Protein hydrolysates *in pet health and nutrition*

Anne Lepoudère
Vivae R&D Manager

Agenda

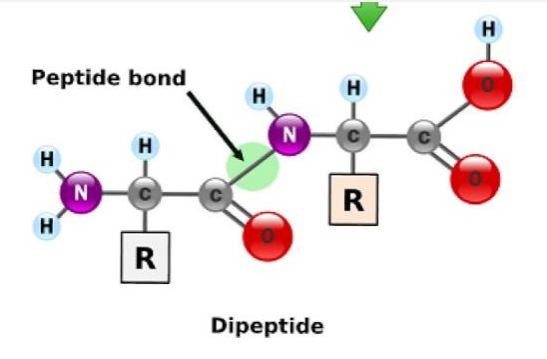
1. **Protein nutrition** in Cats and Dogs
2. **Protein hydrolysates:** a high quality protein source
3. **Benefits of Protein hydrolysates for Senior Pets**
4. **Protein hydrolysates to limit Adverse Food Reactions**
5. **Bioactive Peptides:** a new edge for Protein hydrolysates
6. **Take home messages**

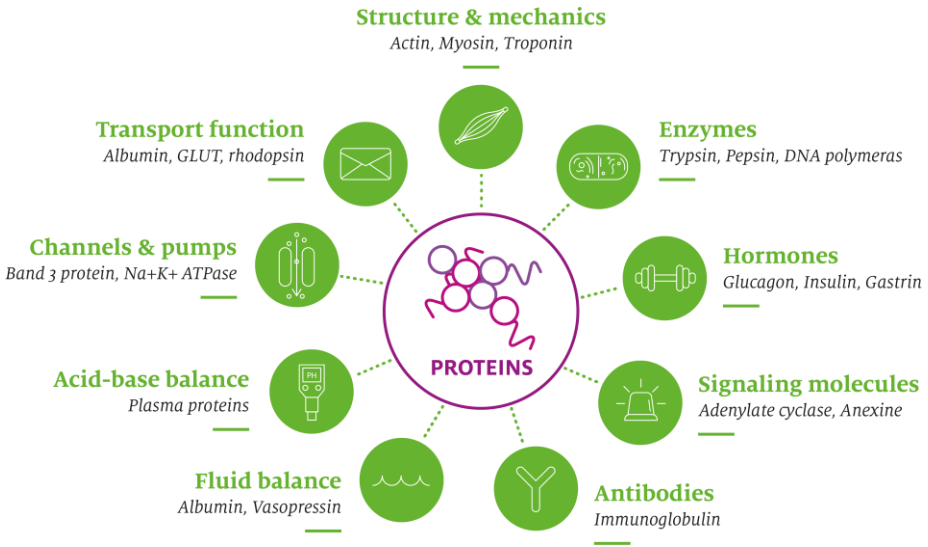
Protein nutrition in Cats & Dogs

Proteins are essential for Pet nutrition
Functions of body proteins

Proteins are essential macromolecules, polymers of amino acids (AA)

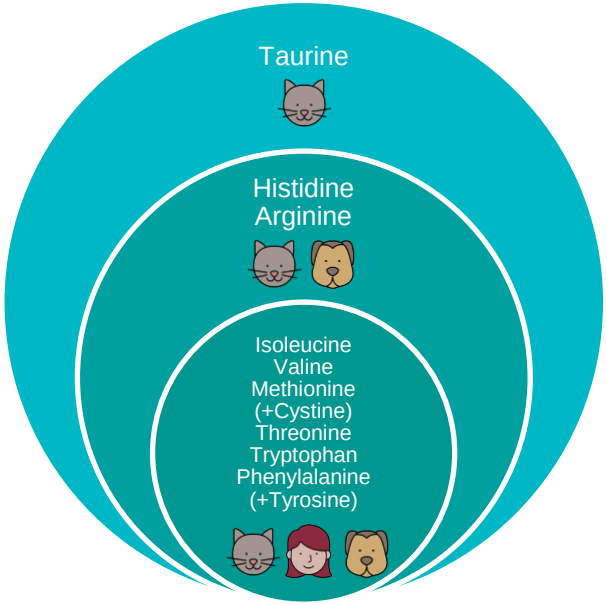
SOURCE OF PEPTIDES AND AMINO ACIDS



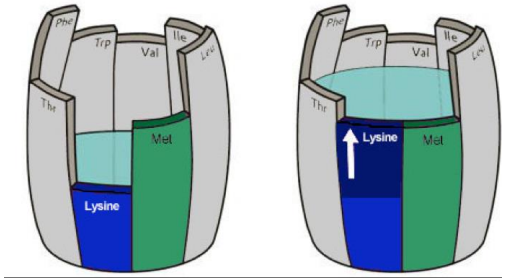


Proteins & Amino Acids are essential for PETS
Essential and Limiting Amino Acids

Classification of AA for Cats and Dogs



Limiting Amino Acids



*The essential AA present in the lowest quantity (relatively to requirement) will **limit the protein synthesis***

Proteins are essential for PETS
Protein requirements

PROTEIN REQUIREMENT =
ESSENTIAL AA + NON ESSENTIAL AA

QUANTITY OF PROTEIN needed to be absorbed by the body to maintain a healthy physiological state

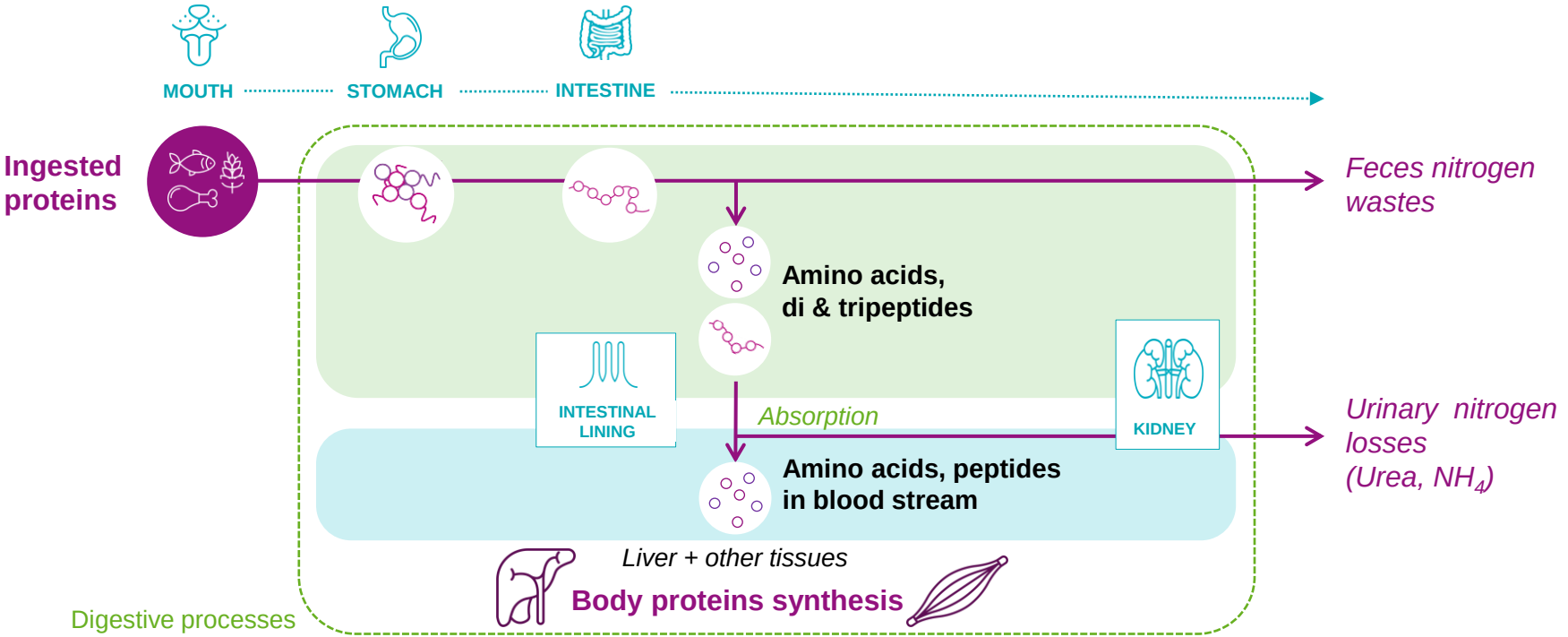
MINIMAL REQUIREMENT
Protein losses = Protein intake

ALLOWANCE
requirement + safety margin

	G/kg *for 4000kcal/kg food*	G/kg body weight$^{0,67/0,75}$
	**80** (min requirement) **100** (allowance)	**2,62** (min requirement) **3.28** (allowance)
	**160** (min requirement) **200** (allowance)	**3,97** (min requirement) **4.96** (allowance)

Source: NRC 2006

Protein digestion and metabolism
From Proteins to Peptides and Amino Acids



Intestinal absorption of Peptides & Amino Acids
Supply of Amino Acids and Peptides

PROTEINS

Digestive hydrolysis

INTESTINAL LUMEN

EPITHELIAL BARRIER

BASO LATERAL SIDE

BLOOD STREAM

Free amino acids oligopeptides

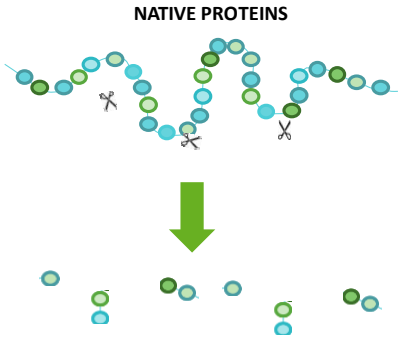
Oligopeptides

Free amino acids

Protein hydrolysates: a high quality protein source

Key attributes of Protein Quality: Process
Enzymatic versus chemical hydrolysis

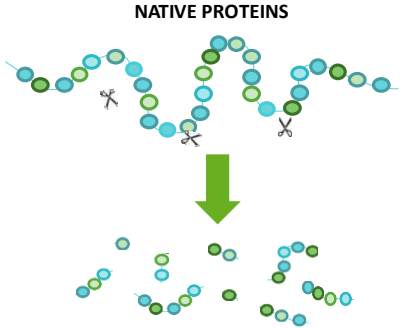
CHEMICAL HYDROLYSIS
Drastic conditions (strong acids or bases)



Mix of short peptides and free AA

- *High content in short peptides and free AA may alter stool quality*
- *Possible high mineral and salt content*
- *Reduction of nutritional value and reduced functional properties (tryptophan is sensitive to acid hydrolysis, as well as sulfur AA)*

ENZYMATIC HYDROLYSIS
Specific enzymes (proteases) cleaving peptidic bounds

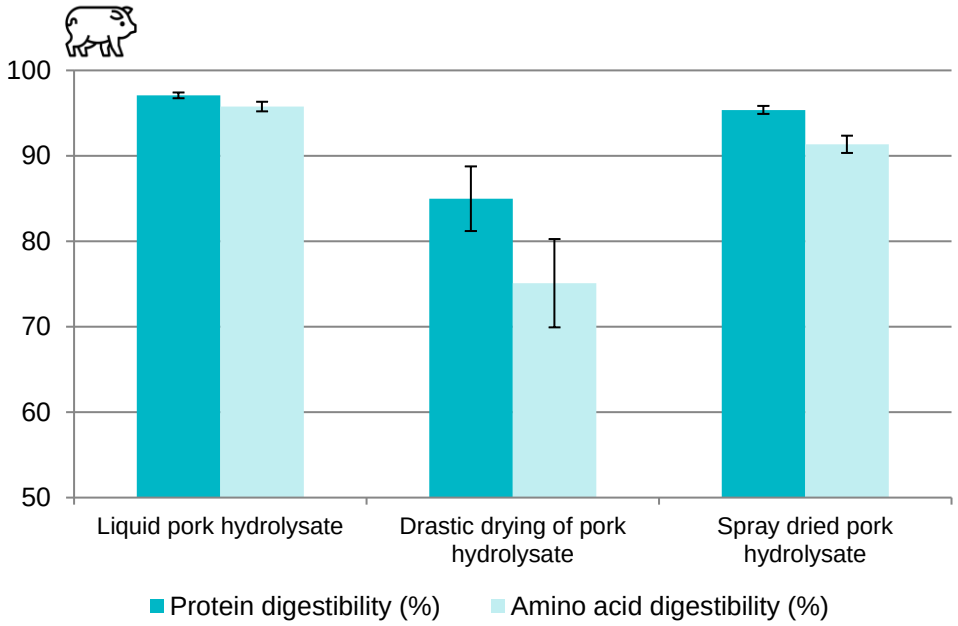


Peptides with targeted MW and free AA

- *Possible control of free AA and proportion of small size peptides*
- *Reproducibility*
- *Preservation of AA*

Key attributes of Protein Quality: Digestibility
Alternative measurements

Key attributes of Protein Quality: Process impact
Protein and AA digestibility in caectomized roosters



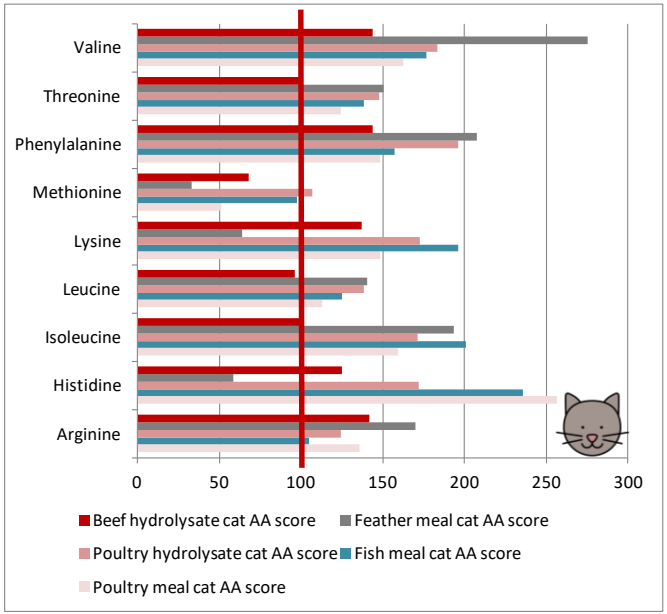
Improvement of food safety and nutritional properties with drying
Microbiology, anti-nutritional factors

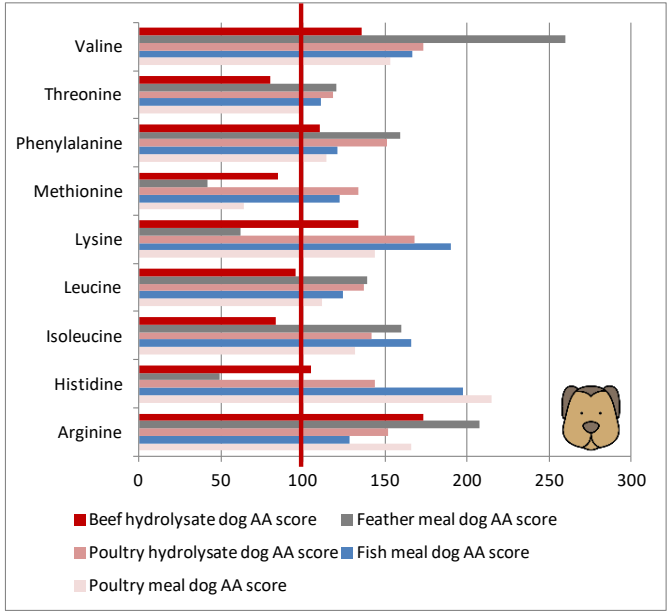
However, if too excessive, proteins and AA can undergo chemical reactions
Crosslinking, racemization, glycation, SAA oxidation, etc.

DECREASE OF PROTEIN AND AA DIGESTIBILITY

Key attributes of Protein Quality: AA scores
Comparison of different Protein sources

AA score expressed in relation to minimum requirements *(Kerr 2013, Bosch 2014)*





Basis 100:
Minimum requirements of AA for puppies and Kittens (NRC 2006)

Choice of raw materials is key to optimize AA score of protein hydrolysates

Key attributes of Protein Quality: Palatability
Dog palatability – Poultry hydrolysate

Dog palatability test

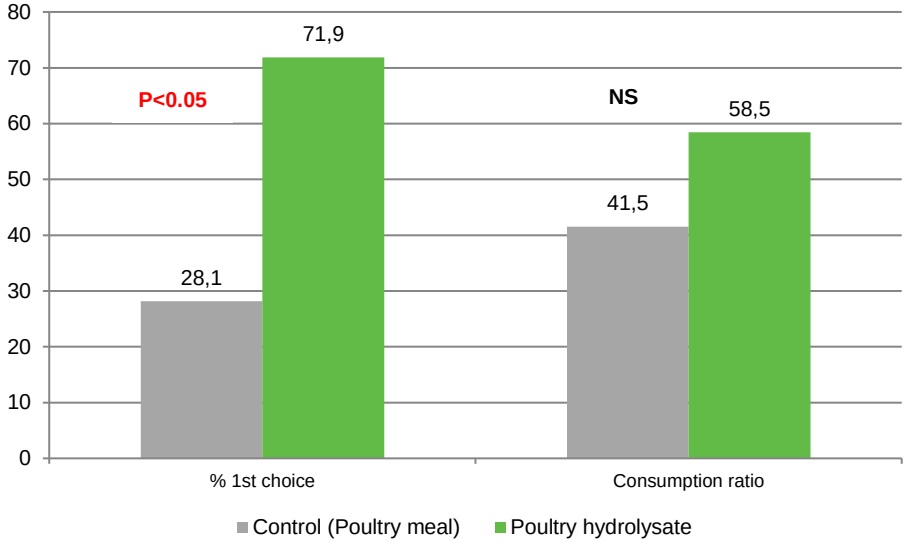
- 26% Poultry meal diet versus 26% Poultry hydrolysate
- 4000 Kcal/kg, 22% protein
- Same coating (6% fat, 2% palatant)

Experimental design

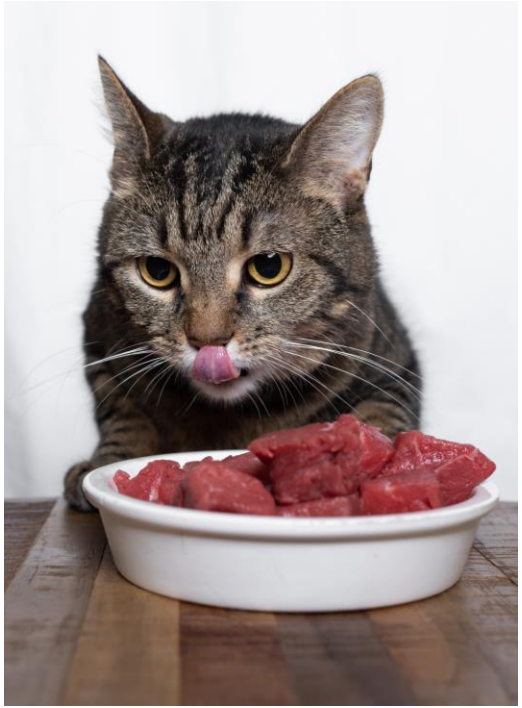
- 40 dogs
- 2 bowl test for 1 meal

Palatability of poultry hydrolysate superior to poultry meal

No generation of bitterness



Protein hydrolysates as a protein source
Key attributes



RAW MATERIAL AND PROCESS

MACRONUTRIENT CONTENT

Protein
Ash
Fat

PALATABILITY

PROTEIN DIGESTIBILITY

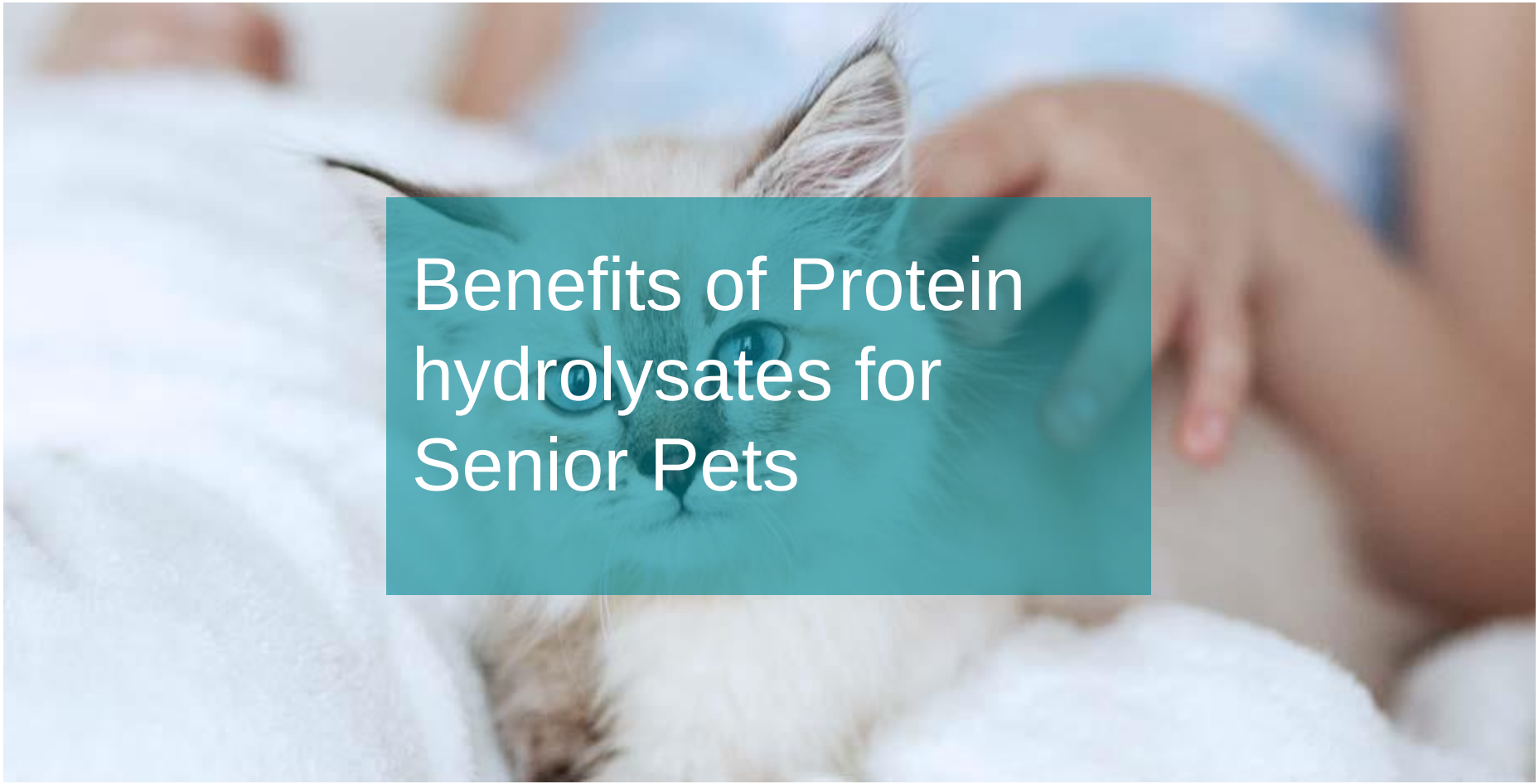
Digestibility (in vitro, in vivo)

MOLECULAR WEIGHT PROFILE

Peptides distribution

AA PROFILE

Amino acid score calculated on the basis of minimal puppy & kittens requirements
Kerr et al., 2013



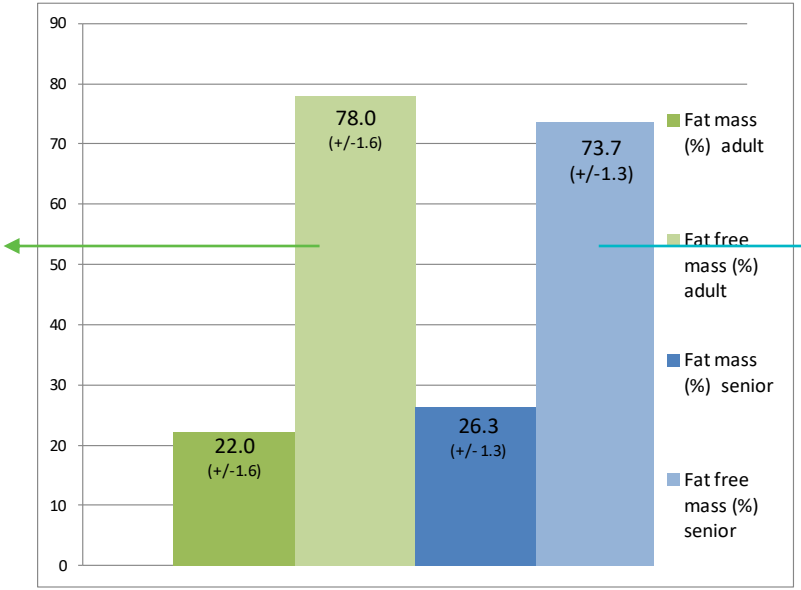
Benefits of Protein hydrolysates for Senior Pets

Effect of Aging in Dogs
Evolution of body weight and composition

Body composition of 2 cohorts of Beagle dogs
Controlled environment, internal data

ADULTS

n=10, 1 -7 y
Mean BW: 11.8 kg
BCS: 5.0



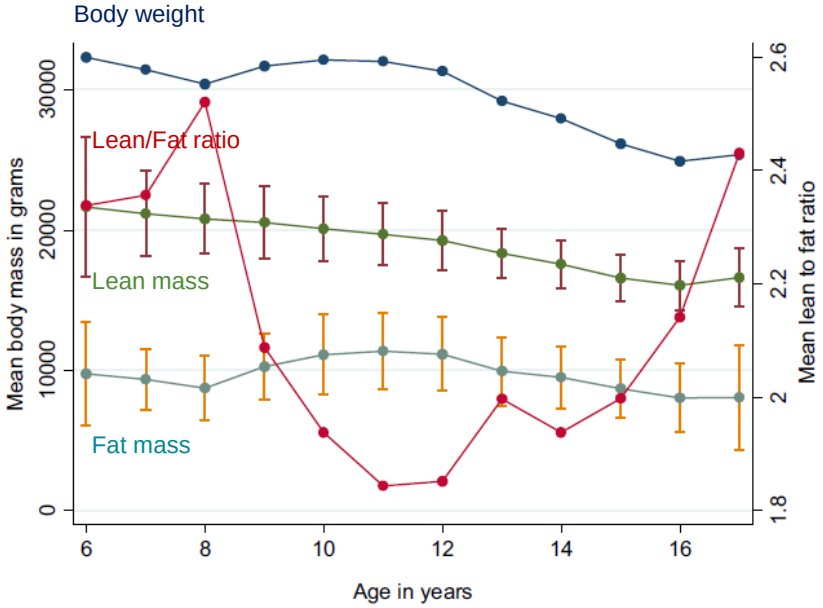
SENIOR

n=10, 11-17 y
Mean BW: 13.4 kg
BCS: 5.7

Effect of Aging in Dogs
Evolution of body weight and composition

Evolution of body weight and composition in 39 retriever Labrador dogs from 6.5 y. to end of life

Prospective cohort study – Penell 2019



Evaluation of protein hydrolysates in Senior Dogs
Material & Methods: Nitrogen Balance

2 GROUPS OF BEAGLE DOGS

10 adult dogs, 1 to 7 y.

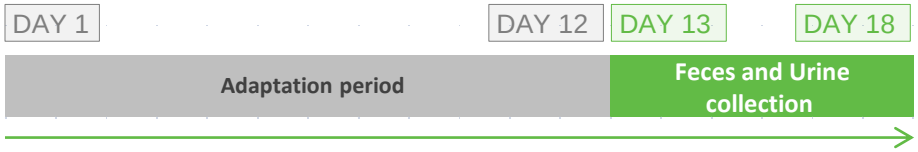
10 senior dogs,11 to 17 y.

3 EXPERIMENTAL DIETS (NRC 2006)

- Poultry meal
- Poultry hydrolysate 1 (medium hydrolysis)
- Poultry hydrolysate 2 (extended hydrolysis)

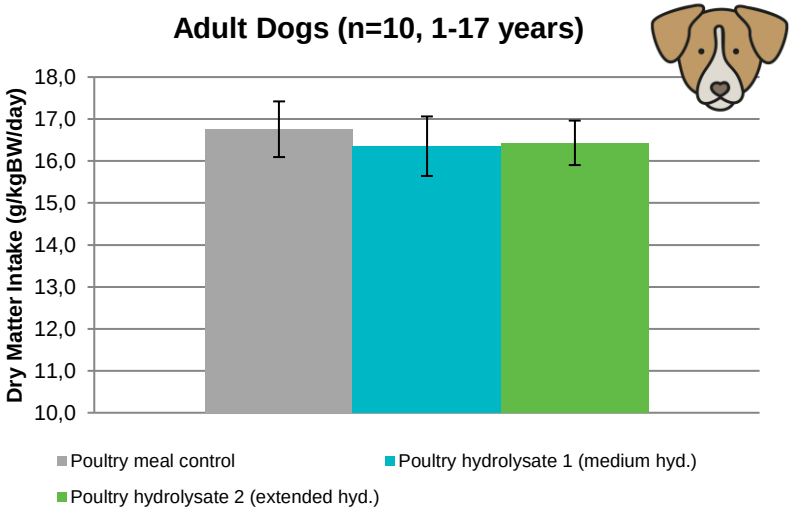
2 LATIN SQUARES

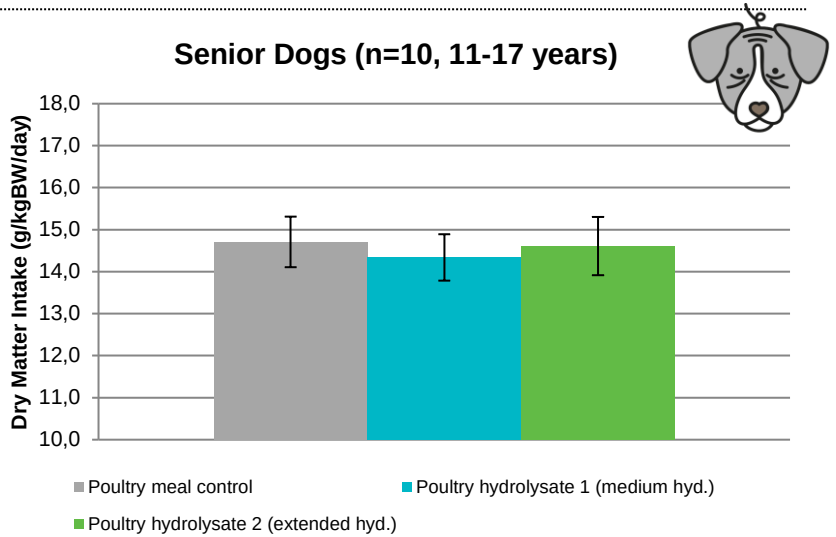
- *(1 for adults, 1 for aged)* for Nitrogen balance evaluation



	Control	Poultry hydrolysate 1	Poultry hydrolysate 2
Characteristics of Protien hydrolysate			
Degree of hydrolysis (% protein)		29%	49%
Diet composition (DM basis)			
Crude protein (%)	16.1%	16.8%	16.2%
Fat (%)	16.8%	17.1%	18.0%
Gross Energy (Kcal/kg)	4888	4935	4845

Evaluation of protein hydrolysates in Senior Dogs
Dry matter intake (g/ kg BW/ day, internal data)





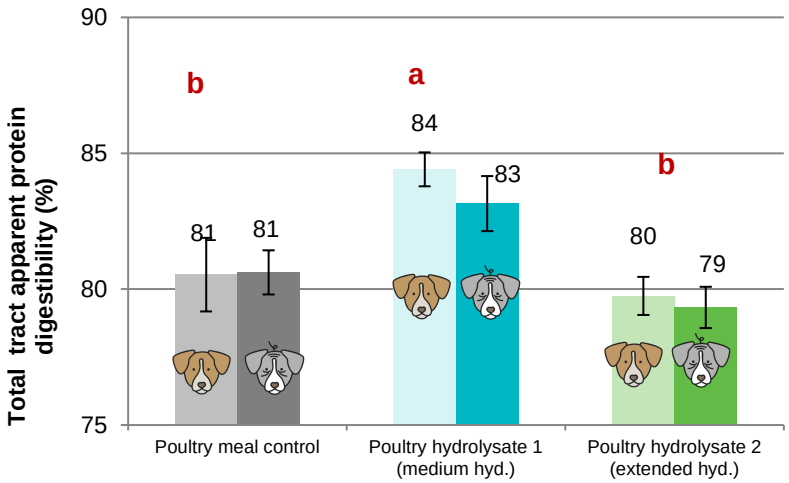
Decrease of food intake in senior dogs versus adult dogs (-12%)

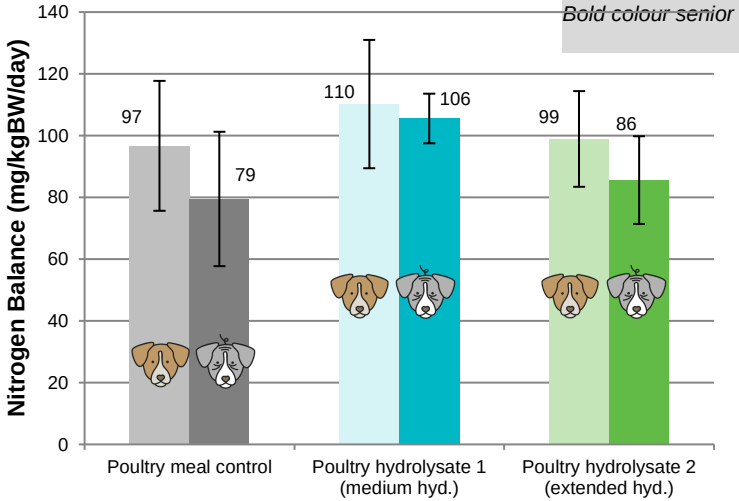
Evolution in energy requirements in relation to aging: decrease by 25% after 7 years of age

ESSENTIAL TO PROVIDE HIGH QUALITY & QUANTITY OF PROTEINS FOR SENIOR, COMPENSATING FOOD INTAKE DECREASE

Sources: Laflamme 2005, 2012 & 2018; Groves, 2019

Evaluation of protein hydrolysates in Senior Dogs
Digestibility and Nitrogen balance





Protein hydrolysates with medium hydrolysis are of interest to improve protein digestibility (adult and senior dogs)

PROTEIN HYDROLYSATES MAY SUPPORT HIGHER AA SUPPLY IN SENIOR DOGS, WHICH IS OF INTEREST WHEN CONSIDERING AGE-RELATED DECREASE OF FOOD INTAKE

Interest of Protein Hydrolysates
for Senior Dogs

DIETARY PROTEIN SUPPLY
by PROTEIN HYDROLYSATES
+ EXERCISE

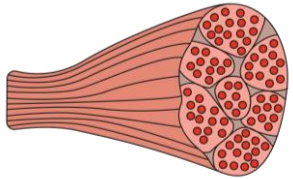
➔ **Protein hydrolysates can improve diet protein digestibility in senior dogs**
Easily digested and absorbed protein sources

➔ **Protein hydrolysates have a good palatability**

➔ **Further investigations are needed to explore their interest for promoting higher muscle protein synthesis**
Source: Koopman et al., 2009; Landi et al., 2013

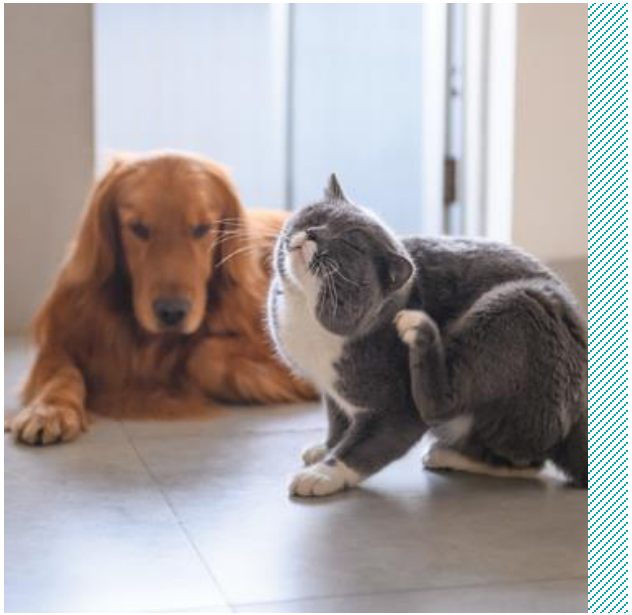
HYPOTHESIS FOR ADDITIONNAL BENEFITS
OF PROTEIN HYDROLYSATES
FOR **FIGHTING SARCOPENIA**

ANABOLIC
STIMULUS



MUSCLE SYNTHESIS > MUSCLE DEGRADATION

Background: Adverse Food Reactions (AFR)
Frequency in Cats and Dogs



➔ Common cause of **non seasonal pruritus**

- 3rd most common allergic skin disease in dogs, after
  -flea allergy dermatitis
  -atopic dermatitis
- 2nd frequency in cats, after flea allergy dermatitis

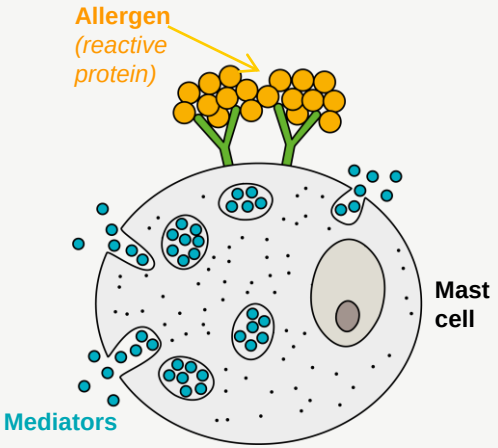
➔ Incidence of AFR in pets has been reported as 1% of all skin diseases and 10% of allergic skin diseases
(Scott et al. 2001)

Origin and triggering of food allergy
IgE mediated food allergy

When oral tolerance fails, immunological response towards food

DIGESTION	• Sensitization induced by specific pattern of dietary protein or peptides in the intestine -> Epitope
SENSITIZATION	• Allergen triggers the production of specific IgE
ALLERGY	• Allergen recognized by 2 adjacent IgE upon repeated allergens exposure = cross-linking • Degranulation process triggering the release of mediators
SYMPTOMS	• Inflammation • Local IgE production = Intestinal hypersensitivity ↑ • Systemic Ig production = Pruritus or GI troubles

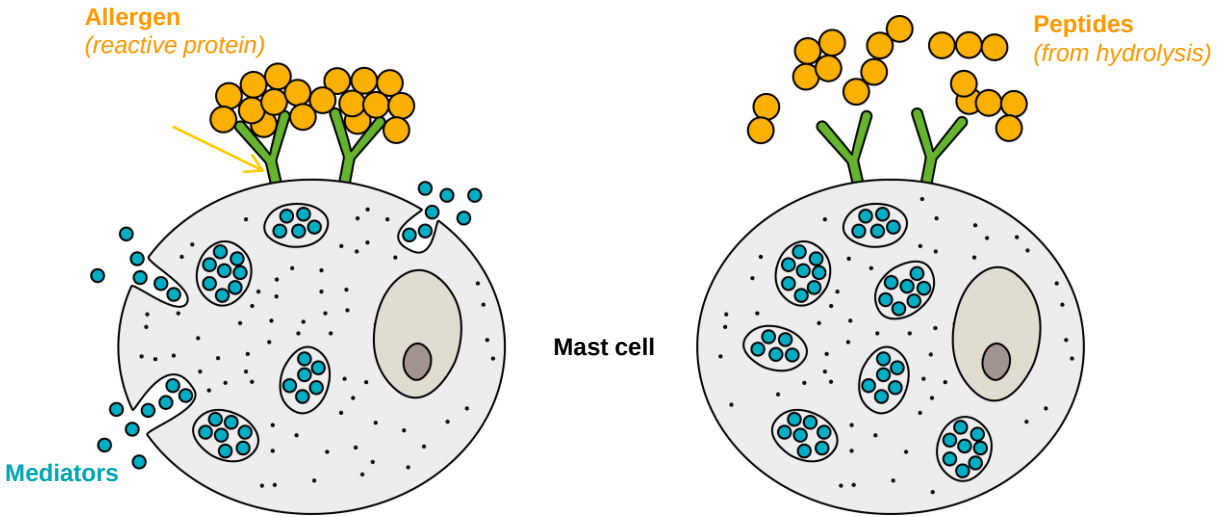
Heather Bax et al. 2012



Interest of protein hydrolysates to tackle food allergy
Target low molecular weight peptides

Mast cell activation

2 allergen specific IgE recognize the food allergen and binds to it, trigerring the degranulation process (mediator liberation)



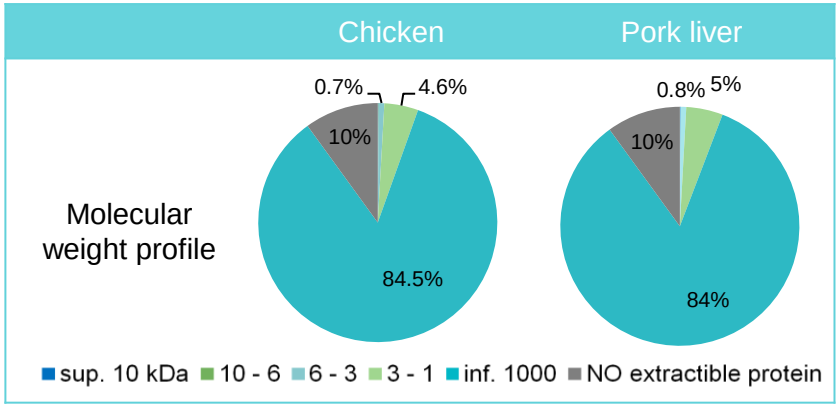
If the allergenic protein is sufficiently hydrolysed, IgE cross-linking may not occur and therefore, mast cells do not degranulate

Protein hydrolysates: Evaluation of hypoallergenicity
Protocol

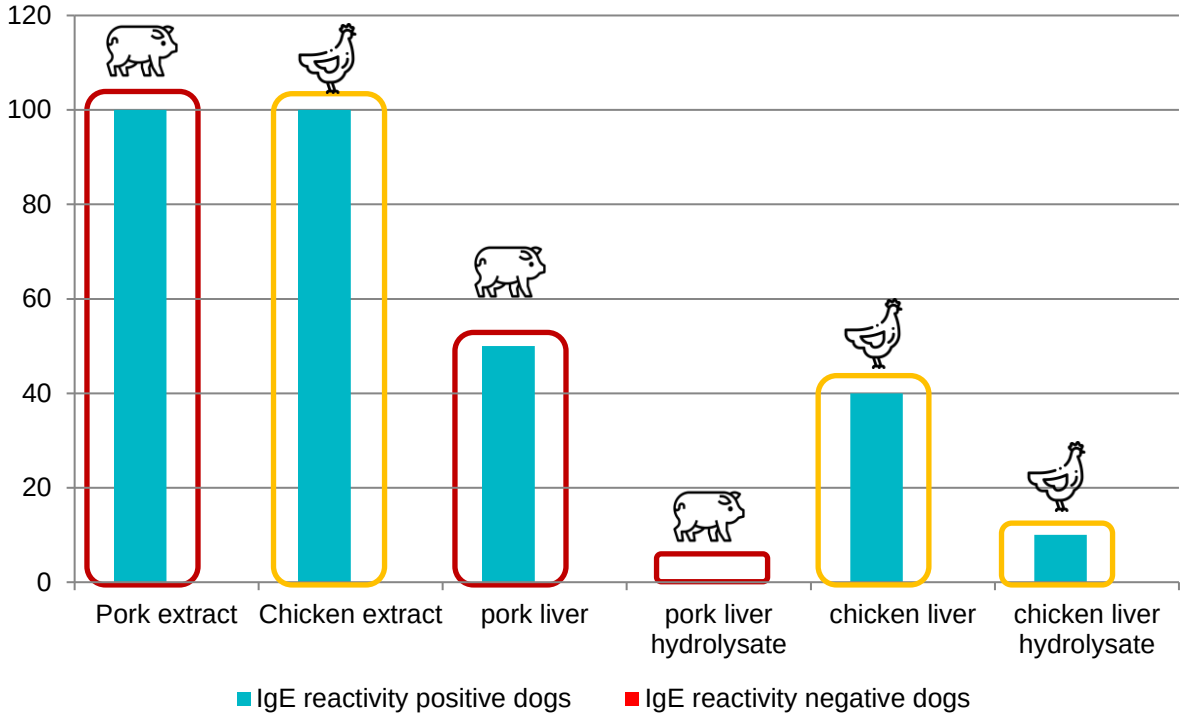
Protocol to determine Hypoallergenicity

MATERIALS

➔ Protein hydrolysates of 2 origins (90% soluble protein)

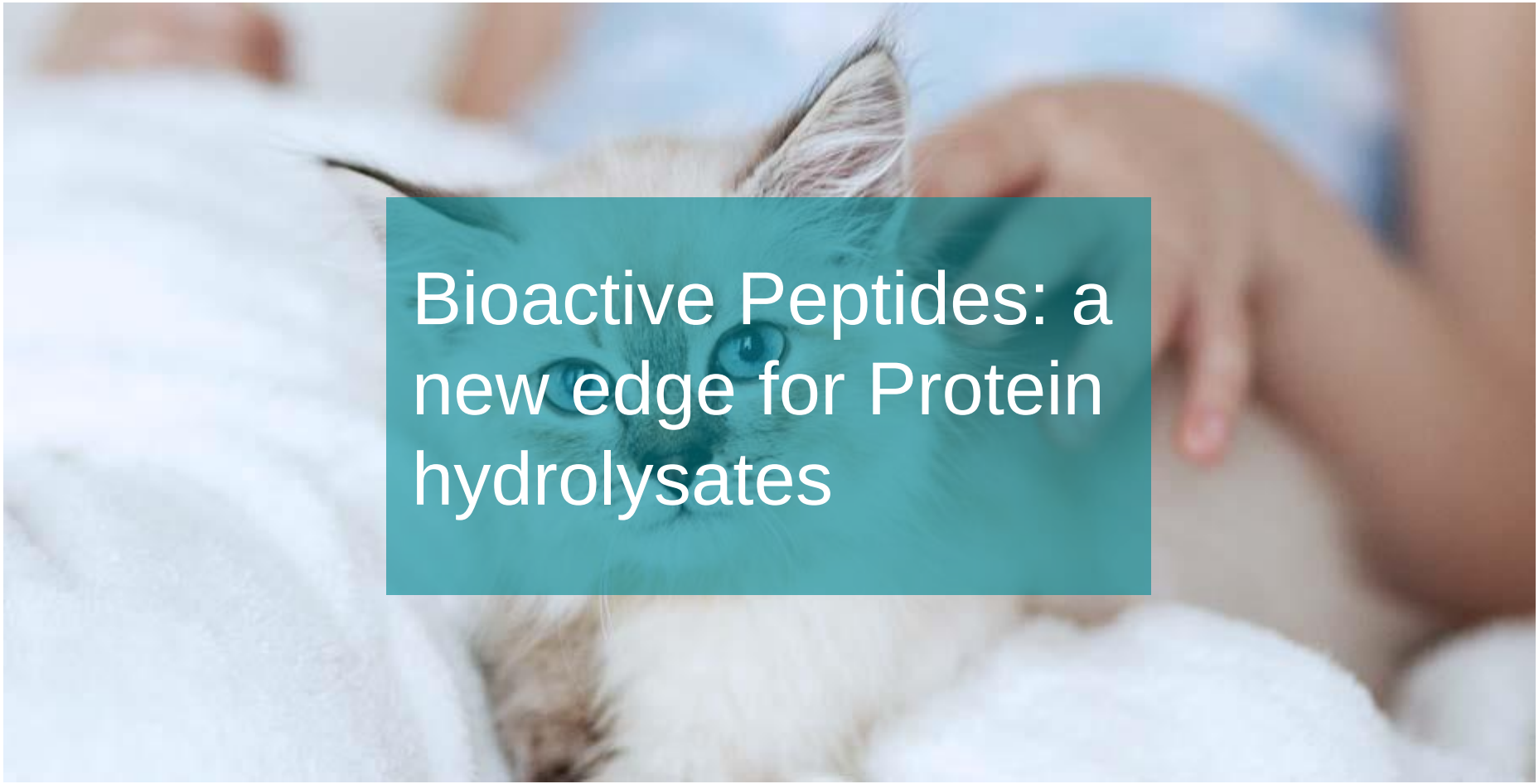


Protein hydrolysates: Evaluation of hypoallergenicity
IgE reactivity results



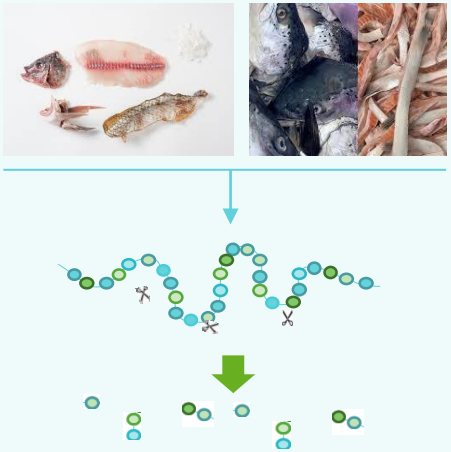
Hydrolysis of protein chain may be the only way to prevent epitope identification by the immune system

Selection of hydrolysed protein sources with **low hypoallergenicity**



Bioactive Peptides: a new edge for Protein hydrolysates

Context: What are Bioactive Peptides?

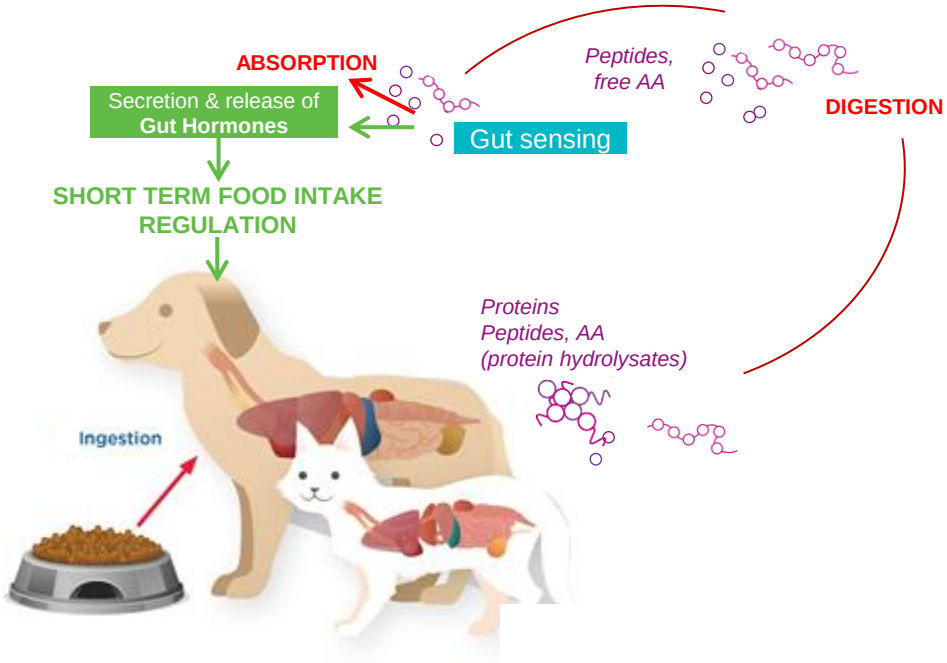


STANDARDIZED RAW MATERIAL & PROCESS

➔ Specific AA sequences
(2 to 20 Amino Acids)

Toldrá et al. 2018; Wang et al., 2018.

Context: Short term regulation of Food intake
Evaluation of a Protein hydrolysate



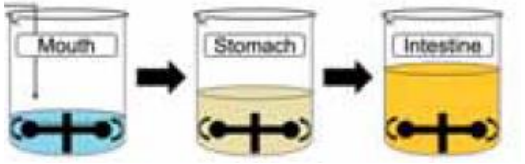
Adapted from Wernimont et al. 2020

Impact of Digestion on Peptide composition

FBP

FBPH

SIMULATED GI DIGESTION

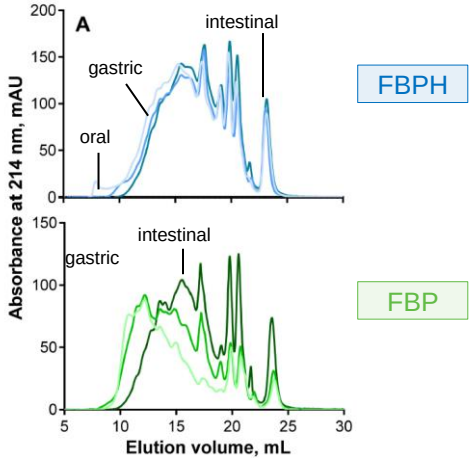


Mouth
2 min 39 °C

Stomach
2 h 39 °C
pH 2

Intestine
4 h 39 °C
pH 6,8

PEPTIDE PROFILE
(size exclusion chromatography-fast protein liquid chromatography, sec-fplc)



FBP *Fish Byproduct Protein (raw material)*

FBPH *Fish Byproduct Protein Hydrolysate*

FBPH had a consistent peptide profiles across the digestive process, conversely to FBP

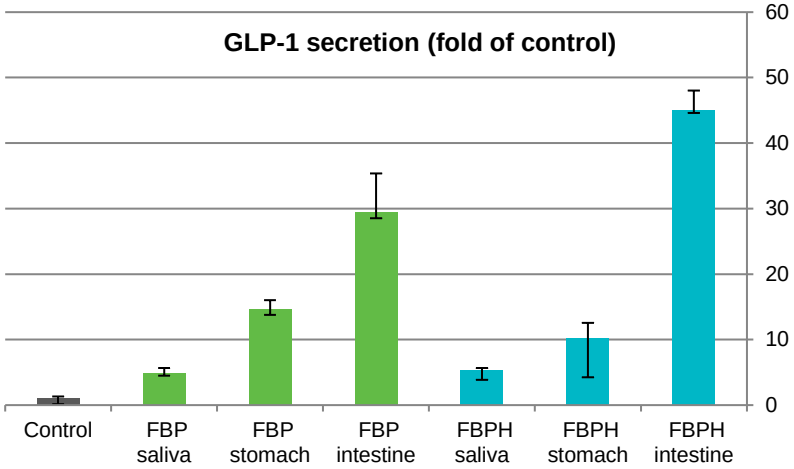
Bioactive Peptides for Energy homeostasis regulation

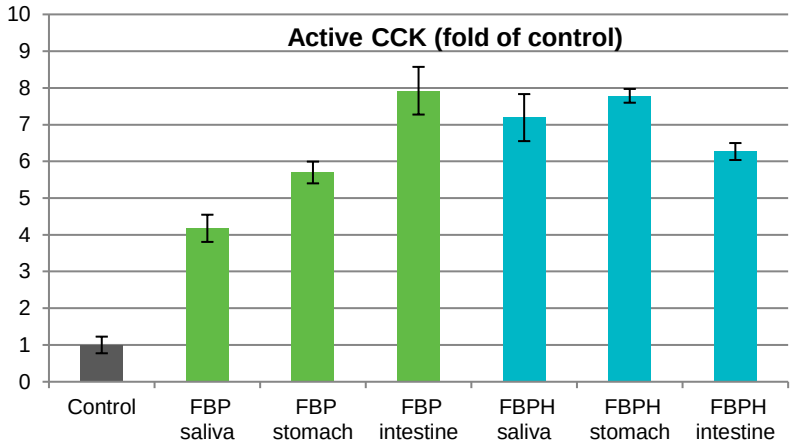
Evaluation of FBP or FBPH after simulated digestion (10 mg/mL)

2h, 37°C >

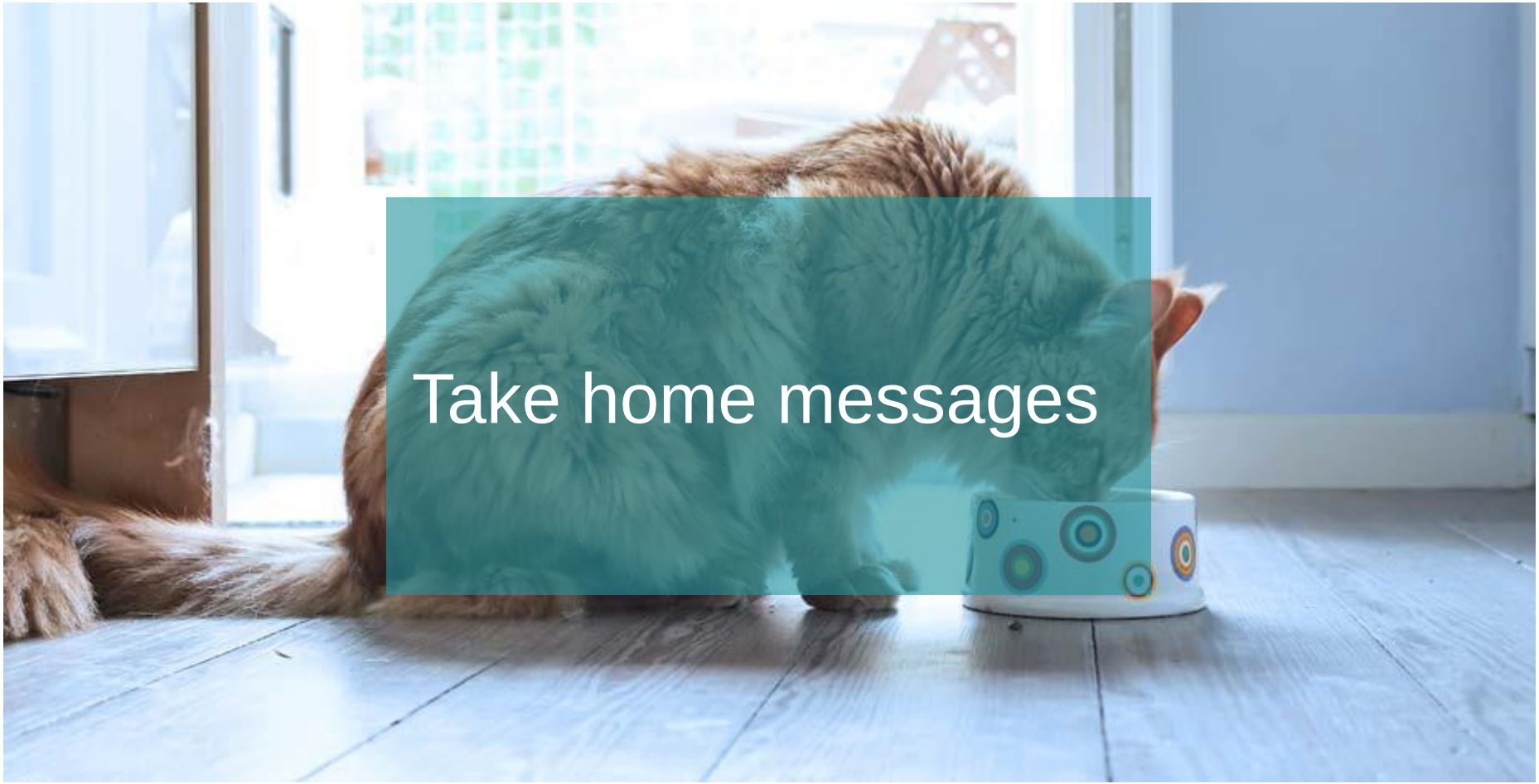
CCK & active GLP-1 secretion
RIA detection

STC-1
Enteroendocrine cells

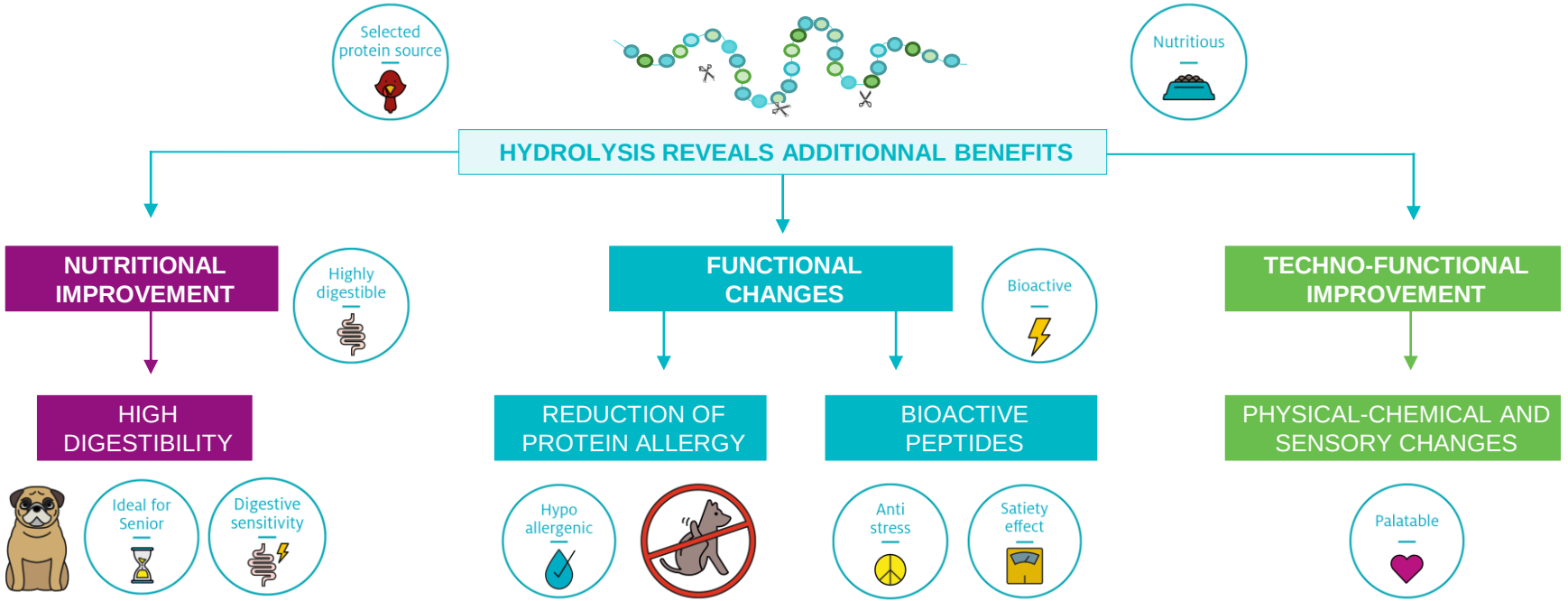




Interest of FBPH: **digestion resistant bioactive peptides for the stimulation of satiating hormones**
Superiority to FBP after digestion for GLP-1 bioactivity



Protein hydrolysates are high quality protein sources



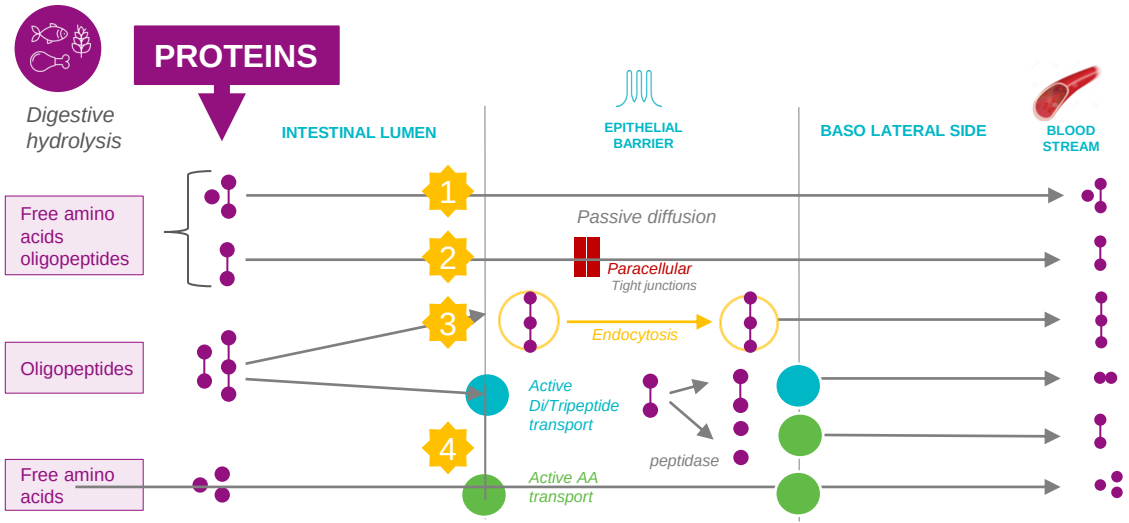
Adapted friom Tavano et al. 2018

inspired by pets,
creative by nature

dianapetfood

THANK YOU
for your attention

Intestinal absorption of Peptides & Amino Acids
Supply of Amino Acids and Peptides



DEPENDING ON PEPTIDES AND AMINO ACIDS PHYSICOCHEMICAL PROPERTIES:

1. Passive diffusion for lipophilic AA and peptides
2. Paracellular transport for smaller and hydrophilic peptides
3. Endocytosis for larger peptides
4. Active transport for di/tri peptides or AA
   e.g. PepT1 (active, H+), some peptides hydrolysed in free AA