Hydrolysed protein diets: where are we today?

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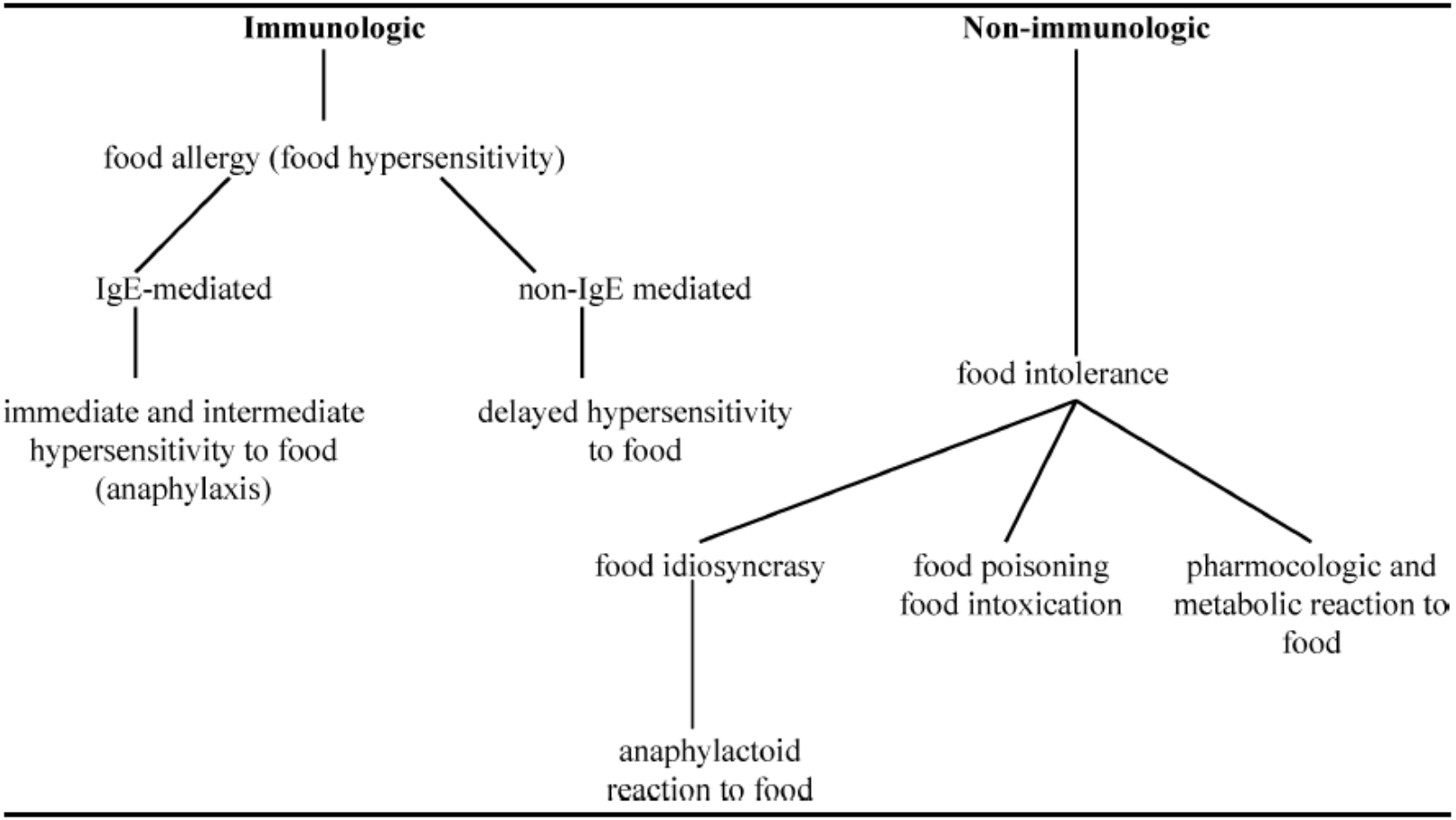
Introduction

Table 1 Classification of adverse reactions to food (adapted from Guilford, 1996a; Roudebush et al., 2000)



Prevalence adverse reaction to food: ARF

- Dermatology center: ARF + cutane Sn: 7,6-12%
- Allergic dogs: 9-36% cutane ARF (CARF)
- CARF + GI Sn: 10-31% (often milde Sn)
- Atopic Dermatis + food -> 26% GI Sn
  - food -> 10% GI Sn

Gaschen & Merchant, 2011

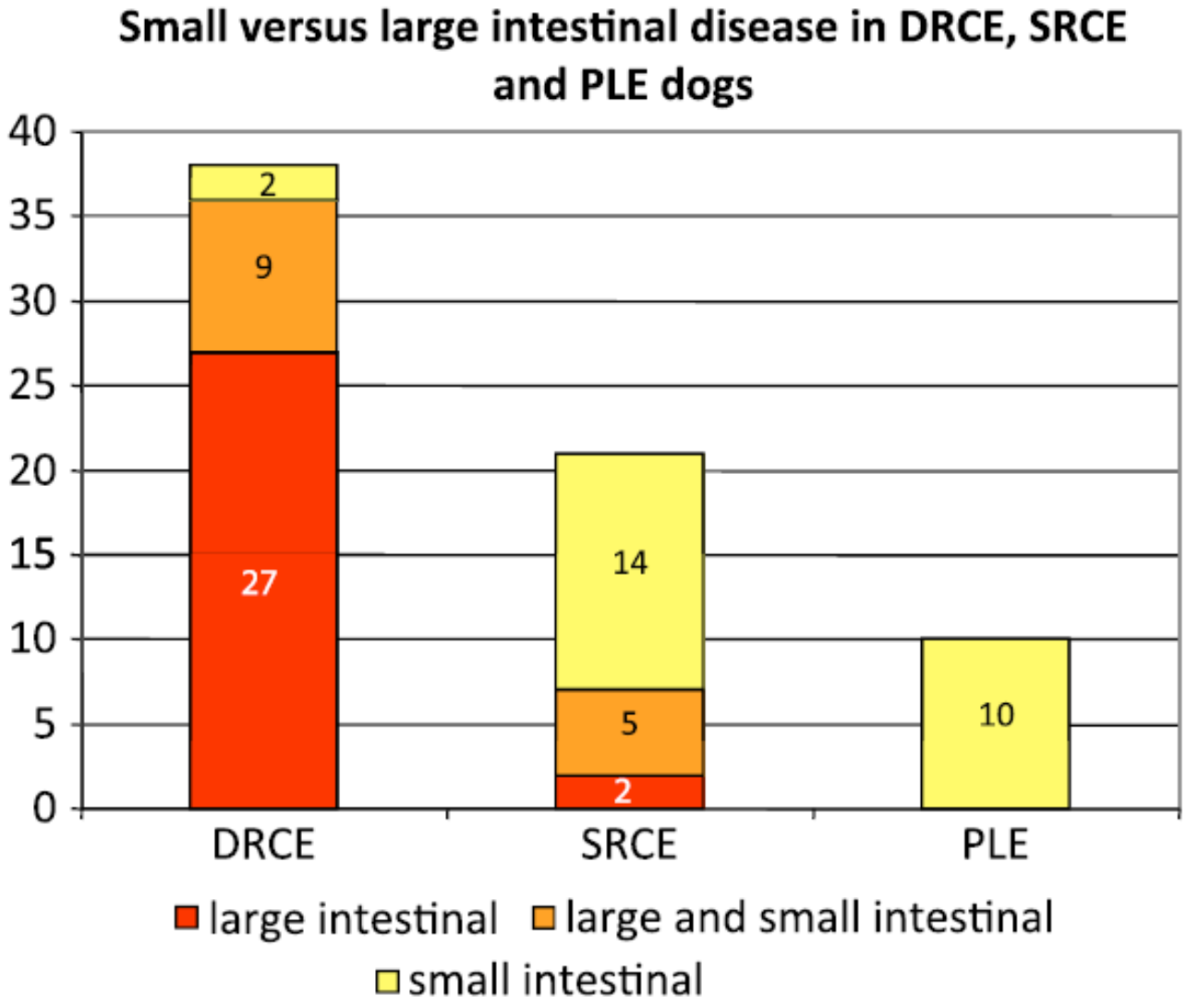
Symptoms

- Age: dog:4m – 14y; often started before 1 year (33-51%)
  cat: 6m-12y, 50% younger < 2y

- pruritis (medium to severe),non seasonal, often general

- distribution ~ atopy

- gastro-intestinal Sn: 10 à 30%



Allenspach et al., 2007

Ethiopathogenesis food allergy

- Mucosal barrière

- Oral tolerance: GALT

Table 2 Components of the mucosal barrier (adapted from Sampson, 1991)

Nonimmunologic barriers
Block penetration of ingested antigens
– intestinal peristalsis
– intestinal mucus coat (glycocalyx)
– intestinal microvillous membrane composition
– intact mucosa
Breakdown ingested antigens
– gastric acid and pepsins
– pancreatic enzymes, intestinal enzymes
– intestinal epithelial cell lysozyme activity
Immunologic barriers
Block penetration of ingested antigens
– antigen-specific secretory IgA (sIgA) in gut lumen: elicited at the Gut Associated Lymfoid Tissue (GALT)

Ethiopathogenesis: **Characteristics allergens**

- Proteins, often glycoproteins
- MG: 10 000 à 70 000 dalton
- Humans: cross reactivity
- Animals???, no cross reactivity between beef and milk
- Sensitivity reaction type 1, 3 en 4

Table 4 Common food allergens in the dog

	Beef	Dairy	Wheat	Lamb & mutton	Egg	Chicken	Soy	Pork	Rabbit	Fish	Canned foods[1]	Dry foods[1]	Diverse[2]	Number of animals
Walton (1967)	13	22	11	6	3			4	1	2	17		5	82
Jeffers et al. (1991)	12	5	4		2	3	3					2		13
Harvey (1993)	6	11	8	1	4	1							1	25
Denis and Paradis (1994)	8	4	1	1	2	2		1	1				2	14
Paterson (1995)	13	2		5	4	2	1	2					6	20
Jeffers et al. (1996)	15	7	6		5	7	8						3	25
Chesney (2002)	5	4				4		1				10	3	19
	72	55	30	13	20	19	12	8	2	2	17	12	20	198
%	36	28	15	6,6	10	9,6	6	4	1	1	8,6	6	10	

[1]Canned foods and dry foods: commercial foods in which the exact causative food allergen was not identified.
[2]Diverse: corn, rice, "biscuit," chocolate, gluten.

Verlinden et al., 2006

Table 5 Common food allergens in the cat

	Beef	Dairy	Fish	Poultry	Rabbit	Commercial foods[1] (D/C)	Egg	Lamb	Barley/ Wheat	Additives	Diverse[2]	Number of animals
Walton (1967)	5	7	1	1	1	1C					2	18
Stogdale et al. (1982)	1		1	1								1
Medleau et al. (1986)						1C						1
White and Sequoia (1989)		2	6			4*		1				13
Guaguère (1993)	4	3	2				1					17
Reedy (1994)								1				1
Guilford et al. (1998)	5		1	1		4C 6D		3		1	3	22
Guilford et al. (2001)	3	1	1	1		1C 5D		1	4	1	5	16
	18	13	12	4	1	22	1	6	4	2	10	89
%	20	14,6	13	4,5	1	24,8	1	6,7	4,5	2,2	11	

[1]Commercial foods (D = dry foods, C = canned foods): the exact food allergen was not identified.
*Not specified commercial food.
[2]Diverse: penicilline, 'Brand's essence', gluten, viscera.

Verlinden et al., 2006

Food anamnesis

- Regular food
- Snacks
- Table scaps
- Supplements
- Chew bones.
- Other? Flavoured medication,…

Some caes=> diary with daily food intakes

Diagnosis

- provisional diagnosis: elimination diet for 8-12w very strict!!!
- final diagnosis: re-introduction old diet & supplements (7-14d)
- Provocation: introduction of suspected Ag's (7d)

Allenspach et al., 2007

70 dogs + chronical enteropathy

Elimination diet 10d

44% negative

56% positive

No relaps: 79%

relapse = ARF: 21%

Provocation with old diet

Elimination diet

NEED for PROVOCATION!

Provocation with beef, chicken, lam, milk

+ in only 2 dogs = VA

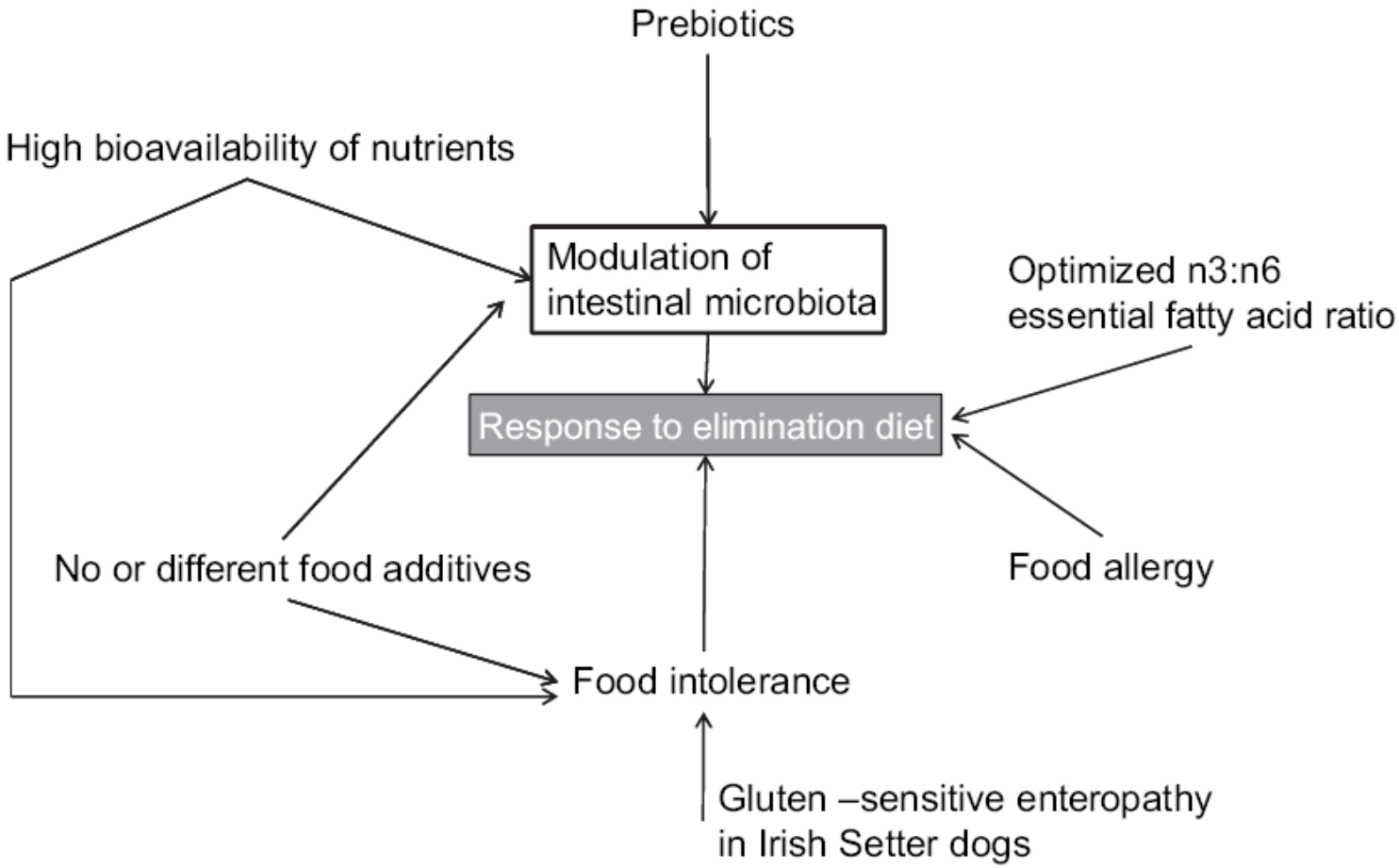


Fig. 2. Canine and feline diet-sensitive enteropathy. Possible mechanisms for positive response of the inflamed GI mucosa to an elimination trial with a commercial veterinary prescription novel protein diet or a hydrolyzed diet.

Gaschen & Merchant, 2011

Ideal elimination diet

- New or hydrolysed protein source
- One protein source
- Meeting but not exceeding protein req too much
- Highly digestible
- Low in additives, conservatives and vasoactive amines
- Complete and balanced

Treatment

- Avoidence of allergen(s)

tolerance individually different
Threshold? -> other allergy's

- Future research:

role GI flora: probiotics, n-3 FA, partial gehydrolysed proteins,…

Content

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Introduction

Term ‘hypoallergenic diets’

Substantial reduction in allergenicity, tolerated by patients that are hypersensitive to the intact protein

Diagnosis and treatment of food allergy

- Novel protein diets
- Hydrolyzed protein diets

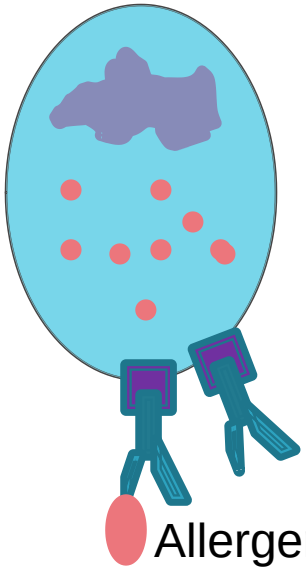
Table 3 Comparison of different types of hypersensitivity reactions (Roitt, 1991)

Hypersensitivity reaction	Type I anaphylaxis	Type II cytotoxic	Type III immune complex mediated	Type IV cell-mediated
Antibody	IgE bound to mast cell	IgM and IgG ± CF*	Humoral antibodies ± CF*	T-cell receptor
Origin of antigen	Exogenous	Cell surface	Extracellular	Associated with MHC-molecule on macrophage or target cell
Response to intradermal antigen				
– Maximal reaction	After 30 minutes	—	After 3–8 h.	After 24–48 h.
– Form of appearance	Urticaria	—	Erythema en oedema	Erythema and induration
– Histology	Degranulation of mast cells Oedema	—	Acute inflammation Mainly polymorphonuclear cells	Perivascular inflammation Mainly monomorphonuclear cells

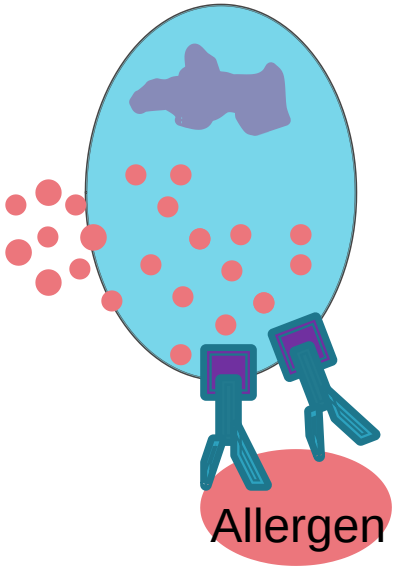
*CF: complement fixation.

Mast cell degranulation

Type I hypersensitivity mast cell



Type I hypersensitivity mast cell



Importance of the size of the allergen

Food allergens

- All proteins: antigenic <-> only few: allergenic
- Influenced by immunogenicity + gut permeability
- Molecular weight of peptides
  max: 70kDa <-> enteric absorption
  min:10kDa; 3-5kDa; 1-1.4kDa

Food allergens

Food allergen	Molecular weight (kDa)
Dairy products (casein, lactoglobulin)	14.4–27
Egg white (chicken)	28–77.7
Peanut	17–63.5
Soybean	20.5
Fish	12.3
Grains	7.8–66.5

Sampson , 1993

Reduction of antigenicity

- Disruption 3 dimensional structure
- Altering AA side chains
- Cleaving peptide bonds

Methods

- Heat treatment => denaturation <-> heat stable Ag
- pH manipulation
- Enzymatic hydrolysis + filtration

Enzymatic hydrolysis

- Cleavage of protein into smaller peptides

If within Ag peptide sequence=> immune inactive

- By proteases: endo-peptidase +/- exo-peptidase
- Selection! Often by trial and error + in vitro evaluation

- Degree of hydrolysation (DH): Partially or extensively hydrolysed -> AA

- Ultrafiltration: residual AA sequences, trace of enzymes, **removal of larger fragments**

Enzymatic hydrolysis

Changes in functional properties

- Improved digestibility
- Reduction in allergenicity
- No decrease in nutritional value

But also: cleaving -> disruption of 2°/3° structure

- Exposing hydrophobic parts --> taste
- ↗ number of ions --> osmolarity

- **Change in DH -> may have a different effect on the different properties!**

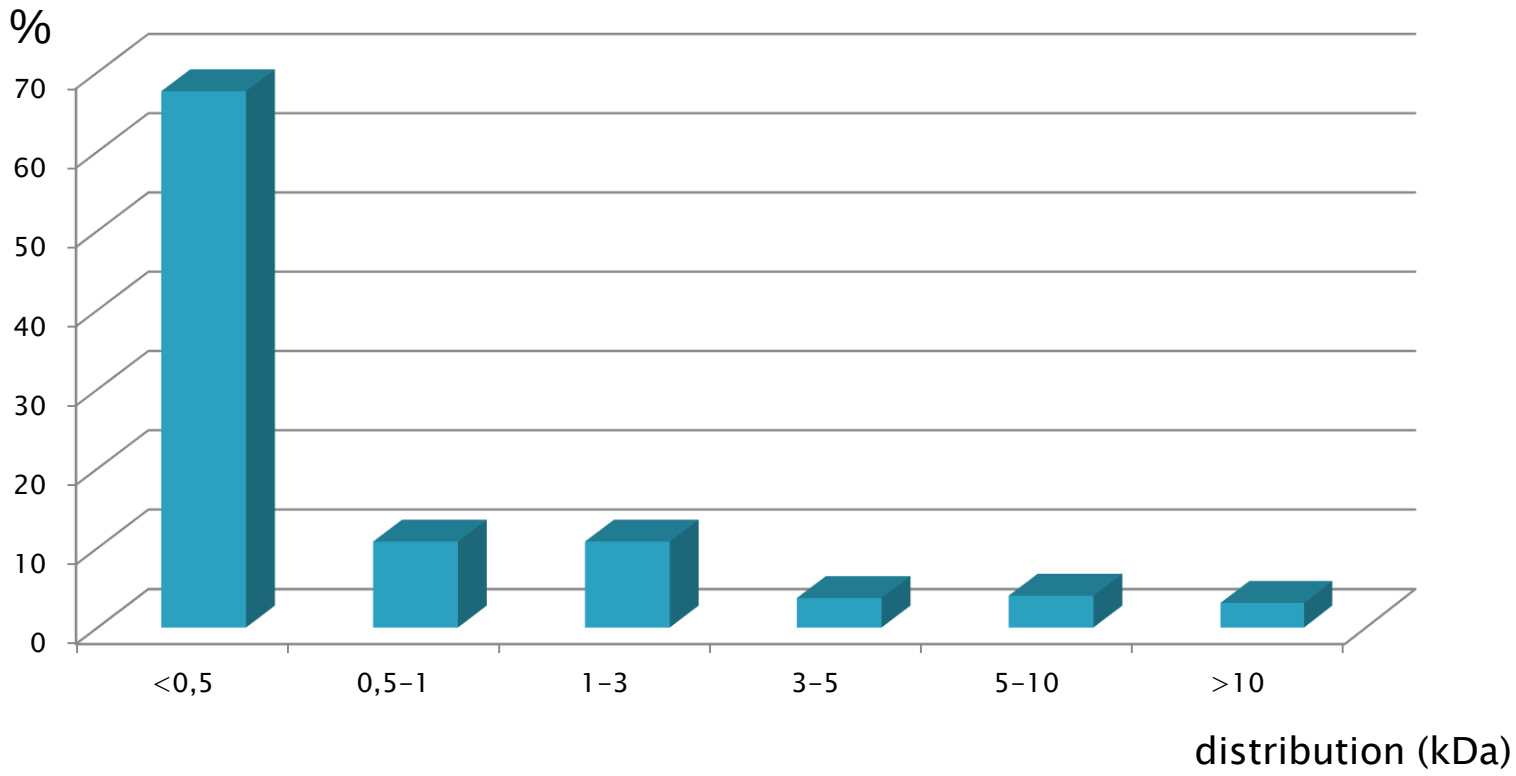
Boumans, 2001

Immunological evaluation

- In vitro
  - Molecular weight profiles
  - Competitive inhibition essays (ELISA)
  - Others
- Clinical trials!!

Cave et al. 2004

Molecular weigth distribution of chicken hydrolydate



Cave et al., 2004

Degree of hydrolysation?

- In vitro

binding of canine soy specific IgE to **native, complete and partially hydrolyzed** soy protein

- In vivo

ID test in experimentally soy hypersensitive beagles

Serra et al., 2006

Degree of hydrolysation?

In vivo

- Concentration of 100ug/ml and hydrolysation of 10-30kD: weal area of 17.9mm² in 3 dogs

- Conclusion: **hydrolysation of soy protein < 10kDa (or at least < 30kDa)**

Serra et al., 2006

Clinical studies

Study	Reference	Sponsor	Experimental or spontaneous sensitizations	Subjects	Relevant outcome measures
Groh and Moser 1998	32	Not specified	Spontaneous	29 client-owned dogs with pruritus suspected of having cutaneous adverse food reactions	Unclear
Olson *et al*. 2000	28	Iams	Experimental	14 laboratory dogs: 11 sensitized to casein, soy and chicken liver + 3 controls	IDT, skin lesions, pruritus
Sousa *et al*. 2000	33	Purina	Spontaneous	24 client-owned dogs with signs of cutaneous adverse food reaction	Pruritus
Beale and Laflamme 2001	34	Purina	Spontaneous	10 client-owned dogs with corn or soy sensitivity	Pruritus
Jackson *et al*. 2003	13	Purina	Spontaneous	14 laboratory dogs hypersensitive to corn and soy	Skin lesions*
Biourge *et al*. 2004	35	Royal Canin	Spontaneous	60 client-owned dogs with pruritus suspected of having skin hypersensitivity	Pruritus
Loeffler et al. 2004	36	Hill's	Spontaneous	63 client-owned dogs suspected of allergic skin or ear diseases	Pruritus
Loeffler *et al*. 2006	37	Hill's	Spontaneous	181 client-owned dogs with nonseasonal pruritus (includes those of Loeffler *et al*. 2004)	Pruritus
Puigdemont *et al*. 2006	29	Royal Canin	Experimental	12 laboratory dogs: 9 sensitized to soy + 3 controls	IDT, skin lesions, pruritus*
Serra *et al*. 2006	30	Royal Canin	Experimental	8 laboratory dogs: 6 sensitized to soy + 2 controls; same dogs as in Puigdemont *et al*. 2006	IDT, immunoblotting
Ricci *et al*. 2006	31	Purina	Spontaneous/ Experimental	26 (phase I) or 12 (phase II) laboratory dogs with clinical response to intact soy or chicken	Skin lesions, pruritus†

Mandigers et al., 2010; Mandigers et a., 2011; Ricci et al., 2011

Clinical studies

- Large group of dogs (63, 181) + spontaneous food reaction -> commercial chicken hydrolysate *(Loeffler et al. 2004, 2006)*
- 36 dogs with adverse food reaction + soy hydrolysed rice diet *(Biourge et al. 2004)*
- Lack of information: dogs hypersensitive to which parent protein? => **no conclusions on the effect of hydrolysation**

Clinical studies

- Hu: test hydrolysed whey formula:

double blind placebo controlled -> tolerance of 90% in children with **proven** cows milk allergy

(Am Acad Pediatrics, Giampietro et al., 2001)

- Dogs: *Jackson et al., 2003; Puigdemont et al., 2006; Ricci et al., 2011*

Clinical studies

- 14 dogs: soy + corn hypersensitivity -> hydrolysed soy protein (12.2kD) + corn starch

- 3/14 dogs adverse cutaneous reaction to test diet, 2/3 reacted to corn starch

-> soy hydrolysate was well tolerated

Jackson et al., 2003

Clinical studies

- Double blind controlled study
- Experimentally induced type I hypersensitivity<-> soy protein
- 6 sensitised dogs, 2 controls
- Oral challenge in cross over with soy isolate or soy hydrolysate

Puigdemont et al., 2006

Clinical studies

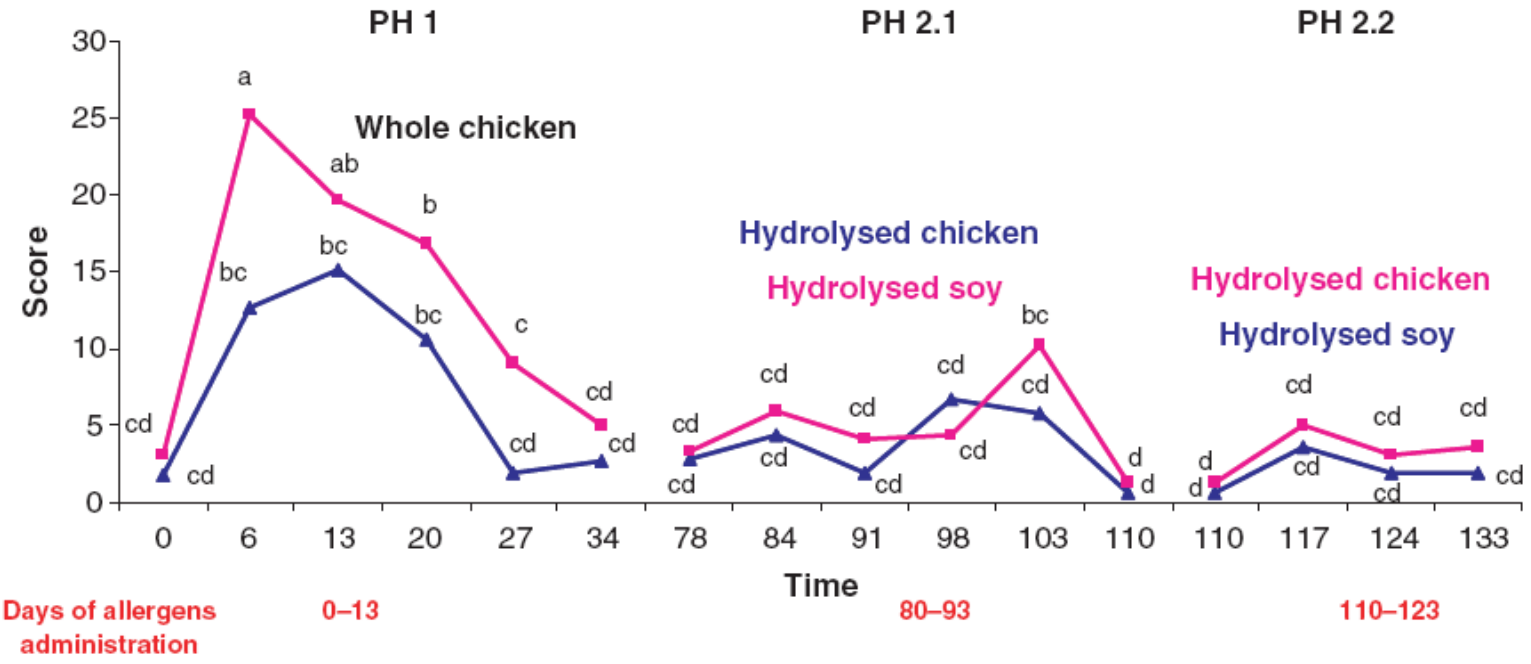
- 3/6 dogs: GI reaction after native soy protein
- 0/6 dogs: clinical signs or reactions after hydrolysed soy protein

Puigdemont et al., 2006

Clinical Studies

Ricci et al., 2011

- Dubbel blind placebo controlled
- 12 honden cutane reactions to chicken
- Cross-over 14d hydrolysed chicken & soy versus intact chicken
- Significant decrease score hydrolysed versus intact chicken in 11/12 dogs; 8/11: 80% reduction
- No effect on serum IgG & IgE



Possible problems

- Palatability: bitterness

<−> Loeffler et al., 2004; Biourge et al., 2004, Verlinden et al., 2006

- Persistent immunogenicity

Hydrolysed diets <-> purified diet: AA and small peptides

- Osmolarity

682mOsm/L (hydrolysed diet) <-> 293mOsm/L (standard diet)

- Expensive

Cave et al., 2006

Possible advantage

- Partially hydrolyzed proteins

Induction of oral tolerance?

Rats + induction of cow's milk hypersensitivity + partially or extensively hydrolysed cow's milk during induction

-> induction of antigen specific oral tolerance by partially but not by extensively hydrolysed cow's milk

Fritsché et al., 1997

Commercial dry diets for dogs

Diet	Protein source	Carbohydrate source	Lipid source
Hill's z/d ultra	chicken	corn starch	soy oil
Purina HA	soy	corn starch,	coconut, rapeseed oil
Royal Canin Hypoallergenic	soy, poultry liver	rice	Poultry, pork fat, soy, borage, oil
Specific FA management CYD	salmon, rice protein*	rice	pork fat, sunflower
Trovet σ HD	chicken, fish, soy	rice	fish, chicken, borage, flaxseed, beef
Affinity Advance hypoallergenic	soy	corn starch,	coconut, rapeseed, corn oil

Commercial dry diets for dogs

Diet	Protein source	Mean size	max size
Hill's z/d ultra	chicken		10kDa
Purina HA	soy	12.79kDa	
Royal Canin Hypoallergenic	soy, poultry liver	10-12kDa	
Specific FA management	Salmon+ rice*		10kDa
Trovet σ HD	Chicken, fish, soy		10kDa
Affinity Advance hypo allerginic	soy	13kDa	

* Intact protein

Conclusion

- Use of commercial hydrolysed diets
- Consideration of carbohydrates and lipids source as potential **protein** allergens
- More research needed!

Difficulties in practice

Adverse reactions to food

- Puppy/kitten? <-> **hypoallergenic feed adult**

- IBD -> hypoalbuminemia <-> **protein%**
  -> low BCS <-> **Fat%**

- ARF +…..

Remember

- 16% Belgian vet will never advise a home made diet
  (De Smet en Poels, 1995)

- 90% of home made elimination diets for dogs & cats are inadeqaute for adults (Roudebush et al. 1992)

TABLE 1 Nutritional screening risk factors

	Check (✔) if present
History	
Altered gastrointestinal function (eg, vomiting, diarrhea, nausea, flatulence, constipation)	
Previous or ongoing medical conditions/disease	
Currently receiving medications and/or dietary supplements	
Unconventional diet (eg, raw, homemade, vegetarian, unfamiliar)	
Snacks, treats, table food > 10% of total calories	
Inadequate or inappropriate housing	
Physical examination	
Body condition score (9-point scale): any score less than 4 or greater than 5	
Muscle condition score: mild, moderate or marked muscle wasting	
Unexplained weight change	
Dental abnormalities or disease	
Poor skin or hair coat	
New medical conditions/disease	

WSAVA guidelines, 2011

9-year-old, intact male, Briard