HEALTHY LIVING

A SCIENTIFIC SUPPORT PAPER

The Hypoallergenic Healthy Living diet provides a 75% reduction in free radicals to support a healthy immune system.

University of Liège – 2024 Study.

CONTENTS

WHY ARE HEALTH AND WELL-BEING IMPORTANT?

Overall health and well-being are crucial for dogs for several reasons, all of which contribute to their quality of life and longevity.

Companion animals have specific nutritional requirements depending on their species and life stage (FEDIAF, 2024).

Pet parents are increasingly aware of the importance of high-quality food to ensure that their pets develop, grow and continue to age in good health. As a result, many countries now have a substantial ageing pet population as well as a human one, which accelerates market demand for multi-functional pet nutrition. Advances in veterinary care and companion animal health requirements have dramatically increased life expectancy in pets.

However, with this, there is an increase in the prevalence of several common health concerns and diseases among companion animals, which largely mirror the increase observed in similar diseases in humans. Such increases are of particular concern because many of the most common health conditions in dogs are largely avoidable through nutritional management and preventive care (Spofford et al., 2013).

WHY ARE PETS LIVING LONGER?

Each pet is different; however, researchers have identified key factors that are driving the overall increase in life expectancy, such as an improved understanding and awareness of health needs and better-tailored nutrition.

Three broad factors are thought to influence how long an individual dog lives: the genetic make-up of the individual, the living environment and conditions, including diet from a puppy throughout adulthood, and finally, the occurrence of disease.

Healthy development, living and ageing are lifelong concepts referring to the preservation of optimal physical and mental well-being in puppies and adult dogs and are characterised by a long health span during which an individual is generally healthy and free from serious disease. This is most likely to be achieved through the promotion of optimum everyday health and well-being (Adams et al., 2018).

WHAT IS AN ALL LIFE STAGES RECIPE?

An "All Life Stages" recipe can be suitable for both puppies and adult dogs because it is formulated to meet the nutritional requirements of the most demanding life stage—typically growth and reproduction.

According to FEDIAF Nutritional Guidelines, if a pet food meets the nutrient profile for puppies, it also meets or exceeds the needs of adult dogs. These recipes are more nutrient-dense, often containing higher levels of protein, metabolisable energy and other essential minerals and fatty acids to support healthy development in puppies.

As a consequence of the increase in life expectancy of pets, **adult and senior individuals represent an increasing proportion of the total dog population.** During the ageing process, numerous metabolic and physiological changes occur, including oxidative stress and inflammation

Early preventive nutrition promoting protective ingredients may therefore be an effective strategy to limit the development of age-related disorders and ensure healthy living and ageing of pets.

WHAT CONSTITUTES GOOD OVERALL HEALTH IN PUPPIES AND ADULT DOGS?

Good overall health in puppies depends on a combination of proper nutrition, veterinary care, growth, and environmental factors. A healthy puppy should be fed a balanced, age-appropriate diet rich in essential nutrients as per the FEDIAF (European Pet Food Industry Federation) Nutritional Guidelines.

Puppies and adult dogs have different nutritional requirements because they are at distinct stages of physiological development, and this is clearly reflected in FEDIAF Nutritional Guidelines.

Puppies are in a rapid growth phase and require higher levels of energy, protein, fat, and specific minerals such as calcium and phosphorus to support the development of muscles, bones, organs, and the immune system.

FEDIAF highlights that proper calcium-to-phosphorus ratios are particularly important in puppies, especially large breeds, to ensure healthy skeletal growth.

Digestive health is critically important in puppies because their digestive systems are still developing and play a vital role in their overall growth, immune function, and well-being.

A healthy gastrointestinal tract allows puppies to efficiently break down food and absorb essential nutrients such as amino acids, fatty acids, vitamins, and minerals, all of which are **crucial for proper skeletal, muscular, neurological, and immune development.**

Research shows that the **digestive efficiency in puppies is lower than in adult dogs**, particularly for nutrients like protein and fat, making the composition and digestibility of their diet especially important (Meyer & Zentek, 2005).

If digestion is compromised, **puppies may suffer from malabsorption or nutrient deficiencies**, even when fed a complete diet, potentially impairing development and growth.

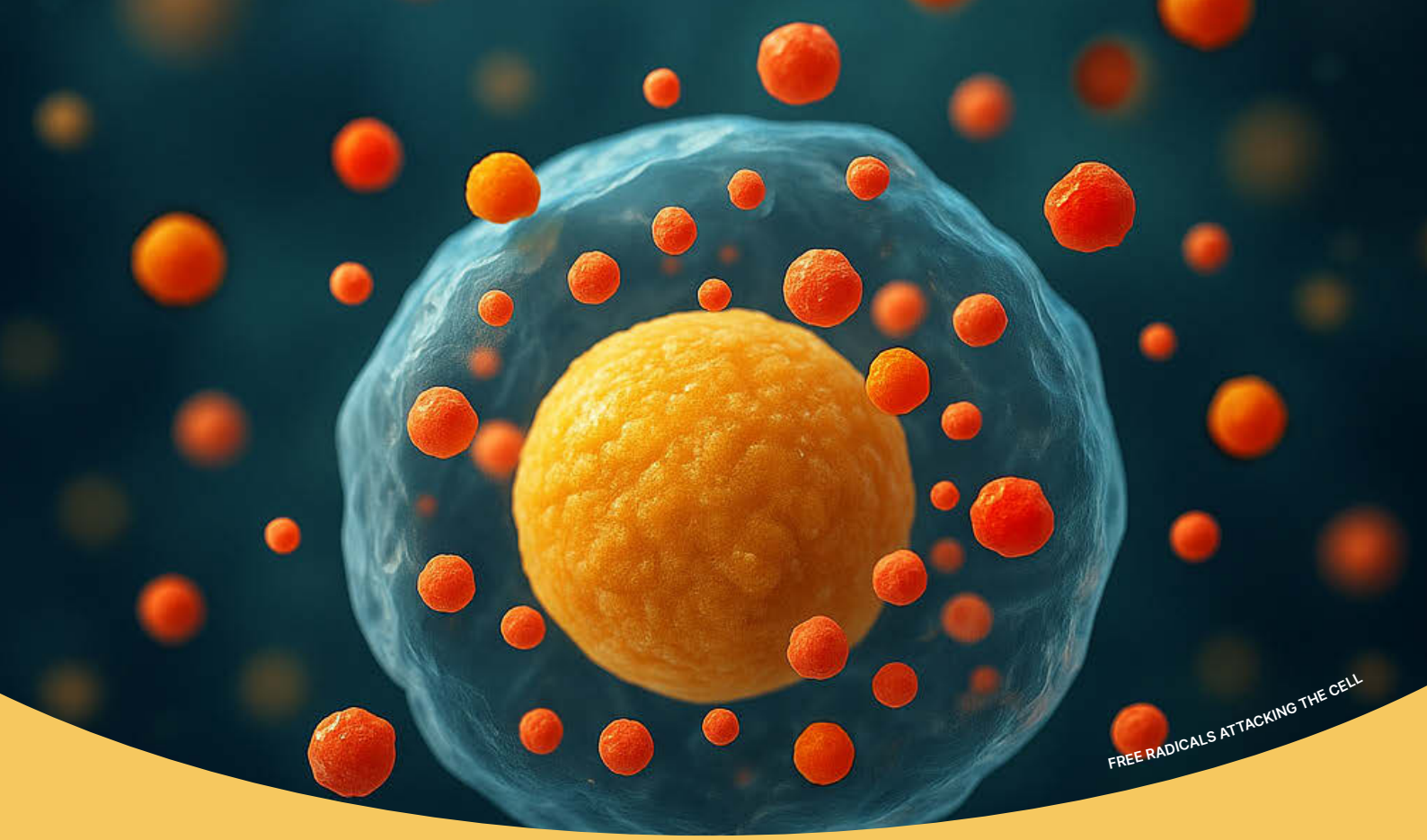
Several key factors influence good overall health in adult dogs to ensure their physical and mental well-being, for example:

A healthy digestive system is very important to the overall health of dogs since its primary role is to digest food and absorb the nutrients so the body can use them for energy, growth, maintenance and repair.

A dog's skin and coat can be perceived as an immediate indicator of their health and well-being. Both the skin and coat are integral to providing a physical barrier that protects a dog from external objects as well as physical, chemical and environmental stressors that may cause harm internally.

The major objectives of a feeding program designed for adult pets should include **maintaining health and optimal body weight**, and preventing or slowing chronic disease. The maintenance of a lean body condition has been proven to **increase both the quantity and quality of life in dogs** (Kealy et al., 2002).

Secondly, there is a **clear link between excess weight and joint problems in dogs**. Excessive weight will apply additional pressure on joints. **When a joint is overloaded, this can cause cartilage breakdown and increase the risk of joint damage.**



FREE RADICALS ATTACKING THE CELL

WHAT IS OXIDATIVE STRESS?

Oxidative stress is defined as excessive production of reactive oxygen species **(ROS),** also known as **free radicals,** in cells and tissues when antioxidant defences are not present in sufficient amounts to neutralise them.

The term “free radical” is used to describe a highly reactive molecule, having one or more unpaired electrons in the last electron layer (Halliwell & Gutteridge, 2015).

At high concentrations, ROS can interact with the biomolecules that are present in tissues, cell membranes, and organelles, causing cell damage.

Under normal conditions, the organism can neutralise the effects of the free radicals by using its antioxidant defences.

However, in situations of imbalance between oxidant and antioxidant agents, the production of free radicals exceeds the neutralising capacity of organic compounds, resulting in oxidative stress (Halliwell & Gutteridge, 2015).

Of all the cellular components compromised by the harmful effects of ROS, cell membranes are the most severely affected owing to lipid peroxidation, which invariably leads to alterations in the membrane structure and permeability.

Oxidation is an autocatalytic reaction which has the potential to produce both large quantities and varieties of oxidation products

Lipid peroxidation results in the accumulation of end products, for example, malondialdehyde, which has known **harmful effects on health** (Halliwell & Gutteridge, 2015).

Another **major consequence of oxidative stress is DNA damage**, which includes base modifications, nucleotide oxidation, loss of bases and strand breaks.

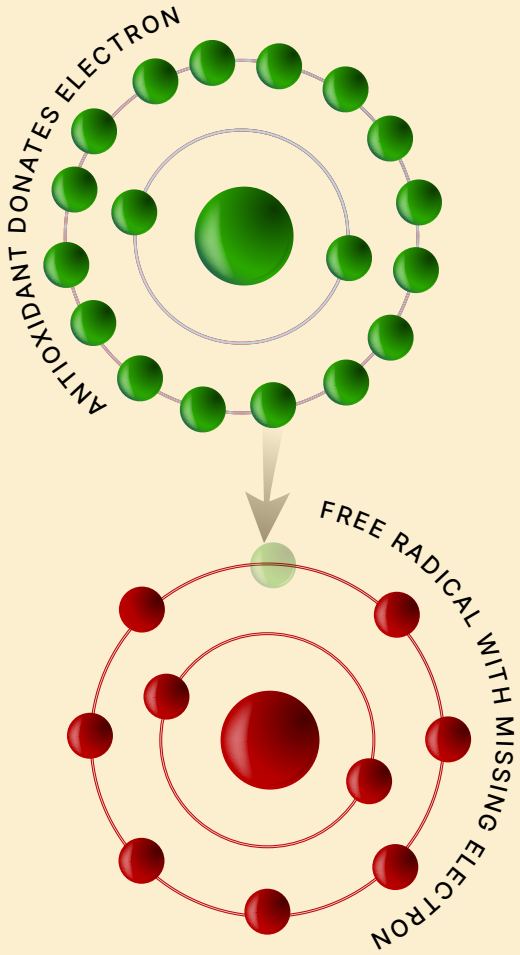
These examples will cause modification to the structure of DNA, which may alter cell metabolism, such as replication or transcription.

Not all ROS affect DNA similarly; superoxide and hydrogen peroxide at physiologically-relevant levels do not readily react with intact DNA (via redox chemistry); however, they can react with other molecules and produce ROS such as hydroxyl radicals, which readily react with DNA (Gonzalez-Hunt et al., 2018).

Nonpathological conditions in which oxidative stress also occurs in dogs include free radical production during physical activity.

A significant increase in the levels of the antioxidant glutathione peroxidase enzyme was observed in dogs submitted to intense exercise for a short period of time (Pólózel, 2011).

In that study, the increased levels of glutathione peroxidase were related to an increased production of free radicals, a secondary effect of the higher oxygen consumption by the body during physical exertion. This demonstrates the importance of antioxidants in dogs.



WHAT ARE ANTIOXIDANTS?

Antioxidants are substances that **can delay or prevent the oxidation of nucleic acids, proteins, lipids, or carbohydrates**.

In the scavenging process, antioxidants produce a more stable compound after they react with a free radical. The basic concept of antioxidant scavenging of ROS involves the antioxidant donating a single electron to a free-radical species (Halliwell & Gutteridge, 2015).

Reducing free radicals through antioxidant activity plays a crucial role in supporting a healthy immune system.

While ROS are naturally produced during immune responses, excessive ROS can be harmful to the immune system itself. High levels of ROS can damage immune cells, including T lymphocytes, B cells, and antigen-presenting cells, by oxidising cellular membranes, proteins, and DNA, leading to impaired proliferation, signalling, and even apoptosis (Dröge, 2002).

In dogs, antioxidant supplementation has been shown to preserve immune cell viability, reduce markers of inflammation, and improve clinical outcomes in conditions associated with oxidative stress, such as canine osteoarthritis, dermatitis, and infectious diseases (Miller & Tainter, 2020; Fascetti & Delaney, 2021).

Furthermore, excessive ROS can dysregulate immune signalling pathways such as NF-κB, **leading to chronic inflammation or immune suppression**.

Antioxidants help maintain redox balance and support controlled immune activation, which is essential for an effective yet non-damaging immune response.

In addition, ROS can compromise mucosal and epithelial barriers, such as the gastrointestinal tract, which plays a major role in canine immune defence.

By reducing oxidative damage, **antioxidants help maintain gut integrity, reducing pathogen entry and supporting a robust immune barrier** (Ogun, 2015; Hall et al., 2011).

Therefore, maintaining antioxidant capacity in dogs is essential to protect immune cells, regulate immune responses, and preserve physical immune barriers, ultimately contributing to overall immune system resilience and health.

THE IMPORTANCE OF BIOAVAILABLE AND BIOACTIVE PEPTIDES TO SUPPORT HEALTH IN DOGS DURING ALL LIFE STAGES

Proteins are large molecules made up of individual 'building blocks' called amino acids, and they are essential for dogs at all life stages.

After eating food containing protein, the process of protein digestion begins as enzymes released in different parts of the gastrointestinal tract break it down into protein hydrolysates: short chains of amino acids called peptides and free amino acids.

This enables these building blocks to be absorbed into the body where they can be recombined to build new proteins (such as skin, hair, muscle, antibodies, enzymes, hormones, etc).

Historically, it was believed that only free amino acids were absorbed from the gastrointestinal tract by specific amino acid transporters. In contrast, it is now recognised that the majority of amino acids are absorbed from the intestine as di- and tri-peptides by the broad-specificity peptide transporter PepT1 (Fei et al., 1994).

Di-peptides and tri-peptides are most abundant in the molecular weight range of 0.2–0.25 kDa and 0.3–0.4 kDa, respectively.

Research has shown that the intake of proteins that have already been hydrolysed (peptides) is more readily absorbed from the digestive tract than intact protein and even individual amino acids.

This ensures an ideal supply of amino acid building blocks required for the renewal and synthesis of key peptide hormones and proteins (Maebuchi et al., 2007; Zhao et al., 1997).

Protein hydrolysates produced from several seafood processing by-products possess several bioactive properties, including antioxidant and antimicrobial activity (Chalamaiah et al., 2012).

Fish by-products, such as skin, heads, dark muscles, viscera, and bones, are **rich in protein and are major sources of collagen and can be converted into peptides** through enzymatic protein hydrolysis.

Several studies have documented **bioactive properties of peptides, including antioxidant, anticholesteremic and antimicrobial in various species such as salmon.**

Salmon protamine hydrolysate was found to possess antioxidative activity against hydroxyl, 2,2-diphenyl-1-picrylhydrazyl and superoxide anion radicals

Through consecutive chromatographic methods including size exclusion, ion exchange chromatography and reverse-phase high-performance liquid chromatography (HPLC), a series of peptide fractions with high antioxidative activities were obtained (Wang et al., 2008).

Water-soluble peptides with a molecular weight below 10 kDa were isolated from residual materials of cod (liver, skin, and mix) and salmon (skin and mix) using cut-off filtration.

Liquid chromatography-mass spectrometry (LC-MS/MS) identified bioactive peptide motifs in all samples, including those with potential benefits for type 2 diabetes, cardiovascular health, immunomodulation, prolyl endopeptidase (PEP) activity, and antioxidant activity.

Antioxidant potential was confirmed through two assays: hydroxyl radical scavenging activity (HRSA) and ABTS radical cation decolourisation.

Salmon samples showed higher antioxidant activity than cod, with all samples (except cod skin) surpassing the antioxidant activity of alanine-histidine (AH), a known antioxidant dipeptide (Pampanin et al., 2016).

Black Soldier Fly (*Hermetia illucens*) Larvae protein derivatives (including proteins and protein hydrolysates) **contain a significant amount of short-chain peptides, known for their antioxidant properties**. This study evaluated the in vitro antioxidant potential of BSF protein derivatives using five different models.

Chicken meal and fishmeal, commonly used in pet food and aquaculture feeds, were used as industry benchmarks. The results revealed that **chicken meal and fishmeal provide little to no protection against oxidative damage** caused by neutrophils and myeloperoxidase activity. In some models, these meals even exhibited pro-oxidant effects.

In contrast, the BSF protein derivatives demonstrated effectiveness in protecting animal cells from oxidative damage due to immune responses (Mouithys-Mickalad et al., 2020).

The hydrolysed salmon present in the Hypoallergenic Healthy Living recipe contains naturally occurring antioxidants that can help protect against oxidative damage associated with ageing in dogs.

COLLAGEN PEPTIDES

Collagen is a protein found exclusively in animals, especially in the skin, bones and connective tissues of mammals, birds and fish. **Collagen provides and maintains the structural integrity of various tissues throughout the body.**

Type I collagen is the most abundant collagen, making up more than 90% of the protein content of bone, and is the major collagen of tendons (this type of connective tissue attaches muscles to bones) and ligaments (this type of connective tissue attaches one bone to another bone – holding joints together), providing structure and strength to these tissues.

Type II collagen is the predominant component of cartilage, the extremely strong, flexible and semi-rigid support tissue found at points where two bones meet, providing a smooth surface that allows joints to move easily and a 'cushion' effect to absorb the shock of impact, especially on the ends of weight-bearing bones (e.g., hip, elbow joints).

Collagen is essential for bone health. It provides the protein matrix ('scaffolding') on which calcification (bone mineralisation) can occur. Bone collagen undergoes continual breakdown, repair and renewal, so providing nourishment through dietary collagen or collagen peptides is important to help maintain lifelong strong, healthy bones.

In arthritic dogs supplemented with type II collagen, a significant increase in peak vertical force (N/kg body weight) and impulse area (N s/kg body weight) was reported, indicative of a **decrease in arthritis associated pain** (Gupta et al., 2012).

Supplementation with collagen peptides was proven to be **beneficial in dogs with osteoarthritis** that had not previously responded to osteoarthritic treatments. Results indicated a statistically **significant reduction in lameness** compared to the beginning of treatment. Pet owners also reported an i**mprovement in the daily routines of their dog**, including **significantly decreased discomfort in standing up and a clear reduction in contact pain.**

Collagen peptides naturally occurring within the hydrolysed salmon present in the Hypoallergenic Healthy Living recipe aim to support cartilage metabolism for the growth of healthy joints in puppies and help to maintain these healthy joints in adult and senior dogs.

WHAT MAKES THE HYPOALLERGENIC HEALTHY LIVING DIET SO UNIQUE?

The development and formulation of the Hypoallergenic Healthy Living recipe has centred around the 'Power of Peptides' using the latest Freshtrusion HDP® technology.

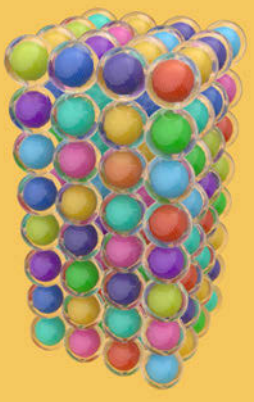
HDP freshtrusion® HIGHLY DIGESTIBLE PROTEIN

Freshtrusion HDP® (Highly Digestible Protein) is the unique process of cooking fresh meat and fish ingredients in the presence of a natural enzyme, which digests (hydrolyses) the protein into a mixture of peptides and free amino acids.

This increases the digestibility and bioavailability of the protein and improves palatability, through what we like to refer to as the Goldilocks Principle:

THE GOLDILOCKS PRINCIPLE

Instinctively, it would be assumed that intact protein would be best for a dog to digest as it contains all the nutritional elements together as one. Similarly, individual amino acids, broken down as small as possible, might be considered to be much easier to absorb. However, it has been proven in research studies that the ideal digestibility and absorption rates occur in small-chain peptides (≤ 3kDa). We like to refer to this as the 'Goldilocks principle'.



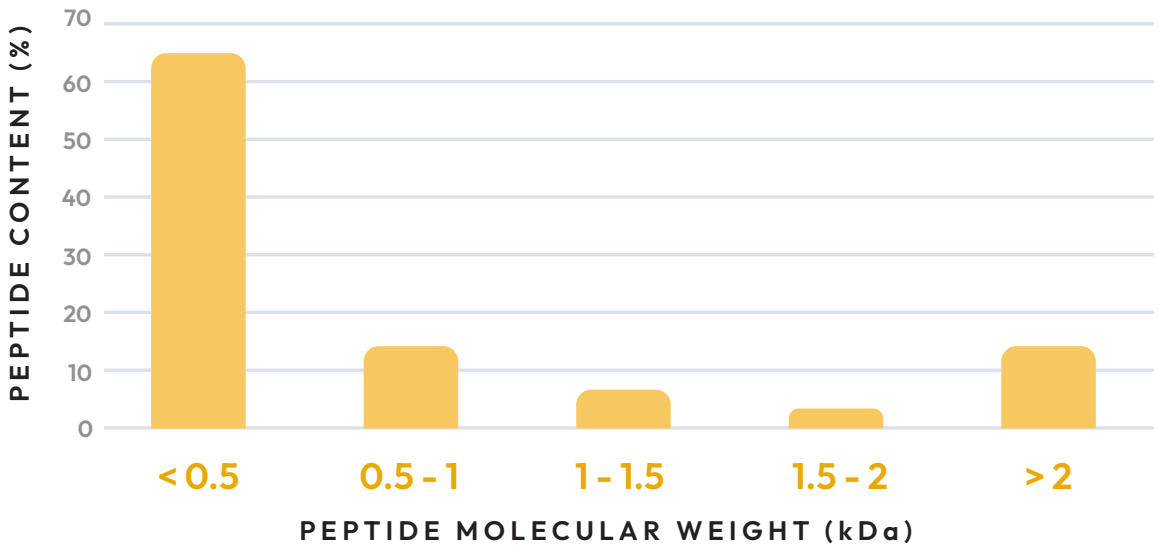
INTACT PROTEIN

DI AND TRI-PEPTIDES

SINGULAR AMINO ACIDS

TOO BIG JUST RIGHT TOO LITTLE

HYPOALLERGENIC HEALTHY LIVING : PEPTIDE CONTENT (%)



A minimum of 64% of the peptides in this recipe are < 0.5 kDa, with just 13% of the peptides > 2 kDa.

These results show the majority of peptides in the finished kibble fall into the < 0.5 kDa category, which includes the highly digestible and nutritionally beneficial dipeptides and tripeptides, achieving the Goldilocks Principle.

THE POWER OF THE PEPTIDES FOR HEALTHY LIVING

- ✔ **Increases the digestibility and bioavailability of the protein**
- ✔ **Improves the palatability of the recipe**
- ✔ **Ensures an ideal supply of amino acid building blocks required for the renewal and synthesis of key antioxidant proteins and structural proteins such as collagen**
- ✔ **Helps to support and maintain healthy joints and regain mobility**

WHAT OTHER INGREDIENTS ARE BENEFICIAL IN MAINTAINING GOOD HEALTH AND WELL-BEING?

In addition to the inclusion of hydrolysed protein, the Hypoallergenic Healthy Living diet includes a range of functional ingredients, including Coconut Oil, L-Carnitine and our unique Wellness Blend, which includes Seaweed, Turmeric, Orange, Carrot, Camomile, Linseed Fructooligosaccharides and Mannanoligosaccharides.

Feeding dogs medium-chain triglycerides (MCT) found in coconut oil, alongside fish oil, and L-carnitine-enriched foods, was found to mitigate age-related changes in serum fatty acids (FA) and carnitine metabolites. Forty-one healthy Beagles, with an average age of 9.9 years, were fed either a control or treatment diet for 6 months. The treatment diets included added L-carnitine and varying amounts of fish oil, along with MCT and reduced animal fat in treatment food.

Supplementation with fish oil and MCT led to increased levels of eicosapentaenoic and docosahexaenoic acids, as well as lauric and myristic acids, while reducing saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and arachidonate. Overall, the treatment diets helped counteract the effects of ageing on serum FA and carnitine metabolite concentrations (Hall & Jewell, 2012).

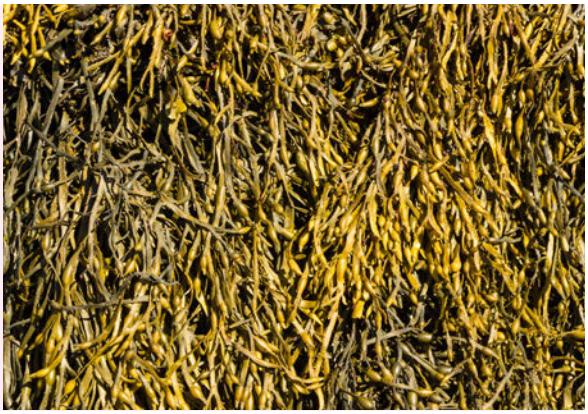
Beagle dog trials

Aged Beagle dogs were divided into control and treatment groups, with the treatment group receiving a diet supplemented with 5.5% MCT for 8 months. Cognitive tests assessing learning ability, visuospatial function, and attention were conducted before and after supplementation. The MCT-supplemented group performed significantly better on most tests, particularly on more difficult tasks.

Additionally, the MCT group had elevated levels of β-hydroxybutyrate, a ketone body. These findings suggest that long-term MCT supplementation can enhance cognitive function in aged dogs by providing an alternative energy source for the brain (Pan et al., 2010).

Seaweed

The seaweed *Ascophyllum nodosum* is commonly used in dental products for dogs. Although the exact mechanism of its action is still unclear, the results of our studies suggest that seaweed changes the composition of saliva in supplemented dogs by inhibiting or turning off some pathways that could enhance plaque or calculus development (Gawor et al., 2021).



Turmeric

Turmeric is reported as an effective therapeutic agent in traditional medicine for treating and preventing various diseases. It has demonstrated a broad spectrum of biological and pharmacological effects in drug delivery.

Turmeric has been actively used to treat ageing-related conditions, such as cardiovascular diseases, atherosclerosis, neurodegenerative disorders, cancer, rheumatoid arthritis, ocular diseases, osteoporosis, diabetes, hypertension, chronic kidney diseases, chronic inflammation, and infections.

The functional applications and therapeutic potential of curcumin in addressing ageing-associated diseases are well-documented in scientific literature (Kumar et al., 2018).

L-carnitine

Additionally, L-carnitine supplementation has been shown to promote weight and fat loss in overweight dogs.

The inclusion of L-carnitine in diets fed to dogs firstly enhances energy conversion by increasing fatty acid oxidation, which helps to reduce body fat stores (Sunvold et al., 1998).

L-carnitine may prevent the loss of lean muscle mass during increased activity and weight reduction, which is important for the long-term maintenance of optimum body condition and weight (Varney et al., 2017).

Orange (Naringin)

Naringin is a bioflavonoid, exceedingly abundant in citrus species, including orange. In literature, naringin has been scientifically well documented for its beneficial effects in various neurological disorders, in particular the protective role of naringin against oxidative stress-induced neurological disorders in rodents (Viswanatha et al., 2017).

Carrot

Carrot-based products have demonstrated significant nutritional and digestive benefits when incorporated into canine diets.

As a pectin-rich fibre source, this product enhances digestive health by improving the apparent faecal digestibility of total dietary fibre, phosphorus, and magnesium.

Additionally, its inclusion in pet food formulations has been shown to increase faecal short-chain fatty acids, particularly acetate, which plays a crucial role in gut health and metabolic regulation.

Furthermore, the high carotenoid content in carrots provides antioxidant properties, which may contribute to immune system support and overall well-being in dogs (Eisenhauer et al., 2019).

Camomile

Traditionally, chamomile has been used as an anti-inflammatory and antioxidant agent. Additionally, it has been frequently used as a solution to calm nerves and reduce anxiety in animals, including dogs (Alex & Srivastava, 2019).

Linseed

Linseed is a natural source of omega-3 fatty acids; these essential fatty acids have an integral role in skin and coat health. Alpha-linolenic acid (ALA) may offer health benefits that extend beyond its role as a precursor for endogenous EPA and DHA production (Burron et al., 2024).

Fructooligosaccharides (FOS) and Mannanoligosaccharides (MOS)

The prebiotics fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) occur naturally in plants, such as chicory, and are isolated from yeast cell walls, respectively.

Prebiotics have been defined as non-digestible oligosaccharides that stimulate the growth and activity of a limited number of resident colonic bacteria (Gibson & Roberfroid, 1995), which can have a beneficial impact on factors including digestive health, immune function, and faecal quality.

In dogs, FOS supplementation increased the apparent total tract digestibility of several minerals (Ca, Mg, Na, Zn and Fe) (Pinna et al., 2018).

Similarly, Beynen et al. (2002) reported significantly increased magnesium and calcium absorption in dogs fed an oligofructose-supplemented diet. A possible mechanism of action for the increased mineral absorption is that a decrease in ileal pH (i.e., an increase in acidification) raises the solubility of the minerals, making them more available for absorption by the small intestine.

A study designed by Kore and colleagues (2012) to assess the effect of dietary supplementation of MOS on nutrient digestibility, hind gut health indices, and plasma metabolic profile found that supplementation of MOS at 1% of diet dry matter positively influenced feed intake, fibre digestibility, and indices of hindgut health.

The study used five adult dogs in a complete crossover design; the dogs were fed a homemade diet alone or one supplemented with MOS (at 1% level).

A digestion trial, conducted at the end of each period, revealed that the intake of feed dry matter and other nutrients increased when supplemented with MOS. Digestibility of fibre was improved in the MOS-supplemented group, while that of other nutrients was not affected.

Higher faecal concentration of total SCFAs due to MOS supplementation was also recognised, and the addition of MOS tended to reduce faecal coliforms with an associated elevation in lactobacilli count compared to the control group.

What are the results?

As part of the development of the Hypoallergenic Healthy Living recipe, an independent study was conducted by the University of Liège in Belgium.

This study aimed to investigate the bioactive and beneficial role of fresh hydrolysed salmon, which is present in our Hypoallergenic Healthy Living recipe. In particular, the study aimed to evaluate the potential anti-radical and antioxidant properties of salmon peptides.

For the generation of free radicals, sodium persulfate aqueous solution was mixed with 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) and incubated overnight in the dark to obtain a dark-coloured solution. The aqueous test samples were then added.

During this process, the blue-green ABTS radical cation is converted back into its colourless neutral form in the presence of the potential antioxidant molecule.

The results showed that hydrolysed fresh salmon exhibited strong antioxidant potential by inhibiting free radical activity, as hydrolysed fresh salmon neutralised 75% of the free radicals compared to the control.

This is beneficial for the immune system as reducing excess free radicals protects immune cells from oxidative damage, maintains proper immune signalling, and aids in reducing chronic inflammation.

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