of Veterinary Medicine, Volume 2015, Article ID 412501, 9 pages 2015.

• Vauzour D., Rodriguez-Mateos A., Corona G., Oruna-Concha M.J. and Spencer J.P.E. Review, Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action. Nutrients 2010, 2, 1106-1131.

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