

Recent Advances in Pet Nutrition

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I. Laue, D., II. Tucker, L.A.

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Antioxidant considerations for companion animal, with special reference to immunity

P. F. Surai

Introduction

Human and animal defence against various diseases depends on the efficacy of the immune system which is responsible for elimination of foreign substances (e.g. parasites, bacteria, moulds, yeast, fungi, viruses and various macromolecules) or the creation of specific inhospitable conditions within the host for a wide range of pathogens. This protective capacity is based on the effective immune system, which is considered to be an important determinant of animal health and well-being. In that sense, a remarkable ability of components of the immune system to distinguish between self and non-self is a great achievement of animal evolution.

It is difficult to avoid nutritional or environmental stresses which are responsible for immunosuppression and increased susceptibility to various diseases. For example, mycotoxins are among major immuno-suppressive agents in animal diet. In such situations immuno-modulating properties of certain macro- and micronutrients are important. Research from the last 10 years indicates that selenium is a major immuno-stimulating agent and its' true physiological level exceeds the requirement for growth and development. Its immuno-modulatory effects have been observed in a variety of species when administered in excess of established dietary requirements. Selenium forms an essential component of selenocysteine-containing proteins involved in most aspects of cell biochemistry and immune cell activity. Selenium concentration drops during acute infections compared with the values after the recovery (Sammalkorpi *et al.*, 1988), and deficiency weakens the host immune response, thereby increasing the risk of bacterial and viral infections.

Immune system of animals

There are two major types of immune function: natural and acquired

immunity (Figure 1). Natural immunity (the 'innate' system) includes physical barriers (e.g. skin, mucus lining of the gut), specific molecules (e.g. agglutinins, precipitins, acute-phase proteins, lysozyme), phagocytosis (macrophage and heterophil cells), and lysing activity ('natural killer' lymphocytes (NK cells) (Table 1).

Figure 1. General scheme of the immune system (adapted from Surai 2002)

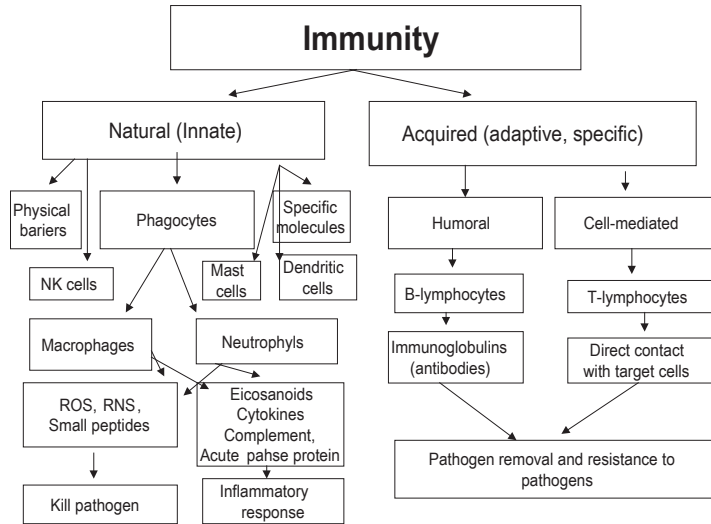


Table 1. Key elements of the immune system (adapted from Kolb, 1996; Lydyard *et al.*, 2000)

Cells	Significance
Monocytes, macrophages	Phagocytosis, synthesis of interleukins 1,6, 8 and other substances
Neutrophils	Phagocytosis of bacteria, viruses and toxins
Eosinophils	Destruction of parasites
Basophils	Initiation of inflammatory processes
Mast cells	Release of inflammatory mediators
B cells (B lymphocytes)	Production of plasma cells (immunoglobulins = antibodies), antigen-specific; 10% of total lymphocytes
T helper cells (Helper T lymphocytes)	Antigen-specific, produce cytokines: IL 2, IL3, IL4, IL5, IL9 and IL10; 55% of total lymphocytes
Cytotoxic T cells (T lymphocytes)	Destruction of tumour cells and virus-infected cells; antigen-specific, 25% of total lymphocytes
Suppresser T cells (T lymphocytes)	Inhibition of immune reactions (development of autoimmune diseases)
Natural killer cells	Destruction of tumour cells and virus-infected cells, 10% of total lymphocytes

Table 1.
Contd.

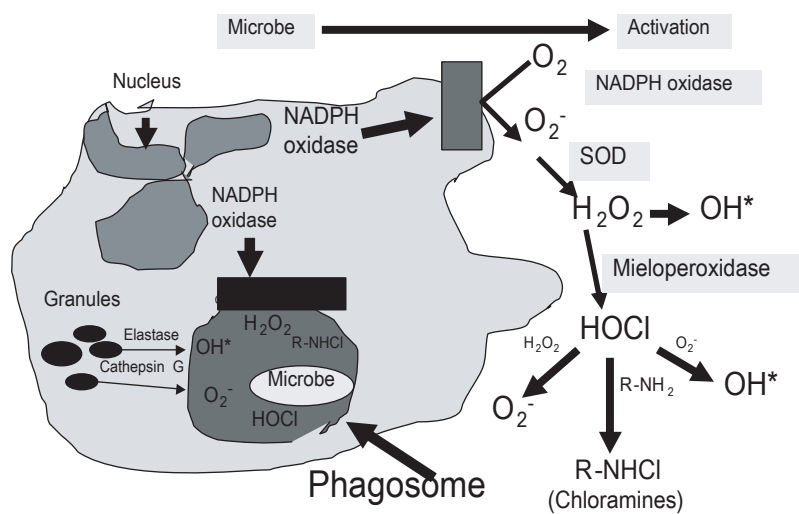
<i>Cells</i>	<i>Significance</i>
Macromolecules	
Immunoglobulins	Binding of foreign cells and proteins; Promotion of their ingestion by Phagocytes
Interferons (IFN- α ; IFN- β ;IFN- γ)	Activation of macrophages (γ - interferon); Inhibition of viral replication
Complement system: a set of over 20 soluble glycoproteins	Destruction of foreign cells
Interleukins	Regulation of specific types of leukocytes
Leucotrienes	Promotion of inflammatory process
Lysozymes	Dissolution of bacterial membranes
Collectines- a group of carbohydrate-binding proteins	Act as opsonins in non-adaptive immune Response to pathogen
Acute phase proteins- a group of plasma proteins produced in the liver in response to microbial stimulus	Maximise activation of the complement system

Macrophages are important immune cells as they perform a range of functions, including phagocytosis of foreign particles, destruction of bacterial or tumour cells, secretion of prostaglandins and cytokines and regulate activity of lymphocytes and other macrophages (Qureshi, 1998). Phagocytosis, the engulfing and destruction of foreign bodies, is the major mechanism by which microbes are removed from the body

Macrophage activation and phagocytosis of foreign particles are regularly accompanied by a so-called 'respiratory burst', where oxidising materials (reactive oxygen or nitrogen species (ROS, RNS)), are deliberately produced to kill pathogenic microbes (Figure 2). Macrophages and other phagocyte cells (leukocytes such as neutrophil, monocytes and eosinophils) can synthesize toxic oxygen metabolites to achieve this (Zhao *et al.*, 1998). In general, the production of ROS and RNS is a characteristic for both mammalian and avian macrophages (Qureshi *et al.*, 1998) and comprise the major metabolites produced by macrophages (Dietert and Golemboski, 1998). Macrophages bind, internalize, and degrade foreign antigens (e.g. bacteria) quickly; it takes 15 minutes for chicken macrophages to kill more than 80% *Salmonella* (Qureshi *et al.*, 1998).

Natural immunity works rapidly, gives rise to the 'acute inflammatory response' observed in response to challenge by disease. Macrophages also secrete a great number of immune communication molecules, such as cytokines (including the pro-inflammatory interleukin 1 (IL-1) and interleukin 6 (IL-6)), and tumour necrosis factor- α (TNF). They produce cytokine inhibitors, endocrine hormones and neurotransmitters (Klasing, 1998a) which regulate specific immunity, initiating and directing immune systems and inflammation, and amplifying responses, both by communicating with, and presenting parts of pathogens to other immune cells.

Figure 2. Respiratory burst in neutrophils (adapted from Kettle and Winterbourn, 1997; Nordberg and Arner, 2001)



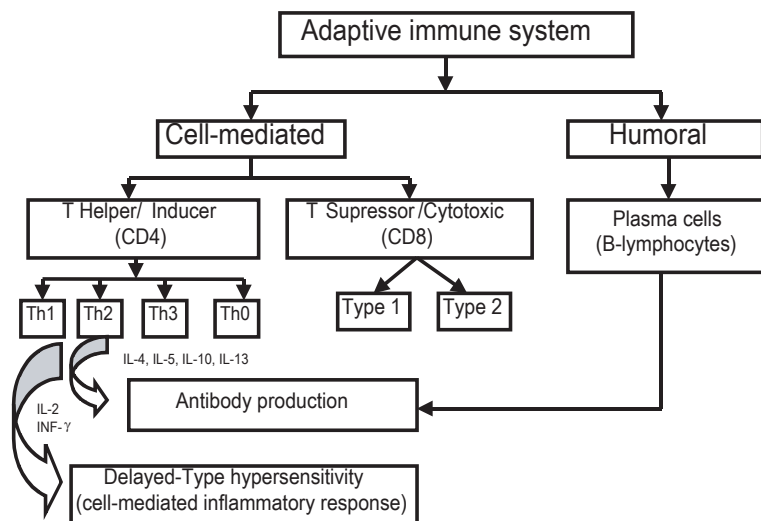
The purpose of immune cells and their metabolites is to destroy invading organisms. Excessive or inappropriate production of these substances can cause mortality. Oxidising materials enhance IL-1, IL-8, and TNF production in response to inflammation, but can also cause harmful effects, including killing host cells, damaging membranes and tissue directly via degradation of essential cellular components (such as membranes), more indirectly by altering the enzyme (protease) balance that normally exists within tissue (Conner and Grisham, 1996) and by causing cell mutations (Weitzman and Stossel, 1981).

Acquired ('specific') immunity includes 'humoral immunity' and 'cell-mediated immunity' (Figure 3). Humoral immunity is mediated by antibodies that are released by B-lymphocytes into the bloodstream. This immunity is based on the production of immunoglobulins (Table 2) which recognise and eliminate antigens by binding and removing invading organisms or toxins.

Table 2.
Main immunoglobulin classes
(adapted from Lydyard *et al.*, 2000)

Immunoglobulin	Characteristics
IgG1; IgG2; IgG3; IgG4	Largest quantity; provide the bulk of immunity to the most blood borne infectious agents; the only antibody class to cross the placenta to provide humoral immunity to the infant; vaccination asks the immune system to produce IgG specific for a particular antigen
IgA	A first line of defence against microbes entering through mucosal surfaces directly communicating with the environment; synthesised by plasma cells; prevents colonisation of mucosal surfaces by pathogens
IgM	The first antibody produced in an immune response in large quantity
IgD	Present in humans, not documented in animals; functions as an antigen receptor on B cells
IgE	Involved in allergy development and in Immediate hypersensitivity syndromes such as hay fever and asthma

Figure 3.
General scheme of adaptive immune system
(adapted from Field *et al.*, 2001)



Cell-mediated immunity is based on specific antigen recognition by thymus-derived T-lymphocytes. Cells infected with a foreign agent, e.g. a virus, are detected and destroyed via direct contact between an activated T-cell and its target (the infected cell) (Qureshi *et al.*, 1998). Cell-mediated immunity is responsible for 'delayed-type hypersensitivity' (DTH) reactions, where the body launches an immune attack against an innocuous or beneficial agent such as a foreign graft (Wu and Maydani, 1998).

Interactions between T and B-cells, as well as antigen presenting cells, are responsible for the development of specific immunity. These defence mechanisms are stimulated by exposure to foreign substances and are specific for distinct macromolecules, and increase in magnitude with each successive exposure to a particular macromolecule (Miles and Calder, 1998). In comparison to natural immunity, this type of immunity takes longer to develop, but is highly specific for antigens and has 'memory' in case of future re-infection (Table 3).

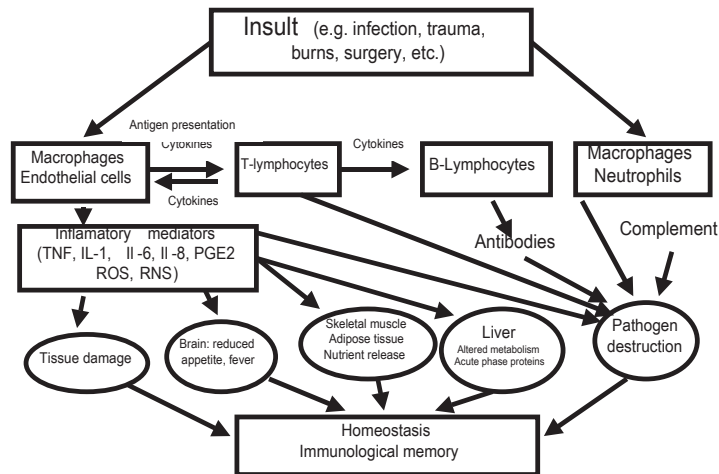
Table 3.
Key features of innate and adaptive immunity (adapted from Hansson *et al.*, 2002; Calder, 2001).

	<i>Innate</i>	<i>Adaptive</i>
Appearance in evolution	Primitive organisms	Vertebrates
Induction time	Fast (hours to days)	Slow (days to decades)
Recognizes	Common 'pathogen-associated microbial patterns' (PAMPs)	Unique epitopes on each pathogen/antigen
Cellular components	Phagocytes (macrophages and neutrophils); NK cells; mast cells; dendritic cells	T and B cells
Generation of specificity	Encoded in germline; Has some specificity, no memory	Somatic rearrangement; Highly specific and has memory
Effector Mechanisms	Complement (Alternative pathway); cytokines; chemokines; cell-mediated cytotoxicity	Antibodies; cytotoxic T cells (CTL); classical complement activation; antibody-dependent cell-mediated cytotoxicity; cytokines; chemokines
Soluble mediators	Macrophage-derived cytokines	Lymphocyte-derived cytokines
Characteristic transcription factors	NF- κ B (+JNK/AP1)	Jak/STAT, NF- κ B, etc.
Physiological Barriers	Skin mucosal membranes Lysosome Stomach acid Commensal bacteria	Cutaneous and mucosal immune systems Antibodies in mucosal secretions

These two parts of the immune system work together via direct cell contact and interactions involving chemical mediators such as cytokines and chemokines (Figure 4). For correct function, the animal's immune system requires the co-operation of macrophages, B-lymphocytes and T-lymphocytes with various other types of immune cells. Correct response to infection requires cellular proliferation (T-lymphocytes), enhanced protein synthesis (including immunoglobulin synthesis by B-lymphocytes and acute phase

protein synthesis by liver) and inflammatory mediator production. Physiological changes resulting from stimulation of the immune system include fever, anorexia and loss of tissue (Grimble, 1997).

Figure 4.
The natural and adaptive immunity interactions (adapted from Calder, 2001)



Immuno-modulation and nutrition

Nutrition plays an important role in modulation of the animal's immune system. The majority of scientific literature published on the interaction of nutrition and the immune system examines the effects of deficiencies or excesses of single nutrients (Schoenherr and Jewell, 1997). However, for effective immuno-competence, a balance of nutrients is required.

Energy

Immune response is usually energetically expensive. In healthy adult pets, energy deficiencies are uncommon, indeed up to 40% of the dogs and cats seen by veterinarians in the USA are overweight (Grieshop, 2003). To maintain an effective immune system it is an important task for nutritionists to ensure that energy consumption and expenditure are in balance. Judicious caloric restriction beginning even in adult life, can have a rejuvenating effect on immune response capacity of companion animals (Sheffy and Williams, 1981).

Protein

Protein synthesis is a key attribute of the immune response, making it important to balance dietary protein. Maintaining an adequate supply of essential amino acids is crucial, as an excess of essential amino acids in the diet of companion animals may prove detrimental

for immuno-competence. Changes in the immune system have been observed in cats fed a diet containing 5% cysteine with and without dietary taurine (Schuller-Levis et al., 1991). Pathological examination of regional lymph nodes, livers, and spleens showed abnormalities in cats fed cysteine to excess.

Fat and fatty acids

Dietary fat participates in a range of physiological functions including immuno-competence. Whilst fat is an important source of energy, lipids are shown to play a key role as constituents of membranes as well as precursors of active substances such as hormones and eicosanoids.

Certain fats, such as polyunsaturated fatty acids (PUFA), are important for immune cell metabolism. PUFAs consist of two major families: the omega-3 or n-3 and omega-6 or n-6 fatty acids. Their classification (nomenclature) is based on the location of the double bonds in the hydrocarbon chain. If the first double bond is located between C3 and C4 (counting from the methyl end), it is known as the omega-3 (n-3) fatty acid. Likewise, if the first double bond is between C6 and C7, it is called omega-6 (n-6) fatty acid (Ahmed, 1998). Omega-3 and omega-6 fatty acids are not inter-convertible in animals, as the source compounds differ in structure. Feed compounds contain different proportions of these fatty acids, with green leafy vegetables, grass, linseed and rapeseed oils being good sources of n-3 a linoleic acid (LNA) and grains and plant oils such as corn, soya bean and sunflower providing rich sources of n-6 linoleic acid (LA).

Cats require pre-formed long-chain polyunsaturated fatty acids such as arachidonic acid (AA) and docosahexaenoic (DHA) acid in the diet, derived from LA and LNA respectively. Approximately 25% of the fatty acids found in the plasma membranes of immune and inflammatory cells are sourced from AA, and it also forms the precursor of prostaglandins and leukotrienes, which have pro-inflammatory and immuno-regulatory properties (Calder, 1998). Eicosanoids are involved in regulation of major metabolic processes in the body, and functions of immuno-competent organs are dependent on a supply of essential fatty acids. Whether the difference in fatty acid profile translates into improvement of health and productive characteristics is difficult to quantify, and requires further investigation. n-3 PUFA can affect inflammation and immunity by altering lymphocyte, monocyte, macrophage, neutrophil and endothelial cell functions. Such changes in immune system can affect protective efficiency of the body. However, data on n-6/n-3 balance in companion animal nutrition are limited and sometimes controversial. For example, feeding a diet containing

an (n-6):(n-3) fatty acid ratio of 5:1 had a positive, rather than an expected negative, effect on the immune responses of both young and geriatric dogs (Kearns *et al.*, 1999). Supplementing dogs with n-3 fatty acids did not affect IL-1, IL-6 or TNF-alpha production, but increased certain prostaglandin production from peritoneal macrophages. Conversely dogs consuming low concentrations of n-3 fatty acids with medium concentrations of vitamin E had the largest delayed-type hypersensitivity (DTH) skin test response (Hall *et al.*, 2003).

Lipid oxidation in companion animal diets is a major problem. In a recent study it was shown that oxidized lipids negatively affect growth, antioxidant status, and certain immune functions in dogs. Detrimental effects were evident at 100 ppm aldehyde in the diet, which represents a rather moderate level of oxidative stress. In one experiment, three groups of eight, two-month old Coonhound puppies were pair-fed diets for 16 weeks. The control diet contained < 50 ppm aldehydes, and two treatment diets contained thermally oxidized lipids targeted to deliver 100 ppm aldehydes (medium-oxidation) and 500 ppm aldehydes (high-oxidation). Dogs fed the high-oxidation diet weighed less than those from the medium-oxidation and control groups at the end of the study (Turek *et al.*, 2003). Oxidized lipids reduced serum vitamin E levels, total body fat content, and bone deposition. Furthermore, blood neutrophils and monocytes from dogs fed the high oxidation diet had reduced 'killing' capacity (less superoxide and hydrogen peroxide production) when stimulated compared to the control group, and lymphocyte production was suppressed by dietary oxidized lipid.

Taurine

Taurine has a special role in cat nutrition. This sulphur-amino acid (2-aminoethane sulphonic acid) is synthesised from methionine and cysteine in the presence of vitamin B6, and is found in almost all tissues in mammals. It is the most abundant intracellular amino acid in humans which is not incorporated into proteins. However, cats have limited ability to synthesise taurine and are unable to recycle it via bile salt production unlike other mammals, making it essential in feline diets. Seafood and meat are good sources of taurine which is implicated in numerous biological and physiological functions (Bidri and Choay, 2003; Schuller-Levis and Park, 2003; Lourenco and Camilo, 2002; Degim *et al.*, 2002) including:

- antioxidation
- bile acid conjugation and cholestasis

- xenobiotic detoxification
- modulation of intracellular calcium levels
- participation in retinal development and function
- endocrine/metabolic effects
- osmoregulation, neuro-modulation and stabilisation of the membranes
- modulation of cell proliferation, inflammation and collagenogenesis reproduction by preservation of the motility of the spermatozoa, support of their capacitation, improvement of the chances of success of fertilization and the early embryonic development
- immuno-modulation

Retinal degeneration is a common symptom of taurine deficiency (Hayes et al., 1975), as is compromised reproduction (Sturman et al., 1985; 1986). Taurine physiological levels in cats decrease during certain clinical conditions, e.g. in cats with dilated cardiomyopathy, plasma taurine concentration have been recorded at only 38% of the normal value in healthy subjects (Fox et al., 1993).

Taurine, and its precursor, hypotaurine, are located the cytosolic compartment of neutrophil cells. The ratio of taurine to hypotaurine is approximately 50:1 (Green et al., 1991). It is considered to be the most abundant free amino acid in certain immune cells (Schuller-Levis and Park, 2003), and comprises more than 50% of the free amino acid pool of lymphocytes (Redmond et al., 1998). The antioxidant function of taurine in macrophages is of great importance (Paseses-Morales and Cruz, 1985). Hypochlorous acid (HOCl), the major oxidant produced during phagocytosis, reacts with free amino groups ultimately causing loss of important thiol proteins (Carr et al., 2001). Taurine is an efficient scavenger of HOCl, preventing the neuronal damage it causes (Kearns and Dawson, 2000). These findings suggest an important role of oxygen-dependent mechanisms in the cell to maintain an appropriate oxidant-antioxidant balance (Wedi et al., 1999). Depletion of this particular amino acid is potentially deleterious to lung macrophages and pulmonary tissue (Zhang and Lombardini, 1998).

Administration of taurine has been shown to reduce inflammatory bowel disease in rats by increasing defending capacity against oxidative damage (Son et al., 1998) and has been shown to protect guinea pig heart tissue from oxidative stress (Raschke et al., 1995). In rats, the host inflammatory response induced by transplantation of neurons was studied and it was reported that taurine facilitated graft survival (Rivas-Arancibia et al., 2000).

Taurine at normal cell concentrations can inhibit oxidative damage to DNA (Messina and Dawson, 2000). When neutrophils were stimulated with a monoclonal antibody in the presence or absence of the amino acid, apoptosis at 18 h was inhibited and intracellular calcium levels were maintained (Condrón et al., 2003). This phenomenon has been observed in human and other cells. (Verzola et al., 2002; Wu et al., 1999; Watson et al., 1996).

Age-related decline in the cat's immune system (Harper et al., 2001) is of great importance, and mechanisms regarding a decrease in total plasma antioxidant capacity of older cats (Harper and Frith, 1999) await further clarification. Experimental infection with feline immunodeficiency virus demonstrated that aged cats developed more severe disease than young adult cats (George et al., 1993).

Vitamin E

Vitamin E is the major antioxidant in biological systems, and the minimum dietary requirement of cats is 30 IU/kg dry matter with diets high in PUFAs requiring up to four times these levels for stability (NRC, 1986). Immuno-modulating properties of this vitamin are well known, however, the results are not always consistent. Following a 12 week feeding trial, dogs consuming low concentrations of vitamin E (17 mg/kg) had lower percentages of CD8+ T cells, compared with dogs consuming medium (101 mg/kg) or high (447 mg/kg) alpha-tocopheryl acetate concentrations (Hall et al., 2003). Dogs consuming low vitamin E diets had higher CD4+ to CD8+ T cell ratios. On day 4 of week 15, the percentage of CD8+ T cells was highest in dogs fed medium concentrations of vitamin E; however, the CD4+ to CD8+ T cell ratio was higher only in dogs fed low concentrations of vitamin E with high concentrations of n-3 fatty acids (Hall et al., 2003). It is interesting that vitamin E alone in doses up to 4.3 IU/g does not improve immune status of cats (Hendriks et al., 2002). However, a mixture of antioxidants including lycopene, β -carotene, lutein, vitamin E, taurine and ascorbate were shown to improve antibody response to vaccination in cats (Harper et al., 2001). It seems likely that antioxidant compounds, including organic selenium, vitamin E, carotenoids and taurine in combination with other immuno-modulators such as omega-3 fatty acids, could be beneficial in cats.

Vitamin A and carotenoids

Vitamin A plays an important role in immuno-modulation, as it regulates many cellular functions relevant to immuno-competence. Maintenance of epithelial surfaces is an important function of this

vitamin, and affects the ability of the immune system to recognise foreign bodies. Impairment of the synthesis of cell surface glycoproteins as a result of vitamin A deficiency is shown to play a crucial role in immunosuppression (West et al., 1991). Glycoproteins are essential components of receptors and are also involved in the regulation of gene expression.

It seems likely that carotenoids have specific immuno-modulating properties. The immuno-modulatory action of lutein has been demonstrated in domestic cats (Kim et al., 2000a). Female Tabby cats (10-month old) were supplemented daily for 12 weeks with 0, 1, 5 or 10 mg lutein, and increased response to vaccination were observed in a dose-dependent manner in Week 6. Compared to control, cats fed lutein also showed enhanced immune cells proliferation. Supplementation increased the percentages of CD4 + and CD21 + lymphocytes at Week 12, and plasma IgG was higher in cats fed 10 mg lutein in Weeks 8 and 12 (Kim et al., 2000a). Similarly, dietary β -carotene has been shown to increase cell-mediated and humoral immune responses in female Beagles (Chew et al., 2000). Compared with unsupplemented dogs, those fed 20 or 50 mg of β -carotene had higher CD4 + cell numbers, CD4:CD8 ratio, and plasma IgG concentration. Furthermore, the delayed-type hypersensitivity response to phytohemagglutinin (PHA) and vaccine was heightened in β -carotene-supplemented dogs. However immune response was impaired in dogs classified as 'low β -carotene absorbers'. Dietary lutein stimulated immune responses in domestic dogs in a similar fashion (Kim et al., 2000). Female Beagles (17-18-month old) were supplemented daily with 0, 5, 10 or 20 mg lutein for 12 weeks, and results showed that lutein-supplemented dogs had a heightened DTH response to PHA and vaccine by week 6. Furthermore, dietary lutein increased lymphocyte proliferative response to mitogens and increased the percentages of cells expressing CD5, CD4, CD8 and major histocompatibility complex class II molecules. The production of IgG also increased in lutein-fed dogs after the second antigenic challenge. (Kim et al., 2000).

Zinc

Zinc is the second most abundant trace element in mammals and is a component of over 300 enzymes and taking part in:

- antioxidant defence as an integral part of SOD
- hormone secretion
- keratin generation and epithelial tissue integrity
- nucleic acid synthesis

- protein synthesis
- sexual development and spermatogenesis
- immune function

Zinc is required as a catalytic, structural and regulatory ion for enzymes, proteins and transcription factors, and is thus a key trace element in many homeostatic mechanisms of the body, including immune responses. Low zinc bioavailability results in limited immuno-resistance to infection, especially in aging animals (Ferencik and Ebringer, 2003). A variety of *in vivo* and *in vitro* effects of zinc on immune cells depend on its concentration. Important immune cells show decreased function after zinc depletion, e.g. monocyte functions are impaired, whereas in natural killer cells, cytotoxicity is decreased, and in neutrophil granulocytes, phagocytosis is reduced (Ibs and Rink, 2003). Furthermore, the normal functions of T cells are impaired and B cells undergo apoptosis. Impaired immune functions due to zinc deficiency are shown to be reversed by an adequate supplementation. However, high dosages of zinc can have negative effects on immune cells and, as often noted with minerals, show symptoms that are similar to zinc deficiency (Ibs and Rink, 2003). Organic Zn is characterised by improved bio-availability in comparison to inorganic sources and is considered to be better digested, stored and utilised in the body.

Copper

Copper is an essential component of a range of physiologically important metalloenzymes and taking part in:

- antioxidant defence as an integral part of SOD
- cellular respiration
- cardiac function
- bone formation
- carbohydrate and lipid metabolism
- immune function
- connective tissue development
- tissue keratinisation
- myelination of the spinal cord

The immune system requires copper to perform several functions, although little is known about its direct mechanism of action (Percival, 1998). For example, some of the recent data from various studies showed that interleukin 2 is reduced during copper deficiency, as is T cell proliferation. It is important to note that, even during marginal deficiency, immune cell proliferation responses and interleukin concentrations are reduced (Percival, 1998). Copper deficiency is associated with decreased number of neutrophils, along with reduced ability to generate superoxide anions to kill ingested pathogens. In many experiments it has been proven that Cu deficiency lowers antibody production, however cell-mediated immunity is more resistant to Cu deficiency. Copper deficiency appears to reduce production of interferon and tumour necrosis factors by mononuclear immune cells (Spears, 2000).

Inorganic copper has a strong pro-oxidant effect and, if not bound to proteins, can stimulate lipid peroxidation in feed or, even more importantly, in the intestinal tract. Organic copper does not possess these damaging properties, and can be fed to improve the copper status of animals

Iron

Iron has a vital role in many biochemical reactions, taking part in:

- antioxidant defence as an essential component of catalase
- energy and protein metabolism
- haem-respiratory carrier
- oxidation/reduction reactions
- electron transport system

Iron is an important metal required for the proliferation of all cells including those of the immune system. Indeed iron plays an essential role in immuno-surveillance, because of its growth promoting and differentiation-inducing properties for immune cells and its interference with various immune pathways and activities (Weiss, 2002). It is also crucial for the proliferation of tumour cells and micro-organisms, due to its role in mitochondrial respiration and DNA synthesis. As a result, deficiency causes several defects in both the humoral and cellular areas of immunity, one of the most profound being a reduction in peripheral T cells and atrophy of the thymus (Bowlus, 2003). Growing evidence suggests that T cells may regulate iron metabolism, perhaps through interactions with the major histocompatibility complex gene.

Iron is a strong pro-oxidant and, if not chelated to proteins, can stimulate lipid peroxidation. This is especially relevant to the digestive tract where lipid peroxidation can be stimulated causing enterocyte damage, and decreasing absorption of nutrients especially other antioxidants. If iron is included in premix in inorganic form it can stimulate vitamin oxidation during storage. Organic iron supplementation avoids these problems and improves iron reserved in the animal.

Selenium

Selenium is the 'chief executive' of the antioxidant system, involved in a regulation of major antioxidant defences in the body (Surai, 2004). It is proven that Se supplementation improves natural and adaptive immunity in both humans and animals, and companion animals are not an exception to this rule.

Animal feed formulations include supplementation with selenium as a safety margin to prevent deficiency and to maintain good health and high reproductive performance. Reproductive success in all animals depends on antioxidant status, since spermatozoa are rich in polyunsaturated fatty acids which require antioxidant protection. It has been suggested that, during evolution, all animals adapted only to the organic form of selenium (Surai, 2002), as feed ingredients, such as plant materials, contain selenium only in its organic form, which are mainly as various seleno-amino acids including selenomethionine. As the digestive system of the animal has adapted to this form of selenium, inorganic selenium (selenite or selenate) is not a natural form for it to utilise, leading to important differences in absorption and metabolism between different forms of selenium. Organic selenium is actively and preferentially absorbed in the intestine as an amino acid, employing similar processes as methionine. In cats, similar to other animals, the absorption of selenomethionine is accelerated by specific amino acid active transport mechanisms in the gut mucosa. By contrast, inorganic selenium is passively absorbed in the gut as a mineral ion. Selenomethionine can build Se reserves in the body (mainly in muscles), but no Se reserves exist in the body when inorganic selenium is fed. Other forms of seleno-amino acids (for example, Se-cysteine) are not a reserve form of the element, hence organic selenium is more effective than inorganic sources, especially under stressed conditions where higher antioxidant demands need to be met.

Se concentration in companion animals' blood serum appears to be substantially higher than that in humans or farm animals (Table 4).

In a recent study with cats, effects of different Se levels were examined, using four treatment diets ranging from 0.95 to 1.03 mg/kg Se (Hedriks et al., 2002). Kittens fed a low-Se diet had significantly reduced plasma Se concentration and GSH-Px activity. They showed compromised thyroid hormone metabolism, where the plasma total thyroid hormones T4 increased and T3 decreased significantly (Yu et al., 2002), effects which can affect thermoregulation, growth and development and ultimately lead to increased mortality in young animals. Se metabolism in cats has some specific features, with concentration in cat's serum being 50-70% higher than that in dogs (Wedekind et al., 2003). Kitten requirement in Se has been recently estimated to be around 0.15 mg Se/kg diet (Table 5; Wedekind et al., 2003).

Table 4.
Reference values of serum selenium concentration in Switzerland, mmol/L (Forrer et al., 1991)

Species	Range
Cat	3.60-10.09
Dog	1.90-4.31
Pig	1.97-3.32
Chicken	1.68-4.28
Humans, 20-60 years	0.78-1.48
Humans, 60-100 years	0.61-1.73
Horse	0.36-1.68
Goat	0.14-1.42
Calves, 3-9 month old	0.19-0.65
Cattle, >9 months old	0.10-0.82
Sheep	0.09-0.54

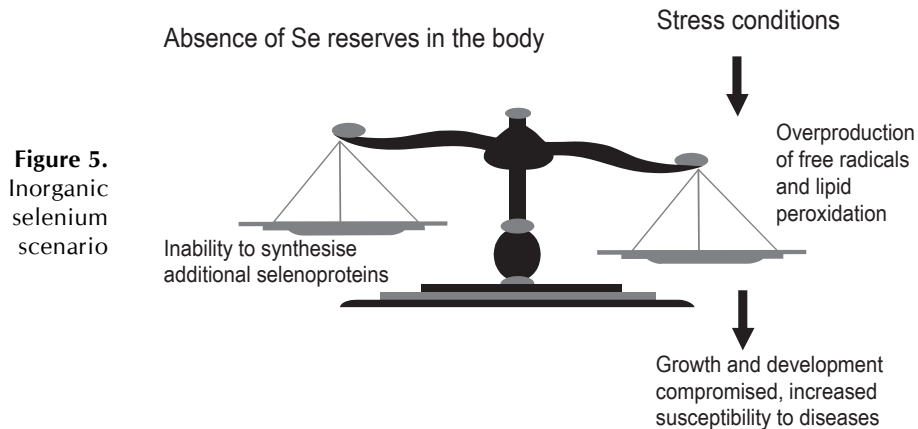
Table 5.
Response of kittens fed graded levels of sodium selenite (adapted from Wedekind et al., 2003)

Treatment	Dietary Se, mg/kg	GSH-Px, Plasma, U/ml	RBC, GSH-Px, U/10 ⁶ cells	Plasma Se, μM/l	T3, nmol/l
1	0.027	0.31	1.90	0.22	0.73
2	0.073	1.03	3.85	0.99	0.86
3	0.100	1.38	5.23	1.56	0.92
4	0.122	1.72	5.92	2.06	0.91
5	0.210	2.32	5.65	4.12	0.91
6	0.314	2.60	5.81	4.61	1.27

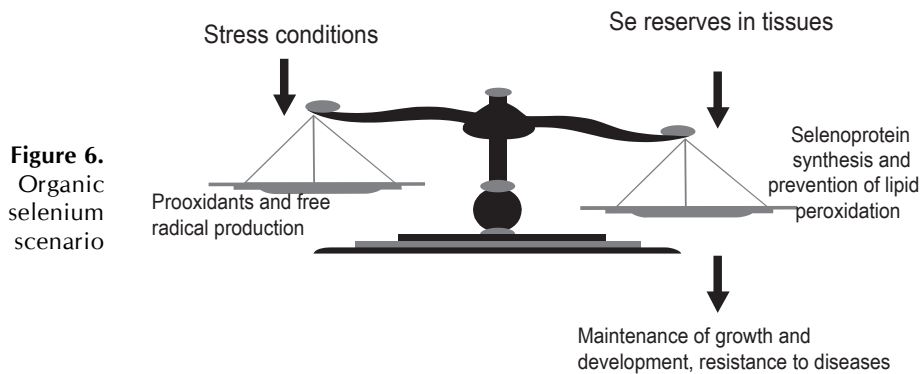
Two scenarios of using selenium in companion animals

Let's consider two different scenarios of antioxidant defence and immuno-modulation in cats and dogs. The first scenario, the most common one, is for animals when inorganic selenium is included in the diet (Figure 5). Under stressed conditions (such as disease or

hard physical work), the body responds by mobilising antioxidant reserves in the body and by synthesising additional seleno-proteins. In this scenario the main limitation is inadequate selenium reserves and restricted ability to synthesise additional seleno-proteins, resulting in poor antioxidant protection when overproduction of free radicals occurs as a result of oxidative stress. In such a scenario it would be expected that immunity and health would be compromised and reproductive performance decreased. It is necessary to realise that we are not speaking about dramatic differences, but cumulative or a succession of stresses which can clinically affect animal behaviour and health, especially important in newly born kittens and puppies, as their antioxidant system is immature and depends on maternal antioxidant transfer via colostrum and milk. As inorganic selenium is not transferred to the milk in any considerable amounts, we would not expect an improvement in antioxidant availability through this route in this scenario.



Consider the same case when organic selenium is used in the diet (Figure 6). Benefit is derived from selenium reserves accumulated in the form of selenomethionine in tissue. Under stressed conditions, protein catabolism releases Se allowing synthesis of additional seleno-proteins to prevent damaging effect of free radical overproduction. Adequate storage of antioxidant reserves is especially important, as many stresses are associated with a reduction in food intake, resulting in a reduced supply of anti-oxidants such as vitamin E. Synthesised seleno-proteins prevent lipid peroxidation, and benefiting the animal by maintaining immuno-competence and reproductive capacity. Since selenomethionine is transferred efficiently through colostrum and milk, newly born animals have a ready supply of antioxidants for protection against oxidative stress. Furthermore, seleno-dependent enzymes involved in thyroid hormone activation would ensure correct thermoregulation in very young.



We need to realise that this scenario has limitations in terms of level of stresses we are considering. For example, when very high levels of oxidising toxins present in the diet or environmental stresses are too high, the body response would not be sufficient to prevent patho-biological changes in the animal. Within the range of everyday stress conditions however, this model/scenario would be effective.

Everyday stresses for pets include:

- **Oxidized Fat In The Diet.** A complete diet for animals contains a range of fat ingredients and some of them are oxidized during feed storage, producing toxic peroxides which further stimulate lipid peroxidation.
- **Mycotoxins** or any other toxic compounds in the feed. It is generally recognised that more than 25% of the world's grain production is contaminated with fungal mycotoxins, making it difficult to avoid them. In many cases they are not readily detectable, but this does not guarantee no contamination. Mycotoxins are considered strong pro-oxidants, responsible for lipid peroxidation. Pet food quality control is typically strict, and tries to ensure only high quality ingredients are used in the diet. However, since environmental pollutants such as heavy metals or mycotoxins are ubiquitous it is almost impossible to completely avoid their presence in raw materials. An analytical survey of 42 elements in 31 commercial canned pet foods was conducted (Furr et al., 1976) found that arsenic, bromine, cadmium, chromium, mercury, selenium were highest in fish-containing cat foods. Lead was consistently high in chicken products. Barium, nickel and tin were also high in some samples. A survey of mutagens (nitrosamines, polychlorinated biphenyls) and toxic elements, as well as the protective constituents zinc, selenium, and vitamin C, from 48 pet foods (Mumma et al.,

1986) found high concentrations of fluoride and iodide in some samples, and high concentrations of mercury and selenium in certain cat foods containing fish. Polychlorinated biphenyls were detected in one cat food.

The occurrence of ochratoxin A (OTA) in canned (26 samples) as well as dry pet foods (17 samples) for cats and dogs has been reported (Razzazi et al., 2001). OTA was detected in 47% of the pet food samples at levels of 0.1-0.8-ng/g. Higher levels of OTA were detected in two pet food samples (3.2 and 13.1 ng/g food). OTA was also detected in 62% of cat kidneys (0.35-1.5 ng/g tissue). When one hundred samples of pet foods including 35 samples of cat food were analysed, low levels of the mycotoxins aflatoxin B1, OA and fumonisins were detected in some of them (Scudamore et al., 1997), and aflatoxin B1 has been reported as being higher in cat food compared to dog food (Sharma and Marquez, 2001). Recently, the fungal profiles of 21 dry pet foods (12 belonging to dogs and 9 to cats) corresponding to 8 commercial brands made in Argentina and imported into Europe were analysed (Bueno et al., 2001). Ten genera of fungi were identified; predominantly *Aspergillus* (62%), *Rhizopus* (48%), and *Mucor* (38%). The most prevalent were determined to be *Aspergillus flavus*, followed by *Aspergillus niger* and *Aspergillus terreus*, which are all potential sources of mycotoxins. There are also other contaminants in cat's food, e.g. the concentration of bisphenol A ranged from 13 - 136 ng/g in canned cat food (Kang and Kando, 2002).

Mycotoxins are considered to be major immuno-suppressive agents in animals. Using adsorbents to bind mycotoxins and prevent their detrimental effects appears to be an important strategy in immuno-modulation (Surai, 2004). Supplementing with proven products such as Mycosorb™ (Alltech Inc, USA) is an effective means of safeguarding growth, health and reproductive performances in various species.

- **Exercise.** Lack of, or over-exercising increases ROS production and both are physiologically stressful for companion animals
- **Disease challenge.** This one is the most important stresses. Immune cells themselves produce free radicals as an important weapon to kill pathogens. Therefore disease challenge substantially increases free radical production and, if not protected adequately, healthy tissues can be damaged. In addition, Se is considered to have a specific role in immune system regulation, which could be independent on its antioxidant functions.
- **Vaccination** is also a substantial stress, and in some cases using vitamin E as a vaccine adjuvant can help by improving vaccination efficiency.

- Various **medications** in the diet. Some can interfere with antioxidant absorption or assimilation
- **Psychological stress**, like lectures, anxious fear

The exposure to potential stresses varies from one animal to another, but overproduction of free radicals and the established need for antioxidant protection are common factors for all of them.

As discussed for selenium, animals have evolved to utilise the organic form of minerals in their diets, and their digestive system has adapted to these forms of Se, Fe, Zn, Cu and Mn. As a result their assimilation from the diet is more efficient compared to inorganic sources.

The beneficial effect of organic selenium for companion animals may be even higher when it is used in combination with other organic minerals; Zn, Cu, Fe and Mn. It is proven that these minerals are more effectively absorbed and metabolised in the body, which could be a major advantage for companion animals. The general relationship of antioxidants with fertility and immunity of companion animals is shown in Figure 5.

Antioxidant defences and the anti/pro-oxidant balance in the body are major determinants of many physiological functions in companion animals, and more attention should be paid to this important issue. New forms of trace minerals, in conjunction with stabilised forms of vitamin E available on the market today can substantially improve antioxidant defences, especially in cats, resulting in an improvement in health and wellbeing.

Conclusion

Undoubtedly, the nutritional status of the animal plays an important role in resistance mechanisms against disease-causing organisms, and may influence the outcome of disease in infected animals. When the diet is balanced in major nutrients, the immune response is optimised, however, in many cases, requirements for the antioxidants, vitamin E or selenium, to maintain immuno-competence are much higher than those for optimal growth and development. Protection against the toxic effects of mycotoxins (major dietary immuno-suppressors) by using such adsorbents as Mycosorb® is an important strategy of immuno-modulation. Organic selenium in the form of Sel-Plex® has been proven effective in maintaining antioxidant defences of animals, which are directly related to immuno-competence. Using organic minerals such as

Bioplex® copper and iron in the diet of the companion animals is also an important option to prevent negative interactions with other minerals and potential free radical proliferation in the digestive tract. Indeed, immuno-modulation starts from the digestive tract where dietary antioxidants, pro-oxidants and other nutrients interact with each other. The result of such interactions can be positive (improved immuno-competence) or detrimental (immuno-suppression) depending on their balance.

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