# Immunonutrition – fact or fiction?

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1. Introduction
   - Immune reaction and nutrition
   - Immune system: gut and general
2. Immunonutrition
   – Basic nutrition
   – Functional supplements
3. Therapeutic applications

- Systemic stress response to illness-induced anorexia or hypermetabolism

  - Metabolic rate ↗
  - Protein catabolism ↗
  - Cell mediators ↗
    - cytokines, prostaglandins, leukotrienes…
  - Oxidative stress ↗

- "Immunonutrition" = need for…
  - Proliferation, production
    - Empirical
    - Factorial
  - Example lysine requirement (% of intake [chicken])
    - Development 1 - 2
    - Conservation 0.5 - 2
    - Reaction 7 - 10
    - Expression of markers (e.g. nutrient transporters)

Humphrey 2010

# Immune system

- Gut = biggest immune organ
- Surface

  - Microbiota
  - Feed antigens
  - Environment

How does the immune system learn tolerance?

# Immune development in puppies

- Few data about immunological development in puppies available (Greeley et al. 1996 + 2001, Faldyna et al. 2001 + 2005, Toman et al. 2002)

- No investigation in food tolerance- or - sensitization-development during postnatal development in puppies

- Knowledge about mechanisms from conclusion by analogy from human medicine or other animals (e.g. pigs, mice)

Thesis, Patricia Phillip

# Hypothesis

1. Dietary protein sources influence the development of oral tolerance against food antigens in puppies

2. Dogs with develop oral tolerance against food antigens have a higher $T_{Reg}$-Population and IL-10 concentration than sensitized dogs

# Study design

| age of the puppies | group | diet |
|---|---|---|
| 3 weeks to 11 weeks | 1 | mixed protein source |
| | 2 | single protein source |
| cross –over | | |
| 11 weeks to 19 weeks | 1 | single protein source |
| | 2 | mixed protein source |

mixed protein source: soy protein + poultry meal + fish meal
single protein source: poultry meal

## Results

- **Differences of immune traits were not found**
- After the cross-over, the stimulation indices for the food associated proliferation assay for the fish, chicken and soyprotein were sigificantly higher compared to before the cross-over
- This was accompanied by slightly decreased numbers of CD4+/Foxp3+-T-lymphocytes
- Age-dependent effects more likely than food-dependent effects

- Intestinal microbiota
  - Bacteria ~ 55 divisions
    - Dominating: Bacteroidetes and F
    - Proteobacteria, Actinob
      Verrucomicrobi acteria
  - Archaea ions
    anobacteriales
    Yeasts, moulds
  - Protozoa
  - Viruses

No developped immune system without intestinal microbiota

RESEARCH ARTICLE

# Humoral immune responses against gut bacteria in dogs with inflammatory bowel disease

**Sirikul Soontararak, Lyndah Chow, Valerie Johnson, Jonathan Coy, Craig Webb, Sara Wennogle, Steven Dow***

## Abstract

Inflammatory bowel disease (IBD) in dogs is associated with clinical signs of intestinal dysfunction, as well as abnormal lymphocytic and myeloid cell infiltrates in the small and/or large intestine. Thus, in many respects IBD in dogs resembles IBD in humans. However, the factors that trigger intestinal inflammation in dogs with IBD are not well understood and have been variously attributed to immune responses against dietary antigens or intestinal antigens. Previous studies in humans with IBD have documented increased production of IgG and IgA antibodies specific to intestinal bacteria, and this abnormal immune response has been linked to disease pathogenesis. Therefore, we investigated the humoral immune

greater levels of antibody binding in dogs with IBD. Based on these findings, we concluded that antibody binding to commensal gut bacteria was significantly increased in dogs with IBD, that particular phyla were preferential targets for gut antibodies, and that anti-bacterial antibody responses may play an important role in regulating gut inflammation.

- Actinobacteria + Collinsella → antibody binding in dogs with IBD ↗
- Anti-bacterial antibody responses may play an important role
- No systemic response

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# Immune system depends on/ can be influenced

- Adequate supply of
  1. Protein (source, quantity) and amino acids
  2. Fat and fatty acids, n-3/n-6
  3. Minerals, vitamins, trace elements
- Functional components, supplements
  1. Fermentable carbohydrates
  2. Antioxidants, carotenoids
  3. Pro- and prebiotics

- Allergy/Intolerance

  - Mostly dietary proteins
  - Rarely other feed ingredients
    - Additives
    - Mites

Does protein influence the immune reaction depening on intake and specific AA?

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# Food … Dogs and Cats: A…

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*Food allergy (FA) is defined as "all immune-mediated reactions following food intake," in contrast with food intolerance (FI), which is non-immune-mediated. Impairment of the mucosal barrier and loss of oral tolerance are risk factors for the development of FA. Type I, III, and IV hypersensitivity reactions are the most likely immunologic mechanisms. Food allergens are (glyco-)proteins with a molecular weight from 10–70 kDa and are resistant to treatment with heat, acid, and proteases. The exact prevalence of FA in dogs and cats remains unknown. There is no breed, sex or age predilection, although some breeds are commonly affected. Before the onset of clinical signs, the animals have been fed the offending food components for at least two years, although some animals are less than a year old. FA is a non-seasonal disease with skin and/or gastrointestinal disorders. Pruritus is the main complaint and is mostly corticoid-resistant. In 20–30% of the cases, dogs and cats have concurrent allergic diseases (atopy/flea-allergic dermatitis). A reliable diagnosis can only be made with dietary elimination-challenge trials. Provocation testing is necessary for the identification of the causative food component(s). Therapy of FA consists of avoiding the offending food component(s).*

**Keywords** adverse food reactions, clinical signs, diagnosis, hypoallergenic diet, therapy

# Functions of some key amino acids in the gut

| | Energy substrates | Immunity and health | Antioxidative defense | Protein synthesis |
|---|---|---|---|---|
| Arginine | Precursor of Creatine | Precursor of Nitric Oxide | | • Modulates mTOR activity<br>• Precursor of polyamines |
| Glutamine | Metabolic fuel for enterocytes | Metabolic fuel for lymphocytes | Precursor of glutathione | Modulates mTOR activity |
| Glycine | | | Precursor of glutathione | |
| Threonine | | Rich in immunogloulins and mucins | | |
| Cysteine | | Rich in mucins | Precursor of glutathione | |
| Tryptophan | | Critical for macrophage and lymphocyte function | | |
| BCAA/Leucine | Precursor of glutamine | | | Modulates mTOR activity |

# Arginine and ornithine

- Mitogen-stimulated proliferative activity of blood leukocytes revealed a quadratic effect
- Phagocytic granulocytes linearly decreased

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STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access ACVIM American College of Veterinary Internal Medicine

## Effects of arginine and ornithine supplementation to a high-protein diet on selected cellular immune variables in adult cats

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**Abstract**

**Background:** Dietary protein and amino acid intake and composition can modulate immune function.

**Objectives:** To evaluate the effects of high-protein intake and arginine and ornithine supplementation on feline immune cells.

**Animals:** Ten healthy cats.

**Methods:** Experimental study. Cats received a high-protein basal diet as a single daily meal. A crossover design was applied with treatments being basal diet (w/o); basal diet with arginine supplementation (+50, 75, 100% compared to the arginine provision by the basal diet; Arg 1-3); and basal diet with ornithine supplementation (+100, 150, 200% compared to the arginine provision by the basal diet; Orn 1-3). Blood samples were collected at the end of each 11-day treatment period.

**Results:** Mitogen-stimulated proliferative activity of blood leukocytes revealed a quadratic effect for the dietary supplementation of arginine ($P$ = .02) and ornithine ($P$ = .03) (means for ConA-stimulation: w/o = 6.96; Arg 1 = 9.31; Arg 2 = 11.4; Arg 3 = 8.04; Orn 1 = 15.4; Orn 2 = 9.43; Orn 3 = 9.28; pooled SEM: 0.96). The number (% gated) of phagocytic granulocytes linearly decreased with increasing dietary concentrations of arginine ($P$ = .05) and ornithine ($P$ = .03) (means: w/o = 95.5; Arg 1 = 93.0; Arg 2 = 92.5; Arg 3 = 92.6; Orn 1 = 92.6; Orn 2 = 92.6; Orn 3 = 91.5; pooled SEM = 0.44).

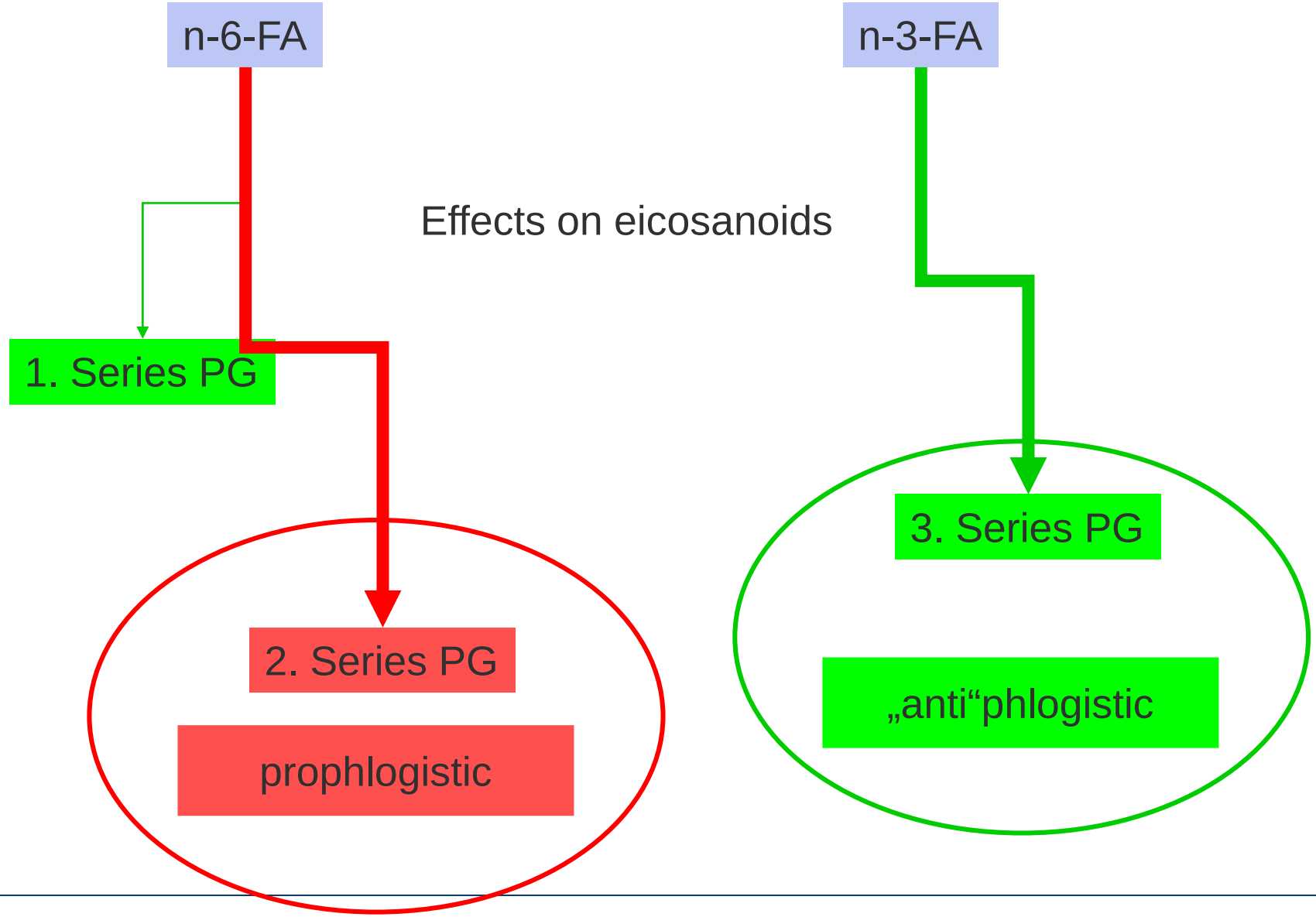
**Conclusions and Clinical Importance:** This study could demonstrate immunomodulating properties of dietary arginine and ornithine in cats.

**KEYWORDS**
feline, immune system, phagocytic granulocytes, proliferation

# Conclusion 1

- Give adequate protein
- High quality, „non-allergenic“
- Amino acids are of high interest in low protein diets

# n-3-Fatty acids



# n-3-Fatty acids

- Flax oil
  - α-Linolenic acid
  - Dogs and cats: limited capacity to metabolize α-linolenic acid to EPA or DHA

- Fish oil
  - EPA and DHA
  - Proven antiinflammatory effects

# Conclusion 2

- Select fat sources depending on disease condition
- Add fish oil if immune reaction is „high“
- Consider composition of the normal diet: fat and fatty acids

Fish oil

-> 0.2 - 0.5 g/kg BW

# Trace elements

- Zinc → 1000 µg/kg BW
  - Ubiquitous role in immune functions
    - Dogs with genetic disease
    - Detary deficiency leading to severe immune deficiency
- Selenium → 5 µg/kg BW
  - Humoral immune response
- Copper → 100 µg/kg BW
  - Lymphoid tissues

# Conclusion 3

- Consider trace element supply in subacute and chronic diseases
- Use a balanced supplement (Zn, Se, Cu…)
- Your advantage as future diplomate…

# Immune system depends on/ can be influenced

- Adequate supply of
  1. Protein (source, quantity) and amino acids
  2. Fat and fatty acids, n-3/n-6
  3. Minerals, vitamins, trace elements
- Functional components, supplements
  1. Fermentable carbohydrates
  2. Antioxidants, carotenoids
  3. Pro- and prebiotics, nucleotides derived from yeast

- Fermentable fibre

  - Blood

    - CD4/CD8 ratio ↗
    - B cells ↘

  - T cell mitogen responses

    - Intraepithelial lymphocytes ↗
    - Peyer's patches ↘
    - Lamina propria ↘

Field et al. (1999)

Colonic barrier-related proteins

Mucins

Immuno-globulin A

Complex gene regulation

Innate and adaptive immune system

Short chain fatty acids → immune programming

# Dietary Fibre

| | Diet[1] | | | | |
|---|---|---|---|---|---|
| Ingredient, % | BLP | BHP | CLP | CHP | BCLP |
| Rice flour | 61.1 | 40.0 | 61.7 | 40.6 | 69.1 |
| Poultry meal, low ash | 9.50 | 24.3 | 8.70 | 23.9 | 12.2 |
| Greaves meal | 3.20 | 13.3 | 6.70 | 16.8 | 3.70 |
| Brewer's spent grain | 14.8 | 15.1 | - | - | 1.70 |
| Carrot pomace | - | - | 10.8 | 11.0 | 1.60 |
| Rapeseed oil | 6.70 | 4.40 | 7.30 | 4.90 | 6.90 |
| Bone meal | 1.70 | 0.10 | 1.90 | 0.20 | 1.70 |
| Mineral and vitamin premix[2] | 1.60 | 1.70 | 1.60 | 1.70 | 1.70 |
| Potassium hydrogen carbonate | 1.30 | 0.90 | 1.00 | 0.60 | 1.20 |
| Titanium dioxide | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |

- 10 Beagle
- Brewers spent grain
- Carrot pulp

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COMPANION ANIMAL NUTRITION

## Effects of Brewer's spent grain and carrot pomace on digestibility, fecal microbiota, and fecal and urinary metabolites in dogs fed low- or high-protein diets[1]

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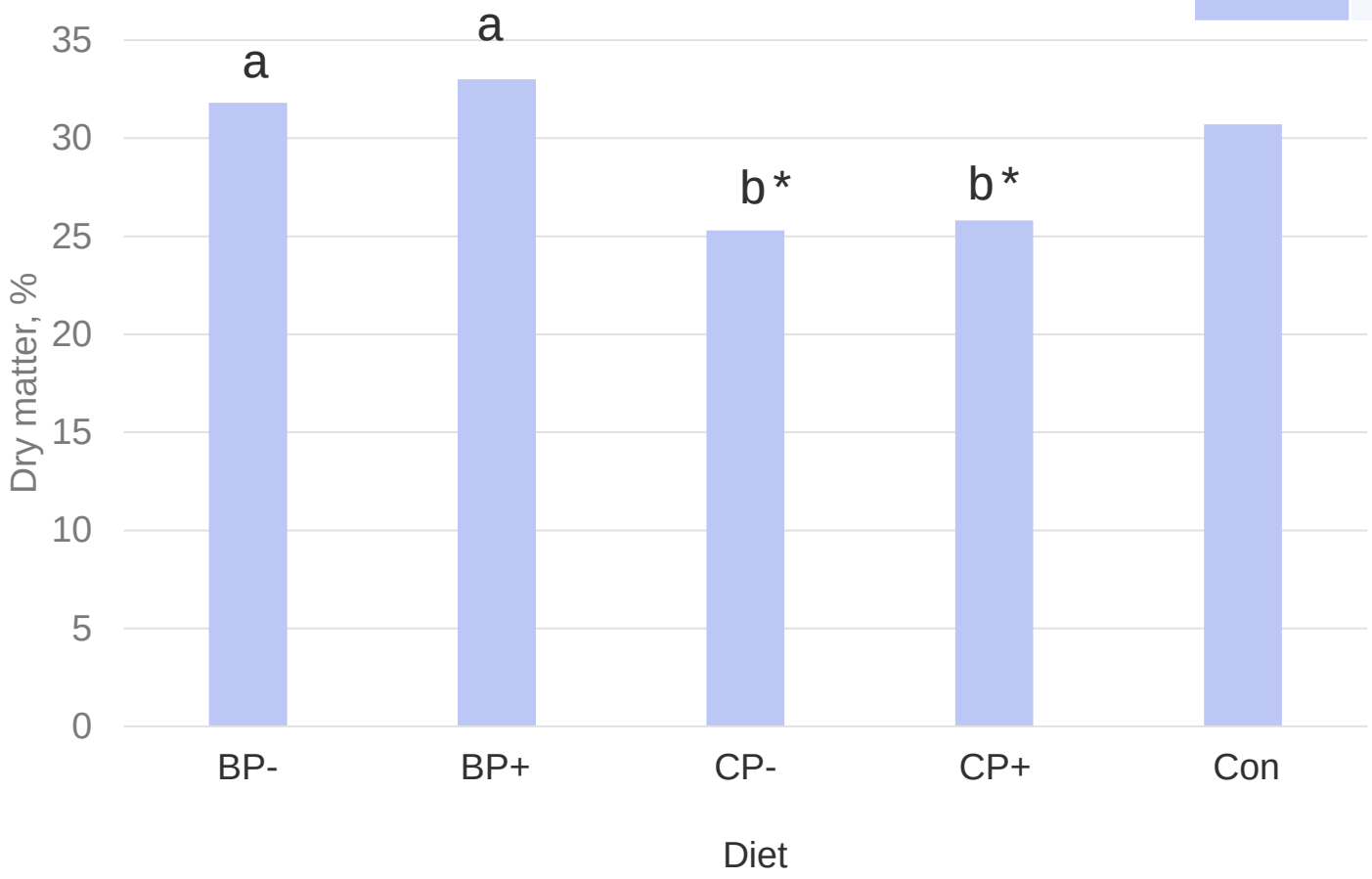
- Faecal dry matter

| SEM | P | | |
|---|---|---|---|
| | Protein | fibre | Protein × fibre |
| 0,58 | 0,36 | <0,001 | 0,657 |

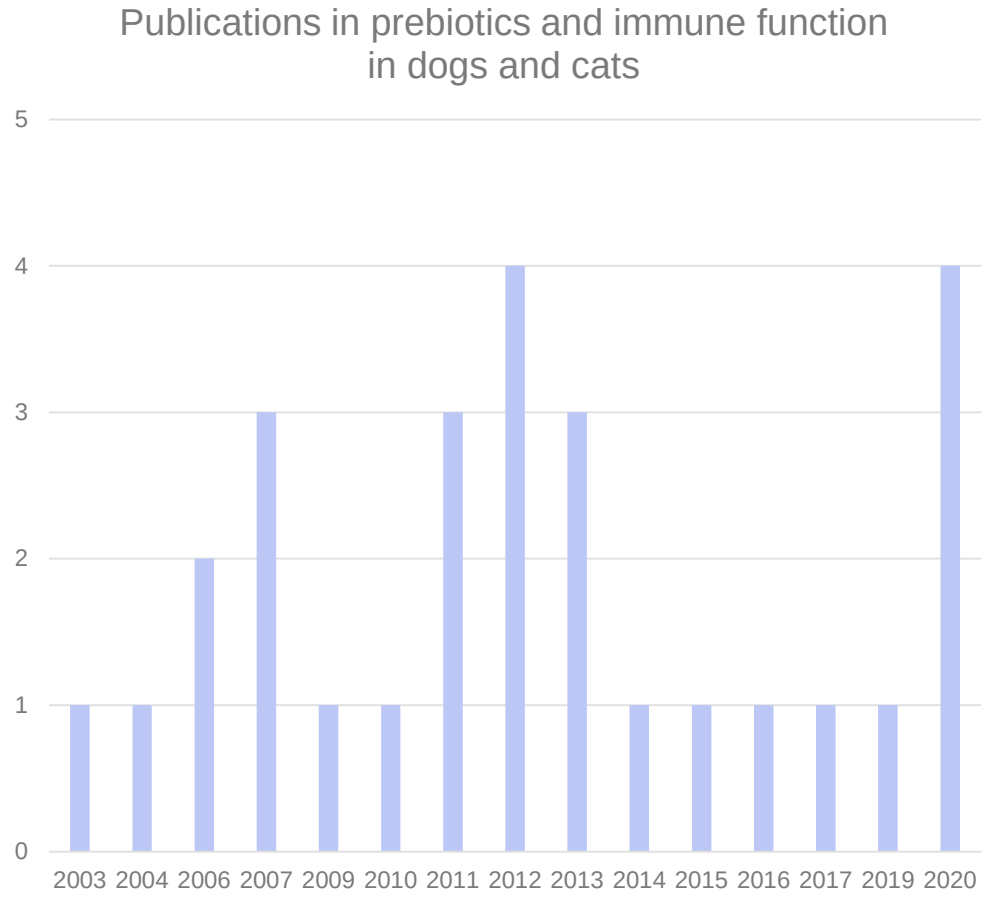


# Prebiotics

- Prebiotics are non-digestible carbohydrates
- Fermented by the intestinal microflora
- By theory, they favour the growth of beneficial bacteria and inhibit pathogens

- Oligosaccharides / prebiotics

  - scFOS (Field et al., 1999)
  - Mannanoligosaccharides (MOS) + scFOS
  - sIgA ↗ (Swanson et al., 2002)
  - Colostrum and milk IgM ↗, puppies IgM against B. bronchiseptica ↗(Adogonoy et al., 2007)



- Oxidative stress

  - Cell damage
  - Inflammation

Isaksson et al. 2011

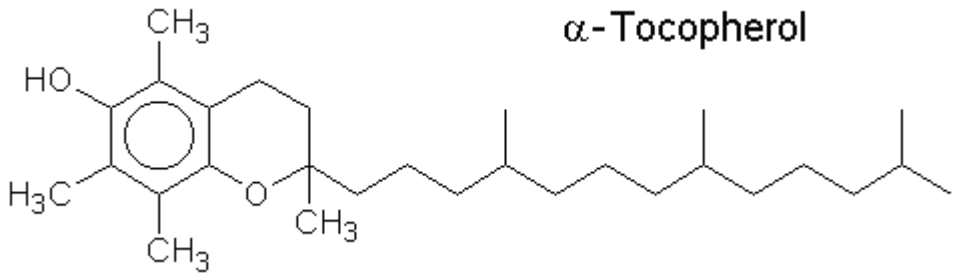
# Antioxidants

- Vitamin E and C
  - Neutrophils increased bactericidal activity
  - Altered gene expression (Hall et al. 2011)
- Vitamin C
  - No clear effects (Hesta et al. 2009)
- Carotenoids
  - Astaxanthin enhanced cell mediated and humoral immune response
  - Decreased DNA damage and acute phase protein production (Chew et al. 2011)
  - Impact on immune response through cell-mediated and humoral actions (Park et al. 2016)

# Conclusion 4

- Consider AOX supplements in critical ill patients
- Use vitamin E or combine with other AOXs

1-2 mg vitamin E /kg BW




http://www2.chemie.uni-erlangen.de/

- Gut microbiota in carnivores
  - different to other species:

    Clostridia ↑, Bifidobacteria ↓

- Type of diet
  - Strong influence on the intestinal microbiota
    - Protein: quantitiy, quality
    - Fibre
    - CHO: wet vs. dry diets

- Theory

  - Modulation of the intestinal immune response

    - Gut associated immune system

    - General immune system

- Some effects on cytokines in ex vivo cultures

  - Increased mRNA and protein levels of IL-10
  - Ratio of TNF alpha -/IL-10, IFN- gamma /IL-10, and IL-12p40/IL-10 decreased
  - Favorable effects of PC on regulatory cytokines relative to inflammatory cytokines

Sauter et al. 2006

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- Immunonutrition in companion animals

  - Gastroenterology

    - Inflammatory bowel disease
    - Food intolerance, sensitivity
    - Food allergy

  - Dermatology

  - Chronic inflammatory disorders

  - Support during therapy

  - Preventive: newborn or aged animals

# Conclusion

1. Diet ingredients and nutrient profiles affect the immune function of dogs and cats
2. Consider patients with low feed intake
3. P[illegible] of major interest and should be adjusted

Be critical, evidence based studies in patients are still scarce

„Immunonutrition“ is a critical part of clinical nutrition…