GASTROINTESTINAL DIGESTIVE CARE

A SCIENTIFIC SUPPORT PAPER

The Digestive diet was proven to display 95% protein digestibility

Ghent University Vet School - Feeding Study

CONTENTS

WHY IS DIGESTIVE HEALTH IMPORTANT?

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

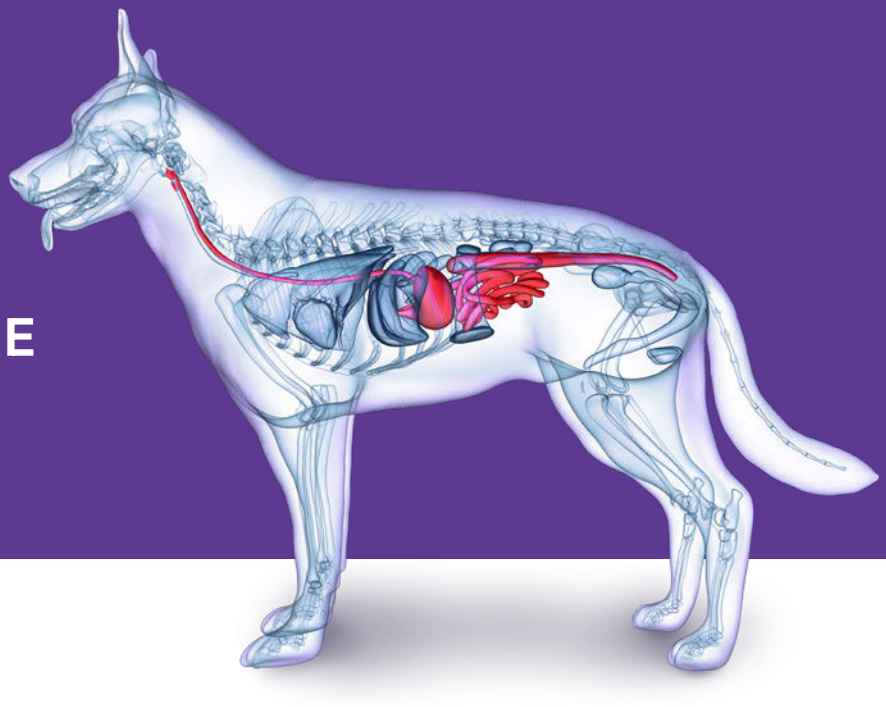
The gastrointestinal (GI) tract also provides a barrier between external factors and the dog's internal environment, keeping out potentially pathogenic organisms and harmful substances.

Gastrointestinal conditions can occur due to a number of factors, such as stress, illness, eating something inappropriate, inflammation and food sensitivity or allergy.

Poor digestive health can cause discomfort for dogs, with common signs including frequent loose stools/diarrhoea, abdominal discomfort, bloating, constipation, lack of appetite and vomiting.

Not surprisingly, this can also lead to stress and concerns for owners and potentially result in a visit to the vet.

IMPORTANT ROLES OF THE DIGESTIVE SYSTEM



Essential nutrients can't be made by the body and must be provided in the diet. It is important that the epithelial cells of the GI tract are healthy in order to carry out their nutrient absorption role effectively.

Dog foods are composed of different ingredients which provide a complex mixture of nutrients. Some nutrients are present in food as large molecules (e.g. protein, fat, starch) that need to be broken into smaller pieces (digested) so that they can then be absorbed.

Other nutrients (e.g. vitamins and minerals) are already small enough but need to be delivered to the right part of the digestive tract in order to be absorbed.

MECHANICAL DIGESTION

The first stage of **digestion** – mechanical digestion – begins when food is chewed in the mouth and physically broken into smaller pieces. This helps increase the surface area of the food, providing easier access for the digestive enzymes that are released lower down in the gastrointestinal tract to break down protein, fat and starch.

ENZYMATIC DIGESTION

Enzymatic digestion of protein begins in the stomach, where the presence of hydrochloric acid provides the ideal low-pH environment to activate the enzyme pepsin, which starts to digest dietary proteins.

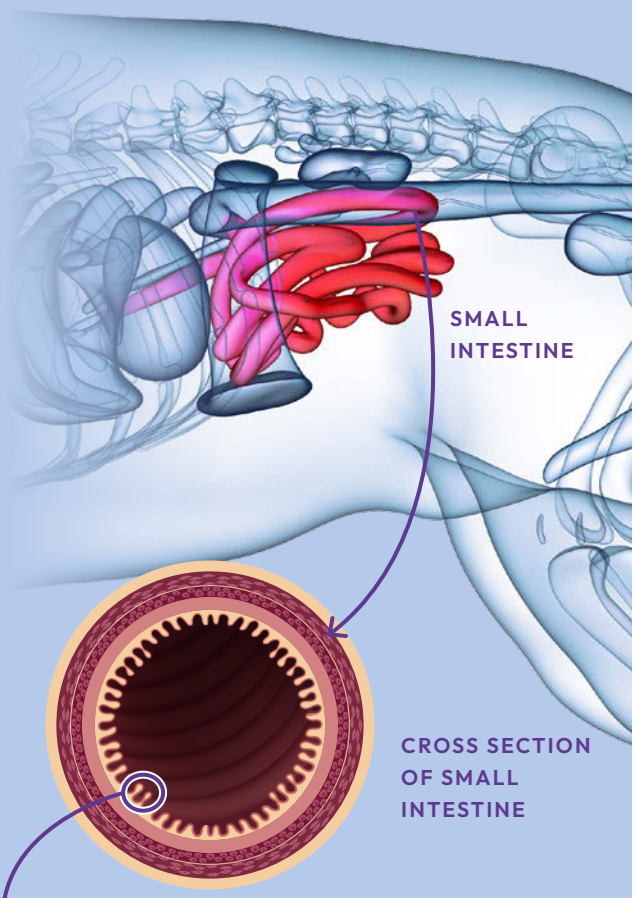
Partially digested food leaves the stomach and enters the small intestine, where the pancreas releases further enzymes – e.g. trypsin, lipase and amylase – to digest protein, fat and starch, respectively.

SMALL INTESTINE

Digestion continues along the small intestine, breaking down protein, fat and carbohydrates into peptides / amino acids, monoglycerides / fatty acids, and monosaccharides (e.g. glucose, fructose) that can then be absorbed.

The **small intestine** is specially adapted for the absorption of nutrients – the lining of the small intestine is highly folded into finger-like projections called villi, and specialised epithelial cells (**enterocytes**) that line the villi have even smaller projections, called **microvilli**, on their surface which together increase the surface area available for **nutrient absorption**.

The digested nutrients are taken up from the lumen of the small intestine into the enterocytes via special transport proteins (e.g. amino acid transporters, di/tripeptide transporter (PEPT1), sodium-glucose transporter 1, fatty acid transport proteins) (Goodman 2010).

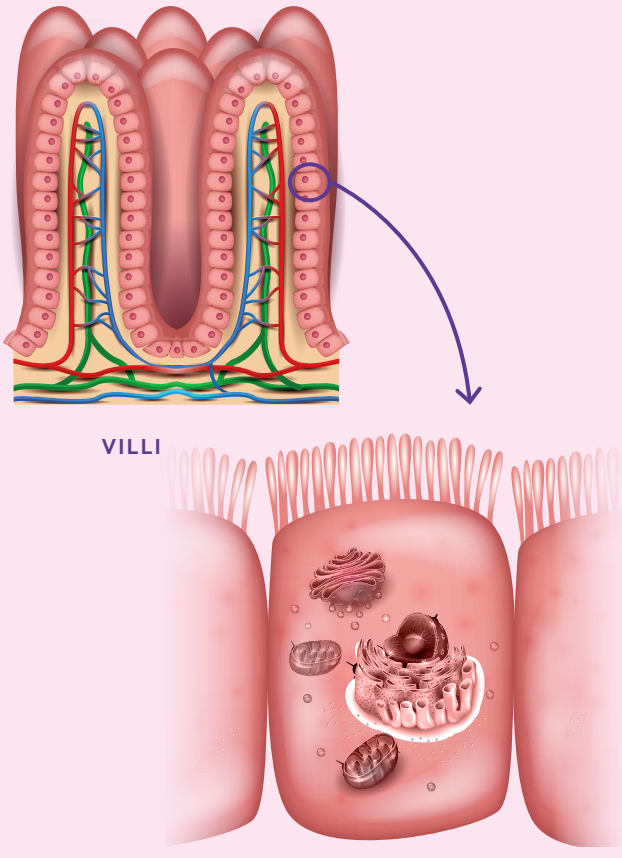


THE VILLI

The villi have a rich supply of blood capillaries where the water-soluble nutrients in the enterocytes (e.g. amino acids, glucose, B vitamins) can diffuse or be transported into the blood to be carried around the body to be stored or used as needed.

Fat and fat-soluble vitamins (A, D, and E) are 'packaged' into chylomicrons in the enterocytes and then transferred into the lymphatic vessels (called lacteals) that lie alongside the capillaries within the villi.

The chylomicrons are transported through the lymph system, which drains back into the bloodstream to supply the tissues with fat absorbed from the diet.



PHYSICAL BARRIER

The GI tract provides a physical and immunological barrier to keep out toxins and microorganisms. It is important that the cells of the GI tract are healthy to provide an effective barrier.

The mainstay of the **intestinal barrier** is the single layer of intestinal epithelial cells that line the GI tract. This epithelium is composed of several different cell types – e.g. enterocytes, goblet cells, enteroendocrine cells, etc - each with specific functions.

The individual epithelial cells are anchored to an underlying basement membrane and are attached to their neighbours through **tight junctions** - assemblies of different proteins.

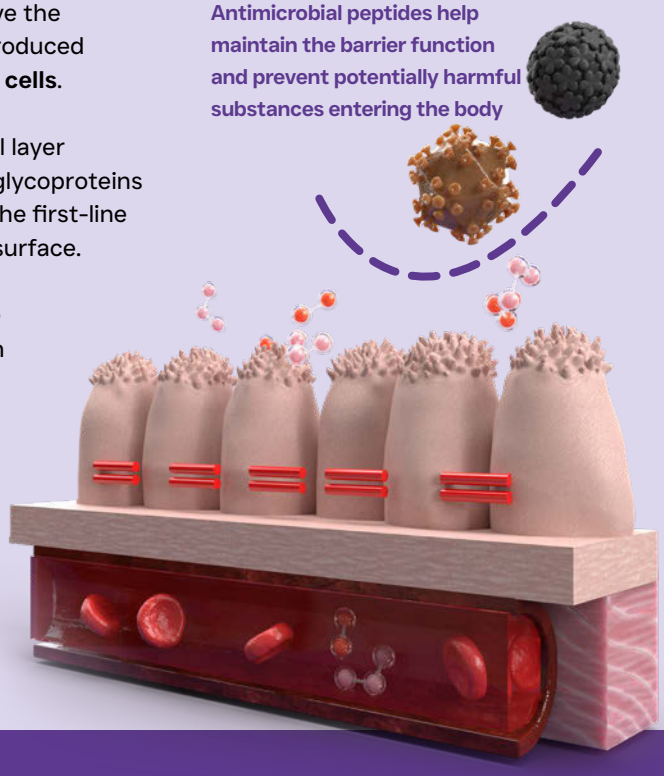
The tight junction proteins, including **occludin, claudins, zonula occludens (ZO) and junctional adhesion molecules**, are crucial for the maintenance of epithelial barrier integrity (Chelakkot et al., 2018).

A **mucus** layer sits above the intestinal epithelium, produced and secreted by **goblet cells**.

Intestinal mucus is a gel layer composed of complex glycoproteins (mucins) important as the first-line barrier of the mucosal surface.

To further minimise the risk of contact between virulent bacteria and the epithelium, the small intestinal mucus contains **antimicrobial peptides (AMPs)** such as defensins and cathelicidins and other host defence proteins produced and secreted into the mucus by intestinal epithelial cells.

Antimicrobial peptides help maintain the barrier function and prevent potentially harmful substances entering the body



IMMUNE DEFENCE

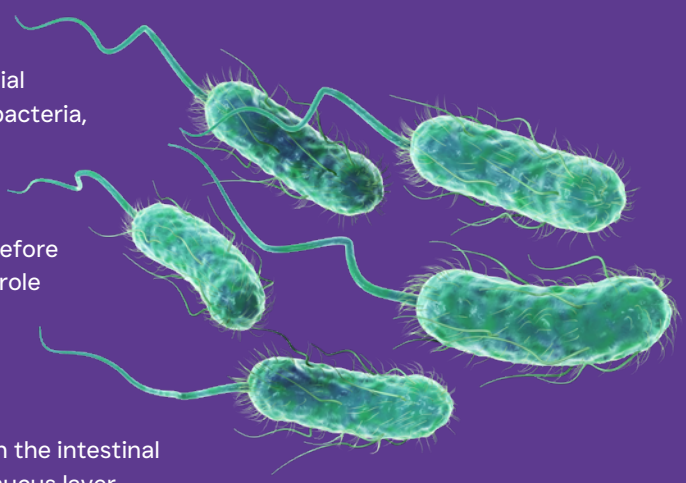
A major proportion of the immune system is found in the GI tract. The cells of the immune system need to be well nourished to be at their most effective in fighting off pathogens in the GI tract.

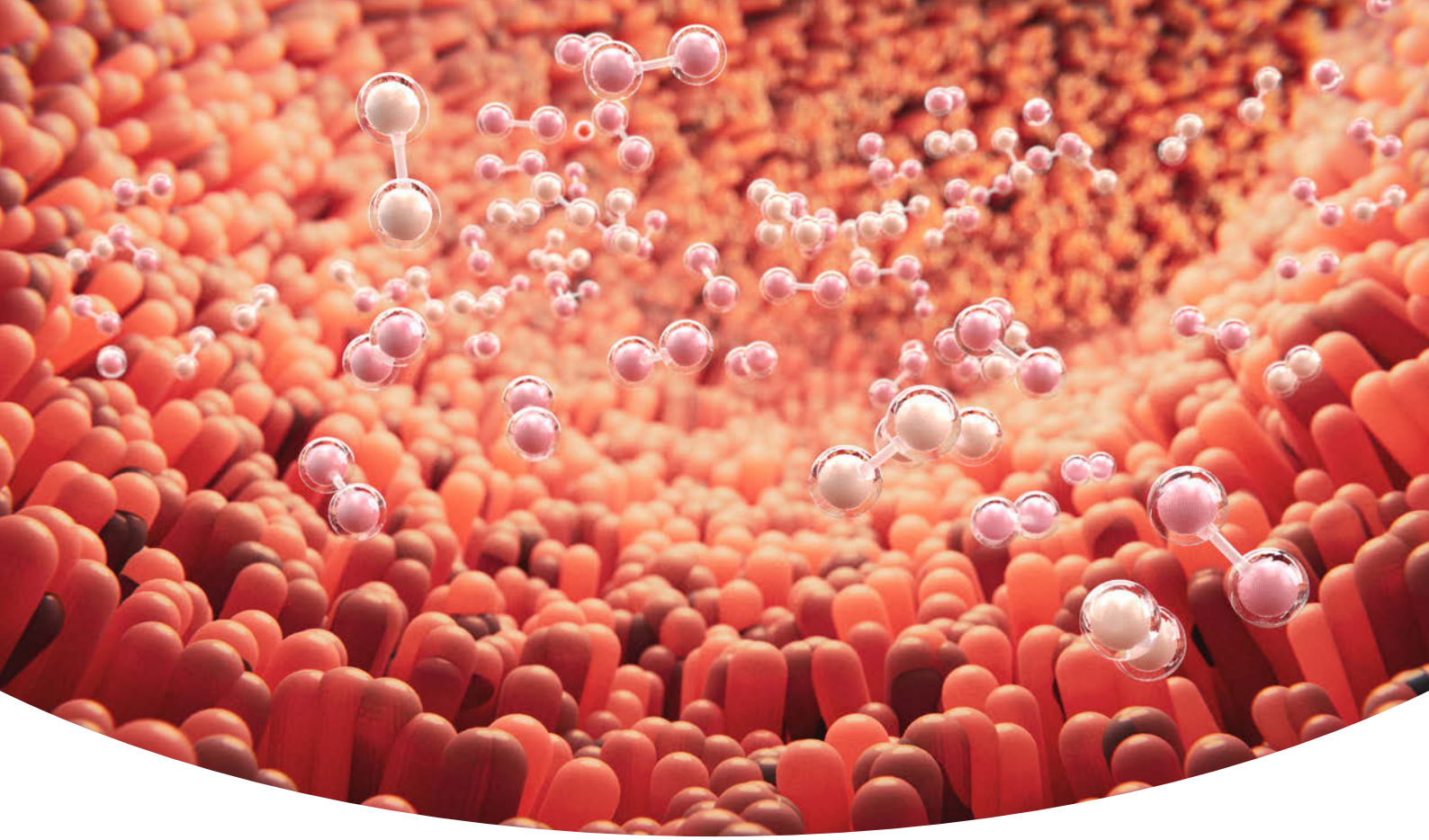
The immune system of the GI tract is chronically exposed to antigens from the intestinal lumen and, therefore, must be able to distinguish which antigens should be tolerated (e.g. self-antigens, food, symbiotic microbes) or 'attacked' (e.g. pathogenic microorganisms, toxins).

To help with this immunological surveillance and contribution to the barrier function of the intestinal mucosa is an array of immune cell populations, including T and B lymphocytes, plasma cells, dendritic cells, and components of the innate immune system such as macrophages, mast cells and neutrophils. This intestinal immune system lying just below the intestinal epithelium basement membrane, is sometimes referred to as **gut-associated lymphoid tissue (GALT)**.

Another component of the immunological barrier is secretory Immunoglobulin A (IgA), produced by plasma cells, which binds to bacteria in the intestinal lumen and prevents microbial invasion by coating bacteria, inhibiting adherence to epithelial cells and neutralising bacterial toxins, therefore playing a significant role in maintaining barrier function (Camilleri et al., 2019).

Interactions between the intestinal epithelial cells, the mucus layer and the GALT enables a robust barrier system which selectively allows the absorption of water and essential nutrients while protecting against adverse health effects from ingested or endogenous toxins.





GUT MICROBIOME

The gut microbiome is made up of beneficial and potentially harmful bacteria. A healthy gut microbiome can provide nutritional support to GI cells and contribute to barrier function and immune defences.

The lower small intestine and, particularly, the large intestine (colon) house a huge number and variety of microorganisms, including bacteria, fungi, protozoa and viruses, which together constitute the intestinal microbiome. However, it is the bacterial populations that are the most well-studied (Sekirov et al., 2010).

The so-called gut commensal bacteria have been described as one component of the intestinal physical barrier since they promote resistance to the colonisation of harmful or pathogenic bacteria species by competing for nutrients, occupying attachment sites and stimulating the production of antimicrobial factors such as IgA and AMPs by intestinal immune cells and intestinal epithelial cells (Sekirov et al., 2010).

Additionally, the gut microbiota plays a role in the fermentation of undigested as well as non-digestible components of food, which can result in potentially detrimental or beneficial effects on the host animal.

For example, an undigested protein that escapes absorption in the small intestine may be fermented by bacteria in the large intestine, resulting in the generation of different metabolites.

On the one hand, microbial fermentation of amino acids can give rise to the production of beneficial **short-chain fatty acids (SCFAs)**, including butyrate, which is an important fuel source for colonocytes.

However, fermentation of amino acids can generate products such as ammonia, phenols, indoles, amines and hydrogen sulphide, which not only contribute to faecal odour but may also have detrimental effects on colonocytes (Diether & Willing, 2019).

For non-digestible substrates in food, such as dietary fibres, fermentation by gut microbes results in the production of butyrate (and other SCFAs), the preferred fuel source for colonocytes.

In addition, SCFAs appear to have a role in the effect of Gram-positive commensal bacteria to stimulate the proliferation and migration of intestinal epithelial cells, a key mechanism to maintain the homeostasis and structural integrity of the intestinal epithelial barrier (Park et al., 2016).

The Digestive Health recipe has been developed with specific processes and ingredients to support digestive health and maintain effective intestinal barrier functions.

THE IMPORTANCE OF BIOAVAILABLE AND BIOACTIVE PEPTIDES TO SUPPORT DIGESTIVE HEALTH

Proteins are large molecules made up of individual 'building blocks' called amino acids.

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, which 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) are more readily absorbed from the digestive tract than intact protein and even individual amino acids (Maebuchi et al., 2007; Zhao et al., 1997).

This has the benefit of minimising the amount of undigested protein reaching the large intestine, where it could undergo fermentation by intestinal bacteria, therefore reducing the formation of potentially harmful and odour-forming compounds.

Intestinal epithelial cells have an extremely short lifespan (approx. 3-5 days). In a normal healthy gut, an equilibrium exists between the loss of 'old' epithelial cells at the tip of the villus and the generation of new cells in the crypt (basement region of the villus), which migrate up the villus to replace the shed cells (Williams et al., 2015).

In a setting of intestinal cell damage such as infection, inflammation, etc., the equilibrium of epithelial cell loss and replacement may be disturbed, and, in such conditions, proliferation is key to the recovery of the epithelium after disruption by different insults that lead to intestinal inflammation.

Therefore, intestinal epithelial cell proliferation rate is very important to maintain intestinal barrier function (Martínez-Augustin et al., 2014).

Enterocytes were discovered to have a receptor (GPR93) that was activated by a meat peptide, resulting in the stimulation of intracellular cellular signalling pathways associated with cell proliferation and differentiation (Choi et al., 2007).

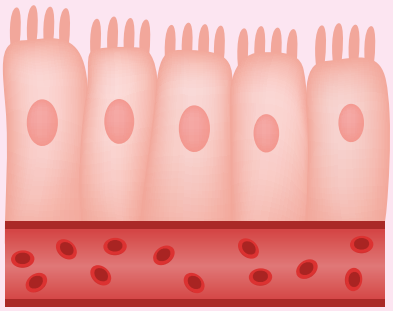
CELL REPAIR

In cells from two different regions of the intestinal tract (intestinal epithelial cells and colon cells), tested in vitro, peptides were shown to stimulate the proliferation of both cell types (Fitzgerald et al., 2005).

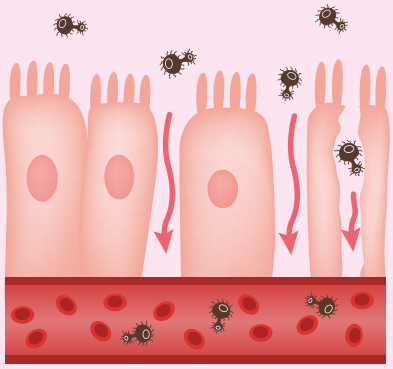
Furthermore, cell migration in 'wounded' colon cells in vitro was significantly increased, suggesting a beneficial role of the peptide in repairing intestinal damage. The peptide was also shown to be effective in vivo, having a protective effect in animal models of intestinal injury (Fitzgerald et al., 2005; Marchbank et al., 2009).

INTESTINAL EPITHELIAL CELLS

The intestinal epithelial cells form tight links (known as tight junctions) between the cells, which help to maintain the barrier function and stop potentially harmful substances from the environment from entering the body.



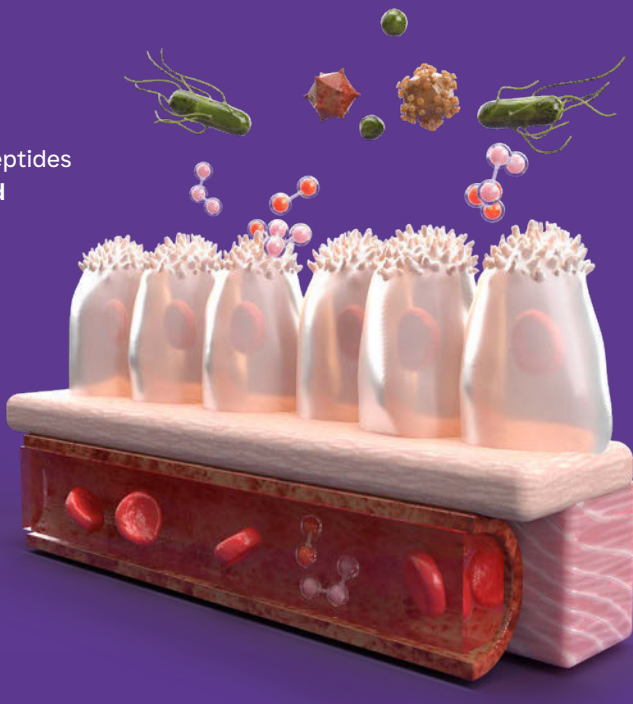
Normal tight junctions serve to protect against harmful substances from the environment entering the body through the bloodstream.



Leaky, inflamed, or damaged junctions may potentially fail to prevent harmful substances from entering the body.

Many different proteins are involved in the formation and function of tight junctions. If the tight junctions become damaged (e.g. bacterial infection or inflammation), the intestinal barrier function can become compromised ('leaky'), which can result in gastrointestinal problems and potentially other health problems.

POSITIVE EFFECTS OF PEPTIDES ON EPITHELIAL CELLS & TIGHT JUNCTIONS



Several studies have demonstrated the beneficial effects of peptides on intestinal barrier function. A dairy-derived **peptide inhibited the passage of ovalbumin across the membrane of human intestinal epithelial cells** (Caco-2 cells) in vitro (Tanabe et al., 2006).

It was subsequently shown that the effect of the peptide on **reducing epithelial permeability (i.e. increasing barrier function)** was associated with increased expression of the tight junction protein occluding, **indicating a positive effect of a food-derived peptide on epithelial barrier function** (Yasumatsu & Tanabe, 2010).

Using Caco-2 cells stimulated with an inflammatory cytokine (tumour necrosis factor-α, TNF-α), **collagen peptides were shown to reduce the barrier dysfunction associated with inflammation** by stopping the breakdown of the tight junction proteins ZO-1 and occludin (Chen et al., 2017).

A poultry-derived **peptide was shown to increase tight junction protein levels and down-regulate the expression of inflammatory cytokines to protect the intestinal barrier**, contributing to the alleviation of colitis in animals (Li et al., 2020).

Similarly, **other animal peptides decreased the disease activity index (DAI) score and colon tissue injury in an animal colitis model.** The protective mechanisms of the peptide were associated with reduced infiltration of lymphocytes, down-regulation of pro-inflammatory cytokines (TNF-α, interleukin-6) coupled with increased levels of anti-inflammatory cytokines (transforming growth factor-β1, interleukin-10) and up-regulation of anti-oxidative genes (Wei et al., 2022).

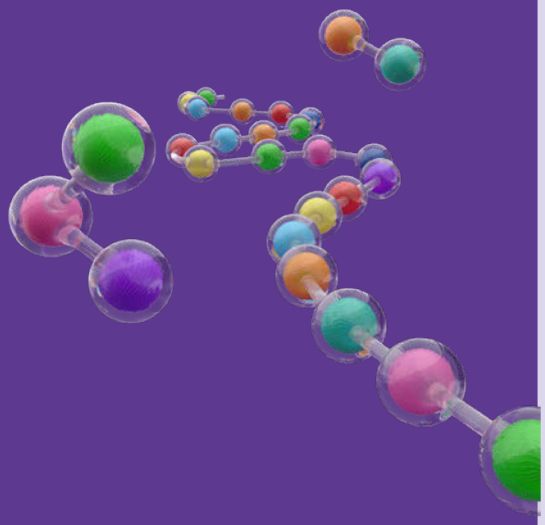
THE IMPORTANCE OF PEPTIDES FOR DIETARY ALLERGY MANAGEMENT

A food allergy is an inappropriate immune response to a normal food or ingredient (e.g. a protein in the food), which can result in gastrointestinal (e.g. diarrhoea, vomiting) and/or dermatological (e.g. red itchy skin) signs in dogs (Verlinden et al., 2006).

BREAKING DOWN OF PROTEIN FOR THE POSITIVE EFFECT

The ability of a protein to induce an immune-mediated hypersensitivity (allergic) response is dependent on the size and structure of the protein.

By using controlled enzymatic hydrolysis, proteins can be partially or extensively broken down into smaller peptides that can be too small to be detected by the immune system, meaning the hydrolysed proteins have a lower allergenic potential, and therefore making them beneficial for dogs with an allergy to intact dietary protein.



HYDROLYSED PROTEIN HELPS REDUCE FOOD-RELATED ALLERGIC REACTIONS

Ensuring that **a hydrolysate has no peptides greater than 3 kDa or even 1 kDa** would ensure the greatest chance of eliminating any residual allergens (Cave, 2006).

The effectiveness of protein hydrolysis as a means to help reduce food-related allergic reactions has been shown in a study of 12 dogs with adverse skin reactions after consumption of poultry meat; when fed with poultry peptides, **all but one showed a reduction in clinical scores** (Ricci et al., 2010).

WHAT MAKES THE GASTROINTESTINAL DIGESTIVE CARE RECIPE SO UNIQUE?

The development and formulation of the Gastrointestinal Digestive Care recipe has centred around the 'Power of Peptides' using the latest Freshtrusion HDP technology.

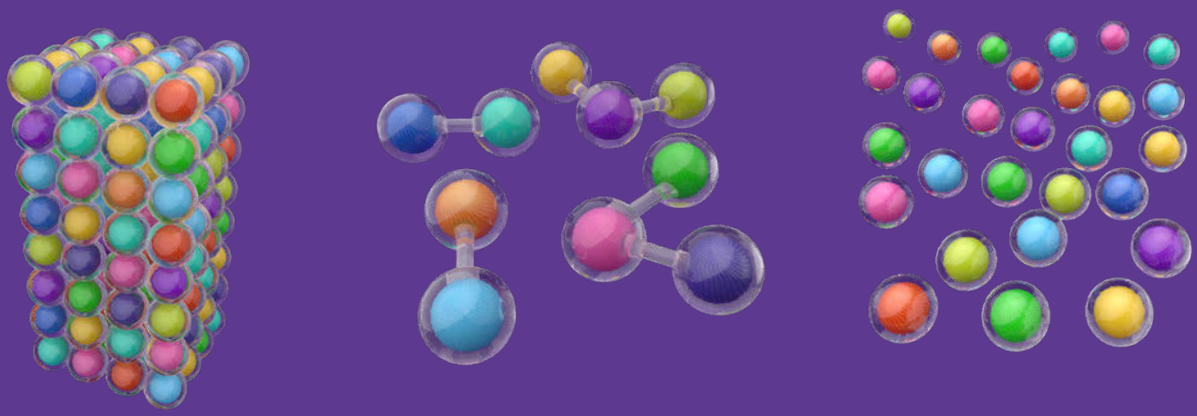
HDP freshtrusion HIGHLY DIGESTIBLE PROTEIN

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, improves palatability and reduces the allergenic potential of the protein, through what we like to refer it as the Goldilocks Principle:

THE GOLDILOCKS PRINCIPLE

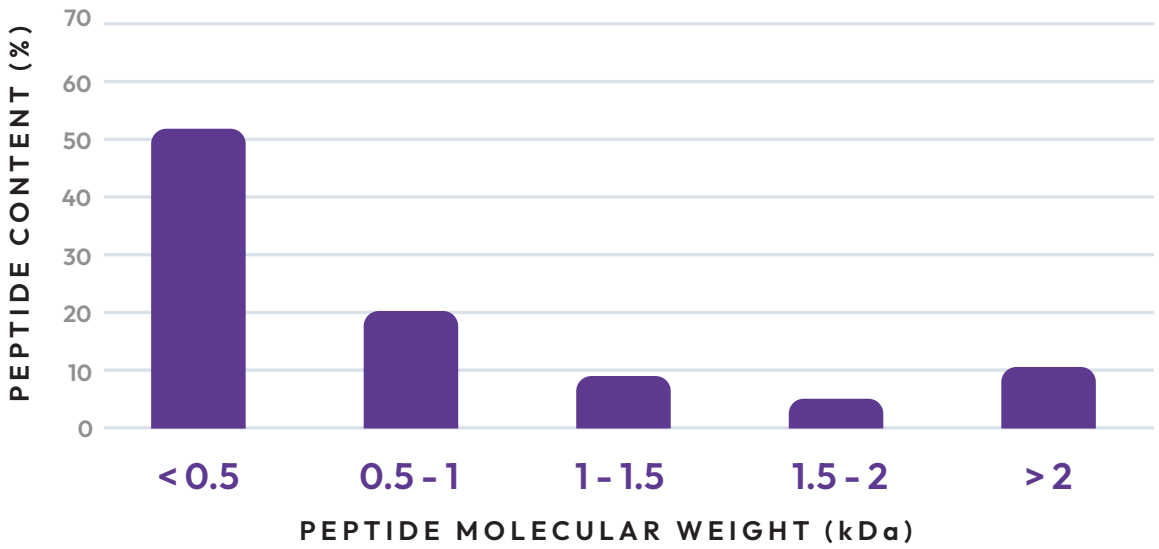
Instinctively, it would be assumed that intact protein would be best for a dog to digest as it contains all the nutritional elements together as one. Similarly, individual amino acids, broken down as small as possible, might be considered to be much easier to absorb. However, it has been proven in research 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

GASTROINTESTINAL DIGESTIVE CARE RECIPE: PEPTIDE CONTENT (%)



A minimum of 52% of the peptides in this recipe are < 0.5 kDa with just 10% of the peptides > 2 kDa

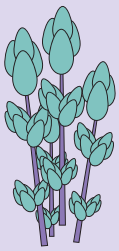
These results show the majority of peptides in the finished kibble fall into the < 0.5 kDa category, which includes the highly digestible and nutritionally beneficial dipeptides and tripeptides - achieving the Goldilocks Principle.

THE POWER OF THE PEPTIDES FOR DIGESTIVE HEALTH

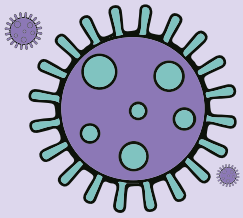
- ✔ **Increases the digestibility and bioavailability of the protein**
- ✔ **Improves the palatability of the recipe**
- ✔ **Ensures an ideal supply of amino acid building blocks to support renewal of intestinal epithelial cells**
- ✔ **Helps maintain effective intestinal barrier function by increasing tight junction protein levels**
- ✔ **Reduces the allergenic potential of the protein to aid sensitive digestion**

In addition to the inclusion of hydrolysed protein, the Gastrointestinal Digestive Health recipe includes an added postbiotic that has been shown to have beneficial effects on digestive health and immune function in dogs.

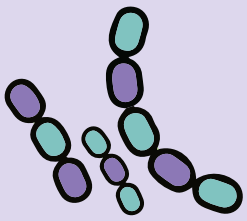
POSTBIOTICS & DIGESTIVE HEALTH



PREBIOTICS
Dietary fibres that feed good bacteria



PROBIOTICS
Live beneficial "good" bacteria that support gut health



POSTBIOTICS
Metabolites produced by the good bacteria to support digestive health

What are postbiotics?

The International Scientific Association of Probiotics and Prebiotics (https://isappscience.org) defines postbiotics as "a preparation of inanimate microorganisms and/or their components that confers a health benefit to the host".

Postbiotics can be produced by bacteria or yeast using precise fermentation inputs and processes to create beneficial metabolites.

TruPet™

The TruPet™ postbiotics used in the Digestive Health diet include residual yeast cells, yeast cell wall fragments and beneficial fermentation metabolites produced during the precise fermentation of specific material by yeast, Saccharomyces cerevisiae.

The metabolites and bioactive compounds present in postbiotics include amino acids, vitamins, SCFAs, nucleotides and more. A research study carried out in dogs demonstrated the following beneficial effects on digestive health and immune function in dogs (Lin et al., 2019).

There was a beneficial shift in the microbiota of dogs that received TruPet™ compared to the control group, with an increase in the abundance of Bifidobacterium, which is associated with a healthy gut in terms of reduced pathogenic bacteria and enhanced immune function (Araya-Kojima et al., 1995).

Immune cells from postbiotic-supplemented dogs exhibited increased pathogen surveillance potential based on increased proportions of B cells and monocytes that expressed MHC class II molecules on their cell surface (Lin et al., 2019), which is important for recognition of foreign invaders and initiation of effective adaptive immune responses.

MHC class II molecules are required to present antigens and stimulate helper T cells to trigger an appropriate immune response.

The T helper cells appeared primed to react to threats as populations of interferon-γ (IFN-γ) secreting helper T cells and IFN-γ-secreting cytotoxic T cells increased in response to immune stimuli from dogs supplemented with TruPet™ compared to control.

Anti-inflammatory effect

Although the postbiotic treatment increased the potential responsiveness of the immune system to mount a response to an invading microorganism, the finding that the production of the inflammatory cytokine TNF-α following stimulation in vitro with different lymphocyte activators was reduced in TruPet™-supplemented dogs suggests a potential anti-inflammatory effect of the postbiotic.

Stool odour reduction

Supplementation with TruPet™ postbiotic also resulted in a reduction in compounds that contribute to faecal odour and may also be detrimental to gut health - faecal phenol and total faecal phenol + indole concentrations were reduced (Lin et al., 2019).

WHAT ARE THE RESULTS?

In a study with the University of Ghent we aimed to test and assess the apparent digestibility of Gastrointestinal Digestive Care recipe by an in vivo digestibility trial in 8 dogs.

Daily macronutrient intakes and faecal excretions were calculated and apparent digestibility of those nutrients were calculated. All dogs consumed their dietary portion completely.

Based on the FEDIAF Nutritional Guidelines only a digestibility ≥ 80% can be considered normal. **The average protein digestibility of the Gastrointestinal Digestive Care recipe was 95%**, which can be considered high protein digestibility.

The University of Ghent concluded the protein digestibility "excellent" and that the diet meets the quality standards for digestibility.

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