Università degli Studi di Milano

GRADUATE SCHOOL OF VETERINARY SCIENCES
FOR ANIMAL HEALTH AND FOOD SAFETY
Director: Prof. Valentino Bontempo.

Doctoral Program in Animal Nutrition and Food Safety

Academic Year: *2015-2016*

Interactions between diet and gut health in dogs

Ana Elena Valdez Lumbreras

Tutor: **Prof. Valentino Bontempo**

Coordinator: **Prof. Giovanni Savoini**

INDEX

CHAPTER 1

Foreword

1. Foreword

Intestinal health is one of the principal reasons why owners take their pets to the veterinarian, the problem in puppies and adult dogs is diarrhea frequency without an apparent cause.

Diet is a factor that can extremely vary in terms of quantity, quality, frequency and duration of meals in individuals, but that can also be modified with targeted dietary interventions to produce the best results for any individual. There are growing numbers of compositional foods under development are emerging in the market that target specific nutritional deficiencies beyond the traditional pet-food market.

In commercial pet food, different additives can be used to implement and guarantee the gut health: micronutrients, anti-oxidants, prebiotics, probiotics, enzymes, amino acids and different bioactive compounds.

Developing and maintaining a healthy intestinal tract is a pre-requisite for general health and prevent disease. The gut epithelial cells lining of the intestinal lumen is the first point of contact between intestinal contents and the rest of the body. It is at this interface that nutrition, environment and genetics come together to determine gut health. The intestinal mucosa is lined by a monolayer of intestinal epithelial cells joined together at their apical poles by tight junctions, and supported by an extensive and complex immune system and transcellular pathways for nutrient absorption. The mucosal surface of the gastrointestinal tract forms a barrier that separates the luminal contents from the effector immune cells underneath. Moreover, many immune cell types affect the function of the intestinal epithelium. For example, any abnormal activation of macrophages overproduces the inflammatory cytokines that cause cell damage to the intestinal epithelial monolayers and subsequent mucosal inflammation. Cellular injury from reactive oxygen species (ROS) is implicated in a wide variety of gut diseases and pathologic conditions. Newborn and the elderly animals are more prone to oxidant injury.

If we go to the beginning of dog's life we can discover that maybe they are caused by an inadequate warning. For these reasons, the weaning must be gradual in order to allow the change from the milk to a suitable diet for the growth stage. A newborn puppy faces the environment being sterile and during a relatively short time acquires the typical microflora for the species. The higher quality colostrum guarantees a good development of an immunological system and therefore a potential healthy dog, but when this is not the case we need to found other options to solve the problems and fortunately we actually rely on

probiotics and prebiotics but we count only a few studies about companion animals.
The last years supplements such as bovine colostrum are known for promoting a healthy immune system and treatment or prevention not only for reducing the post-weaning disturbances, but also for any forecoming gastrointestinal diseases linked to immunological system deficit.

There are studies where Colostrum ingestion in neonatal puppies dramatically increases serum alkaline phosphatase and gamma glutamyl transferase. Serum alkaline phosphatase and gamma glutamyl transferase concentrations might be more readily available. It is worth to note that bone growth also elevates serum alkaline phosphatase, but growth-related serum concentrations of alkaline phosphatase seldom exceed four times average adult concentrations.
A decrease of immunoglobulin absorption may be accomplished in a variety of ways, such as competition between intestinal microbes and immunoglobulins for a common receptor on the intestines on the intestinal epithelial cell.

On the other hand, the digestive system of the dog is constantly affected by harmful environmental factors, not only by pathogenic microorganisms or by a bad weaning; also it is affected by changes in immune system due to allergenic food. For this reasons we have to start to implement new strategies to treat these dogs. Recent studies demonstrated that a fiber like the prebiotic one is to be considered as a possible immune response. In fact, some authors argue that bacterial products are able to overcome the mucosal barrier and activate immune cells.

The type of dietary fiber is important for its potential to change the number and types of GI bacteria. Changes in the normal bacterial counts, due to changes in diet, use of antibiotics or other factors may increase the risk of gastrointestinal and nutritional problems, and the risk of disease.
Normally in canine diets are three types of fiber. The first type, the highly fermentable fiber, decomposes rapidly and is digested by bacteria in the GI tract of the dog. Pectin is highly fermentable fiber. Ingestion of excessive amounts of highly fermentable fibers may result in liquid or unformed stools and gas production in dogs.

The second type is the moderately fermentable fiber. GI bacteria digest these fibers at a moderate speed. The incorporation of moderately fermentable fiber, such as beet pulp, in a dog diet helps maintain a healthy GI tract lining and producing moist, well-formed stools. The moderately fermentable fiber sources also feed beneficial bacteria, helping to outnumber the harmful species.

The last type is the short fermentable fiber. Large gut bacteria do not digest these fibers such as cellulose. Although many believe that these fibers may help in case of constipation.

1.1 Alimentation and immune system.

Nutrition directly affects the immune response in three ways:
1. Increase or exaggerated response
2. Cancellation or restriction of the response
3. Change in the nature of the response.

Whether such a change represents a benefit or not depends on the stage of the disease and the subject itself.
Attenuation of the immune response can be beneficial in cases of hypersensitivity (such as atopic dermatitis) or at a heightened immune activation (as in the inflammatory response syndrome or SIRS). Similarly, an increase in immune response can be useful in the prevention or elimination of infection or the development of antitumor immunity.
Conversely, the modulation of immunity may be harmful or even fatal to the host. Immunosuppression may aggravate morbidity and even induce sepsis in case of infectious reached.
An increase in immunity can amplify a state of self-destruction in situations where immune activation has been already excessively or poorly regulated (SIRS, hypersensitivity). It is clear that a single food may not suit all cases.
To understand how nutrition modulates immunity we have to understand the nature of immunity.

1.2 Role of immune system

The immune system has evolved in order to defend the body against infectious agents, viruses, bacteria, fungi, the largest multicellular parasites. Immune responses vary from nonspecific responses, complex and adapted destruction or which can include the elimination of the pathogen. The perfect response to infection is theoretically eliminated without damageing itself. However, the immune responses are never perfect and do damage.
At worst they can be fatal. This basic concept is fundamental when interpreting the effects of nutrition in immunity.

Natural immunity
The anatomical and physiological mechanisms in place before the first exposure contribute to qualified immunity of innate. Many of these mechanisms are

primitive (lysozyme, phagocyte), while other more complex ones only exist in vertebrates and are even more sophisticated in mammals.
In mammals the initial role of immunity is eliminating microorganisms where this is possible. When an infection occurs, the natural response results in one or all the following:
1. Elimination of infection
2. Limiting the spread of infection.
3. Stimulation of acquired immunity by producing an early inflammatory response to infection.
Natural immunity thus provides the "Danger Signal" that gives the alert and activates acquired immune responses.

Recognition of microbes

The natural immune cells have evolved receptors available that recognize phylogenetically conserved molecules. These molecular profiles are called pathogen associated molecular patterns or PAMPS. Examples of PAMPS are lipopolysaccharides (LPS), which are contained in the walls of gram-negative bacteria, lipoteichoic acid in the walls of gram-positive bacteria and the double chain RNA viruses. Receptors include the scavenger receptors PAMPS (scavenger receptors), the mannose receptors and the Toll-like receptor family (TLR) (Akira, 2003). So far, 10 TLRs are known in mammals, but the expression of these 10 types of TLR has not been described in cats yet.
Most TLR are membrane proteins, although TLR9 binds to an intracellular ligand (bacterial DNA). Fixing a TLR ligand to it leads to the appearance of the nuclear transcription factor NF-kB, which enters the nucleus and binds to specific sites on the DNA of the host cell, therefore leading to the transcription of various pro-inflammatory genes. In macrophages and neutrophils these genes encode cytokines (tumor necrosis factor or TNF-a, IL-1 and IL-12), adhesion molecules (E-selectin), the cyclooxygenase (COX), the nitric oxide synthase (iNOS), and macrophage costimulatory molecules, CD80 and CD86, expressed on the surface of macrophages.

The result TLR signals leads to the migration of leukocytes to inflamed tissues, increased disposal microbes or infected cells, and the production of cytokines and inflammatory chemokines that alert and activate immune system cells acquired.

The destruction of phagocytosed microbes

Phagocytosed microbes remain within the phagosome in the cytoplasm. Once internalized, these phagosomes fuse with lysosomes containing preformed several proteases (such as elastase). In addition, activation of phagocytes (via signals from TLR) results in the assembly of multi-subunit of the machine of NADPH oxidase in the membrane of the phagosome and in the plasma membrane. This enzyme complex catalyzes the reduction of oxygen diatomic (O_2) in the superoxide radical (O_2-). O_2- is then changed to hydrogen peroxide, a strong oxidant partially responsible for microbial killing. However, the myeloperoxidase in phagosomes uses peroxide ion to produce an even more powerful antibacterial, hypochlorous acid (HOCl). This production of powerful oxidants (following activation and phagocytosis by neutrophils and macrophages) consumes the available oxygen and it refers to as oxidative burst (DeLeo et al, 1999).

Upon activation of phagocytes the inducible form of nitric oxide synthase (iNOS) is also synthesized, therefore leading to the production of free nitric oxide radical (NO) which reacts with superoxide to form the metabolite toxic peroxynitrite (Eiserich et al, 1998). These various oxidants exist not only in the phagocyte but are also released in the extracellular medium where they contribute to the destruction of microbial nearby. Inevitably this results in oxidative damage to the surrounding tissue.

To protect themselves against oxidation phagocytes require high concentrations of the cytosolic antioxidants (aqueous) and membrane (lipophilic) because they are degraded and need to renew quickly during oxidative stress. The most important cellular antioxidants are glutathione, ascorbic acid, tocopherol and taurine. Neutrophils cats contain high intracellular concentrations of taurine: it constitutes 76% of the cytosolic pool of amino acids, compared to 44% in the lymphocytes (Fukuda et al, 1982). Removal of HOCl by converting taurine chloramine protects the cell against the auto oxidants that had formed. Taurine chloramine may also act as an intracellular signal molecule thus limiting the production of O_2 • - and • NO. In cats showing a taurine deficit in their diet, phagocytosis and oxidative burst are deleted therefore confirming the major antioxidant role of taurine (Schuller-Levis et al, 1990).

<u>Acquired immunity</u>

Acquired immunity is stimulated by infection and by signals from the innate immune system. During re-exposure to the infectious organism, the magnitude, the specificity and the speed of response increase, hence the term of acquired immunity. Acquired immunity is the area of T and B-lymphocytes generating humoral (antibodies) and cells of specific molecules, the so-called antigens.

<u>Eicosanoids</u>

Eicosanoids are a group of lipid messengers synthesized from polyunsaturated fatty acids (PUFAs) 20-carbons, dihomo-g-linolenic acid (DGLA; 20: 3 n-6), arachidonic acid (ARA; 20 : 4n-6) and eicosapentaenoic acid (EPA; 20: 5n-3). Eicosanoids include prostaglandins (PGs), thromboxane (TXs), leukotrienes (LTs), lipoxin, the Roxy-eicosatetraenoic acid hydroperoxide (HPETEs) and hydroxy-eicosatetraenoic acid (HETE).

<u>Nutritional needs of the immune system</u>

During the development period

The first and perhaps the most significant effect of nutrition on immunity occurs during the development of immune system cells (Cunningham-Rundles et al, 2005). This development takes place during intrauterine life, but it is followed by an important period of maturation soon after birth continuing throughout life. Zinc, proteins, essential amino acids, vitamin A and copper are examples of nutrients that are able to compromise the immune system development in animal growth during food deprivation. Micronutrient deficiency disturbs the natural and acquired immune responses. Maternal zinc deficiency may greatly reduce the number of spleen and thymus cells. The post-vaccination antibody secretion in young animals may be affected by maternal deficiencies in zinc, iron, copper, selenium and magnesium.

During development, malnutrition may alter the microbial colonization of mucosal surfaces as well as the response to commensals and pathogens, increase susceptibility to infections and reduce the ability to fight against infection once it is established. Such abnormalities may persist farther beyond the initial period of malnutrition and life deteriorates the immune phenotype of the animal.

1.3 Consequences of malnutrition on immunity

A simple deprivation of food leads to atrophy of lymphoid organs, decreases the number and functions of circulating leukocytes and physically and functionally alterates the epithelial barriers. This results in an increased susceptibility to infections from endogenous commensals such as the skin or intestine and as nosocomial exogenous organisms.

In dogs food deprivation results in a decrease of the number of circulating lymphocytes, in lymphocyte proliferation in response to stimulation and impaired ability to produce a specific T cell response or B antigen following the administration of exogenous antigens. Neutrophil chemotaxis and liver production of acute phase proteins are reduced (Dionigi et al, 1977). Specific nutritional deficiency may lead to several anomalies, e.g. vitamin E deficiency reducing lymphocyte proliferation in dogs, though this effect is partially reversible by supplementing with other antioxidants (Langweiler et al, 1983). Although the effects of malnutrition on immunity are not specifically evaluated in cats, they probably are not very different in this case. Serum albumin is strongly correlated with the body condition cats show in veterinary clinics and it is likely that the same situation may occur for immune function (Chandler and Gunn-Moore, 2004).

-Impact of Immune responses on nutritional status

Immune responses to infections, tumors, or secondary to an immune-mediated disease may affect the subject's nutritional status (Table 1).

Lower food intake	Mechanism	Examples of secondary effects.
Decreased food intake	IL-1, IL-6, TNF-α: effects on central nervous system and peripheral.	Weight loss, loss of lean mass, melting fat, nutritional deficits.
Malabsorption of nutrients	Intestinal villi atrophy, enteritis.	Decreased absorption of fat soluble vitamins, vitamin B12 deficiency
Increased nutritional loss	Enteritis, increased of glomeruline permeability	Hypoproteinemia, vitamin A deficiency
Increased nutritional needs	Fever, leukocytes replication, tissue reparation.	Needs increased by: glutamine, tocopherol, folic acid, vitamin A, energy requirements
Metabolism and systemic transport altered		Insulin resistance and hyperglycemia, hyperlipidemia, decreased serum glutamine

Table 1: The Impact of Nutritional Status on the Immune Response.

Immune response to antigens

Immunological basis of food tolerance.

Foreign dietary antigens interact with the intestinal immune system to prevent unnecessary or even harmful immune reactions. Accordingly systemic immunity does not react during the passage of the same antigen to the general circulation. This lack of reactivity of orally absorbed antigens is known as oral tolerance. It is generated in an active manner, specifically with a specific antigen, and involves the induction of a unique immune response.

Peyer plates are primary induction areas of the intestinal immune system.

The M cells present in the epithelium on the surface of lymphoid follicles capture insoluble particulate antigens, and whole microorganisms (Brandtzaeg, 2001). Antigens and bodies are then transported to leukocytes in the basal membrane invaginations, represented by B cells, macrophages and dendritic cells. In the normal intestine antigen-presenting cells (APCs) are devoid of costimulatory molecules such as CD80 and CD86. The antigens processed by the non-activated APC were then presented to naive B and T cell follicle, which then weakly rent PROLIFE. These phenomena occur in a different local microenvironment from other areas of the body; this results in the induction of hypo-responsive T cells, Th3 or Th2 (Kellermann and McEvoy, 2001). The activated cells progress then through the lymphatic system and arrive in the systemic circulation after having gone through the mesenteric lymph nodes.

Then they bind to the mucosa using the cell adhesion molecules (CAMs) specifically expressed by endothelial venules mucosal tissues. B and T cells activated integrated into the lamina propria so expect a second meeting with their specific antigen.The activated cells are capable of secreting cytokines, but the complete differentiation into effector T-cells or plasma cells cannot take place without a second exposure.

The intestinal epithelial cells are responsible for the uptake of antigens, their release to professional APCs, and a presentation limited to MHC class II cells in the mucosa. In the normal intestine, these side CPAs cells, like their predecessors are devoid of costimulatory molecule, which contributes to a tolerogenic environment. Clones of effector T cells resident in the normal gut produce Th2 and Th3 cytokines, especially IL-10 and TGF-b, which guide the B cells to plasma cells synthesizing IgA secreting while inhibiting the development of Th1 cells and the production of IgG.

It is important for the immune system to reserve the right to react quickly to pathogens. This ability to recognize the pathogenicity is based on the production of "danger signals" by PAMPs receptors such as TLRs.

Expression of TLR2 and TLR4 is low to non-existent in the mucosal cells of the normal human intestine, but can be quickly produced in response to inflammatory cytokines (Abreu et al, 2001). The absence of these "danger signals" results in a relatively inefficient antigen presentation by APCs intestinal greatly reduced or absent Production of TNF-a / IL-1 / IL-12 and the absence of expression of the molecule costimulatory CD80 / 86. The T cells activated by such APCs divide less due to the fact that more clones undergo apoptosis, while the surviving memory cells tend to secrete IL-10, TGF-b, or no cytokine (Jenkins et al, 2001). This association between apoptosis, malfunctions surviving clones and T cells secreting anti-inflammatory cytokines that tend to the production of IgA is the basis of tolerance to luminal antigens.

Oral tolerance is therefore based on a delicate balance between induction of IgA, T-cell deletion, energy and immunosuppression, and the presence of antigen-specific lymphocytes capable of responding to invasive pathogens change Isotype antibodies to IgM production, IgE or IgG, and the production of inflammatory cytokines like IFN-g, IL-12, and IL-6.

<u>Loss of tolerance to dietary antigens</u>

The loss of tolerance to a food antigen produces an immune response, though conventional generating undesirable side effects such as local inflammation or other anatomic sites. This response is characterized by one or more of the following:

- Local cell-mediated inflammation: the resulting chronic stimulus may lead to intestinal lymphocytic infiltrates characteristic of chronic inflammatory bowel disease;
- Local production of IgA antibodies and the production of IgE results in activation of mast cells and bowel hypersensitivity, i.e. food allergy with gastrointestinal symptoms (vomiting and/or diarrhea);
- Systemic antibody production: circulating IgE causing mast cell degranulation in extra-intestinal sites, causing dermal hypersensitivity reactions, i.e. with a food allergy accompanied by pruritus.

Initiating events that lead to the loss of oral tolerance, or preventing it from developing, are not described in cats and remain poorly understood regardless of the species. The suggested mechanisms are:

- Increased permeability mucosa after an injury in the mucosa or neonatal status;
- Coad ministration of a mucosal adjuvant: which activates and induces a phenotypic change in intestinal dendritic cells, such as bacterial enterotoxins;
- Parasites: intestinal parasitism in cats leads to an exaggerated systemic humoral response including increased production of IgE (Gilbert and Halliwell 2005).

The importance of the infections causing an immune response through the Th1 cytokines is currently being discussed in the context of the prevention of type 1 hypersensitivity reactions in humans. "The hygiene hypothesis" postulates that in children the SI maturation defect slowing the passage of a Th-2 type response Th-1 may be due to insufficient microbial pressure in Western societies (Romagnani, 2004). According to this theory bacterial and viral infections during childhood would encourage the immune system to produce a Th-1 type response, thus reducing the possibility of allergic reactions via the Th-2. The reduction of the microbial load in the environment would be responsible for the persistence of neonatal response Th-2 type and thus promote allergies.

The particular role of parasites in modulating allergic reactions, food or not, was discussed for half a century. Several old-enough studies in humans suggest that, as in cats, the infected individuals are more likely to develop allergies (Warrell et al, 1975; Carswell et al, 1977; Kayhan et al, 1978). In contrast the incidence of allergies is very high in Western populations and growing in developing countries. Elevation of anti-inflammatory cytokines, such as IL-10, occurring during chronic helminth infestation, is inversely correlated with allergies. It was suggested that the response of the host to the parasite determines its predisposition to develop allergic diseases and the induction of a good anti-inflammatory response (e.g. IL-10) during constant stimulation of the immune system helps to explain the inverse relationship between many infections and allergies (Yazdanbakhsh et al, 2002). Before applying the hygiene hypothesis to cats the role of parasites and other infections and the development of food hypersensitivity reactions should be put into perspective. Since in most cases, IgE do not seem involved in the immunological mechanisms of adverse reactions to food, the problem immediately appears as apparently very complex.

1.4 References.

Brandtzaeg P. (2001). Nature and function of gastrointestinal antigen-presenting cells. *Allergy 2001 ; 56 Supp 67 : 16-20.*

Brandtzaeg P. (2001) Nature and function of gastrointestinal antigen-presenting cells. *Allergy; 56 Supp 67 : 16-20.*

Carswell F, Merrett J, Merrett TG, et al. (1977). IgE, parasites and asthma in Tanzanian children. *Clin. Allergy. 7: 445-53.*

Chandler ML, Gunn-Moore DA. (2004). Nutritional status of canine and feline patients admitted to a referral veterinary internal medicine service. *J Nutr 134 : 2050S-2052S*

Cunningham-Rundles S, McNeeley DF, Moon A. (2005). Mechanisms of nutrient modulation of the immune response. *J. Allergy Clin Immunol 115 : 1119-1128; quiz 1129.*

DeLeo FR, Allen LA, Apicella M, et al. (1999)NADPH oxidase activation and assembly during phagocytosis. *J Immunol 163 : 6732-6740.*

Dionigi R, Ariszonta, Dominioni L, et al. (1977). The effects of total parenteral nutrition on immunodepression due to malnutrition. *Ann Surg 185 : 467-474.*

Fukuda K, Hirai Y, Yoshida H, et al. (1982) Free amino acid content of lymphocytes and granulocytes compared. *Clin Chem 28 : 1758-1761.*

Jenkins MK, Khoruts A, Ingulli E, et al. (2001) In vivo activation of antigen-specific CD4 T cells. *Annu Rev Immunol 19:23-45 : 23-45.*

Jenkins MK, Khoruts A, Ingulli E, et al. (2001) In vivo activation of antigen-specific CD4 T cells. *Annu Rev Immunol 19:23-45 : 23-45.*

Kayhan B, Telatar H, Karacadag S. (1978) Bronchial asthma associated with intestinal parasites. Am. *J. Gastroenterol. 69: 605-6.*

Kellermann SA, McEvoy LM. (2001) The Peyer's patch microenvironment suppresses T cell responses to chemokines and other stimuli. *J Immunol 167 : 682-690.*

Romagnani S. (2004)The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both. *Immunology. 112: 352-63.*

Schuller-Levis G, Mehta PD, Rudelli R, et al. Immunologic consequences of taurine deficiency in cats. *J Leukoc Biol 1990 ; 47 : 321-331.*

Stechmiller JK, Childress B, Porter T (2004). Arginine immunonutrition in critically ill patients: a clinical dilemma. Am *J Crit Care 13: 17-23.*

Weatherill AR, Lee JY, Zhao L, et al. (2005). Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4. *J Immunol 174 : 5390-5397.*

Yazdanbakhsh M, Kremsner PG, van Ree R. (2002) Allergy, parasites, and the hygiene hypothesis. *Science. 296: 490-4.*

CHAPTER 2

Objectives

2. Objectives

The general objective of this thesis was to underline the strict relation between diet and gut. In fact, the adequate nutritional regime can prevent enteric disease and promotes the correct development of the immune system but also represents an important therapy for some gastrointestinal disorders.

2.1 Microcrystalline cellulose inclusion in dog diets with acute enteritis.

It was to evaluate the effects of the inclusion of microcrystalline cellulose in the diet of canine subjects suffering from acute diarrhea on nutritional status (weight, BCS, MCS), some of serological markers, the trend in CCECAI changed, and the performance of some fecal marker.

2.2 Effects of bovine colostrum supplementation in weaning puppies.

The aim of this study was to evaluate the effect of bovine colostrum dietary supplementation in Golden Retriever puppies on growth and microbiological composition of feces.

2.3 Nutritional strategies to control recurrent diarrhea in adult dogs: clinical cases.

The objective of this part was to formulate veterinary diets in order to control recurrent diarrhea in adult dogs.

CHAPTER 3

Microcrystalline cellulose inclusion in dog diets with acute enteritis.

3. Microcrystalline cellulose inclusion in dog diets with acute enteritis

3.1 Introduction

Carbohydrates are a group of molecules more or less complex, the composition of which elementary is given of carbon, hydrogen, and oxygen, often enriched by other elements, such as phosphorus or nitrogen, characterized by the chemical formula (CH2O) n. To date, there are various classifications in the literature depending on the characteristic examined.
If we consider the chemical structure, one can distinguish simple sugars, such as monosaccharides (e.g. Glucose) and disaccharides (eg. Sucrose), and complex sugars, such as oligosaccharides, consisting of 3-9 sugar units, such as, and polysaccharides, consisting of, for more than 9 units such as amylase, amylopectin, and glycogen. The structure and chemical bonds of some of these are shown in Table 2.

Name	Molecular structure	Type of link
Lactose saccharides	Ga- Gu- Fr	Β
Rafinose	Ga- Gu- Fr	α-1,6
Galactose saccharides	$(Ga)^n$- Gu	β- 1,4- β- 1,6
Fructose oligosaccharides	(Fr) ^{n}- Gu	β- 1,2
Soya oligosaccharides	$(Ga)^n$- Gu – Fr	α- 1,6
Isomaltooligosaccharides	$(Gu)^n$	α- 1,6
Xylooligosaccharides	$(Xy)^n$	β- 1,4
Palatinose oligosaccharide	$(Gu- Fr)^n$	α- 1,6
Glucosaccharides	$(Gu)^n$ -Fr	α- 1,4
Maltooligosaccharide	$(Gu)^n$	α- 1,4
Cyclodextrins	$(Gu)^n$	α- 1,4 cycle structure.
Gentilooligosaccharides	$(Gu)^n$	β- 1,6

Table 1: Structure and chemical bonds of oligosaccharides with knock-on effects of the bifidobacteria in food for cats and dogs (NRC, 2006).

Further viable subdivision part of dietary carbohydrates is that which separates in sugar of high and low molecular weight (Bach- Knudsen, 1997). Among the low molecular weight sugars include glucose, fructose, lactose, sucrose and maltose, which result be quickly hydrolyzed by digestive enzymes. The high sugar is low molecular weight such as starches, non- amylase structural polysaccharides (NSPs) or dietary fiber are characterized by a large number of

monosaccharides joined together to form large molecules and complex type of linear or branched poorly soluble in water (e.g. Starch, glycogen).
Among these one can include the structural polysaccharides such as celluloses not amylase, hemicelluloses, gums and pectins, equipped with different solubility in water.

Carbohydrates	Functions	Digestion part	Digestion products
Amide **Glycogen**	Storage polysaccharides of plants and animals Structural parts of cellular plant walls.	Small intestine (Enzymatic)	Mono and disaccharides (glucose and maltose)
Hemicellulose **Cellulose**	Associated substances at cell wall.	Large intestine (microbe fermentation)	Volatile fatty acids (acetic, propionic, butyric).
Lignin, cutin, waxes, gums, **mucilage,** **pectins.**	Polysaccharides presents in nature in plants.	Not digested or fermented large intestine (Fermentation microbial)	Excreted in the stool: carbon dioxide, methane, hydrogen, volatile fatty acids.

Table 2: Classification of carbohydrates and their digestibility (Gross et al, 2010).

The main purpose of the presence of carbohydrates in the diet of dogs and cats, and in specifically, in the pet food it is to provide energy. According to their degree of polymerization and digestibility (Table 2) (Nantel, 1999) can be distinguished in:

Absorbable carbohydrates (monosaccharides);
Digestible carbohydrates (disaccharides, oligosaccharides and polysaccharides some non-structural);
Fermentable carbohydrates (lactose, some oligosaccharides, dietary fiber and starch resistant);
Non-fermentable carbohydrates (some dietary fibers).

Among the absorbable carbohydrate you may include monosaccharides (glucose, fructose and galactose) (Hassid, 1970) and polyols (sugar alcohols such as sorbitol, mannitol and xylitol) (BeMiller and Whistler, 1996). These compounds are characterized from being able to be absorbed directly and you do not need to be hydrolyzed by gastric enzymes. Disaccharides and starch may be included in the group of digestible carbohydrates. With the term "disaccharides" identify those sugars such as lactose, sucrose, and maltose. They are low molecular weight sugars and are easily hydrolyzable by enzymes such as α-amylase, lactose and sucrose of the gastrointestinal tract. It is, however, necessary to emphasize that in some situations, the absorption in the small intestine of some of these compounds (e.g. Lactose) can be reduced, because of the different enzymatic capacity individual, linked to several factors (age), favoring in this way the fermentations at the level of the colon. Starch is a polysaccharide not structural (α-glucan) and is the main carbohydrate reserve found in cereals. It consists of molecules of amylose (linear chain of molecules glucose combined with α-1,4 glucosidic bonds) and amylopectin (polymer glucoside branched characterized by the presence of bonds and α-1,4-1.6). Numerous studies have highlighted such as dogs and cats (Meyer and Kienzle, 1991; Gross et al., 2010; Walker et al., 1994; Schunemann et al., 1989) can easily digest starch contained in cereals present in the pet food.

From these studies, it was shown that dogs fed on where a share of between 30 to 57% came from extruded corn, barley, rice or oat starch all the cereals was almost completely digested in the small intestine. Fermentable carbohydrates are considered some oligosaccharides, fermentable fibers, resistant starches and, in certain cases, lactose. As such, they may resist digestion hydrolytic enzymes from the small intestine and be fermented by microorganisms present in the last intestinal tract of dogs and cats.

Oligosaccharides are carbohydrate polymers made up from three to nine monosaccharides residues, joined by glyosidic bonds. These molecules resistant to enzymatic digestion, they include fructans like inulin, the galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), the mannan-oligosaccharides (MOS), the isomalt-oligosaccharides, lactulose, raffinose and stachyose (Lee and Prosky, 1994, 1995, 1999). Some oligosaccharides (FOS, GOS and MOS) are known by the term of probiotics, or non-digestible foods that have a beneficial effect, stimulating the growth and activity of a bacterial population or of a limited number of bacteria already present in the intestine guest (Grizard and Roberfroid, 1999). They can be also called functional foods (Van Loo et al., 1999).

Lactose and sucrose, in fact, can escape to digestion by endogenous enzymes, in addition, they are fermented in the large intestine. In this regard, they can be considered both disaccharide resistant to fermentable carbohydrates. In particular, the activity of lactase in the small intestine of dogs and cats, adults is lower than that of young people, while the activity of sucrose is reduced in young people and adults and can be influenced by the composition of the diet (Welsh and Walker, 1965; Kienzle, 1993). By the term resistant starch or Resistant Starch (RS) will come to identify the proportion of starch and its degradation products, which escape digestion and absorption in the small intestine and are to be fermented by the bacteria last intestinal tract (Englyst, 1989; Muir and O'Dea, 1993). Resistant starch can be classified by the rapidity with which the glucose is to be released from the starchy source in:

RS1: fraction of resistant starch, which is in a form physically inaccessible in the granule and which it is released in the course of technological treatments or mastication (found in unrefined grains and legumes);

RS2: is the starch in granular form and resistant to enzymatic digestion that can be found in the raw potato, the banana and tapioca;

RS3 : major fraction of resistant starch composed mainly of amylose regressed, formed after cooking or during heating and cooling cycles. This fraction is completely resistant to digestion by pancreatic amylase (present in cooked foods such as bread, cornflakes, and potatoes);
RS4: modified starch through chemical or physical treatments (this type of starch is not found in nature) (Lineback, 1999; Bird et al., 2000).

The fractions RS1 and RS2 represent the residues starches that are digested very slowly and incompletely in the small intestine, while the share RS3 is highly resistant to Carbohydrates: the fiber 5 enzymatic digestion bowel, which is fermented at the level of the colon by bacteria. Brown and coworkers (2001) showed that, from a physiological point of view, the RS could have different functions related to their properties similar to those of dietary fiber, including the ability to decrease the glycemic index of food, decrease the glucose response and insulin to food and to maintain the well-being of the intestine. Dietary fiber is defined as what is left of the polysaccharides of the cell wall edible plants, lignin and associated substances after digestion by gastrointestinal enzymes Human (Trowell et al., 1976). The fiber is given by the plant cell wall polysaccharides (cellulose, hemicellulose and some pectins) non-cellulosic polysaccharides (pectin, gum and mucilage) and polysaccharides non-structural (lignin) (Englyst, 1989; BNF, 1990; Eastwood, 1992; Theander et al., 1994). Among them, we can distinguish the fermentable and non-fermentable fibers. The fermentable fibers are characterized by the more or less rapid production of volatile fatty acids in Short chain (Short Chain Fatty Acids - SCFAs) by intestinal bacteria through fermentation processes. The extension of this process is extremely important from a physiological point of view: the higher the rate of fermentation, the transit time inside of the gastrointestinal system, it tends to decrease, decreases fecal bulk and it increases the fecal excretion of bile acids. Finally, the term non-fermentable carbohydrates are indicated dietary fiber not fermented by the microorganisms present in the intestines of dogs and cats, which are an example, the cellulose contained in the wheat bran. These compounds contribute directly to the reduction of the intestinal transit time and the formation of feces.

3.1.1 Fiber

The term fiber refers to a large family of compounds, such as structural carbohydrates, including the cellulose, hemicelluloses, pectin and gum. The cellulose, in particular, is a polymer of glucose consisting of β -1,4 bonds that can only be degraded by microbial enzymes (Figure 1).

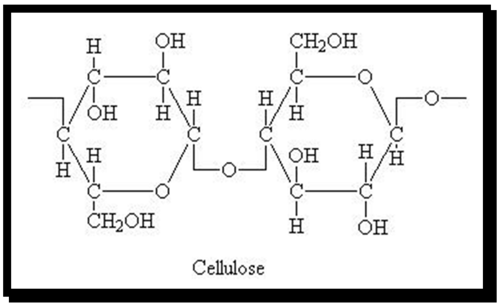


Fig.1: Cellulose structure (Encyclopædia Britannica, 2008).

It is also the most abundant polysaccharide in plants. It is not soluble in water, but it can hold and can be fermented by bacteria in the colon slowly. The hemicelluloses are polymers consisting of glucose, galactose, mannose, arabinose and uremic acids. Such compounds are made up of glucose units joined by β -1,4 bonds glycosidic (Figure 2).

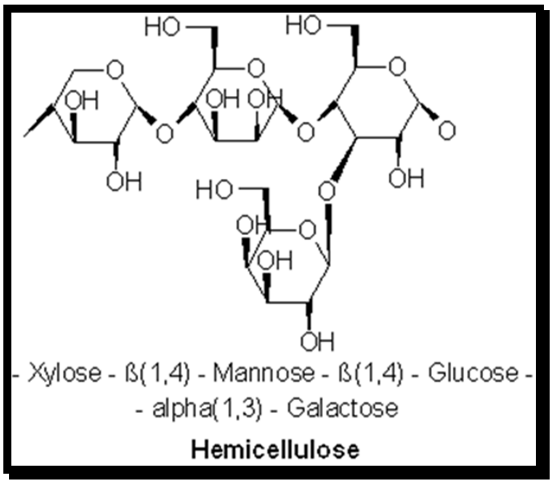


Figure 2: Hemicellulose structure (Scheller HV and Ulvskov P, 2005).

Pectins are composed of a linear chain of galacturonic acid, whose units are linked by α-1,4 glycosidic bonds. For tires, it means a heterogeneous group of polysaccharides with different solubility and viscosity, and then degrees of fermentation variables. The lignin, which chemically is a polyphenolic compound, while not being considered a carbohydrate from the viewpoint of chemical structure, is still included between the fibers, since it constitutes the structural fraction of the cells of plants and is not digested at the level intestinal. There are several classifications of the fiber present in the literature depending are taken into account their degree of fermentation, the water solubility, the ability to water retention and stickiness (Table 3) (Gross et al., 2010).

Carbohydrate and fiber fractions	Method	Fiber solubility	Total dietary fiber analysis	Crude fiber analysis
Fructans, galactans, mannans, mucilages	Rapidly fermentable	Soluble fiber	Total dietary fiber	
Pectin				
Hemicellulose	Moderately fermentable	Insoluble fiber		Crude fiber
Cellulose	Slowly fermentable			
Lignin	Not digested or fermented			
Resistant starch	Moderately fermentable			
Starch	Enzymatically digested			
Mono- and disaccharides	Absorbed			

Table 3: The physico-chemical and analytical of the components of dietary fiber (Gross et al., 2010).

If it consider the degree of fermentation of the fiber can be distinguished fibers to rapid or slow fermentability. As already mentioned, the fibers rapidly fermentable damage origin to short-chain fatty acid and gas in a shorter period than the sources more slowly fermentable fiber (Sunvold et al., 1994, 1994a).
In fact, as the rate of fermentation of the fibers increases, it reduces the time of intestinal transit and stool bulk, while increasing the excretion of bile acids. Between the fibers rapidly fermentable may include citrus fruits, pectin contained in apple and most of the tires. The sources of fiber containing mixtures of pectin, cellulose and hemicellulose (for example, rice bran, oat bran, wheat bran, soybean fiber, soybean mill run and beet pulp) are, however, moderately fermentable. The sources of slowly fermentable fibers used in food for dogs and cats. They contain mainly cellulose and hemicelluloses, including the purified cellulose.
In relation to its ability to be water soluble, the fiber can be divided into two categories: insoluble fiber, such as cellulose, hemicellulose and lignin and the fibers soluble such as, for example, pectins, gums and mucilages (Anita and Abraham, 1997). One must remember that all the fibers retain water to a certain extent; however, the fibers soluble have a greater ability to retain water and can form gels and solutions viscose within the gastrointestinal tract.
The ionic concentration, the pH, the particle size and the different property of retaining water polysaccharide affect the viscosity of the fibers. An increase in viscosity in the gastrointestinal tract may slow gastric emptying, nutrient absorption, and delaying bowel movements, reduce the interactions of the

particles of food with digestive enzymes and epithelial surfaces and reduce postprandial blood glucose (Gross et al., 2010).
The degree of fermentation of a fiber interacts with its ability to retain water and the viscosity influences the fecal mass. For fermentable fibers (moderately and slowly), the volume of the fecal mass is related to the ability to retain water.
The slowly fermentable fibers, such as cellulose, retain their structure longer and are therefore able to bind water. For rapidly fermentable fibers, the inevitable rise of the water reduces the fecal mass. In fact, the fermentable fibers have laxative effects and can cause diarrhea if included in the diet high quantities. However, an increase in fecal mass has been proposed for the treatment and prevention of irritable bowel syndrome, constipation and other gastrointestinal disorders (Gross et al., 2010).

3.1.2 Role and Functions of the fiber

Fiber plays several important functions in an organism. The primary function of a proper and beneficial presence of dietary fiber in the diet of a subject is to gain mass and water in the intestinal contents (Leib, 1990; Twedt, 1993; Gurr and Asp, 1994). The fiber is also able to modulate the intestinal transit time, reducing it in dogs with normal or slow transit time and to extend it in those with more rapid transport of the ingestion (Burrows et al., 1982). Together, these properties help to promote and regulate bowel function. The typical products of microbial fermentation (acetate, propionate and butyrate) are important in maintaining the well-being of the colon. Among these, butyrate appears to be the product most widely used as an energy source by the colonocytes, rather than glucose or amino acids (Roediger, 1982). These products, from the microbial fermentation of fiber, make possible the lowering of the pH luminal. This aspect allows the creation of hostile to pathogens environment: by reducing their number, favorable to the beneficial microbial flora, the increasing in resistance of the intestine against colonization by pathogenic bacteria or potentially extremely important in the prevention and recovery from intestinal disorders (Twedt, 1993; Gurr and Asp, 1994; Burrows et al., 1982; Salter et al., 1993).

We can therefore speak of "prebiotic fiber", which are defined as indigestible food ingredients that selectively stimulate a limited number of bacteria in the colon to improve the health of the host (Gibson and Robertfroid, 1995). This definition implies that the prebiotic fibers are resistant to hydrolysis by the enzymes present in the digestive system, and are able to support the growth of useful bacteria in the host.
Dietary fibers classified as prebiotics promote the multiplication of beneficial bacteria guest at the expense of those pathogens. In addition to this important

activity, the prebiotic fibers may reduce fecal odor by changing the concentrations of the final products of intestinal fermentation and, via their carbohydrate residues, improve immune function. There are different roles and the effects of the presence of fiber in the diet of individuals at different stages of life. In particular, during the growth phase the presence of a suitable amount of dietary fiber can modulate the energy intake favoring a harmonious growth (Delorme et al., 1985).
According to a study by Monsan and Paul (1995), amounts of less than 1% of non-digestible oligosaccharides, can induce weight, gain weight, food intake and an improvement of the health of chickens, rabbits and pigs. As for specific data for dog and cat in this regard, the literature it is lacking (NRC, 2006).
With aging dogs and cats can benefit from the use of foods high in fiber. Indeed, it allows maintaining the functionality and intestinal motility appropriate, further to a lower calorie intake by the subject (Hodgkins and Markham, 1989).

The presence of fiber in the diet also allows better management of the post-prandial glycaemia, favoring the action of insulin. At the level of small intestine, in fact, the dietary fiber acts as a barrier, slowing down the absorption of nutrients, (Eastwood, 1992; Annison and Topping, 1994). This is extremely interesting in the control of glucose metabolism and insulin (Crapo et al., 1976).
In fact, the dietetic carbohydrates, due to the action of specific enzymes (salivary amylase, pancreatic and intestinal hydrolases), are converted into monosaccharides in the gut and then absorbed in the intestinal mucosa. Their absorption in enterocytes is mediated by sodium-dependent transporters and requires the use of ATP.
Glucagon-like peptide 1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP 1-3) are intestinal hormones, which play an important role in regulating blood glucose.
Massimino et al. (1998) have described a correlation between fiber fermentability and the concentration of a peptide similar to GLP-1 in the intestinal mucosa of dogs fed diets with high levels of fermentable fiber. Already Sunvold and Hayek in 1997 had shown an improvement of glucose metabolism following administration of diets with a high content of fiber. In fact, during the study, these authors have reported an increase of GLP-1 in subjects who received a diet containing high fiber fermentability (e.g. Tire, beet pulp and fructo-oligosaccharides) compared to subjects receiving a diet containing fiber less fermentable such as cellulose. Beyond that, we have seen increases in the subjects who took high fiber fermentability increases insulin response. However, the blood glucose concentration, however, did not suffer any influence from the fiber type administered to the subjects.

At the level of the gastrointestinal this consists of an integrated defense system (Winkler et al., 2007), able to differentiate and contribute to the selection of an appropriate microbial flora from birth (Buddington and Sangild, 2011).

The gastrointestinal defenses, in fact, are represented by different components, such as innate and acquired. Among the first may include secretion of acids, antimicrobial peptides, lysozyme and mucus, the tight junctions between enterocytes act as a physical barrier, macrophages and neutrophils and intestinal motility. In what binds passive immunity, which is established as a result of the absorption of antibodies by enterocytes within hours of birth (Van de Perre, 2003).
Acquired immunity is represented by organized lymphoid tissue and associated with enteric tract, Peyer's patches and mesenteric lymph nodes, the B and T lymphocytes and dendritic cells presenting antigens (Rumbo and Schiffrin, 2005).
In this context are the few publications in the literature about the action of the fiber intestinal immunity in dogs.
Some studies have shown to occur as a lower passage of bacteria through the intestinal wall in subjects fed diets rich in cellulose (Deitch et al., 1993; Spaeth et al., 1994; Frankel et al., 1995; Xu et al., 1998).
On the other, hand has not yet been completely clarified the mechanism of action of fermentable fiber. There are, in fact, several assumptions, such as direct contact between the lactic acid bacterial or products of the bacterium with the intestinal immune cells or the production of acids short chain volatile fatty (SCFAs) in addition to the modulation of the production of mucin.
Even the use of fiber as a prebiotic has been considered as a possible agent in the immune response. In fact, some authors argue that bacterial products are able to overcome the mucosal barrier and activate immune cells (De Simone et al., 1987; Perdigon et al., 1988; Solis Pereyra and Lemonnier, 1993; Takahashi et al., 1993; Takahashi et al., 1998; Tejada-Simon et al., 1999). Other authors have shown that the same administration of probiotic bacteria is able to allow some of them to cross the intestinal wall and contact the cells of the GALT activating (Berg, 1985; De Simone et al., 1987; Link-Amster et al., 1994; Schiffrin et al, 1995).

3.1.3 Digestion and use of fiber in monogastric

The dog and the cat have a colon relatively short, which represents less than 20% of the total length of the intestine; it is characterized by a lower use of dietary fiber than other non-ruminants (Kienzle, 1993; Maskell and Johnson, 1993). Although there is a need for dietary fiber by itself, its inclusion in the diet of pets is required for proper operation and for the health of the gastrointestinal tract. The fermentable fibers do not increase the volume of the ration favoring the sense of satiety of the animal and maintain the normal intestinal transit time as well as motility. Those fermentable exert different effects on gastric emptying and from their fermentation operated by the bacteria are formed SCFAs.

In particular, both the dietary fiber as resistant starches and certain oligosaccharides, may exceed the small intestine without being digested and then be fermented by the flora of the large intestine, which is followed by the formation of gases, and short chain fatty acids (SCFAs), mainly acetate, propionate and butyrate (Roediger, 1980; Robertfroid, 1993).
The short-chain fatty acids are readily absorbed by the intestinal mucosa passively to the liver *via* the hepatic portal circulation, where they are degraded. Some studies have shown the presence of acetate also in the systemic circulation (Buckley and Williamson, 1997) probably used by the muscles as a source of energy through oxidation reactions (Pouteau et al., 1998a, b; Remesy et al., 1992). The propionic acid from the portal circulation at the level of the liver is then converted into succinyl-coenzyme A. Butyric acid is mainly oxidized by the cells of the intestinal mucosa and acts as an energy substrate for colonocytes (Roediger, 1980; Firmansyah et al., 1989; Trial work by Drackley and Beaulieu, 1998; Blottiere et al., 2003). Lactic acid is a precursor of gluconeogenesis.
In addition to the activities described, short chain fatty acids also seem to possess in vitro the ability to determine the contraction of the smooth muscle cells of the longitudinal colon, where it is assumed that in vivo may have positive effects on intestinal motility (Rondeau et al., 2003).
The molecular structure and the chemical bonds that connect the sub-monomer units of the carbohydrate, affect their resistance to hydrolysis (Roberfroid, 1993). Moreover, the presence of bacteria in the small intestine can cause fermentation of the fiber before it reaches the colon (Willard et al., 1994; Sparkes et al., 1998). However, this fermentation appears to be affected by the type and the amount of carbohydrate consumed. Among the main factors that can affect the digestibility of the fiber, there are the source and the degree of solubility.

The treatment suffered by the food can play an important role in the use of the fiber. The same technological process, in fact, can facilitate the redistribution of insoluble to soluble fiber (Camire et al., 1990). In particular, studies conducted in vitro by Bednar and collaborators (2001) in a canine model, have highlighted such as extrusion can increase the solubility of the substrate and decrease the production of volatile fatty acids (SCFAs). However, since the non-fermentable carbohydrates contain mostly insoluble fiber, it is unlikely that this will happen for cellulose and wheat bran.

In addition to the same digestibility, the type and amount of fiber in the food are able to influence the digestibility of other nutrients. In general, foods containing slowly fermentable fibers have a dry matter digestibility lower than those who have none, or those with easily fermentable fibers. Moreover, as the share of fiber present in the diet digestibility of the food tends to decrease.
Different factors related to fiber reflected at the level of nutrient availability, since the fermentable soluble fiber or due to its chelating properties can interfere with the absorption of some macronutrients (carbohydrates and lipids) reducing their levels in the blood (e.g. Cholesterol).
The non-starch polysaccharides are fermented in the enteric tract and determining the formation of short chain volatile fatty acids (SCFAs), which are mostly absorbed in the intestine. The energy derived from the oxidation of such compounds, does not correspond to the total energy contained in non-starch polysaccharides, since in the course of the fermentation, some of this energy is lost in the form of another gas and heat (Wisker et al., 1997).
Because there are no data in the literature reference on caloric values of carbohydrate fermentation for the dog and cat, reference is made to the following values of the man (NRC, 2006):

- Non-starch polysaccharides from 0 to 2.4 kcal * g-1 (0 to 10,08kj * g-1) (Schneeman, 1994; Molis et al., 1996).

- Fructosyl unit of oligofructose from between 1 to 1.5 kcal * g-1 (4.18 and 6.28 kj * g-1) (Robertfroid et al., 1993).

- The resistant starches were estimated at 2 kcal * g-1 (8,37kj * g-1) (Brown et al., 2001).

- The non-fermentable cellulose being possesses energy values of zero for dogs and cats.

From a chemical point of view, the SCFAs produced are of weak anions and as such exert an osmotic pressure in the colon and attracting water (Schneeman, 1987; Robertfroid, 1993). Their rapid absorption by the intestinal mucosa, however, balances this trend and at the same time promotes the absorption of water from the intestinal lumen (Herschel et al., 1981; Bowling et al., 1993).

The physical properties and the concentration of fermentable carbohydrates in the diet may have different physiological effects, as reported in Table 4.
These effects are manifested on:

• Intake of the food;
• Intestinal transit time;
• Absorption of nutrients;
• Stool volume.

Carbohydrates, such as dietary fiber, non-digestible oligosaccharides and resistant starches, act in the colon through their physical presence, the products derived from the fermentation or through the modification of the bacterial flora.

Effects	Absorbable	Digestive	Fermentable	Non fermentable
Physicochemical				
Ability to bind to water	NI	NI	X	X
Viscosity	NI	X	X	X
Osmotic	X	X	X	NI
Increase mass	NI	NI	X	X
Physiologic				
Food intake	NI	X	X	X
Transit time	NI	NI	X	X
Fecal excretion	X	NI	X	XX
Nutrient available	NI	X	X	X
Growth performance	NI	X	NI	X
Reproductive performance	X	X	X	NI
Control weight	NI	X	X	X
Hierarchical nutrition	NI	NI	NI	NI
Related to welfare				
Wellness gastrointestinal	NI	X	X	NI
Glycemic control		X	X	NI
Immune function	NI	NI	X	NI

X: Information available publicated.

N/A: Non available information.

Table 4: Summary of main effects on different classes of carbohydrates.

The addition of fiber in the diet can go to influence food intake. In fact, it was noted as the incorporation of dietary fiber may compromise the palatability, promoting satiety, thereby reducing the amount of food taken from the subjects.
The transit time in the gastrointestinal tract is influenced by fermentable carbohydrates. In fact, the increase of the viscosity of the gastrointestinal contents, typical of diets rich in polysaccharides viscous, was associated with a prolonged gastric emptying time, to an increase of the intestinal transit time and to a slowed absorption of nutrients (Schneeman, 1987; Lewis et al., 1994). In a study in dogs, Russel and Bass (1985) showed that the increase in the concentration of fermentable fiber and viscosity of the diet appeared a slowing of gastric emptying.
The cellulose influence, finally, the digestibility of dry matter and organic matter, which decreases proportionally to the percentage of non-fermentable fiber added (Sunvold et al., 1995).

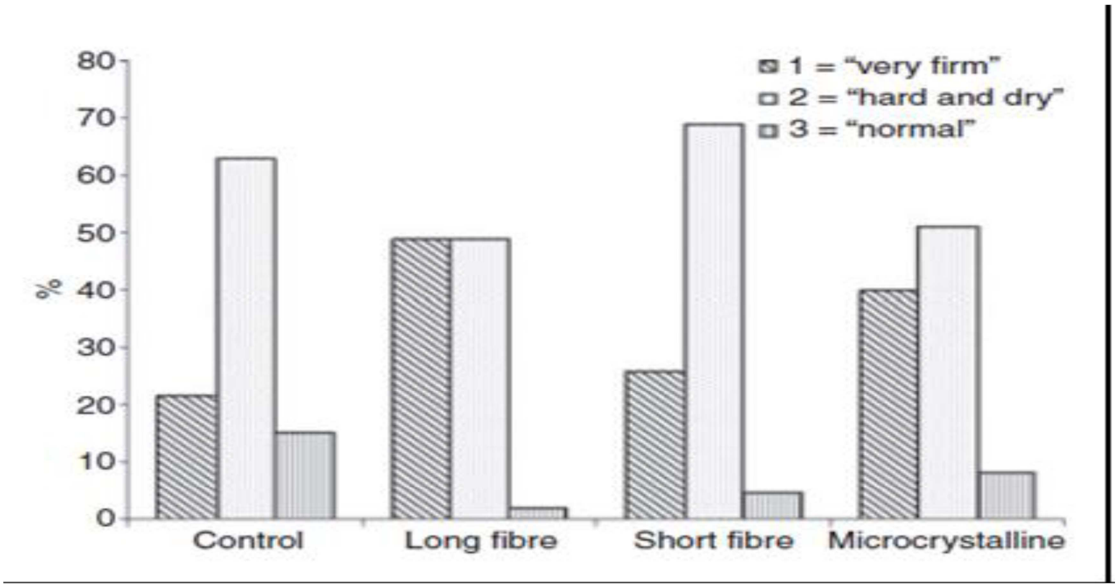


Figure 3: Share of different fiber appearance and stool consistency (Prola et al., 2010).

More recent studies on the action of the cellulose in the cat, show how the long fiber produces more solid feces while not decreasing the water content and dry matter (parole et al., 2010) (Figure 3).
As regards the absorption of nutrients, also the metabolizable energy may vary, as shown by studies conducted by Jewell and Toll (1996) in dogs fed diets containing different levels of fiber. The subjects fed with high dietary fiber content, in fact, showed a lower consumption of metabolizable energy.
The concentration of SCFAs is responsible for the final water content of the fecal mass depending on whether it is a soluble fiber (e.g. Gums and pectins) or insoluble fiber. The presence of soluble fiber is capable of forming a compound gelatinous in intestinal compartment, which increases the viscosity of the contents slowing the intestinal emptying. In the case of those insoluble, they absorb large quantities of water by increasing the volume of stool, the amount and making them stronger. This allows stimulating the speed of transit in the intestinal lumen and, consequently, decreasing the absorption of nutrients.
The very presence of fiber in the diet can go to affect the weight and volume of stool issued by carnivores. Due to the dry matter contained in their feces for 40-55% are represented by bacterial cells (Cummings and Macfarlane, 1991) and the remaining portion of the fiber is not fermented and undigested substances, it is

easy to see how the weight of the same faces can change as the added fiber in the diet.
In particular, an important feature of the cellulose is its ability to bind water. Indeed, it can retain from about 3.5 to 10 times its weight in water. This property has no effect on the formation of stool and intestinal transit time (Meyer and Tungland, 2001).

The size, in particular, the particle size of the sources of cellulose in food is closely correlated with the water retention capacity. As the length of the fibers increases in direct proportion to the ability to bond with water.
Products colloidal gel based on cellulose may increase the viscosity of the intestinal luminal contents and decrease the rate of transit (Reppas et al., 1991).
The ability of the cellulose to create volume, meaning the ability to increase the mass of the ingestion, is exploited in the food industry within foods, low-calorie as an agent of satiety. In humans, the use of cellulose leads to an increase of the stool weight and a decrease in the transit time.
The use in the diet of the dog of substances rich in cellulose, hemicellulose and lignin, can act in a manner similar to humans, however, it is having a greater tolerance to fiber diet in subjects with a higher lipid content (Dobenecker and Kienzle, 1998).

3.1.4 Importance of fiber intake in companion animals

Some studies have shown the importance of the contribution of fiber in the feeding of dogs and cats in order to maintain the health and optimal function of the entire gastrointestinal tract, especially the colon cells (British Nutrition Foundation, 1990).
In humans, dietary fiber has been used as an aid in the management of obesity, diabetes mellitus, hypercholesterolemia, the irritable bowel syndrome, constipation, colon diverticula and Crohn's disease (British Nutrition Foundation, 1990). Similarly, also in the dog, the fiber was included in the diet in order to improve the management of diabetes, obesity and enteric forms.

Considering that obesity is a disease that affects 25% of dogs and cats in industrialized countries (Burkholder and Tool, 2000), where to be affected are mostly elderly and sterilized (Sloth, 1992), its management is extreme importance. Both the soluble fiber as insoluble that are a good solution in the control of energy intake in overweight subjects. Its main purpose is the dilution of nutrients and promoting satiety gastric (Pappas et al., 1989). The addition of fermentable fiber slowly feeding the dog, in fact, can be very helpful for weight control, for the treatment of obesity as well as promoting satiety (Jewell and Toll, 1996). However, other studies have shown that administration of fibers to rapid fermentability in large quantities to foster a sense of satiety, can cause adverse effects such as excessive attenuation gas production and loss of feces (Fahey et al., 1990).

Another important aspect of the contribution of fiber in the diet lies in the management of diabetes in humans than in dogs and cats. The glycemic index represents the level of carbohydrates based on their effect on blood glucose (Nelson et al., 1998). Both, fibers of rapid fermentation as fibers of slow fermentation, helping to keep under control the concentration of blood glucose in diabetic subjects (Nelson et al., 1991; NRC, 2006).

From the clinical point of view, the pet food containing appropriate quantities of fiber, in particular of cellulose, minimize the variations in blood glucose, which can enable to modulate insulin therapy.

The different types of non-digestible carbohydrates can then exert different effects on the welfare of the intestinal tract of dogs and cats, as shown in Table5.

Prebiotics are non-digestible foods that exert an action on the microflora of the colon and improve the state of health of the host (Gibson and Roberfroid, 1995). Examples are the fructo-oligosaccharides (FOS), the mannan-oligosaccharides (MOS), the gluco-oligosaccharides, the galacto-oligosaccharides (GOS) and Xylo-oligosaccharides.

The inclusion of FOS in the diet can improve the ability of the subject to use the fiber, affecting the production of SCFAs in the enteric level. The fermentation of FOS and inulin determines, in fact, an increase in productivity, as reported by Kripke and collaborators (1989), which is equipped with particular tropism on the functionality of colonocytes.

Fiber type	Fermentability	Viscosity	Structure	Function	Prebiotic
Cellulose	NO	NO	No effect /reduced	No effect	No
Beet pulp	YES	NO	Increased	N/A	N/A
Pectin and arabic gomme	YES	YES	Increased	N/A	N/A
Beet pulp and FOS	YES	YES	Increased	Increased	N/A
FOS	YES	NO	Increased	Increased	YES

Table 5: Summary of the effects of certain carbohydrates on the health of the gastrointestinal tract of dogs and cats.

N/A: Information non available.

Structure: changes in the thickness of the mucosa, villus length and crypt depth;

Function: Indicates changes in the transport and absorption of nutrients.

As indeed, the same fermentable fiber can act directly charged to the intestinal wall resulting in an increase in the dog's length of intestinal villi and a greater affinity for the D-glucose (Massimino et al., 1998).
However, this nutrient activity causes different actions on gut bacterial populations. According to Barry and associates (2010), highly fermentable fibers, integrated diet of dogs and cats, can induce a prebiotic effect, stimulating the growth of *Bifidobacterium* and *Lactobacillus* compared with a decline of Clostridia and *E. coli*. The fiber also has the important function to normalize the intestinal contents of water, absorbing water in case of diarrhea, and attracting water, in case of constipation. The most fermentable fiber (e.g. Rubbers and fibers of soybean) may, in fact, helps animals with diarrhea or constipation to adjust the water content in the feces, thereby making watery stools drier and more compact ones more humid.
If you are considering other properties such as binding and gelling properties of the fiber, they also contribute in improving the management of diarrhea, because the increased viscosity of the ingestion is associated with slow transit and delayed gastric emptying (Russel and Bass, 1985).
In subjects suffering from the phenomena of constipation, the fermentable fiber increases stool weight and the moisture content of softening the stool (Zentek, 1996). Any imbalance in the intestinal flora has been linked to diseases such as intolerance / allergies and enteropathy. The prebiotic fibers, in particular, may help to restore or maintain the balance of beneficial bacteria and prevent the development of the opportunists, who can promote and/or contribute to the onset and continuation of diseases.

3.1.5 The fiber in pet food

The term identifies pet food produced by specially formulated for dogs and cats in order to meet their nutritional requirements and physiological. It can be made from a single ingredient or of a set of several components, which make it comprehensive. The ingredients useful in the formulation of the products are chosen according to their nutrient content, digestibility, palatability, functionality, availability and costs.

There are different types of pet food available in different formulations, but the best known are the forms wet (cans, bags, trays) and dry (kibble, cookies). These are produced according to specific processing methods (baking or extrusion) that have different effects on ingredients. Each food must make the different nutrients needed to meet the requirements of each subject; proteins, lipids, carbohydrates and fiber, vitamins and minerals represent these nutrients.

There are many combinations of protein sources that provide an adequate amount of amino acid (e.g. By-products of poultry meal, corn gluten meal, meat meal and bone meal and soybean meal, wheat, barley, rice, amino acids and vegetable protein sources). The selected ingredients are capable of providing the amino acids not only for the desired amount (crude protein 21% minimum SS), but also for the proper balance of essential amino acids.
Fats added to pet food, mainly including animal and vegetable fats, provide essential fatty acids (linoleic acid), and not required to meet the essential needs of the subjects.
Regarding the sources of vitamins and minerals, these are usually included in the product through a "package" prepared and defined premix is added to the food in very small quantities (Thompson, 1998).
Among the sources of carbohydrates more included in pet food are corn, wheat, rice products, barley, wheat and whole wheat, as well as various wheat flours, rice, oats, sorghum and potatoes and other products of their process. The fiber is a subcategory of carbohydrates, but also describes a large group of compounds. The sources of fibers are important for the health of the intestine and the correct intestinal motility. Among those used for the formulation of pet food are wheat bran, rice bran, soybean hulls, beet pulp, powdered cellulose, chicory root, inulin and fructo-oligosaccharides.

Sources of fermentable carbohydrates in the dog and cat are cereals and legumes. Wheat, barley and other vegetable ingredients (e.g. Chicory) foods are important sources of inulin and fructo-oligosaccharides (Monsan and Paul, 1995; Campbell et al., 1997). In this regard, it must be remembered that in the pet food there are slowly fermentable fibers containing mainly cellulose and hemicelluloses (e.g. Purified cellulose), rapidly fermentable (e.g. The pectins present in the apple) and, finally, the sources of fiber mixtures containing pectin, cellulose and hemicellulose (for example, rice bran, oat bran, wheat bran, soybean fiber, soybean mill run and beet pulp), which are moderately fermentable.
As regards the hemicelluloses, they are easily available in the wheat and rye (4-8%), the β-glucans in oats and barley (2-9%) (Prosky and DeVries, 1992; Cho et al., 1997).

Pectin is present in high amounts in apple pomace, in citrus peel and the pulp of sugar beet (Prosky and DeVries, 1992).
Resistant starch is a variable component of all food starch and is present in high quantities in foods with large particles, in undercooked foods, cooked or refrigerated and as modified starch (Brown et al., 2001). Examples of such foods could be the maize starch with a high content of amylose, the starch raw potato and many legumes.

In addition to dietary fiber is already present in food for pets, you can add pure fiber.
Carrageenans and other rubbers are used for the purpose of gelling agents in canned food for dogs and cats, in an amount less than 5% of the dry matter.
Fibers with low or no fermentable, represented by cellulose, are found in many plants as sugar beet and various brans (Meyer and Tungland, 2001).

The microcrystalline cellulose, also called cellulose gel, cellulose is a purified and partially depolymerized devoid of any food allergen. It is an adsorbing agent widely used in the pharmaceutical industry, as a diluent and a binder for the capsules and tablets, both in the wet granulation that in direct compression. This product also has lubricating properties and disruptive, useful in compression.

The fiber in pet food is presented in the form of fine powder or granular, white or almost white, with a density of 0.25 to 0.45 g / cc, practically insoluble in water, in acetone, in ethanol, in toluene, in dilute acids and in solutions 50g / L of NaOH.
It is provided with a pH between 5.0 and 7.5.
From the viewpoint of chemical-structural, is presented as cellulose, with a conformation in parallel of two anhydroglucose units joined by a single bond β-D glycosidic (Sundararajan and Marchessault, 1983).

It is a colloidal product exploited in various ways industrially, for example, is employed as (Battista et al., 1950; 1962):

- Compacted pellet that is extremely hard, heat-resistant, absorbent, and inert; component of hard structural materials, insulating materials and resistant

- Gels and creams;

- Cellulose derivatives in colloidal form.

Products colloidal gel based on cellulose may increase the viscosity of the luminal contents and decrease intestinal transit time (Reppas et al., 1991).
Its ability to increase the mass of the ingesta, makes it particularly suitable as an agent of satiety in low-calorie foods made by the food industry. In humans, the use of cellulose leads to an increase of the stool weight and a decrease in the transit time.
According Dobenecker and Kienzle (1998), the use in the diet of the dog of substances rich in cellulose, hemicellulose and lignin, can act in a similar way to

man, however, was observed a greater tolerance to fiber diet in subjects with higher lipid content. Recent studies conducted by Prola and collaborators (2010) on the action of the cellulose in the cat, show how the long fiber renders the stools more solid, while not decreasing the water content and dry matter.
According to Wichert and colleagues (2002), the cellulose is regarded as a source of fiber, which is not promptly fermented and which has a moderate capacity to bind water. These two properties combined may determine a water binding capacity in feces than the average. As a result, cellulose is used to improve the quality of the feces, although these effects may differ depending on the type of cellulose used. Several products have different particle size. This parameter appears to be closely correlated to the water retention capacity. As the length of the fibers in fact the particle size of the product, increases in direct proportion to the ability to bond with water.
Since the studies by Wichert and colleagues (2002), it was possible to conclude that the inclusion of cellulose in the diet increased the frequency of forming stools compared to what occurred in subjects who did not receive the cellulose.

3.1.5 The role of diet in acute enteritis

Recent studies have underlined the importance of the value of the diet in the management and prevention of gastrointestinal forms that affect dogs and cats. In this context, the value of diets rich in protein sources that have never been taken from the animal before, fermentable fiber and long-chain polyunsaturated fatty acids (ω-3) is receiving more and more attention (Simpson, 1998).
Despite numerous studies have been conducted, the pathogenesis of gastrointestinal events had not yet been fully understood (Fiocchi, 1998; Hanauer, 1996; German et al., 2003; Hall et al., 1994; German, 2005). Nevertheless, extremely important for the management of these diseases is the diet, nutrients and the role they play in the body by influencing the subjects health. In particular, of considerable importance it is the proteins and amino acids and lipids, and carbohydrates.
Proteins are molecules made by the union of amino acids. The amino acids absorbed are reassembled as new proteins by the spleen, and other tissues. Those derived from food are transported through the spleen and other tissues from serum albumin or amino acids. Part of them, after having been absorbed, may be included between the protein synthesis in the muscle and spleen, or exploited for the synthesis of enzymes, hormones and albumins or, still undergo deamination by exploiting the remaining carbon skeletons to obtain energy. A large fraction is exploited also for the synthesis of red and white cells, epithelial cells of the skin and those of the intestinal mucosa. In addition, all the body's

proteins undergo turnover. These molecules are not stored like carbohydrates and lipids, although there is still a reserve protein in the body, especially in muscle, spleen and serum (Gross et al., 2010).
Lipids are molecules with high-energy content that meet the nutritional needs of the subject and functional, facilitating the absorption of vitamins soluble. They are the most concentrated source of energy in pet food. The excess of lipids in the diet is stored as fat in adipocytes (Gross et al., 2010).
According to a study, management of colitis with a hypoallergenic diet and low in fatty acids showed a significant improvement in clinical signs, a reduction of fecal tenesmus, mucus and blood and improvement in fecal consistency (Simpson et al., 1994, 1995).

The carbohydrates are molecules exploited by the body in order to obtain energy, they are also used as a heat source by the body and, finally, as a raw material to obtain nonessential amino acids, glycoproteins, lactose and vitamin C. Where they were in excess are accumulated in the form of glycogen or converted into fat.
However, there are industrial formulations allow for an adequate management of this problem. These can be found even those types of hyper digestible diets constituted by a lipid source (e.g. Fatty acids), proteins from highly digestible, carbohydrate and fat with good palatability, which ensures optimal absorption of nutrients.
The decrease absorption of these nutrients is common in subjects with enteric diseases, whose etiology is multifactorial. Among the various factors involved are the interactions between drugs and nutrients, the seat of the disease, symptoms and dietary restrictions, which can lead to protein-energy malnutrition and specific nutritional deficiencies. Nutritional abnormalities may result from malabsorption, by declining food intake, medications and / or bowel leakage. These deficiencies can differ from one person to another, depending on the location of the disease and the specific nutrient absorption at these locations, as the different factors that alter the nutritional status in subjects with enteropathy (Table 6) (Eiden, 2003).

Among the nutritional factors important for successful management of acute enteritis, water, the electrolytes, lipids and fiber play an extremely important role. With regard to water, it is of major importance for subjects suffering from acute diarrhea taking into consideration the potential for dehydration, which may arise because of an excess of fluid loss and the inability of the subjects to recover those losses.
Extremely important it appears to be also the maintenance of the correct composition of the intestinal electrolytes, in order to avoid excessive losses that

can determine a concentration of electrolytes abnormal systemic. Dogs and cats who present with diarrhea or vomiting, may have a concentration of sodium, potassium and chlorine lower, normal or higher than normal. This situation depends on several factors, such as severity of the disease, nutritional status and location of the disease.

Regarding the fiber, it is able to exert different effects in the gastrointestinal tract.

They include a modification of gastric filling, normalization of intestinal motility, the filling of toxins in the intestinal lumen, the ability to retain and bind excess water, as well as supporting the growth of the normal intestinal flora and acute enteritis: role of nutrients alter the viscosity of the luminal contents. Various types and different levels of fiber have been used in the dietary management of dogs with acute enteritis.

The alteration factors of nutritional status in subjects with enteropathy
Reduced intake of food- Anorexia.
Afraid to eat.
Nausea, vomiting, abdominal pain, diarrhea.
Restrictive diets.
Side effects of drugs.
Losing appetite, altered taste.
Aphtha's oral ulcers
Loss of protein from inflamed mucosa and ulcerated.
Increase in the needs for healing.
Chirurgic resections
Increased requirements of vitamins and minerals. Bacterial overgrowth. Malabsorption. Blood loss.

Table 6: The alteration factors of nutritional status in subjects with enteropathy.

The traditional approach involves the use of foods with a low fiber content (<5% of dry matter) highly digestible and able to produce low residues at gastroenteric level. An alternative approach suggested, is represented by the use of sources of insoluble fibers are included in the diet in quantities of between 7 and 15% in the dry (Davenport Remillard, 2010).

The dietary management of enteropathy remains a controversial issue (Guilford, 1994), although usually involves the use of elimination diets or hypoallergenic (Nelson et al., 1988; Richter 1992; Simpson 1995). Their use implies that the condition is associated with factors such as an unusual diet. The basic aim is to provide the subject with a protein source, associated with a source of carbohydrates never taken previously highly digestible. The gold standard is the homemade diet, it should be given the exclusion of all other nutrients for at least four weeks before considering an alternative diet. Once the subject has

responded to the elimination diet, the components of the original diet should be reintroduced one at a time and observe the response (Paterson, 1995). In this way, it should be possible to identify the unusual dietary and structure a diet suited to the needs of the subject, in order to ensure the long-term welfare. That method represents the reference procedure. The limit of this nutritional intervention is the fact that there has to be based on the commitment of the owner (Leib et al., 1989).
In addition to homemade diets for this purpose may be employed in the management of enteropathies the so-called hypoallergenic diets mono proteins / mono carbohydrates, with or without the fiber addition (Guilford, 1994; Leib, 1990; Simpson et al., 1994; Willard, 1988). The objective is to select a protein source with which the subject has never come in contact. Currently commercially available diets are formulated with mono protein, comprising several protein sources (rabbit, duck, lamb, venison and fish).
It was recently reported the importance of dietary fiber for colon health and an appropriate ratio of polyunsaturated fatty acids (n-6): (n-3) in order to reduce the severity and chronicity of colitis (Aslan and Triadafilopoulos, 1992; Dimski, 1992; Murdoch, 1996; Sunvold and Reinhart, 1996; Twedt, 1996).
The fiber introduced into the food of the subjects gastrointestinal can have different effects on the intestine depending on the type of fiber included (Burrows and Merritt, 1983). The soluble fiber, for example, retains large amounts of water and gel forms rapidly, which increases the viscosity of the lumen, alters the intestinal transit. Such fibers tend to be fermentable and to alter the absorption of the nutrients. The insoluble fiber, however, retains little water and does not form a gel, making it more rapid intestinal transit, promotes peristalsis of the colon and increases the fecal mass (Dimski, 1992).
Among the industrial diets available for treatment, it includes also those types of diets characterized by a source of energy lipid much easier to digest (including medium-chain triglyceride), which provide a better absorption of nutrients due to the high digestibility of proteins, carbohydrates and fats, with good palatability, in addition to containing a single source of protein hydrolysate.
The fermentability of dietary fiber appears to be important in maintaining the health of the colon (Clemens, 1996; Murdoch, 1996), as well as, the absorption of water and minerals in said seat (Herschel et al., 1981). These effects are due to the anaerobic fermentation of dietary fiber, because of the production of fatty acids such as acetate, butyrate and propionate (Reinhart, 1993; Roediger, 1980). These fatty acids do provide 70% of energy needs to colonocytes (Hague et al., 1997), as well as having various beneficial effects on the colon (Guilford 1994; Hague et al., 1997; McInytre et al., 1993; Finnie et al., 1995) (Table 7).

Some beneficial effects of fermentable fiber for colon health observed in humans, rats and dogs
It protects against colonization of pathogenic bacteria
Mass increases colonic mucosal
It increases circulation of the colonocytes
It protects against cancer
Production of mucins, mucous secretion
It reduces the severity and chronicity of colitis
Improves healing after injury

Table 7: Some beneficial effects of fermentable fiber for colon health observed in humans, rats and dogs (Simpson, 1998).

Therefore, even though the current trend is to employ the hypoallergenic diets in the treatment of colitis, several publications suggest the inclusion of adequate levels of fermentable fiber in the diet.

Acute enteritis: role of nutrients.

The diet, therefore, it should contain a balanced quantity of fermentable fibers (for the production of SCFA) and non-fermentable (to promote normal motility) (Reinhart and Sunvold, 1996).

The inclusion of polyunsaturated fatty acids in the diet and, in particular, the ratio of (ω-6) :(ω-3) as revealed recent research have suggested that diet can have a big influence on the severity of the reaction in inflammatory diseases of the gastrointestinal tract is that the skin (Carey 1995 Reinhart 1995). In particular, fluctuations in the (ω-6) :(ω-3) in the diet can affect the levels of these fatty acids in the lipid membrane of colonocytes as other cell populations.

The liberation of (ω-6) by the phospholipase A during inflammation causes the production of arachidonic acid, the eicosanoids, prostaglandin 3 (PG3), thromboxane 3 (TXA3) and leukotriene 4 (LTB4), which are pro-inflammatory. The liberation of (ω-3) in the same circumstances, however, causes the production of a series of molecules (PG2, TXA2 and LTB5) and of less inflammatory eicosapentaenoic acid. In addition, since both the (ω-6) that the (ω-3) compete for the same enzyme systems, it is possible that the inflammatory response is being altered by administering a diet with a content of (ω-3) above normal.

It has been suggested that a ratio (ω-6) :(ω-3) equal to 5: 1-10: 1 provides the ideal ratio of polyunsaturated fatty acids required in order to reduce the severity of inflammatory processes in general and of the colon in specific (Carey, 1995; Reinhart, 1995).

An important role is also played by diets based on hydrolyzed proteins. The main purpose of such a diet is to reduce the possible allergens introduced to the diet. However, the potential problems include poor palatability, the possibility of diarrhea hyperosmotic and a reduced nutritional value. The main indications that lead to the use of a hydrolyzed protein diet is in case of evidence of elimination for the diagnosis of adverse reactions to food and the initial management of inflammatory bowel disease.

An ideal diet in cases of acute diarrhea in the load of the small intestine should be characterized by high digestibility with adequate protein, hypoallergenic, low fat and low in lactose with a protein requirements increase in the course of protein-losing enteropathy. As regards, however, the intake of dietary fat, it should be minimized during gastrointestinal dysfunction because poorly absorbed fatty acids and bile acids cause secretory diarrhea (Guilford, 2008).

The aim of this study was to evaluate the effects of the inclusion of microcrystalline cellulose in the diet of canine subjects suffering from acute diarrhea on nutritional status (weight, BCS, MCS), some of serological markers, the trend in CCECAI changed, and the performance of some fecal marker.

3.2 Materials and methods

3.2.1 Animals and diet

The test was conducted under the supervision of veterinary surgeons and, in particular, the doctor of the Veterinary Clinic gastroenterologist San Martino.
In the trial, it was included twenty individuals of various breeds, aged between six months and thirteen and any sex (Table 8).

Animal	Race	Age	Sex
1	Boxer	5 years	Neutered female
2	Labrador	8 years and 7 months	Neutered male
3	Mongrel	5 years and 8 months	Neutered female
4	Golden R.	3 years and 8 months	Neutered female
5	Mongrel	2 years	Neutered male
6	Golden R.	8 months	Intact male
7	Mongrel	6 years	Neutered female
8	Mongrel	11 years	Intact male
9	Mongrel	13 years	Intact male
10	Golden R.	1 year and 4 months	Intact male
11	Mongrel	9 years	Neutered female
12	Italian Mastiff	6 months	Intact female
13	Mongrel	8 years	Neutered female
14	Mongrel	5 years	Intact female

15	Hovawart	8 years and 4 months	Neutered female
16	Mongrel	8 years	Intact male
17	Mongrel	12 years	Neutered female
18	Mongrel	3 years	Neutered male
19	Labrador	12 months	Intact female
20	Dobermann	3 years	Neutered male

Table 8: Subjects included in the study.

The subjects included were those that showed the first control acute gastrointestinal symptoms and met the following selection criteria:

- No treatment he made the last 20 days (40 if formulations deposit) with:
- Steroids
- Fans
- Antibiotics
- Modulators of peristalsis
- H2 blockers
- Pump inhibitors
- Investigations blood count and biochemical (basic) within normal limits.

Were excluded from this study, however, the subjects with cancer, and those who had the symptoms for more than three days.

At the first clinic visit was carried out with a careful history, nutritional recording of all data concerning the nutritional management of the dog, the previous diet receiving (food, snacks), the quantities and the number of meals. Based on the results obtained from the nutritional assessment were calculated the energy requirements obtained with the aid of the following formula:

$$130 \times \text{dog body weight (kg)} \wedge 0.75$$

Considering the presence of any factors (including physiological state and age), which may have influences it.

The test had a duration of 30 days, during which each subject received a diet Industrial characterized by highly digestible ingredients with a purified source of carbohydrates and protein, consisting of corn starch, hydrolyzed soya protein, minerals, oil coconut, sugar, oil of seeds of soybean, soybean oil and fish oil. The nutritional values of such diet are shown in Table 9.

Twenty dogs admitted to have been divided into two groups:

-Ten subjects fed with the diet industry (control group);

-Ten subjects fed with a diet enriched with industrial microcrystalline cellulose (group treated).

Specifically, the product used in this study had the following characteristics: a total bacterial count less than / equal to 100 cfu / g, yeasts and molds reach a lower and / or equal to 20 cfu / g; is a product completely free of pathogens (*E. coli, Salmonella, S.* aureus and *P. aeruginosa*), as well as with GMO-free and free from risk of BSE / TSE.

Nutritional values	Dry substance
Humidity	75%
Protein	21%
Oils and fats	9%
- **Omega 6 fatty acid**	1.9%
- **Omega 3 fatty acid**	0.6%
Medium chain fatty acid	1.3%
Carbohydrates	55%
Rought fiber	2%
Zinc	210 mg/kg
Vitamin A	20 000 UI/kg
Vitamin E	300 mg/kg
Metabolizable energy (EM) *	3.2 kcal/g

Table 9: Nutritional value of food industrial use. * *Calculated using the modified Atwater factors.*

The microcrystalline cellulose was administered in the form of fine powder divided in two meals together with the food dry chosen in such amount that the final ration presented a crude fiber content of 10% on dry substance. Each dog received a quantity of food distributed in two daily meals.

3.2.2 Assessment of nutritional status

For the evaluation of the nutritional status of each subject, we were considered the parameters described by AAHA Nutritional Assessment Guidelines for Dogs and Cats (2010), such as body weight, the Body Condition Score (BCS), the Muscle Condition Score (MCS) and diet. These data were collected and placed in special personal files updated during the test at days 0, 7, 14, 21, 30.
Weight: The weights were performed always at the same time using the same tool. Body weight expressed (kg) was evaluated in the various controls on the first visit and subsequent to 7, 14, 21 and 30.

Body Condition Score (BCS): for the assessment of fat mass by visual examination and palpation, has been employed a scale of scoring based on 9 points, in which the values 4:05 represent the ideal body condition scores (Fig. 4).

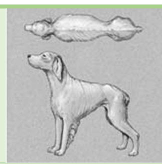
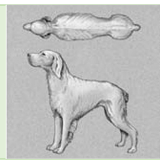
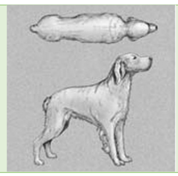
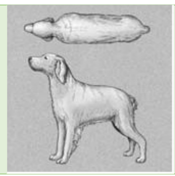
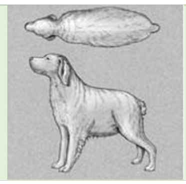
9 Points	Description.	Figure
1/9	Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.	
2/9	Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.	
3/9	Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist. Cats: Ribs easily palpable with minimal fat covering;	
4/9	Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.	
5/9	Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed.	
6/9	Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.	
7/9	Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.	
8/9	Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distensión may be present.	
9/9	Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention.	

Figure 4: Nestlé Purina Body conditional score system.

Muscle Condition Score (MCS): For the evaluation of lean mass by examination visual and palpation at the level of the temporal bones, shoulder blades, ribs, lumbar vertebrae and bones pelvis (Figure 5). It was considered the following scale: 0 = normal, 1 = mild atrophy muscle, 2 = moderate muscular atrophy and 3 = marked muscle atrophy.

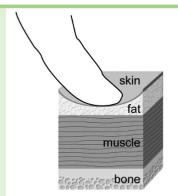
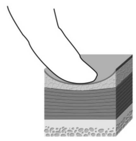
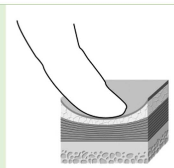
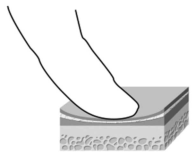
Description	Figure
No muscle wasting, normal muscle mass.	
Mild muscle wasting.	
Moderate muscle wasting.	
Marked muscle wasting.	

Figure 5: Muscle condition scoring (MCS) system.

3.2.3 Clinical indices CIBDAI, CCECAI AND MODIFIED CCECAI

The use of indices of clinical activity is based mainly on the need to assess the severity of clinical signs of disease, in order to determine the control of enteropathy in those affected.
In the present study, it was considered an index for evaluating the severity CCECAI the acute illness elapsing to the intestinal tract. The parameters included in it have contributed to the assessment of the response to the different treatments, in comparison with the studies present in the literature and have allowed making a prognosis based on the studies published. The index CCECAI modified included the assessment of activity, appetite, the vomiting, the consistency of the stool, weight loss and serum concentrations of albumin; each of them has been assigned a numerical value between 0 and 3 (from normal to

pathological) (Table 10). These parameters were detected at the clinic visits made.
Each parameter CIBDAI is evaluated independently of the other as the average time from the development of clinical signs (Jergens et al., 2003).

Activity Index CCECAI modified		
Activity	0 normal 1 decreased slightly 2 moderately decreased 3 markedly decreased	
Appetite	0 normal 1 decreased slightly 2 moderately decreased 3 markedly decreased	
Vomiting	0 normal 1 slight 2 moderated 3 serous	1 episode/weekly 2-3 episodes/weekly >3 episodes/weekly
Consisting feces	0 normal 1 decreased slightly 2 moderately soft 3 liquid	
Frequent stools	0 normal 1 decreased slightly 2 moderately decreased 3 markedly decreased	2-3 times/day blood in feces, mucous or both 4-5 times/day >5 times/day
Albumin levels	0 normal 1 slightly diminished 2 moderately diminished 3 markedly diminished	>20g/L 15-19.9g/L 12-14.9g/L <12g/L

Table 10: Activity Index CCECAI modified.

3.2.4 Serological markers

In correspondence with the first and subsequent clinical visits made at 7 and 21 days were carried out of the blood samples at the level of the cephalic vein of the forearm. On the samples hemochrome cytometric analysis were performed standards, through ProCyte Dx blood cell count (2011), and a biochemical analysis using the system dry chemical Catalyst Dx (2011).

For the blood count, I was taken into account the following parameters:

- Erythrocytes, Hematocrit, Hemoglobin;
- Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean hemoglobin concentration corpuscular (MCHC);
- Distribution of red blood cells (RDW), Reticulocytes;

- White blood cells, neutrophils, lymphocytes, monocytes, eosinophils, Basophils:
- Platelets (PLT), MPV (mean platelet volume), PDW (platelet distribution width), PCT (platelet hematocrit).

Figure 6: blood cell counts ProCyte Dx (IDEXX, 2011).

Regarding the biochemical analysis, however, we were evaluated the following parameters:

- Total Protein, Albumins, globulin and albumin-globulin ratio;
- Cholesterol;
- ALT (GPT);
- Glucose;
- Total Bilirubin;
- urea, creatinine and urea-creatinine ratio;
- Amylase;
- Calcium and phosphorus.

Figure 7: System dry chemical Catalyst Dx (IDEXX, 2011).

3.2.5 Fecal markers

At the first visit and subsequent to 0; 7 and 21 days, were performed examinations of stool samples collected in suitable containers by the owners. They were sought intestinal parasites, by the technique of enrichment, and Giardia using the appropriate test Anigen Rapid Giardia Ag Test Kit.

Figure 8: Enrichment and optical microscope for the identification of intestinal parasites.

Figure 9: Test Antigen Rapid Giardia Ag Test Kit.

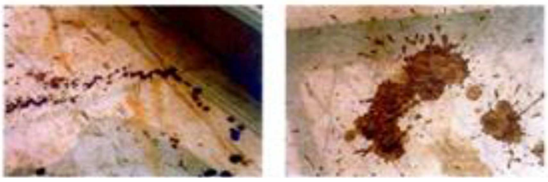
The feces of all subjects included in the study were collected at the 0, 7 and 21 days, before being examined and classified by the scale Fecal Scoring System, as suggested by Steiner (2008). This system uses a score scale between 1 and 3. However, some changes were made as follows:

- Score 0: Firm (ideal). It is well-shaped and cylindrical stools that do not break easily to the collection, but can leave a residue on surfaces and gloves.

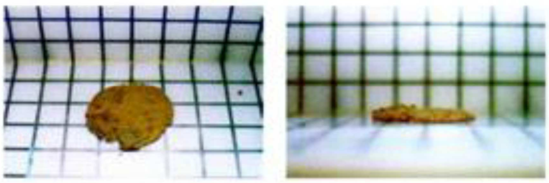
- Score 1: Soft, with shape. They did not break easily and leave a residue on surfaces and gloves. Often they lose their original shape after collection. I am often present in the feces of another score, but may also include the whole stool sample.

- Score 2: it is associated with feces without a definite shape. It is soft stool and it doesn't have a cylindrical shape. It loss easy its original shape and when collected leave residues on surfaces and gloves.

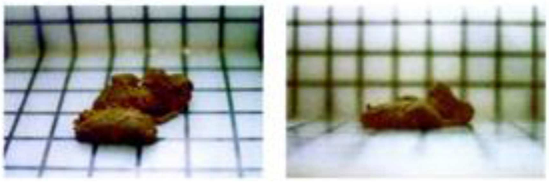
- Score 3: it is associated with liquid stool that cannot contain any particulate matter. These feces are often deposited in more stains, which may also contain mucus or blood. A sample of liquid stool is difficult to collect and always leaves residue on surfaces and gloves.



Score 3: liquid stools



Score 2: Soft stools without definitive shape.



Score 1: Soft stools with shape.

Figure 10: Fecal modified Scoring System (Steiner, 2008).

3.2.6 Statistical analysis

The data collected during the study were analyzed through the statistical program SAS (SAS Institute, version 9.3, 2010).

The effects of dietary microcrystalline cellulose on the health of the dogs examined were evaluated through a system of computerized analysis of variance.
Data on nutritional assessment (body weight, BCS, MCS), the parameters of the index CCECAI changed, the serological markers and feces were analyzed by an application of a mixed procedure, using the contrast option. The statistical model included the following main effects and interactions: treatment, day treatment, treatment day by day.
The significance level considered in this paper is $P < 0.05$. All data are presented as mean and standard error of the mean (± SEM)

3.3 Results and Discussion

3.3.1 Assessment of nutritional status

The twenty subjects included in the study were mostly medium- to large-sized mongrel dogs, although Retriever has accounted for 25% of the breed involved, with a Boxer, a Cane Corso, a Hovawart and a Doberman. They included eight subjects of adult age between 2 and 8 years old, eight Senior (ages 8 to 13) while four were young (between 6 months and 2 years) (Table 11).

Animal	Breed	Age	Sex
1	Boxer	5 years	Neutered female
2	Labrador	8 years and 7 months	Neutered male
3	Mongrel	5 years and 8 months	Neutered female
4	Golden R.	3 years and 8 months	Neutered female
5	Mongrel	2 years	Neutered male
6	Golden R.	8 months	Unneutered male
7	Mongrel	6 years	Neutered female
8	Mongrel	11 years	Unneutered male
9	Mongrel	13 years	Unneutered male
10	Golden R.	1 year and 4 months	Unneutered male
11	Mongrel	9 years	Neutered female
12	Italian Mastiff	6 months	Unneutered female
13	Mongrel	8 years	Neutered female
14	Mongrel	5 years	Unneutered female
15	Hovawart	8 years and 4 months	Neutered female
16	Mongrel	8 years	Unneutered male
17	Mongrel	12 years	Neutered female
18	Mongrel	3 years	Neutered male
19	Labrador	12 months	Unneutered female
20	Dobermann	3 years	Neutered male

Table 11: Breed, age and sex of the patients included in the study.

All subjects maintained their body weight, their Body Condition Score (BCS) and their Muscle Condition Score (MCS) unchanged during the study, except for two subjects, one mongrel and one Golden Retriever, who showed a weight loss, only the case of weight gain due to the growth. Most of the dogs who were

included in the study were large and maintained throughout the test a body weight between 20 and 40 kg, although two subjects were of smaller size and their weight did not exceed 10 kg (small size). Five members of the senior category, the BCS recorded during the test, were higher than ideal (5/9) being between 6 and 7 with an average percentage of overweight of 25%. Most of the adults showed a clinical evaluation of perfect BCS, except for one case of serious overweight (> 20% of ideal body estimated weight) and one of underweight. The young subjects showed good body conditions.
However as regards the MCS no lean mass loss were observed in most of the subjects, except for two Senior subjects, in which from the beginning there was a slight loss of musculature which has not been recovered during the test.

3.3.2 Rating activity index of modified CCECAI enteropathies

In the dogs subject to the test certain parameters were evaluated, such as activity, appetite, vomiting, stool consistency, defecation frequency and level of albumin, in order to check the influence of these parameters on the forms of acute enteritis.
The examined dogs did not present vomiting or weight loss, except for two subjects, one of which showed overweight and one underweight.
From a biochemical point of view, the concentration of albumin remained always in the reference range in both subjects belonging to the concerned control group, or receiving the microcrystalline cellulose supplemented diet.
As regards activity and appetite instead it was possible to highlight the differences in dogs belonging to the two groups. In particular, as regards the activity as the ability to maintain normal physical activities (walking, running and game) it slightly decreased in approximately 90% of subjects during the first visit. However during the study and in particular the second control it showed normal parameters again. Associated with the task, even the appetite reduced in most of the study-involved dogs. In fact, both dogs belonging to the control group and to the treated group showed an appetite reduction (Figure 11). However this condition quickly passed on seven days after the second control, when all dogs were fed normally, consuming all of the food they were offered.

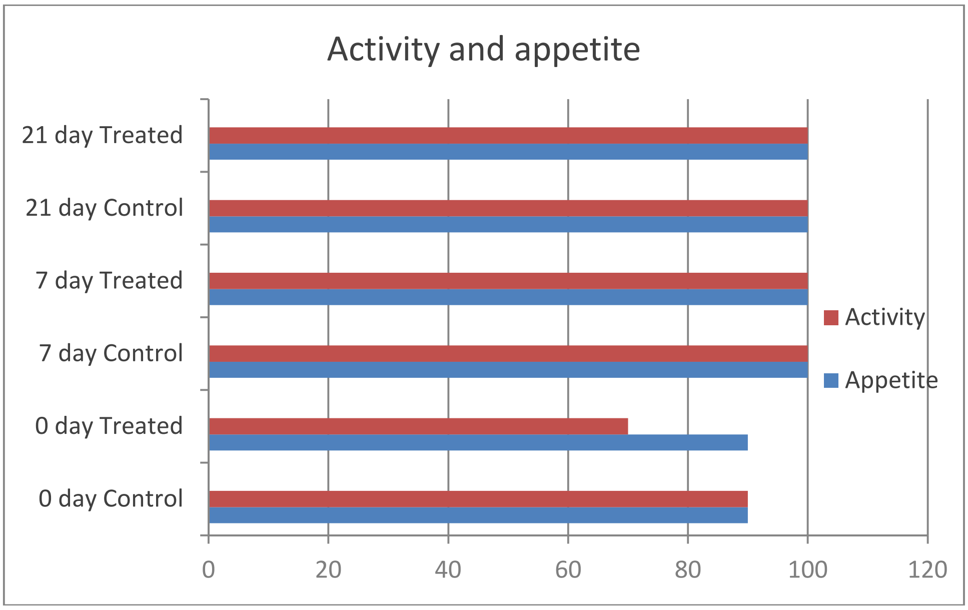


Figure 11: Trends in appetite and activity supported by those receiving the microcrystalline cellulose supplemented diet (Treated) and those receiving a simple diet (control).

Analyzing the associated parameters with the feces and their emissions, the dogs who received the microcrystalline cellulose supplemented diet (treatment group) were affected by an improved fecal score until the return to normal appearance and consistency of stools, although even in the controls there was an improvement of such parameters. It is worth to note that the results we obtained from the defecation frequency where integration with microcrystalline cellulose led to a reduction in the number of defecations. It was possible to show a positive correlation between fecal score and the number of bowel movements after the received dietary treatment. In particular, the consistency of feces and the number of defecations are significantly ($P < 0.001$) related. Indeed, there is a positive match between these two parameters ($R2 = 0.80$). In that condition it is offset by a negative correspondence between the stool consistency and the frequency of bowel movements. In particular, the stool consistency or formed stools was inversely correlated ($R2 = -0.77$) with them. With every day a significant improvement in their consistency occured, whereas the frequency of defecation significantly ($P < 0.001$) reduced. In fact, in subjects who received the microcrystalline cellulose supplemented diet, stool consistency was inversely related to the number of bowel movements.

3.3.3 Evaluation of components in blood chemistry

The blood count of the studied dogs receiving the microcrystalline cellulose supplemented diet did not show any statistically significant differences in the examined components by one of the dogs receiving the staple diet. A load of the different blood components related to the processing of two groups of animals, the values kept almost constant and the reference ranges as shown in Table 12, although the number of reticulocytes progressively lowered along the test compared to baseline in subjects belonging to both groups.

	0 Day		**7 Day**		**21 Day**		**SEM**
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	
Erythrocytes (5.65-8.87 M/µL)	6.95	7.25	7.2	7.04	7.01	6.97	0.24
Hemoglobin (13.1-20.5 g/dL)	16.9	26.71	16.82	16.01	15.92	16.2	0.52
Hematocrit (37.3-61.7 %)	45.06	46.56	46.62	45.35	45.05	45.61	1.65
MCV (61.6-73.5 fL)	64.77	64.08	64.83	64.44	64.23	64.45	0.71
MCH (21.2-25.9 pg)	23.29	23.04	23.43	22.76	22.72	22.91	0.2
MCHC (32.0-37.9 g/dL)	35.95	35.98	36.1	35.36	35.41	35.55	0.29
RDW (%)	18	18.44	17.78	17.98	18.25	17.93	0.48
Reticulocytes(%) (13.6-21.7%)	0.74	0.63	0.56	0.58	0.43	0.45	0.11
Reticulocytes (10.0-110.00 K/µ)	51.88	46.4	39.62	40.72	31.31	32.58	8.63

Table 12: Values of erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, percentage of reticulocytes.

During the study, no significant changes were observed in the population of white blood cells and remained almost constant during all the tests. Similarly, the values of neutrophils and lymphocytes in both groups of animals showed no significant difference, the same goes for monocytes, basophils and eosinophils (Table 13).

	0 Day		**7 Day**		**21 Day**		**SEM**
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	
Leukocyte (5.05-16.76 K/µL)	10.25	10.61	8.53	8.75	8.79	9.68	28.42
Neutrophils (2.95-11.64 K/µL)	7.03	7.2	5.91	5.68	6.08	6.4	0.6
Lymphocytes (1.05-5.10 K/µL)	1.91	2.3	2.11	1.99	1.81	2.37	0.21
Monocytes (0,16-1.12 K/µL)	0.67	0.57	0.53	0.55	0.5	0.68	0.06
Basophils (0.00-	0.03	0.02	0.03	0.02	0.01	0.02	0

0.10 K/µL)							
Eosinophils (0,06-0.23 K/µL)	0.6	0.5	0.59	0.49	0.36	0.61	0.1

Table 13: Values of leukocytes, neutrophils, lymphocytes, monocytes, basophils, eosinophils dogs belonging to the control and the treatment group.

Similarly also the three considered platelet indices remained constant during the test in both groups of animals, as indicated in table 14.

	0 Day		**7 Day**		**21 Day**		**SEM**
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	
Platelets (148-484K/µL)	251.4	375.4	268.6	321.6	266.2	307.1	23.07
MPV (8.7-13.2fL)	9.26	9.8	9.75	9.56	9.8	9.58	0.35
PDW (9.1-19.4fL)	11.59	12.67	12.18	12.08	12.07	12.28	0.63
PCT (0.14-0.46%)	0.33	0.25	0.25	0.3	0.26	0.29	0.02

Table 14: Platelet values and platelet indices as obtained during the test.

Total protein, albumin, globulin and albumin globulin ratio analyzing the profile of total proteins, albumin and globulins of the studied subjects showed differences between the subjects belonging to the control group. The treated group, since the values of the three parameters, remained almost constant during the test, keeping always in the range of physiological values (Figure 12).

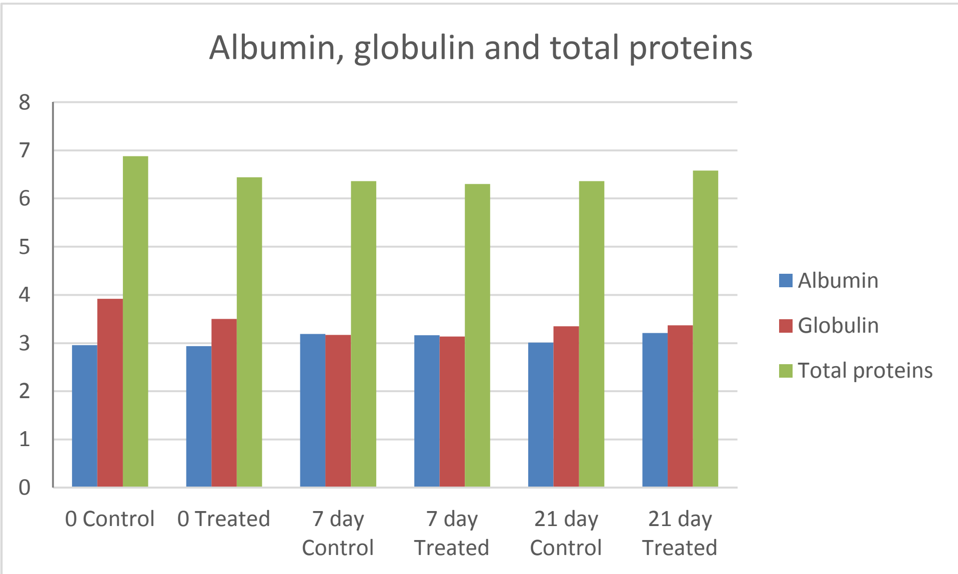


Figure 12: Plasma concentrations of albumin (g/dl), globulins (g/dl) and total protein (g/dl) as obtained during the test.

By analyzing the albumin-globulin ratio we observed statistically significant differences between the two groups during the study. In the third blood collection the values remained unchanged between the two groups. During the first blood sample dogs belonging to the control group showed a mean of 0.77, while the reported mean value of those of the treated group is equal to 0.86. While during the second control all subjects having been fed with basal diet showed average values of 1.04. The treated group accordingly showed very similar average value, equal to 1.05. During the last control at day 21, the control group dogs eventually the showed mean values of 0.85, while the treated group dogs mean value was 0.98 (Table 15).

	0 Day		7 Day		21 Day		SEM
Day	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	
Relationship between albumin and globulin	0.77	0.86	1.04	1.05	0.85	0.98	0.06

Table 15: Relationship between albumin and globulin of the studied dogs.

Urea, creatinine and urea, creatinine ratio.

Regarding the plasma concentrations of urea, no statistically significant differences were found in the control group and treated group dogs values. The concentrations of urea, in fact, were maintained during the test; although this parameter slightly decreased during the second blood collection in treated subjects with an average of 15 mg / dL compared to an average value of 18.46 mg / dL as detected at the first sampling. The plasma urea concentrations detected during the study are shown in Figure 13.

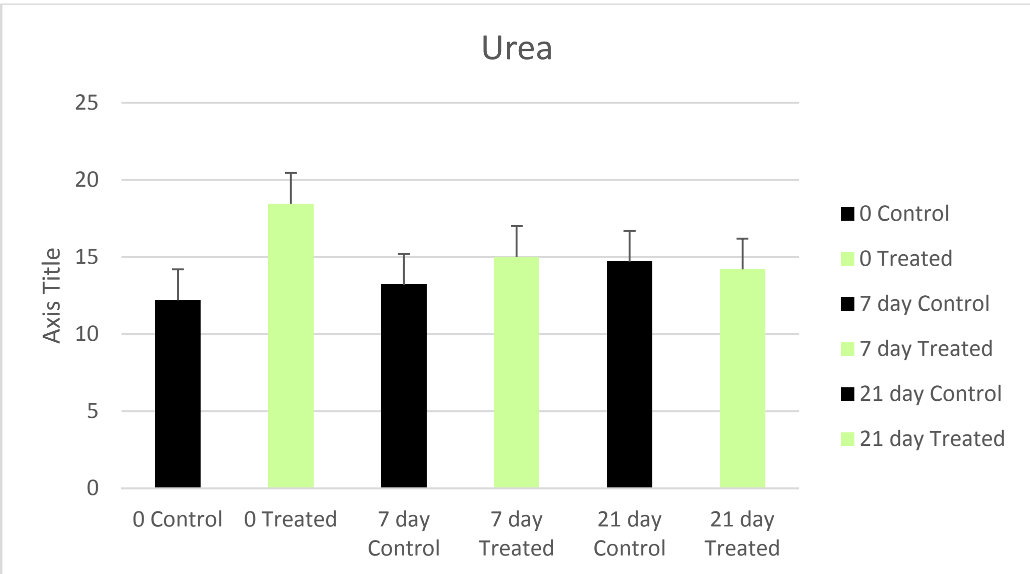


Figure 13: Serum Concentrations of urea (mg / dl) in dogs receiving the control diet and in the treated ones.

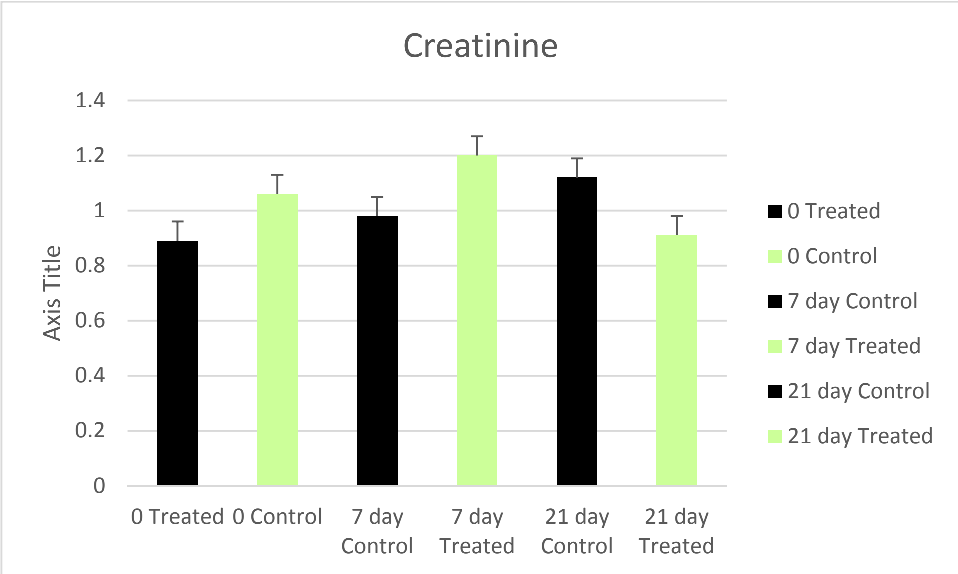


Figure 14: Concentrations of creatinine in serum mg / dl as obtained during the study.

As regards the creatinine concentration of the examined subjects a statistically significant difference were detected between the recorded amount blood in dogs belonging to the group of controls and in the treated ones on day 7. In fact, animals belonging to the control group and receiving the basic diet showed significantly lower average creatinine concentrations (0.98 mg / dL) if compared to those of the animals receiving the supplemented diet (1.2 mg / dL). We observed a difference, though not statistically significant, between the concentrations of this parameter in subjects belonging to the control group and in the treated group in correspondence with the twenty-first day, where the average concentration of the dogs of the treated group showed lower levels of serotonin (0.91 mg / DL) if compared to controls (1.12 mg / DL). On day 0, however, no relevant differences arose between the two treatment groups (Figure 14).

As for the ratio of urea, creatinine statistically significant differences were observed between the two dogs receiving the examined diets. On the occasion of the first drawdown (0 day), as well as on the last (21 days), this ratio was slightly higher in subjects fed with microcrystalline cellulose supplemented diet than in those who were given a basic diet, reflecting what later would be the concentrations of individual parameters constituting the relationship. At the second sampling on day 7, the ratio detected in the control group animals slightly exceeded those of the treated group animals. The trend of this parameter is shown in Table 16.

	0 Day		7 Day		21 Day		SEM
	Control	Treated	Control	Treated	Control	Treated	
Relationship between urea and creatinine	13.9	17.6	14.3	12.6	15.4	17.4	2.11

Table 16: Value urea- creatinine as observed during the study.

Alanine amino transferase ALT (GPT) and other liver parameters.

As for the serum concentrations of alanine amino transferase at day 0, there were no differences between the detected values in animals of the treatment group and those of the control group. At day 7, however, the dogs of the treated group showed serum ALT (59.1 U / L) higher than those of the control group (42.5 U / L), although this difference was not statistically significant. In the third sampling, at day 21, no significant differences were eventually observed between the two groups of animals (Figure 15).

.

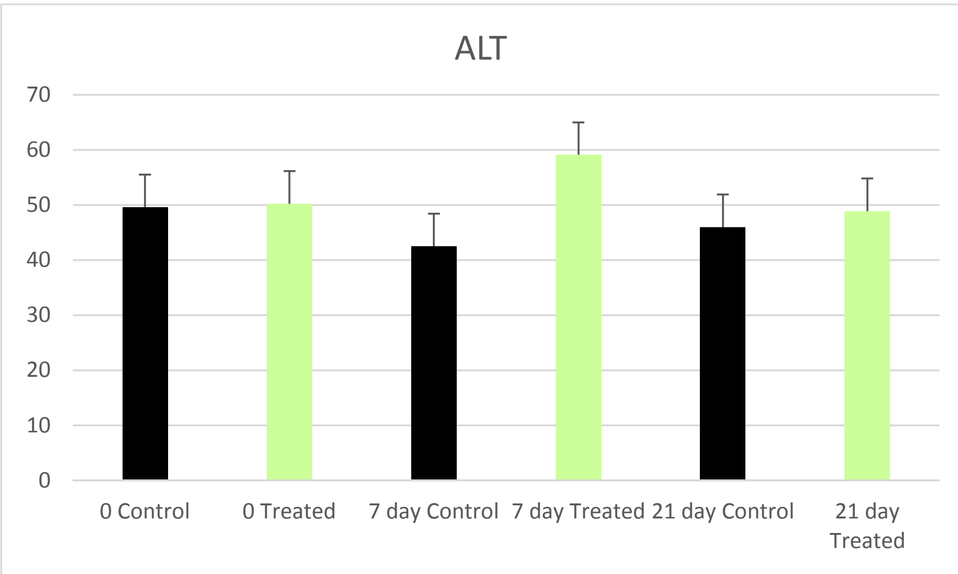


Figure 15: Concentrations of serum ALT expressed in U/L found during the study

Total bilirubin.
Regarding the serum concentrations of total bilirubin, there were no statistically significant differences between the two groups of animals during the test. The values of bilirubin expressed in mg / dL (Figure 16) kept almost constant in both groups of animals during the three levels, as reported below.

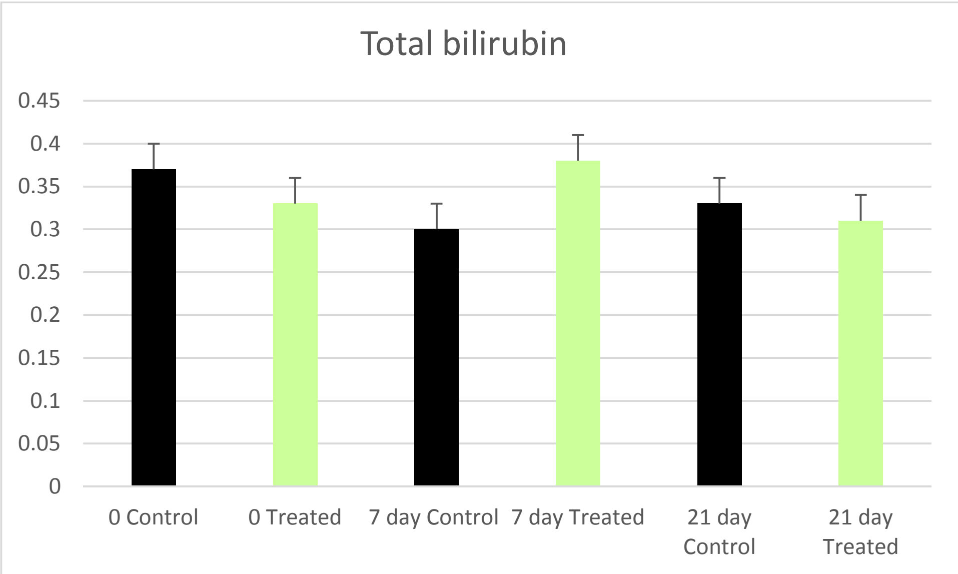


Figure 16: Concentrations of serum total bilirubin, expressed in mg / dL during the test

Cholesterol.
Serum concentrations of cholesterol showed no statistically significant differences between the control group and the treatment group subjects during the test. At the first sampling (day 0) the observed levels were almost uniform in both groups with average values equal to 204.7 mg / dL for the control group and 206.6 mg / dL for the treated group.

After 7 days, at the second sampling, however, a reduction was observed in the plasma cholesterol concentration of the control group dogs if compared to the treated group, dogs with average values respectively of 190 mg / dL and 206, 2 mg / dL. At the last sampling (21 day), the values of both groups were eventually almost similar, with mean serum values equal to 192.1 mg / dL for the control group dogs and 180.6 mg / dL for the treated group. However, during the test it was possible to show a progressive reduction in cholesterol serum in subjects receiving the microcrystalline cellulose diet (Figure 17).

Figure 17: Concentrations of cholesterol serum, expressed in U / L, as detected during the test.

Calcium and phosphorus

During the study, serum concentrations of calcium and phosphorus did not show statistically significant differences between the serum concentrations of these parameters in dogs belonging to the treated subjects group and the control group ones. Serum concentrations remained almost unchanged in both groups of animals during the three blood collections. The trend is shown in Figure 18.

The calcium, phosphorus ratio remained constant, being included in the reference range is physiological (2: 1) throughout the trial in both groups of animals.

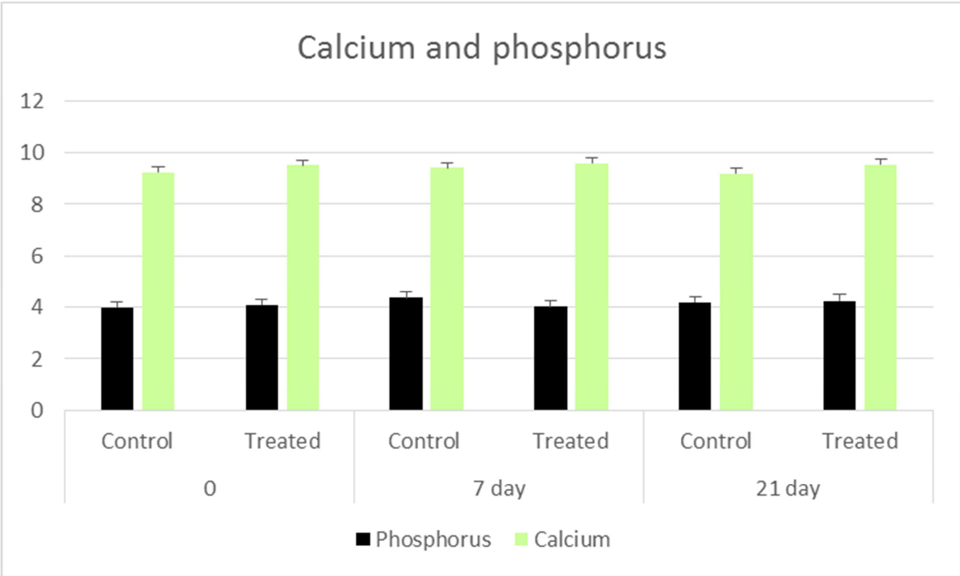


Figure 18: serum concentrations of calcium and phosphorus (mg / dL) as observed during the study.

Amylase.

Analysis of serum amylase levels no statistically significant differences between the two groups of dogs were observed during the study. In the second sampling (day 7) concentrations of this parameter lower (594.3 U / L) compared to that recorded in the first sample (731 U / L) in subjects in the control group.

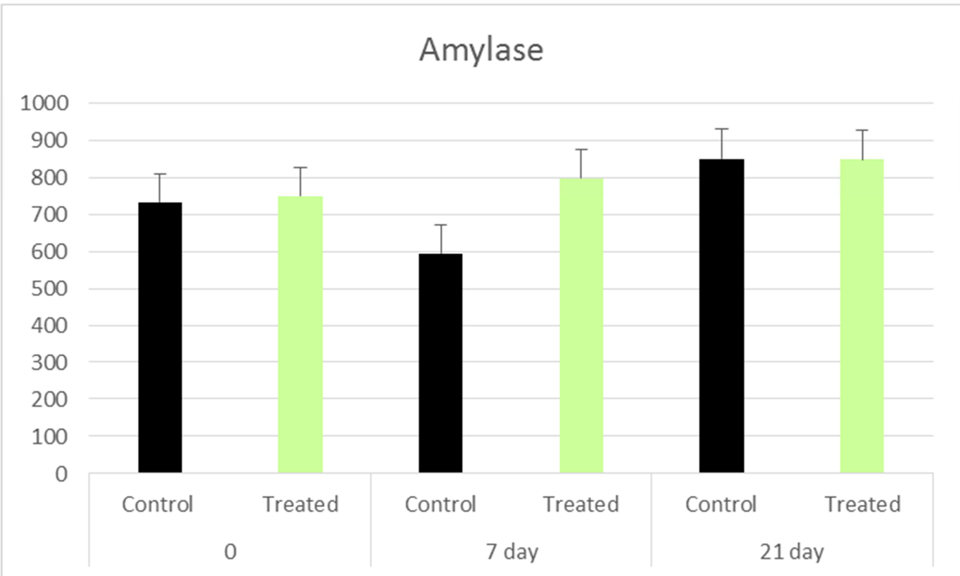


Figure 19: Serum Concentrations of the enzyme amylase (U / L) as detected in blood sample phase during the study.

The serum amylase in dogs receiving the microcrystalline cellulose supplemented diet was maintained during the test recording averages of 749.2 U / L in the first sample (0 days), 737.5 U / L per second (7 day) and 847.3 during the third to twenty-first day. The trend of this parameter is shown in Figure 19.

Evaluation of fecal score.

According to the data collection occurred during the test, the Fecal Score of microcrystalline cellulose enriched diet was better than for subjects who received the control diet. In particular, it was noted an improvement in appearance and consistency of stools, as it is evident from the graph below (Figure 20).

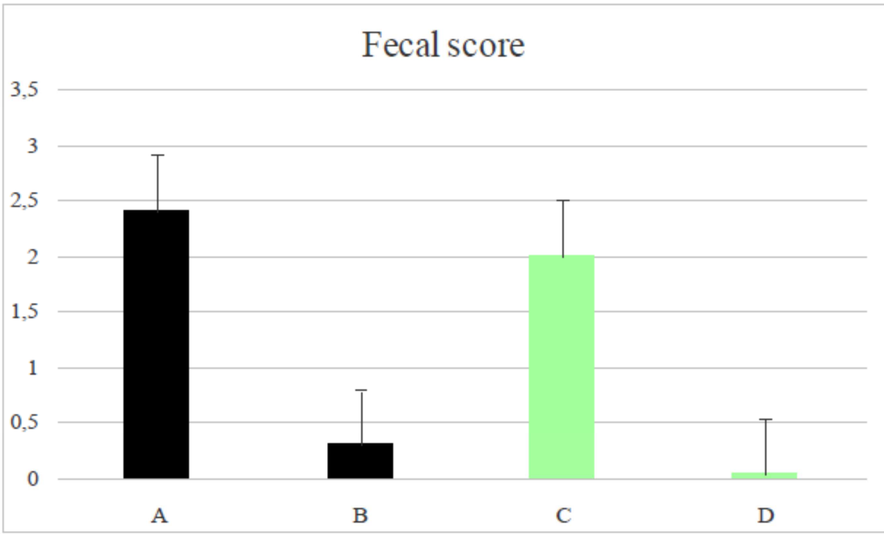


Figure 20: Fecal score subjects at the beginning and end of treatment.

Legend.

A = asymptomatic dogs start control diet without crystalline cellulose.

B = symptomatic dogs at the end of the control diet without crystalline.

C = symptomatic dogs start diet with crystalline cellulose.

D = symptomatic dogs end diet with crystalline cellulose.

By monitoring subjects during the test, it was possible to notice that the dogs receiving the supplemented diet showed an improved fecal score. If at first it was observed only a minimal improvement in the appearance and consistency of stool, during the trial these subjects achieved a reduction of fecal score better than those of the controls did.

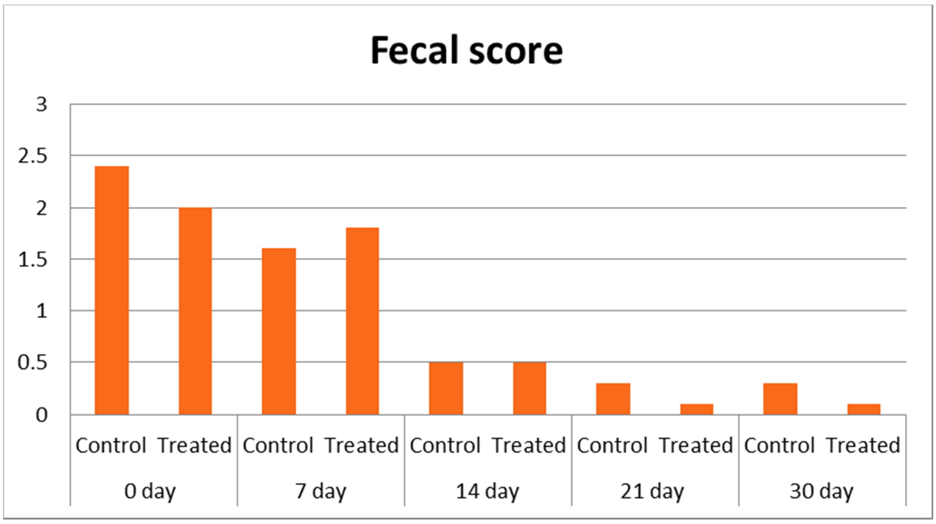


Figure 21: Fecal score detected during the study in symptomatic subjects.

Looking at a sample in the time of trial, the presence of microcrystalline cellulose in the dogs diet resulted in an improvement of fecal score in the treated subjects for up to return to normal appearance and consistency of stool, although in the controls there was an improvement of fecal score.

The 60% of the control group had liquid stools at the start of the test compared with 30% of subjects in the treated group feces, then they showed improvement from day 7 until the end of the test, with the exception of 3 dogs that showed no improvement.

In contrast, 40% of treated group subjects showed shapeless soft stool, but in the following weeks these improved back to normal. As regards the dogs of the control group, 20% presented soft stool without definite form on the occasion of the first control. 30% of treated group dogs showed feces score 1 or with soft form on the occasion of the first visit, remaining the same on at the second visit and then finally normalized and likewise also the 20% of the dogs in the group control presented the same type of feces.

The presence of microcrystalline cellulose in the considered quantities did not affect the main nutritional evaluation parameters.

We need to remember how the same nutritional assessment of the subject is an important part of the dietary anamnesis. For inclusion in the trial for each dog was carried out the evaluation of the last diet, since both the quantity as the profile of nutrients can affect the involved parameters in the nutritional evaluation.

In this context, especially the insoluble fiber has an important role as "a work dilution" of the energy density of the food. Although in this study the inclusion of microcrystalline cellulose was considered "high levels", the presence in the diet could not have influenced the energy density of the food, given the stability of the body weight of the subjects in the time period considered.

It should also be considered the number of meals and the amount, as well as other sources of nutrients, including snacks, food from the table, supplements, food used for administering drugs and medicines palatable, all influential factors conditioning the subject in question.

The dietary management of acute enteric forms is based, as is clear from the literature, the use of diets with different nutritional characteristics, with one or more sources of fiber, which is recommended as a dietary solution ideal for subjects with gastrointestinal symptoms, as well as hyperdigerible diets, hypoallergenic, and elimination (Nelson et al., 1988; Richter, 1992; Simpson 1995).

According to Anderson and collaborators (2009) an increased fiber intake of 14 g/1000 Kcal in the human diet can lead to beneficial effects in the gastrointestinal tract, in addition to reducing the prevalence of certain intestinal diseases, favouring the production of fatty acids short chain is shown to be protective of the mucosa of the entire intestinal tract. These authors also suggest that some soluble fibers (guar gum) are associated with reduced production of stomach acid, thus favouring the protective action. However, in this field finds a use also the insoluble fiber, a time not considered, especially in the quantities used in this study.

In fact, Zentek and associates in 1996 showed that the inclusion of cellulose in the diet of dogs (3% of dry matter) had effects on the digestibility of the ration quota of some nutrients, which was increased. However, such inclusion was going to influence the absorption of sodium, which is a reduced result compared to other types of fibers. In the diets that were used in this study the texture and shape of the stool were improved. While failing to provide data on digestibility, maintaining the BCS and the other parameters of the nutritional assessment of dogs receiving the supplemented diet may indicate that the inclusion to high quantities of cellulose has influenced digestibility. Dietary fiber exerts different effects on the normal intestinal digestion, which is affected by its chemical composition and amounts in the diet. Besides influencing intestinal transit, fiber is also being used as a bacterial substrate and exerts some specific effects on the ration exchange level and on the enteric intestinal absorption, in addition to bile acids and water retention. In particular, these authors (Zentek et al., 1996) have detected as dogs received a cellulose-supplemented diet in order to give formed stools, although the fecal mass and the total excretion of water had increased.

The inclusion of cellulose (4% dry weight) has also allowed a lower organic matter digestibility than other nutrients, although this resulted in an apparent increased digestibility of fat. The fiber was able to affect the availability of minerals by increasing or reducing them. In particular, the cellulose could affect the availability of zinc, iron, calcium and phosphorus. From the obtained data during this study, it was possible to argue that blood concentrations of calcium and phosphorus in dogs receiving a cellulose supplemented diet remained in the physiological range (2: 1).

Integrating the diet with microcrystalline cellulose for the considered individuals in this study allowed an improvement in symptoms, activity, appetite, stool consistency and defecation frequency with a return back to normal appearance and the stool consistency in a shortest period. At the end of the study with microcrystalline cellulose feces occurred more formed, their texture increased and the collected residues were released in smaller quantities than at the beginning of treatment. The frequency of bowel movements in subjects was also regularized.

As it regards the different components of peripheral blood count differences were found in diet treatment in both dogs groups. In fact, the main concerned blood parameters did not change significantly during the administration of the microcrystalline cellulose.

Similarly the biochemical parameters did not change significantly either, except for the plasma concentration of creatinine that appeared significantly lower in the subjects of the compared control group compared to those of the treated group at the second pick. In association with this parameter, the urea-creatinine ratio was also taken into account in order to detect any pathological forms regarding the kidneys. During the study, however, no statistical differences were shown for this parameter.

The albumin-globulin ratio, considered while assessing the nutritional status or the presence of any liver disorders and/or kidney disease did not show any changes in the subjects who received the crystalline cellulose, compared with dogs receiving the control diet.

None of the other considered biochemical parameters such as albumins, globulins and total proteins, alanine amino transferase (ALT), total bilirubin, cholesterol, calcium and phosphorus and amylase, were affected by the use of microcrystalline cellulose in the diet.

Swanson and collaborators (2004) also studied how the diet type affects the concentration of some biochemical parameters, such as cholesterol, and as the concentration of other parameters decrease in dogs that consumed a enriched diet (19% on dry matter) and soybean meal (4% dry weight) when compared with dogs who were fed with a basic diet. This effect, however, was not detected in the course of this study. These authors also suggested that age can influence the concentration of certain biochemical and blood parameters such as red blood cells, hemoglobin, hematocrit, creatinine and calcium, which appear as decreased in the elder, although not the same effects were observed during this study.

With regard to serum cholesterol, it was affected by age. It was observed a lowering of the concentration of this parameter in elder subjects in this study.

3.4 Conclusions

The supplementation of microcrystalline cellulose for 30 days did not affect the nutritional status of the subjects involved in the study.
It is extremely important to assess the quantity and quality of the fiber in the diet. It was observed that the presence of this nutrient can affect the digestibility of other nutrients in our pets. The combination of high fiber in the diet lowering the digestibility of the same was the subject of numerous studies. While failing to provide data on the digestibility it can be said that for the period of use (30 days) the used portion did not affect the body weight, BCS and MCS.
The capacity and the microcrystalline cellulose structure, such as slowly fermentable integrated fiber to high quantities in the diet allowed to be a valid

management agent of acute diarrhea. The consistency and quantity of stools could be improved in animals receiving the fiber diet. In literature, including some studies that were carried out on dogs regarding the beneficial effects of the cellulose on the quality of the feces, on the ability to retain water in the stool, the intestinal transit improved as well as the fecal consistency.
Even the serological markers were considered within the physiological range, although we noticed a decrease in the concentration of creatinine.

3.5 References

Barry K. A., Wojcicki B. J., Middelbos I. S., Vester B. M., Swanson K. S., Fahey G. Jr. (2010). Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. *J. Anim. Sci. 88(9): 2978- 2987.*

Bednar G. E., Patil A. R., Murray S. M., Grieshop C. M., Merchen N. R., Fahey G. (2001). Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *J. Nutr.131(2):276-286.*

BeMiller J. N., Whistler R. L. (1996). Carbohydrates. In Food Chemistry, 3rd Edition, Ed. O. R. *Fennema. pp.157-168. New York: Marcel Dekker, inc.*

Berg R. D. (1985). Indigenous intestinal microflora and the host immune response. EOS *J. Immunol. Immunopharmacol. 4: 161-168.*

Bird A. R., Brown I. L., Topping D.L. (2000). Starches, resistant starches, the gut microflora and human healt. *Curr. Issues Intest. Microbiol. 1(1): 25-37.*

Blottiere H. M., Buecher B., Galmiche J. P., Cherbut C. (2003). Molecular analysis of the effect of short chain fatty acids on intestinal cell proliferation. *Proc. Nutr. Soc. 62(1): 101-106.*

Bowling, T. E., Ralmundo A. H., Grimble G. K., Silk D. A. B. (1993). Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. *Lancet 342: 1266-1268.***British Nutrition Foundation,** (1990). Complex Carbohydrates in Foods. The Report of the British Nutrition Foundation's Task Force on Complex Carbohydrates. London: *Chapman and Hall.*

Brown I. L., Mc Naught K. J., Andrews D., Morita T.(2001). Resistant starch: Plant breeding, application development and commercial use. In advanced Dietary Fiber Technology, B. V. McCleary and L. *Prosky, eds. Pp. 401-412 Oxford, UK: Blackwell Science Ltd.*

Buddington R. K., Sangild P.T. (2011). Companion Animal Symposium: Development of the mammalian gastrointestinal tract, the resident microbiota, and the role of diet in early life. *J. Anim. Sci. 89(5): 1506-1519.*

Burkholder W. J., Toll P.W. (2000). Obesity. Small Animal Clinical *Nutrition, Hand M. S., Thatcher C. D., and Remillard R.L., eds Topeka, Pp. 401-430 Kan.: Mark Morris Institute.*

Burrows C. F. (1992). Canine colitis. Compend. *Contin. Educ. 2: 1347-1354.*

Burrows C. F., Kronfeld D. S., Banta C. A., Merritt A.M. (1982). Effects of fiber on digestibility and transit time in dogs. *J. Nutr. 112(9): 1726-1732.*

Campbell, J. M., Bauer, L. L., Fahey, G. C., Hogarth, A. J. C. L., Wolf, B. W., & Hunter, D. E. (1997). Selected fructooligosaccharide (1-kestose, nystose, and 1F-β-fructofuranosylnystose) composition of foods and feeds. *J. Agric. food chem., 45(8): 3076-3082.*

Case M. S., Daristotle L., Hayek, Raasch. (2011). Canine and Feline Nutrition. A Resource for Companion Animal Professionals. *3 th Edition, eds Elsevier Inc.*

Carpo L. P., A., Reaven G., Olefsky J.(1976). Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes 25:741-747.*

Cho, S., J. W. deVries, and L. Prosky. (1997). Dietary Fiber Analysis and Applications. *AOAC Int., Gaithersburg, MD.*

Cummings J. H., Macfarlane G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol. 70(6): 443-459.*

Davenport D. J., Remillard R. L. (2010). Acute Gastroenteritis and Enteritis. Small Animal Clinical Nutrition**.** 5th Edition Ed. Hand M.S., Thatcher C. D., Remillard R. L., Roudebush P., Novotny B. J. (2010). *pp. 1055-1057. Mark Morris Institute Topeka, Kansas, US.*

Davenport D. J., Jergens A. E., Remillard R. L. (2010). Inflammatory bowel disease. In Small Animal Clinical Nutrition. 5th Edition Ed. Hand M. S., Thatcher C.D., Remillard R. L., 2010. 1066-1076. Mark Morris Institute Topeka, Kansas, US.

De Simone C., Vesely R., Negri R., Bianchi Salvadori B., Zanzoglu S., Cilli A., Lucci L. (1987). Enhancement of immune response of murine Peyer's patches by a diet supplemented with yogurt. Immunoph. and Immunotox. *9(1): 87–100.*

Delorme C. B., Barrette D., Monean R., Lariviere N. (1985). The effect of dietary fiber on feed intake and growth in beagle puppies. Can. *J. Comp. Med. 49(3): 278.*

Dimski D. S. (1992). Dietary fiber in the management of gastrointestinal disease. In: Current Veterinary Therapy XI. Bonagura, *J., ed. Pp. 592–595. W. B. Saunders, Philadelphia, PA.*

Dobenecker B., Kienzle E.(1998). Interactions of cellulose content and diet composition with food intake and digestibility in dogs. *J. Nutr. 128(12): 2674S-2675S.*

Drackley J. K., Beaulieu A.D. (1998). Energetic substrates for intestinal cells. In Recent Advances in Canine and Feline Nutrition: 1998 Iams Nutrition Symposium Proceedings, Reinhart G. A. and Carey D. P., eds. *Pp. 463-472. Wilmington, Ohio 1998: Orange Frazer Press.*

Eastwood M. A. (1992). The physiological effect of dietary fiber: An update. Annu. *Rev. Nutr. 12(1): 19-35.*

Eiden K. A. (2003). Nutritional considerations in inflammatory bowel disease. *Pract. Gastroent. 27(5): 33-54.*

Englyst H. (1989). Classification and measurement of plant polysaccharides. Anim. Feed Sci. Technol. 23(1): 27-42.

Fahey G. C., Merchen N. R., Corbin J. E., Hamilton A. K., Serbe K. A., Hirakawa A. (1990). Dietary fiber for dogs: II. Iso-total dietary fiber (TDF) additions of divergent fiber sources to dog diets and their effects on nutrient intake, digestibility, metabolizable energy and digesta mean retention time. *J. anim. Sci. 68(12): 4229-4235.*

Fahey G. C. Jr, Merchen N. R, Corbin J. E., Hamilton A. K., Bauer L. L., Hirakawa A. (1992). Dietary fiber for dogs: III. Effects of beet pulp and oat fiber additions to dog diets on nutrient intake, digestibility, metabolizable energy, and digesta mean retention time. *J. An. Sci. 70(4): 1169-1174.*

Firmansyah, Penn A. D., Lebenthal E. (1989). Isolated colonocyte metabolism o glucose, glutamine, n-butyrate, and beta-hydroxybutyrate in nutrition. *Gastroenterol. 97: 622-629.*

Frankel W., Zhang W., Singh A., Bain A., Satchithanandam S., Klurfeld D., Rombeau J. (1995). Fiber: effect on bacterial translocation and intestinal mucin content. W. *J. Surg. 19(1): 144-149.*

German A. J., Hall E. J., Day M. J. (2003). Chronic Intestinal Inflammation and Intestinal Disease in Dogs. *J. Vet. Intern. Med. 17(1): 8-20.*

Gibson G. R., Robertfroid M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr. 125: 1402-1412.*

Gross K. L., Yamka Ryan M., Khoo C., Friesen Kim G., Jewell Dennis E., Schoenherr William D., Debraekeleer J., Zicker Steven C. (2010). Macronutrients. In Small Animal Clinical Nutrition. 5th Edition Ed. Hand M.S., Thatcher C.D., Remillard R. L., Roudebush P., Novotny B.J. (2010). *pp. 49-101. Mark Morris Institute Topeka, Kansas, US.*

Guilford W. G. (1994). New ideas for the dietary management of gastrointestinal disease. J. Small Anim. *Pract. 35(12): 620-624.*

Hall E. J., Rutgers H. C., Scholes S.C.E., Middleton D. J., Tennant B.J., King N.M., Kelly D. F. (1994). Histiocytic ulcerative colitis in boxer dogs in the UK. *J. Small Anim. Pract. 35: 509-515.*

Hassid W. Z. (1970). Biosynthesis of sugars and polysaccarides. In The Carbohydrates. *Chemistry and Biochemistry, Pigman W. and Horton D., eds. pp. 301-373. New York: Academic Press.*

Herschel D. A., Argenzio R. A., Southworth M., Stevens C. E. (1981). Absorption of volatile fatty acid and H2O by the colon of the dog. Am. *J. Vet. Res. 42(7): 1118-1124.*

Jergens A. E., Schreiner C. A, Frank D. E., Yosiya Niyo, Ahrens F. E., Eckersall P. D., Benson T. J., Evans R. (2003). A scoring index for disease activity in canine inflammatory bowel disease. *J. Vet. Intern. Med. 17(3): 291-297.*

Jergens A. E., Schreiner C. A., Ahrens F. A. (2000). Laboratory assessment of disease activity in canine inflammatory bowel disease. *J. Vet. Intern. Med. 14: 347 (abstract).*

Jewell D. E., Toll P. W. (1996). Effects of fiber on food intake in dogs. *Vet. Clin. Nutr. 3: 115-118.*

Kienzle E. (1993). Carbohydrate metabolism in the cat. Activity of malta isomaltase, sucrase, and lactase in the gastrointestinal tract in relation to age and diet. *J. Anim. Physiol. Anim. Nutr. 70: 89-96.*

Kripke S.A., Fox A.D., Berman J.M., Gregg Settle R., Rombeau J.L. (1989). Stimulation of intestinal muscosal growth with intracolonic infusion of short chain fatty acid. *J. Parent. Ent. Nutr. 13(2): 109-116.*

Lee S.C., Prosky L. (1999). International survey on dietary fiber: definition analysis, and reference materials. *In Complex Carbohydrates in Food, Cho S. S., Prosky L., and Dreher M., eds pp. 605-608. New York: Marcel Dekker, inc.*

Lewis L.D., Magerkurth J.H., Rodebush P., Morris M. L., Mitchell E. E., Teeter S.M. (1994). Stool Characteristics, gastrointestinal transit time, and nutrient digestibility in dogs fed different fiber sources. *J. Nutr. 124(12): 2716S-2718S.*

Lineback D.R., (1999). The chemistry of complex carbohydrates. *In Complex Carbohydrates in Foods, Cho S. S., Prosky L., and Dreher M., eds. Pp. 115-129. NewYork: Marcel Dekker, Inc.*

Link-Amster H., Rochat F., Saudan K.Y., Mignot O., Aeschlimann J.M.(1994). Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immun. Med. Microb. 10(1): 55-64.*

Markham R.W., Hodgkins E.M. (1989). Geriat. nutrition. Geriat. *Gerontol. 19: 165-185.*

Maskell I.E., Johnson J.V. (1993). Digestion and absorption. In The Waltham *Book of Companion Animal Nutrition, Burger I. H., ed. pp. 25-44. New York: Pergamon Press.*

Massimino S.P., McBurney M.I., Field C.J., Thompson A.B.R., Keelan M., Hayek M.G., Sunvold G.D. (1998). Fermentable dietary fiber increase GLP-1 secretion and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. *J. Nutr. 128(10): 1786-1793.*

Meyer D., Tungland B. (2001). Non-digestible oligosaccharides and polysaccharides: Their Physiological effect and health implications. *In Advanced*

Dietary Fiber Technology, McCleary B.V. and Prosky L., eds. pp. 455-470. Oxford, UK: Blackwell Science Ltd.

Monsan P. F., Paul F. (1995). Oligosaccharide feed additives. in Biotechnology in Animal Feeds and Animal Feeding, Wallace R. *J. and Chesson A., eds. pp. 233-245 New York: VCH.*

Muir J., O'Dea K. (1993). Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. Am. *J. Clin. Nutr. 57(4): 540-546.*

Murdoch D.B. (1996). Large intestinal disease. In: Manual of Canine and Feline Gastroenterology. Thomas, D. A., Simpson, J. W. & Hall, E. J., eds., pp. 151. British Small Animal Veterinary Association, Cheltenham, UK.

Nantel G. (1999). Carbohydrates in human nutrition. Food Nutr. Agric. 24: 6-10.

Pappas T. N., Melendez R. L., Debas H.T. (1989). Gastric distension is a physiologic satiety signal in the dog. *Digest. Dis. Sci. 34: 1489-1493.*

Perdigon G., De Macias M. E., Alvarez S., Oliver G., de Ruiz Holgado A.P. (1988). Systemic augmentation of the immune response in mice by feeding fermented milks with Lactobacillus casei and Lactobacillus acidophilus. *Immun. 63(1): 17-23.*

Prola L., Dobenecker B., Mussa P. P., Kienzle E. (2009). Influence of cellulose fiber length on fecal quality, mineral excretion and nutrient digestibility in cat. *J. Anim. Physiol. Anim. Nutr. 94(3): 362-367.*

Prosky L., DeVries J. W. (1992). Controlling Dietary Fiber in Food Products. New York: *Van Nostrand Reinhold.*

Reinhart G. A. (1993). Fiber nutrition and intestinal function critical for recovery. *DVM. 24: 13-14.*

Reppas C., Meyer J. H., Sirois P. J., Dressman J. B. (1991). Effect of hydroxypropylmethylcellulose on gastrointestinal transit and luminal viscosity in dogs. *Gastroent. 100(5): 1217-1223.*

Richter K. P. (1992). Lymphocytic plasmacytic enterocolitis in dogs. Semin. Vet. Med. Sur*g. 7: 134-144.*

Robertfroid M. (1993). Dietary fiber, inulin, and oligofructose: A review comparing their physiological effect. *Crit Rev. Food Sci. Nutr. 33(2): 103-148.*

Roediger W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut 21(9): 793-798.*

Rumbo M., Schiffrin E. J. (2005). Ontogeny of intestinal epithelium immune functions: Developmental and environmental regulation. *Cell. Mol. Life Sci. 62(12): 1288-1296.*

Russel J., Bass P. (1985). Canine gastric emptying of fiber meals: Influence of meal viscosity and antroduodenal motility. Am. *J. Phisiol. Gastroint. Liv. Physiol. 249(6): G662-667.*

SAS Institute Inc. (2010). User's Guide: Statistics, release 9.3; *SAS Inst. Inc.: Cary, NC, USA.*

Schiffrin E. J., Rochat F., Link-Amster H., Aesclimann J.M., Donnet-Hunghes A. (1995). Immunomodulation of human blood cells following the ingestion of lactic acida bacterial. *J. D. Sci. 78(3): 491-497.*

Schley P. D., Field C. J. (2002). The immune-enhancing effects of dietary fibers and prebiotics. Br. *J. Nutr. 87(2): S221-S230.*

Schneeman B. O. (1987). Soluble vs insoluble fiber. Different physiological responses. *Food Technol. 41: 81-82.*

Simpson J. W. (1995). Management of colonic disease in the dog. *Waltham Focus. 5: 17-22.*

Sloth C. (1992). Pratical management of obesity in dogs and cats. *J. Small Anim. Pract. 33(4): 178-182.*

Solys Pereyra B., Lemonnier D. (1993). Induction of human cytokines by bacteria used in diary foods. *Nutr. Res. 13(10): 1127-1140.*

Sparkes A. H., Papasouliotis K., Sunvold G., Werrett G., Clarke C., Jones M., Gruffydd-Jones T. J., Reinhart G. (1998). Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructooligosaccharides. Am. *J. Vet. Res. 59(4): 431-435.*

Steiner J. M. (1998). Small Animal Gastroenterology. *Pp. 136-137.* Hanover, Germany.

Subcommittee on Dog and Cat Nutrition, Committee on Animal Nutrition and National Research Council. (2006). Nutrient Requirements of Dogs and Cats. *Pp. 49-79. National Research Council. Washington, DC: The National Academies Press.*

Sunvold G. D., Fahey G. C., Merchent N. R., Bourquin L. D., Titgemeyert E. C., Bauer L. L., Reinhart G. A. (1995). Dietary fiber for cats: in vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. *J. Anim. Sci. 73.8: 2329-2339.*

Swanson K. S., Kuzmuk K. N., Schook L. B., Fahey G. C. Jr. (2004). Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. *J. Anim. Sci. 82:1713-1724.*

Takahashi T., Nakagawa E., Nara T., Yajima T., Kuwata T. (1998). Effects of orally ingested Bifidobacterium longum on the mucosal IgA response of mice to dietary antigens. *Biosci. Biotech. Bioch. 62.1: 10–15.*

Takahashi T., Oka T., Iwana H., Kuwata T. & Yamamoto Y. (1993). Immune response of mice to orally administered lactic acid bacteria. *Biosci. Biotech. Bioch. 57.9: 1557–1560.*

Tejada-Simon M.V., Ustunol Z. & Pestka J. J. (1999). Ex vivo effects of lactobacilli, streptococci, and bifidobacteria ingestion on cytokine and nitric oxide production in a murine model. *J. Food Protec. 62.2: 162–169.*

Theander O., Aman P., Westerlund E., and Graham H. (1994). Enzymatic/chemical analysis of dietary fiber. *J. Assoc. Off. Anal. Chem. 77.3:703-709.*

Thompson A., (2008). Ingredients: Where Pet Food Starts. *Topics Comp. Anim. Med. 23.3:127-132.*

Trowell H., Southgate D. A. T., Wolever T. M. S., Leeds A., Gussell M., Jenkins D.A. (1976). Dietary fiber redefined. *The Lancet 307: 7966-967.*

Van de Perre P. (2003). Transfer of antibody via mother's milk. *Vaccine 21(24): 3374-3376.*

Welsh J. D., Walker A. (1965). Intestinal disaccharidase and alkaline Phosphatase activity in the dog. *Proc. Soc. Exp. Biol. Med. 120: 525-527.*

Wichert, B., Schuster, S., Hofmann, M., Dobenecker, B., Kienzle, E. (2002). Influence of different cellulose types on feces quality of dogs. *J. Nutr. 132(6): 1728S-1729S.*

Willard M. D., Simpson R. B., Delles E. K., Cohen N. D., Fossum T. W., Kolp D., Reinhart G. (1994). Effects of dietary supplementation of fructooligosaccharid Barry K. A., Wojcicki B. J., Middelbos I. S., Vester B. M., Swanson K. S., Fahey G. Jr. (2010). Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. *J. Anim. Sci. 88(9): 2978- 2987.*

Bednar G. E., Patil A. R., Murray S. M., Grieshop C. M., Merchen N. R., Fahey G. (2001). Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *J. Nutr.131(2):276-286.*

BeMiller J. N., Whistler R. L. (1996). Carbohydrates. In Food Chemistry, 3rd Edition, Ed. O. R. *Fennema. pp.157-168. New York: Marcel Dekker, inc.*

Berg R. D. (1985). Indigenous intestinal microflora and the host immune response. EOS *J. Immunol. Immunopharmacol. 4: 161-168.*

Bird A. R., Brown I. L., Topping D.L. (2000). Starches, resistant starches, the gut microflora and human healt. *Curr. Issues Intest. Microbiol. 1(1): 25-37.*

Blottiere H. M., Buecher B., Galmiche J. P., Cherbut C. (2003). Molecular analysis of the effect of shortchain fatty acids on intestinal cell proliferation. Proc. *Nutr. Soc. 62(1): 101-106.*

Bowling, T. E., Ralmundo A. H., Grimble G. K., Silk D. A. B. (1993). Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. *Lancet 342: 1266-1268.*

British Nutrition Foundation, (1990). Complex Carbohydrates in Foods. The Report of the British Nutrition Foundation's Task Force on Complex Carbohydrates. *London: Chapman and Hall.*

Brown I. L., Mc Naught K. J., Andrews D., Morita T.(2001). Resistant starch: Plant breeding, applications development and commercial use. In advanced Dietary Fiber Technology, B. V. *McCleary and L. Prosky, eds. Pp. 401-412 Oxford, UK: Blackwell Science Ltd.*

Buddington R. K., Sangild P.T. (2011). Companion Animal Symposium: Development of the mammalian gastrointestinal tract, the resident microbiota, and the role of diet in early life. *J. Anim. Sci. 89(5): 1506-1519.*

Burkholder W. J., Toll P.W. (2000). Obesity. in Small Animal Clinical Nutrition, Hand M. S., Thatcher C. D., and Remillard R.L., eds Topeka, *Pp. 401-430 Kan.: Mark Morris Institute.*

Burrows C. F. (1992). Canine colitis. Compend. Contin. *Educ. 2: 1347-1354.*

Burrows C. F., Kronfeld D. S., Banta C. A., Merritt A.M. (1982). Effects of fiber on digestibility and transit time in dogs. *J. Nutr. 112(9): 1726-1732.*

Campbell, J. M., Bauer, L. L., Fahey, G. C., Hogarth, A. J. C. L., Wolf, B. W., & Hunter, D. E. (1997). Selected fructooligosaccharide (1-kestose, nystose, and 1F-β-fructofuranosylnystose) composition of foods and feeds. *J. Agric. food chem., 45(8): 3076-3082.*

Case M. S., Daristotle L., Hayek, Raasch. (2011). Canine and Feline Nutrition. A Resource for Companion Animal Professionals. *3 th Edition, eds Elsevier Inc.*

Carpo L. P., A., Reaven G., Olefsky J.(1976). Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes 25:741-747.*

Cho, S., J. W. deVries, and L. Prosky. (1997). Dietary Fiber Analysis and Applications. *AOAC Int., Gaithersburg, MD.*

Cummings J. H., Macfarlane G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol. 70(6): 443-459.*

Davenport D. J., Remillard R. L. (2010). Acute Gastroenteritis and Enteritis. In Small

Animal Clinical Nutrition. 5th Edition Ed. Hand M.S., Thatcher C. D., Remillard R. L., Roudebush P., Novotny B. J. (2010). *pp. 1055-1057. Mark Morris Institute Topeka, Kansas, US.*

Davenport D. J., Jergens A. E., Remillard R. L. (2010). Inflammatory bowel disease. In Small Animal Clinical Nutrition. 5th Edition Ed. Hand M. S., Thatcher C.D., Remillard R. L., 2010. *1066-1076. Mark Morris Institute Topeka, Kansas, US.*

De Simone C., Vesely R., Negri R., Bianchi Salvadori B., Zanzoglu S., Cilli A., Lucci L. (1987). Enhancement of immune response of murine Peyer's patches by a diet supplemented with yogurt. *Immunoph. and Immunotox. 9(1): 87–100.*

Delorme C. B., Barrette D., Monean R., Lariviere N. (1985). The effect of dietary fiber on feed intake and growth in beagle puppies. Can. *J. Comp. Med. 49(3): 278.*

Dimski D. S. (1992). Dietary fiber in the management of gastrointestinal disease. In: Current Veterinary Therapy XI. Bonagura, *J., ed. Pp. 592–595. W. B. Saunders, Philadelphia, PA.*

Dobenecker B., Kienzle E.(1998). Interactions of cellulose content and diet composition with food intake and digestibility in dogs. *J. Nutr. 128(12): 2674S-2675S.*

Drackley J. K., Beaulieu A.D. (1998). Energetic substrates for intestinal cells. In Recent Advances in Canine and Feline Nutrition: 1998 *Iams Nutrition Symposium Proceedings, Reinhart G. A. and Carey D. P., eds. Pp. 463-472. Wilmington, Ohio 1998: Orange Frazer Press.*

Eastwood M. A. (1992). The physiological effect of dietary fiber: An update. Annu. *Rev. Nutr. 12(1): 19-35.*

Eiden K. A. (2003). Nutritional considerations in inflammatory bowel disease. Pract. *Gastroent. 27(5): 33-54.*

Englyst H. (1989). Classification and measurement of plant polysaccharides. *Anim. Feed Sci. Technol. 23(1): 27-42.*

Fahey G. C., Merchen N. R., Corbin J. E., Hamilton A. K., Serbe K. A., Hirakawa A. (1990). Dietary fiber for dogs: II. Iso-total dietary fiber (TDF) additions of divergent fiber sources to dog diets and their effects on nutrient intake, digestibility, metabolizable energy and digesta mean retention time. *J. anim. Sci. 68(12): 4229-4235.*

Fahey G. C. Jr, Merchen N. R, Corbin J. E., Hamilton A. K., Bauer L. L., Hirakawa A. (1992). Dietary fiber for dogs: III. Effects of beet pulp and oat fiber additions to dog diets on nutrient intake, digestibility, metabolizable energy, and digesta mean retention time. *J. An. Sci. 70(4): 1169-1174.*

Firmansyah, Penn A. D., Lebenthal E. (1989). Isolated colonocyte metabolism o glucose, glutamine, n-butyrate, and beta-hydroxybutyrate in nutrition. *Gastroenterol. 97: 622-629.*

Frankel W., Zhang W., Singh A., Bain A., Satchithanandam S., Klurfeld D., Rombeau J. (1995). Fiber: effect on bacterial translocation and intestinal mucin content. W. *J. Surg. 19(1): 144-149.*

German A. J., Hall E. J., Day M. J. (2003). Chronic Intestinal Inflammation and Intestinal Disease in Dogs. *J. Vet. Intern. Med. 17(1): 8-20.*

Gibson G. R., Robertfroid M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr. 125: 1402-1412.*

Gross K. L., Yamka Ryan M., Khoo C., Friesen Kim G., Jewell Dennis E., Schoenherr William D., Debraekeleer J., Zicker Steven C. (2010). *Macronutrients.*

Small Animal Clinical Nutrition. 5th Edition Ed. Hand M.S., Thatcher C.D., Remillard R. L., Roudebush P., Novotny B.J. (2010). *pp. 49-101. Mark Morris Institute Topeka, Kansas, US.*

Guilford W. G. (1994). New ideas for the dietary management of gastrointestinal disease. *J. Small Anim. Pract. 35(12): 620-624.*

Hall E. J., Rutgers H. C., Scholes S.C.E., Middleton D. J., Tennant B.J., King N.M., Kelly D. F. (1994). Histiocytic ulcerative colitis in boxer dogs in the UK. *J. Small Anim. Pract. 35: 509-515.*

Hassid W. Z. (1970). Biosynthesis of sugars and polysaccarides. In The Carbohydrates. *Chemistry and Biochemistry, Pigman W. and Horton D., eds. pp. 301-373. New York: Academic Press.*

Herschel D. A., Argenzio R. A., Southworth M., Stevens C. E. (1981). Absorption of volatile fatty acid and H2O by the colon of the dog. Am. *J. Vet. Res. 42(7): 1118-1124.*

Jergens A. E., Schreiner C. A, Frank D. E., Yosiya Niyo, Ahrens F. E., Eckersall P. D., Benson T. J., Evans R. (2003). A scoring index for disease activity in canine inflammatory bowel disease. *J. Vet. Intern. Med. 17(3): 291-297.*

Jergens A. E., Schreiner C. A., Ahrens F. A. (2000). Laboratory assessment of disease activity in canine inflammatory bowel disease. *J. Vet. Intern. Med. 14: 347 (abstract).*

Jewell D. E., Toll P. W. (1996). Effects of fiber on food intake in dogs. *Vet. Clin. Nutr. 3: 115-118.*

Kienzle E. (1993). Carbohydrate metabolism in the cat. Activity of malta isomaltase, sucrase, and lactase in the gastrointestinal tract in relation to age and diet. *J. Anim. Physiol. Anim. Nutr. 70: 89-96.*

Kripke S.A., Fox A.D., Berman J.M., Gregg Settle R., Rombeau J.L. (1989). Stimulation of intestinal muscosal growth with intracolonic infusion of short chain fatty acid. *J. Parent. Ent. Nutr. 13(2): 109-116.*

Lee S.C., Prosky L. (1999). International survey on dietary fiber: definition analysis, and reference materials. *In Complex Carbohydrates in Food, Cho S. S., Prosky L., and Dreher M., eds pp. 605-608. New York: Marcel Dekker, inc.*

Lewis L.D., Magerkurth J.H., Rodebush P., Morris M. L., Mitchell E. E., Teeter S.M. (1994). Stool Characteristics, gastrointestinal transit time, and nutrient digestibility in dogs fed different fiber sources. *J. Nutr. 124(12): 2716S-2718S.*

Lineback D.R., (1999). The chemistry of complex carbohydrates. *In Complex Carbohydrates in Foods, Cho S. S., Prosky L., and Dreher M., eds. Pp. 115-129. NewYork: Marcel Dekker, Inc.*

Link-Amster H., Rochat F., Saudan K.Y., Mignot O., Aeschlimann J.M.(1994). Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immun. Med. Microb. 10(1): 55-64.*

Markham R.W., Hodgkins E.M. (1989). *Geriat. nutrition. Geriat. Gerontol. 19: 165-185.*

Maskell I.E., Johnson J.V. (1993). Digestion and absorption. In The Waltham *Book of Companion Animal Nutrition, Burger I. H., ed. pp. 25-44. New York: Pergamon Press.*

Massimino S.P., McBurney M.I., Field C.J., Thompson A.B.R., Keelan M., Hayek M.G., Sunvold G.D. (1998). Fermentable dietary fiber increase GLP-1 secretion and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. *J. Nutr. 128(10): 1786-1793.*

Meyer D., Tungland B. (2001). Non-digestible oligosaccharides and polysaccharides: Their Physiological effect and health implications. In Advanced *Dietary Fiber Technology, McCleary B.V. and Prosky L., eds. pp. 455-470. Oxford, UK: Blackwell Science Ltd.*

Monsan P. F., Paul F. (1995). Oligosaccharide feed additives. in Biotechnology in Animal Feeds and Animal Feeding, Wallace R. *J. and Chesson A., eds. pp. 233-245 New York: VCH.*

Muir J., O'Dea K. (1993). Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. Am. *J. Clin. Nutr. 57(4): 540-546.*

Murdoch D.B. (1996). Large intestinal disease. In: Manual of Canine and Feline Gastroenterology. Thomas, D. A., Simpson, J. W. & Hall, E. *J., eds., pp. 151. British Small Animal Veterinary Association, Cheltenham, UK.*

Nantel G. (1999). Carbohydrates in human nutrition. *Food Nutr. Agric. 24: 6-10.*

Pappas T. N., Melendez R. L., Debas H.T. (1989). Gastric distension is a physiologic satiety signal in the dog. *Digest. Dis. Sci. 34: 1489-1493.*

Perdigon G., De Macias M. E., Alvarez S., Oliver G., de Ruiz Holgado A.P. (1988). Systemic augmentation of the immune response in mice by feeding fermented milks with Lactobacillus casei and Lactobacillus acidophilus. *Immun. 63(1): 17-23.*

Prola L., Dobenecker B., Mussa P. P., Kienzle E. (2009). Influence of cellulose fiber length on fecal quality, mineral excretion and nutrient digestibility in cat. J. Anim. Physiol. Anim. *Nutr. 94(3): 362-367.*

Prosky L., DeVries J. W. (1992). Controlling Dietary Fiber in Food Products. New York: Van Nostrand Reinhold.

Reinhart G. A. (1993). Fiber nutrition and intestinal function critical for recovery. *DVM. 24: 13-14.*

Reppas C., Meyer J. H., Sirois P. J., Dressman J. B. (1991). Effect of hydroxypropylmethylcellulose on gastrointestinal transit and luminal viscosity in dogs. *Gastroent. 100(5): 1217-1223.*

Richter K. P. (1992). Lymphocytic plasmacytic enterocolitis in dogs. *Semin. Vet. Med. Surg. 7: 134-144.*

Robertfroid M. (1993). Dietary fiber, inulin, and oligofructose: A review comparing their physiological effect. *Crit Rev. Food Sci. Nutr. 33(2): 103-148.*

Roediger W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut 21(9): 793-798.*

Rumbo M., Schiffrin E. J. (2005). Ontogeny of intestinal epithelium immune functions: Developmental and environmental regulation. *Cell. Mol. Life Sci. 62(12): 1288-1296.*

Russel J., Bass P. (1985). Canine gastric emptying of fiber meals: Influence of meal viscosity and antroduodenal motility. Am. *J. Phisiol. Gastroint. Liv. Physiol. 249(6): G662-667.*

SAS Institute Inc. (2010). User's Guide: Statistics, release 9.3; SAS Inst. Inc.: Cary, NC, USA.

Schiffrin E. J., Rochat F., Link-Amster H., Aesclimann J.M., Donnet-Hunghes A. (1995). Immunomodulation of human blood cells following the ingestion of lactic acida bacterial. *J. D. Sci. 78(3): 491-497.*

Schley P. D., Field C. J. (2002). The immune-enhancing effects of dietary fibers and prebiotics. Br. *J. Nutr. 87(2): S221-S230.*

Schneeman B. O. (1987). Soluble vs insoluble fiber. Different physiological responses. *Food Technol. 41: 81-82.*

Simpson J. W. (1995). Management of colonic disease in the dog. *Waltham Focus. 5: 17-22.*

Sloth C. (1992). Pratical management of obesity in dogs and cats. *J. Small Anim. Pract. 33(4): 178-182.*

Solys Pereyra B., Lemonnier D. (1993). Induction of human cytokines by bacteria used in diary foods. *Nutr. Res. 13(10): 1127-1140.*

Sparkes A. H., Papasouliotis K., Sunvold G., Werrett G., Clarke C., Jones M., Gruffydd-Jones T. J., Reinhart G. (1998). Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructooligosaccharides. Am. *J. Vet. Res. 59(4): 431-435.*

Steiner J. M. (1998). Small Animal Gastroenterology. Pp. 136-137. Hanover, Germany.

Subcommittee on Dog and Cat Nutrition, Committee on Animal Nutrition and National Research Council. (2006). Nutrient Requirements of Dogs and Cats. Pp. 49-79. National Research Council. Washington, DC: *The National Academies Press.*

Sunvold G. D., Fahey G. C., Merchent N. R., Bourquin L. D., Titgemeyert E. C., Bauer L. L., Reinhart G. A. (1995). Dietary fiber for cats: in vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. *J. Anim. Sci. 73.8: 2329-2339.*

Swanson K. S., Kuzmuk K. N., Schook L. B., Fahey G. C. Jr. (2004). Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. *J. Anim. Sci. 82:1713-1724.*

Takahashi T., Nakagawa E., Nara T., Yajima T., Kuwata T. (1998). Effects of orally ingested Bifidobacterium longum on the mucosal IgA response of mice to dietary antigens. *Biosci. Biotech. Bioch. 62.1: 10–15.*

Takahashi T., Oka T., Iwana H., Kuwata T. & Yamamoto Y. (1993). Immune response of mice to orally administered lactic acid bacteria. *Biosci. Biotech. Bioch. 57.9: 1557–1560.*

Tejada-Simon M.V., Ustunol Z. & Pestka J. J. (1999). Ex vivo effects of lactobacilli, streptococci, and bifidobacteria ingestion on cytokine and nitric oxide production in a murine model. *J. Food Protec. 62.2: 162–169.*

Theander O., Aman P., Westerlund E., and Graham H. (1994). Enzymatic/chemical analysis of dietary fiber. *J. Assoc. Off. Anal. Chem. 77.3:703-709.*

Thompson A., (2008). Ingredients: Where Pet Food Starts. *Topics Comp. Anim. Med. 23.3:127-132.*

Trowell H., Southgate D. A. T., Wolever T. M. S., Leeds A., Gussell M., Jenkins D.A. (1976). Dietary fiber redefined. *The Lancet 307: 7966-967.*

Van de Perre P. (2003). Transfer of antibody via mother's milk. Vaccine 21(24): *3374-3376.*

Welsh J. D., Walker A. (1965). Intestinal disaccharidase and alkaline Phosphatase activity in the dog. *Proc. Soc. Exp. Biol. Med. 120: 525-527.*

Wichert, B., Schuster, S., Hofmann, M., Dobenecker, B., Kienzle, E. (2002). Influence of different cellulose types on feces quality of dogs. *J. Nutr. 132(6): 1728S-1729S.*

Willard M. D., Simpson R. B., Delles E. K., Cohen N. D., Fossum T. W., Kolp D., Reinhart G. (1994). Effects of dietary supplementation of fructooligosaccharides on small intestinal bacterial overgrowth in dogs. Am. *J. Vet. Res. 55: 654-659.*

Wi**nkler P., Ghandimi D., Schrezenmeir J., Kraehenbuhl J. P.** (2007). Molecular and cellular basis of microflora-host interactions. *J. Nutr. 137(3): 756S-772S.*

Wisker E., Bach Knudsen K. E., Daniel M., Eggum B.O., Feldheim W. (1997). Energy values of non-starch polysaccharides: Comparative studies in humans and rats. *J. Nutr. 127(1): 108-116.*

Xu D., Lu Q., Deitch E. A. (1998). Elemental diet-induced bacterial translocation associated with systemic and intestinal immune suppression. *J. Parent. Ent. Nutr. 22(1): 37-41.*

Zentek. J. (1996). Cellulose, pectins, and guar gum as fiber sources in canine diets. J. Anim. Physiol. Anim. Nutr. 76: 36-45.es on small intestinal bacterial overgrowth in dogs. Am. *J. Vet. Res. 55: 654-659.*

Winkler P., Ghandimi D., Schrezenmeir J., Kraehenbuhl J. P. (2007). Molecular and cellular basis of microflora-host interactions. *J. Nutr. 137(3): 756S-772S.*

Wisker E., Bach Knudsen K. E., Daniel M., Eggum B.O., Feldheim W. (1997). Energy values of non-starch polysaccharides: Comparative studies in humans and rats. *J. Nutr. 127(1): 108-116.*

Xu D., Lu Q., Deitch E. A. (1998). Elemental diet-induced bacterial translocation associated with systemic and intestinal immune suppression. *J. Parent. Ent. Nutr. 22(1): 37-41.*

Zentek. J. (1996). Cellulose, pectins, and guar gum as fiber sources in canine diets. *J. Anim. Physiol. Anim. Nutr. 76: 36-45.*

CHAPTER 4

Effects of bovine colostrum supplementation in weaning puppies

4. Effects of bovine colostrum supplementation in recently weaning puppies

4.1 Introduction

The colostrum represents the secretion accumulated by the mammary gland at the time of the final third of pregnancy. It allows the maturation of the digestive tract. In must be ingested from birth to the first eighteen hours by the newborn babies to fulfill his immunizing role because only 10% of the antibodies pass by the transplacentar way in carnivores.

The colostrum is elaborated and stored in the mammary gland, principally during the last gestation weeks. It is rich in immunoglobulins and other bioactive substances. Many authors affirm the bitch's colostrum is secreted between 24 to 72 hours postpartum. In the primiparous bitches, lactation is generally established later than in multiparous. Lactogenesis occurs most often in 24 hours before parturition in a primiparous, while it is not uncommon that occur up to one week in a prior pluriparous. This early can be a problem because dogs can secrete colostrum during this period when the little ones are not yet born, and then secrete milk at birth (Voldoire, 2002).

In the bitch, the mammogenesis is advanced, with development of the alveolar-lobe is differed to which is taking place at each estrous cycle of gradual growth which in term metoestrus 40 to 50 days after the LH peak. Endocrine sequence controlling lactogenesis is hormonal balance at the end of gestation and involves mainly prolactin, progesterone and estrogens and to a lesser extent, the glucocorticoids and insulin. Therefore, progesterone during the luteal phase exert a negative feedback on the secretion of prolactin. A decrease in the ratio progesterone / prolactin triggers the intracellular secretory activity of mammary acinar (Poffenbarger et al., 1991; Fieni et al., 1999).

At the late pregnancy, the sudden drop in progesterone is accompanied by an increase of estrogen production. This change in the hormonal balance causing the parallel emergence of a prolactin peak and a major increase in activity synthetic breast cells. The maintenance of the Milk secretion occurs through a neurohormonal reflex initiated by suckling puppies. This reflex involves the Adrenocorticotropic hormone or ACTH, prolactin and oxytocin.

4.1.1 Composition of colostrum

Colostrum composition differs from milk (Tables 6 and 7). Colostrum has a distinctive yellow color. As with other species, bitches colostrum is significantly denser than the normal milk: density colostrum bitch is about 1.060 and the refractive index thereof is in the order of 25, whereas the milk, it is only 14.26 (Costachescu et al., 2011).

For comparison, it is 10 for milk cows. Colostrum provides nutrients, water, growth factors, enzymes digestives, vitamins, minerals and maternal immunoglobulins. These contributions are essential for the survival of newborns. The dry matter, content declines during the first three days of lactation. According to Hand and Lewis. (2000), colostrum bitch is rich in dry matter, which makes quite thick and viscous compared to milk, and makes it more difficult for low feeding puppies.

Colostrum is also a very energy secretion. In the bitch, the value energy is 1800 kcal (Adkins et al., 2001).

Protein compounds.

The proteins in the colostrum of bitches increase from 0 to 4.3% 2 days, up to about 7 % in milk at the weaning (Costachescu et al., 2011).

According to studies, the protein content of colostrum varies in the canines (Table 1). This decrease probably corresponds to a change in water content. Protein levels declined significantly between the first and third day. The protein levels in the colostrum rapidly decrease from 12 to 24 hours after parturition, causing a decrease in dry matter.

In normal puppies, the blood protein is remarkably high, exceeding frequently that of a healthy adult (between 60 and 80g/L). This is due to the ingestion of high protein colostrum. However, due to glomerular filtration capacity incompletely developed, we similarly observed a strong physiological proteinuria in puppies. The total proteins in urine then decrease during development 1.64 g /L (24 hours postpartum) to 0.29 g /L (two weeks postpartum), the evolution of excretion of immunoglobulins in urine following this decrease (Schafer-Som et al., 2005b). Dog colostrum is characterized by a high content of immunoglobulins, representing about 50% of the proteins.

Immunoglobulins are effectors of the immune response mediated humoral antibodies support the function. They are glycoprotein nature. In the dog, three classes are currently individualized and characterized with certainty: IgG, IgM and IgA; the existence of IgE is strongly suspected, but no validated test at the international level allows highlighting in carnivores (Heayns, 2012).

The immunoglobulins of all species of higher vertebrates are built on the model of Porter and Edelmann, 4 channels identical polypeptide pairs: two light chains that differ in the types and subtypes, and two heavy chains that are different in classes and subclasses immunoglobulins, and determining by the specific antigenic determinants wear, the specificity of the class. The portion of immunoglobulin, which is reactive with a given antigen, is called fragment antigen binding (Tizard, 2000).

The IgG is the simplest and the best known in terms of the structure. They are composed of a monomeric molecule of formula L2H2. Each light chain L (Light) is formed from a common to all molecules of the IgG constant region (CL) and a part variable (VL) of an IgG molecule to another. Similarly, there are in each heavy chain H (Heavy), three constant domains and a variable part. Disulphide bridges (Figure 1) connect these four channels.

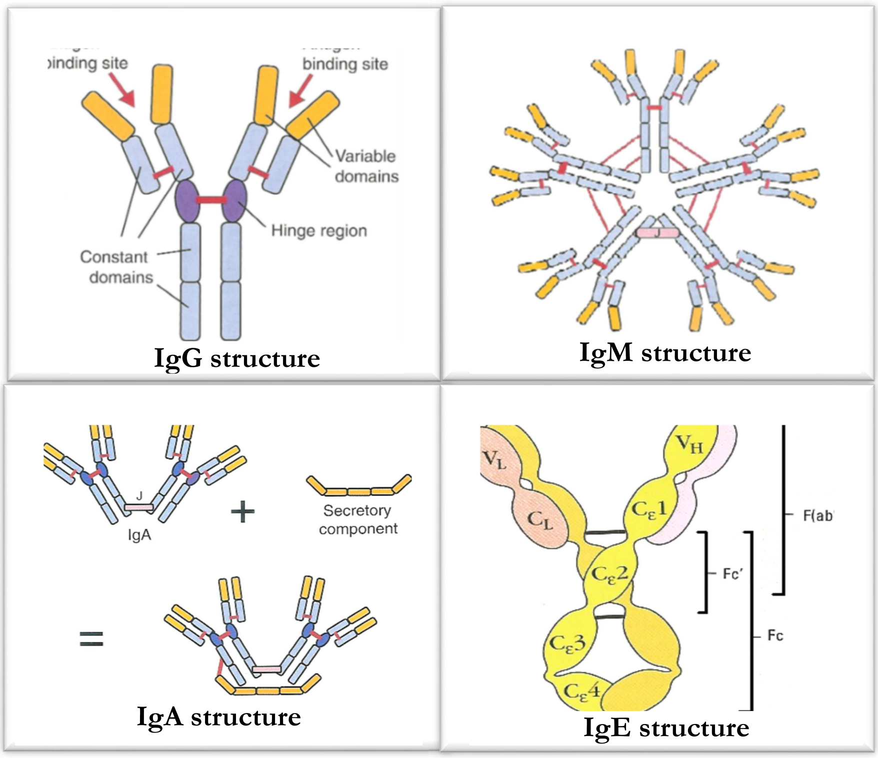


Figure 1. Immunoglobulins structure (Tizard et al., 2000).

IgM is the second immunoglobulin class most represented in the dogs. It is the largest immunoglobulin PM with a million Daltons. IgM is a pentameric molecule consisting of the formula (H2 L2) 5, which is associated with a piece J of junction that appears to play a role in the polymerization of subunits (Figure 1).

The IgM production originates independent humoral response or a T primary thymus-dependent humoral response. It fixes complement very effectively. This is an important agglutinin, it is able to neutralize toxins and viruses, opsonize bacteria and with the help of complement to lyse many microbes and cells. The IgM has a sedimentation constant of 19S (Heayns, 2012).

The secretory IgA are often (the concept of secretory immunoglobulins was established there are more than twenty years), it is also the most common immunoglobulin in bodily secretions. The IgA is dimeric, they are found in the serum and in the secretions in slightly different structures in serum IgA is formed monomers of formula L2 H2 or dimers (H2 L2) 2J, J being the connecting part connecting the two monomers (Figure 1).

In secretions, the dimer form predominates, connected to another protein called secretory piece found only on secretory IgA. Their PM is usually 360 KDa. They provide a primary defense against local infections due to their presence in secretions. They are stronger than the other immunoglobulins proteolysis through their secretory piece. This is a portion of the receiver IgA on the surface of glandular epithelial cells (Figure 1), the formation of secretory IgA therefore occurs in the epithelial cells. IgA have a sedimentation constant of 6.7 or S 11.7, depending on the subclass (Norcross, 1982).

The IgE (Figure 1) are found in low concentrations in the serum and tissues. They have a molecular weight of 190 KDa and a sedimentation constant of 8S. They have not been detected in cats, but some extrapolated human tests detect in dog serum. We did not find evidence of their presence in colostrum, which is possibly due to the difficulty of the highlight.

Colostrum is a milk producing transudate, which anticipates lactation. This is a syrupy, more essential that the milk and in the passive transfer of immunity substance, colostrum is the most rich in antibodies organic liquid. Its concentration in immunoglobulin is significantly higher than that dog serum by a secretion active at the breast, under the influences of estrogen and progesterone. This active secretion is decreasing during lactation (Table 1).

Immunoglobulins in bitch colostrum.

There have been many discussions about the source of the colostrum immunoglobulins; it seems that IgG comes from a strong share from the serum, whereas IgA, IgM, and part of IgG are produced locally. In a study with six bitches make by Schafer et al., 2005, found that IgG concentrations decreased from 72 hours to six weeks after parturition, whereas, IgA and IgM declined after 24 hours. Respect to the queen, one study with 65 queens of Claus et al., (2000), show that IgG and IgA concentrations in colostrum were significantly higher on the day of parturition than 7 days of lactation.

Time from parturition	**Immuglobulins (mg/m/L)**		
Immunoglobulin type	**G**	**A**	**M**
on 24h post-partum (mg/mL)	19.3	9.9	0.6
on 48h post-partum (mg/ml)	13.5	6.0	0.3
on 72h post-partum (mg/mL)	18.9	6.2	0.4

Table 1: Immunoglobulin concentrations in mammary secretions of bitch.

Amino acids.

For most of the amino acids, the greatest variations occur between the first day and on the third day of lactation with decreasing concentrations in colostrum between the first and third day of the mammary secretion. These concentrations increase then during lactation until day 21, but without reaching the value again of their initial concentration in colostrum. After day 21 of lactation, each acid amino evolves in isolation (Table 2) (Adkin et al., 2001).

Amino acid	1 Day	3 Day	7 Day
Alanine	0.170	0.52	0.082
Arginine	0.104	0.032	0.056
Asparagine + Aspartic acid	0.235	0.073	0.117
Cysteine	0.058	0.021	0.032
Glutamine + Glutamic acid	0.426	0.124	0.242
Glycine	0.079	0.020	0.027
Histidine	0.076	0.021	0.041
Isoleucine	0.128	0.037	0.069
Leucine	0.301	0.093	0.169
Lysine	0.128	0.037	0.061
Methionine	0.073	0.022	0.036
Phenylalanine	0.096	0.027	0.048
Proline	0.302	0.083	0.168
Serine	0.138	0.036	0.065
Threonine	0.149	0.045	0.070
Tryptophan	0.007	ND	0.005
Tyrosine	0.068	0.021	0.031
Valine	0.206	0.060	0.102

Table 2 Amino acids composition mmol/L throughout lactation in bitch colostrum and milk.

Other proteins.

The fraction of non-protein nitrogen is 5.7% in colostrum and it ups to 9.9% in milk. The proportion of casein varies during lactation. It represents 60.7% of colostrum proteins and increases to 75.4% in the 3rd day of lactation, and then decreases slowly (Adkins, Lepine e Lonnerdal, 2001). The albumin level is higher in colostrum (20.5 mg / ml on the first day secretion) in milk (Schafer-Somi et al., 2005a).

Other non-protein compounds.
Lipids.

In dogs, the lipid from 2.4% between 0 and 2 days at 5.2% in milk decrease to 2.7% at weaning (Terodemos, 2005). The rate of fat depends on the stage of lactation. It grows in early lactation and varies differently according to the authors during the remainder of lactation (Lonnerdal et al., 1981; Adkins, Lepine e Lonnerdal, 2001).

Lactose.

In dogs, the rate of lactose in colostrum is quite low compared to that of milk mature, it increases until day 28 to 40.2 g / L in the milk concentration, then decreases to 35 g / L, which value remains constant for the remainder of the lactation (Hand, 2000; Adkins, Lepine e Lonnerdal, 2001).

Citrate.

In dogs, there were significant differences in the lactation with 4.8 mm the first day and increased up to 6.6 mm on day 7, then decreased to return to values similar to those of the colostrum to the 28th day (Adkins, Lepine e Lonnerdal, 2001).

In the bitch, the values given below are to be considered subject to their statistical significance, because of the small number of samples taken for the study and strong deviations (Lonnerdal et al., 1981). The authors consider here levies a day like colostrum and those three days like milk. However, some believe that the mammary secretion in the third day is the late colostrum (Adkins, Lepine e Lonnerdal, 2001).

Calcium.

As in most species, the calcium concentration is influenced by the stage of lactation. The concentration is lower in colostrum than in milk, it increases during lactation to reach a peak around the 35th day (Lonnerdal et al., 1981; Adkins et al., 2001). This correlation can be explained by the ability of caseins, the major milk proteins, transport calcium. However, the concentrations found in the literature vary: about 0.98 g / L for Lonnerdal et al., 1981 against 0.360 g / L to Adkins et al., 2001. The ratio colostrum and the concentration of milk are also very variable according Lonnerdal et al., 1981 and only 2 according to Adkins (Adkins, Lepine e Lonnerdal, 2001). These differences may explain the large variations in phosphate ratio in the colostrum, this is between 1.5 - 0.4 (Kirk et al., 2001).

Phosphorous.

The phosphorus concentration increases during lactation: 0.935 g / L colostrum to reach 1.400 g / L on day 28 of lactation (Adkins, Lepine e Lonnerdal, 2001).

Iron.

The authors provide different values for the composition of milk and iron colostrum. In every way, the iron concentration is higher in colostrum and milk dog to other animals. The dog is capable of secreting milk with concentrations, iron are 10 times higher than serum. This phenomenon indicates that the transfer of iron or retention by the breast tissue through mechanisms different from those of other species or iron level is the same or less than that of serum (Lonnerdal et al., 1981). The iron content in the colostrum is higher than that of milk, and it is strongly influenced by the stage of lactation.

Copper.

The copper concentration is not influenced by the stage of lactation. Its values in colostrum dogs are 1.3 mg /L for Adkins et al. (Adkins, Lepine and Lonnerdal, 2001) and 1.8 mg /L for Lonnerdal et al. (Lonnerdal et al., 1981). These values are superior to that of human colostrum (0.3 to 0.6 mg / L) (Lonnerdal et al., 1981).

Zinc.

The values of colostrum and their evolution during lactation differ between authors.

Manganesium.

The manganese concentration is not influenced by the stage of lactation and little about its bioavailability and its importance has known: its concentration in the colostrum is about 0.15 mg / L (Lonnerdal et al., 1981).

Magnesium.

The results are also highly variable for magnesium. According Lonnerdal et al. (Lonnerdal et al., 1981), its concentration is not influenced by the stage of lactation and is about 59 mg /L in colostrum and milk. Concentrations measured by Adkins et al. (Adkins, Lepine e Lonnerdal, 2001) is higher: 128.5 mg / L in colostrum and 85.8 mg /L on day 3. This rate would remain roughly constant during lactation.

Vitamins.

The high content of the colostrum of vitamins A, B1, B2, and C made of a first feed quality for the newborn (Berre, 1996).

Cellular elements.

This product contains a secretion of large amounts of cellular components and especially leukocytes. Some cellular elements, called Donne's corpuscles contain lipid spherules that tend to give a high calorific value to colostrum, as well as many enzymes involved in the digestion of colostrum (Berre, 1996).

Bioactive components.

Insulin Growth factor (IGF).

The 1 and 2 IGF and transporter proteins are found in high concentrations in colostrum, which are above or equal to those of the maternal circulation levels. The rate IGF 1 in dog colostrum is between 40 and 60 ng /mL. It decreases by 5 to 10 times until the third day and then increases gradually the remainder of the lactation. The mammary gland therefore has the ability to synthesize or carry the IGF through the blood mammary and mammary secretions.

Antitrypsin.

The maternal immunoglobulins escape digestion process of proteins, due to the relative immaturity of the enzymatic processes in the newborn, but also because colostrum itself contains trypsin inhibitors. These inhibitors colostrum trypsin protect IgG from proteolytic degradation, which contributes to a higher intestinal absorption (Crawford, Hanel e Levy, 2003).

Transmitting their species immunoglobulins via colostrum a generally inhibitors of trypsin rate high enough during parturition, but decreases rapidly during the first days of lactation (Sandholm e Honkanen-Buzalski, 1979). The trypsin inhibitors found in plasma, unlike those found in colostrum, and are not present in all species of mammals. Inhibitors of trypsin resistant colostral acidity seem to be a characteristic of carnivores, artiodactyls, ruminants and pigs. The trypsin inhibitors in colostrum carnivores seem to find a greater than that of colostrum ruminant concentration (Baintner, 2007).

On the other species, we note that in the bitch, these inhibitors are present in the colostrum and milk during the first two weeks of lactation (Sandholm e Honkanen-Buzalski, 1979).

Growth hormone (GH).

The maximum concentration of growth hormone occurs just after parturition 2413 ± 1642 mg/L of colostrum and then decreases to reach 211 ± 242 mg/L of milk on the 4th day of lactation. These colostral concentrations are 100 to 1000 times greater than those encountered in the plasma of mothers at the same time (2.55 ± 1.1 mg/L). GH concentrations are 10- 100 times higher than in milk compared to plasma. The concentration of hormone growth in puppy plasma is larger than that of the mother for at least 15 first days of life, but the concentrations of growth hormone secretion in breast are not related to plasma concentrations, whether the puppy or the mother (Schoenmakers et al., 1997). This suggests that part of the growth hormone is produced in the breast, and or all of the milk GH tissue is not well absorbed by the gastrointestinal tract intestinal puppy. The physiological significance of this high rate of growth hormone may be to promote the development of the stomach and digestive tract of newborns. Together with other hormones, GH plays a role in the maturation of the gastrointestinal mucosa and closing the transport of antibodies in the intestine (Schoenmakers et al., 1997).

Gamma glutamyltransferase (γ-GT) and alkaline phosphatase (PAL).

Serum GGT activity and PAL (whose origin is probably placental colostrum and/or bowel) are significantly higher in puppies less than ten days' measured in the serum of adult activities (Hoskins, 2001). These enzymes promote intestinal growth and enzyme production.

In adults, they are released by the kidney, liver (and maybe by the bone). But in the puppy, it is highly likely that high serum activities are due to transport colostral enzymes by pinocytosis in enterocytes.

These enzymes have a normal activity in the serum of colostrum in mothers but, PAL activities and γ-GT is higher than in the serum of the mother: they are 10 times higher for PAL, 1000 times higher for γ-GT (Center et al., 1991).

4.1.2 Comparison of colostrum with milk

Colostrum is for most authors to mammary secretion during first two days postpartum, for the third day in some part, but for others it is already a milk or transition between the two, which is justified by the fact that measures of components of the mammary secretion in that time are generally intermediate between the colostrum and milk.

Colostrum is thus characterized by highly divergent from that of the milk composition, two opposite directions from the same compound during lactation. Some components of colostrum are practically absent as milk antitrypsin, immunoglobulins and IGF on dogs. Colostrum also has compounds also present in milk, but higher or lower concentrations depending on the compound and time of lactation which compares the colostrum.

The significant differences in values are noted for the same compound colostrum, which may be due to the inaccuracy of studies on values composed colostrum we think that these values can vary depending on the sampled races, number of lactating mothers, the number of small litters, breasts removed, the immunization status of mothers is exceptionally reported, excluding the small number of animals tested, and methods of analysis (which for some is inaccurate).

4.1.3 Absorption of colostrum

The halving of absorption efficiency is faster in puppies than in kittens where it happens approximately at 16 hours of life (Casal, Jezyk e Giger, 1996). Nevertheless, the important permeability of the digestive mucous membrane during the first 72 hours of life allows the absorption of many of its compounds (Voldoire, 2002).

Evolution of the intestinal permeability.

In dogs, 4-6% of IgG transmitted from mother to newborn, are by the placenta, the rest of these Igs through the colostrum (Schafer-Somi et al., 2005), where an important role thereof in the transmission of immunity. By this route of transmission colostrum, the immunoglobulins must then pass the intestinal barrier to be used the newborn.

The first studies on the intestinal absorption of newborn puppies reported an absorption for ten days after birth (Bardelli 1930) cited by Staley et al. (Staley and Bush, 1985). More recent studies show that immunoglobulins cannot cross the barrier intestinal after 24 hours in puppies, so the ingestion of colostrum is to be early (Berre, 1996), and according Poffenbarger et al. (Poffenbarger et al., 1991), the maximum absorption efficiency colostral immunoglobulins occurs eight hours after birth.

For other authors, immunoglobulins cannot go beyond the digestive barrier a period of 15 hours (Voldoire, 2002) and then to the first 24-36 hours of life (Levy et al., 2001; Arnaud, 2004). After this period, the bowel wall becomes impermeable to the passage of immunoglobulins.

Selective transport.

The acquisition of colostral immunity during the first 24 hrs also occurs thanks to a selective transport, which is carried out by an endocytosis of the immunoglobulins after joining the receptors present on the apical membrane of the cell, then they across the membrane basophilic and a drowning out to become possible the immunoglobulins enter the circulation (Buddington et al., 2003). Thus, the enterocytes have a specific receptor for the transport of IgG. This receptor (FcγRn) transports in a selective way portion FC of IgG of the intestinal light to the circulation without degradation (Crawford, Hanel e Levy, 2003). This receptor has homologies with the antigens of major histocompatibility complex class I (Stefaner, Praetor e Hunziker, 1999).

pH role.

In the dog, the pH of gastric contents is usually neutral at birth. It remains in the newborn at higher than adult values (5-6.5) only for the first 24 hours. The maintenance of a high pH would explain the passage through the gastrointestinal mucous proteins such as immunoglobulins for quite a long time. Then the pH decreases rapidly with the onset of the secretion of hydrochloric acid (Shofer et al., 1990).

Mechanism of intestinal non-permeability to immunoglobulins.

Intestinal non-permeability is defined as the cessation of transport macromolecules from the gut to the blood. The mechanisms that lead to the interruption of the absorption of proteins are poorly known. Some assumptions are based on the normal cycle of renewal of the epithelium intestinal: after birth the intestinal epithelial cells to begin to divide a higher speed at the intestinal crypts and migrate to the top of the villi replacing desquamating cells (Poffenbarger et al., 1991). These new cells are more mature; lose capacity pinocytosis immunoglobulins and the ability of receptor synthesis FcγRn. When the digestive system is stimulated by the ingestion of food, these cells are replaced by others without this capability. Six hours after birth, 50% of the absorption capacity of the protein is still present. After eight hours, this percentage is more than 33%, to be completely useless in 24 hours (Cortese, 2001).

Several factors seem to influence the judgment of the intestinal absorption which colostral factors, the ionic composition of the nutrient solution, the amount and concentration of the immunoglobulin solution. These factors are not well known in carnivores unlike pork or veal. Intestinal permeability decreases rapidly after ingestion of colostrum probably due to release of an endogenous hormone or hydrocortisone adrenocorticotropic hormone (ACTH). The exogenous supplementation of these two hormones in mother within 24 hours before the hand prevents intestinal permeability and therefore absorption of colostrum in the newborn puppy (Hoskins, 2001). They may also be associated with increased insulin levels which occurs after the beginning of the feeding (Voldoire, 2002).

4.1.4 Physiological roles of colostrum

It plays several significant roles at the newborn: helps with the elimination of meconium by its laxative action, useful contributions of nutrients to limit hypoglycemia and to begin the uterine extra growth, protection against hypothermia, systemic transfer of immunity and digestive local immunity.

Maturation function.

Other than nutritive and immune function, colostrum has a mild laxative effect on the newborn, encouraging the passing of meconium, which aids in the excretion of excess bilirubin and prevent jaundice. However, the absorption of colostrum immediately after birth may play a role important in the establishment of postnatal circulatory volume (Case, 2011). The volume of colostrum ingested after birth contributes to the supply of liquid entering the volume of the circulating newborn. An insufficient fluid intake is the cause of circulatory disorder (Voldoire, 2002). The immaturity of the cardiovascular system of the puppy during the first five days of life prevents it from combat against multiple stressors. For this reason, the contribution of liquid, so the production of milk by the mother must be able to maintain the volume blood. In addition, the turnover of water in puppies is very high during the neonatal period. The feeding of a large volume contributes significantly to the maintenance of blood volume puppy immediately after birth. Insufficient fluid intake may cause significant decrease in blood volume resulting in a fatal heart failure (Voldoire, 2002).

Immunological function.

Colostrum contains other protective factors, that is, it comprises high concentrations of antibody, of the chemical mediators of the immune system such as interferons, but also several types of functional immune cells (lymphocytes, dendritic cells, macrophages and neutrophils). These cells are ingested by newborn and help the establishment of a local cell-mediated immunity (Poffenbarger et al., 1991). Conversely function of cells found in colostrum is not completely elucidated. These cells amplify defense mechanisms in the newborn transfer cell mediated immunity. They also have bactericidal and phagocytic activities localized to the digestive tract and increase the activity of lymphocytes (Cortese, 2001).

The protection conferred by passive transfer of immunoglobulins via colostrum is supplemented by innate immunity neutrophils (PMN) are the major actors. They are the first line of defense against bacteria, acting through phagocytosis by the bacterial and oxidative reaction. Their efficiency is facilitated by the deposit of opsonins such as antibodies or complement proteins to the surface of bacteria. Theoretically, lack of colostrum intake may compromise the functionality of neutrophils in the neonate by reducing the amount of opsonins capable of facilitating bacterial phagocytosis. This phenomenon has been reported in foals: the absorption of colostrum increase bacterial neutrophilic phagocytic activity by increasing opsonization (Hanel et al., 2003).

Non-absorbed immunoglobulins, IgA and IgM, present in colostrum, exercise their action locally over the entire length of the digestive tract. They protect the newborn from enteric pathogens. Their half-life in carnivores is 8 to 9 days (Gomet, 2003; Le Bouc, 2005).

Immunological function.

Colostrum has a protective role against pathogens, due to it provides the 95% of immunoglobulins during the first days of life. The puppy receives through the transplacentar way from its mother a humoral protection, systemic or local, about 3 to 20%, according to the authors (most agreeing transfers 5 to 10%). The remainder is brought colostrum (Berre, 1996).

The systemic immunity is acquired by transfer of IgG (having a half-life of 5-10 days through the intestinal tract (Gomet, 2003). The amount of immunoglobulins transferred depends on:

The amount of colostrum ingested by each puppy (members of the same litter may therefore have acquired immunoglobulin variable rate, depending on the colostrum ingested volumes).

Nutritional function.

It is essential for newborn survival, but the exact colostrum composition of carnivorous is not extensively known, and data reported in literature are characterized by high variability (Adkins, Lepine e Lonnerdal, 2001). Furthermore, colostrum contains higher concentrations of vitamins A and E as compared to mature milk. Vitamin E is required to protect the newborn against oxidative stress and vitamin A is fundamental for growth and development, also it permits to increase 25% of its hepatic reserves (Debier et al., 2005).

In 1923, Kuttner and Ratner hypothesized that the permeability of the barrier placental antibody are inversely proportional to the number of layers of tissue existing between mother and fetus. In dogs, the endotheliochorial placentation (4 layers) allows a lower transmission antibody through the placenta, the transmission being essentially colostrum tract. The thickness of the placenta may also represent a protective barrier against pathogens, explaining that the puppies did not develop an immune response in the uterus (Voldoire, 2002).

The aim of this part was to evaluate the influence of bovine colostrum supplemented in of Golden Retriever puppy's diet on zootechnical parameters and the immune system and microbiological composition of feces.

4.2 Materials and methods

4.2.1 Animals and diet

The study was conducted from February 2014 to March 2014 in the "Allevamento San Francesco" breeding kennel.

A total of fourteen puppies (Golden retriever) was randomized into two groups (7 controls and 7 treated) balanced for sex (50% males and 50% females) and homogeneous for weight and birth. Every puppy was identified individually with a microchip. They were fed with two rations during the test phase of the trial. All puppies stayed with their dam in heated whelping boxes (surface: 2 m^2) from 4 weeks of birth to approximately 8 weeks of age.

The puppies were fed the same diet, a dry complete diet balanced for growing dogs reported in table 3. Puppies consumed fresh water ad libitum and were fed in groups (control and treated).

During the 'pre-test' phase, both groups were fed a commercial, nutritionally complete and balanced product (baby milk: approximately 30 % protein, 25 % fat and 0.10 % fiber).

Starter 1	
Ingredients	**Percentage (%)**
Dehydrated chicken and turkey proteins	-
Rice	28
Fresh chicken and turkey meat	15
Animal fats, maize, maize gluten, animal protein hydrolysate, beet pulps, flax seeds, maize oil, dehydrated salmon proteins, brewer's yeast	-
Dehydrated eggs	1
Pea fibre, FOS (Fructoolisaccharides)	0.2
Seaweed (Schizochytrium spp)	0.1
Dried chicory extract (Cichorium intybus)	0.1
Additives	**Per Kg**
Vitamin A	26.000 UI
Vitamin D3	1.355 UI
Vitamin E	405 mg
Choline chloride	1.200 mg
DL- methionine	500 mg
Copper sulphate pentahydrate	50 mg (copper 12.5 mg)
Ferrous carbonate	395 mg (Iron 150 mg)
Calcium iodate	-
Anhydrous	25 mg (Iodate 2.5 mg)
Sodium selenite	20 mg (selenium 0.2 mg)
Zinc oxide	265 mg (zinc 195)
Manganous oxide	55 mg (manganese 35 mg)
Analytical constituents	**Percentage (%)**
Crude protein	32
Crude oils and fats	22
Crude fibres	1.7
Crude ash	6.9
Calcium	1.3
Phosphorus	1
Omega 3 fatty acids	0.9
Omega 6 fatty acids	3.9
Moisture	8

Table 3: Ingredient of the diet Basal diet (www.trainer.eu).

The chemical composition on the label of the basal diet was represented by 8 %, protein 30 %, crude fat 22 %, crude fiber 1.8 % and ash 6.9 %; metabolisable energy: 17,786 kJ/kg (4251 kcal/kg), compliant with FEDIAF 2011 recommendations.

The basal diet was analysed to measure the principal components: dry matter (AOAC method 930.15); crude protein (CP, AOAC method 2001.11); ether extract (EE, DM 21/12/1998); crude fiber, (CF, AOCS method Ba 6a-05);

neutral detergent fiber, (NDF, AOAC method 2002.04) and ash (AOAC method 942.05).

From the 4th week of age the amount of dry diet was increased gradually. The daily ration was calculated based on the number of puppies in the litter and it was administered 3 times a day. One third of the quantity was distributed early in the day, the second, third at midday and the last third at the end of the day. Feeding amounts were calculated from amounts consumed during the previous week and adjusted weekly for the remainder of the study with the aim of maintaining puppies on standard growth curves with ideal body condition scores.

For young puppies 4–6 weeks of age, the dry diet was rehydrated with water just before distribution, and they also received 15g of the formula baby milk (Table 4). Babydog milk was reconstituted in water, creating a totally homogenous formula, according to the instruction of the producer.

Baby milk	
Ingredients	**Percentage (%)**
Skimmed milk powder, whey protein powder, copra oil, whey powder, palm oil, soya protein concentrate	-
Bovine colostrum powder	2
Wheat flour and minerals	-
Additives	**Per Kg**
Vitamin A	25.000 UI
Vitamin D3	1.100 UI
Vitamin E	175 mg
Vitamin B1	24 mg
Vitamin B2	44 mg
Calcium D- pantothenate	78 mg
Vitamin B6	20 mg
Folic acid	4.4 mg
Vitamin B12	0.4 mg
Vitamin C	11 mg
Vitamin K3	0.95 mg
Vitamin PP	182 mg
Biotin	1.4 mg
Taurine	2.200 mg
Ferrous chelate of glycine hydrate	473 mg (iron 110 mg)
Cupric chelate of glycine hydrate	42.9 mg (copper 11 mg)
Zinc sulphate monohydrate	548 mg (zinc 200 mg)
Manganous sulphate monohydrate	200 mg (manganese 65 mg)
Calcium iodate anhydrous	5.1 mg (iodine 3.3 mg)
Sodium selenite	0.88 mg (selenium 0.4 mg)
Analytical constituents	**Percentage (%)**
Crude protein	30
Crude oils and fats	25
Crude fibres	0.1

Crude ash	9.5
Calcium	1.2
Phosphorus	0.8
Sodium	0.4
Lysine	2.4
Methionine	0.95
Methionine + cystine	1.2
Moisture	5

Table 4 : Chemical component of Baby milk formula (www.trainer.eu).

Then, at the end of the treatment of the pre-test phase, groups were fed a commercial, nutritionally complete and balanced extruded dry puppy food 'control diet' (Starter 1 product: approximately 35.5% protein, 23% fat, 2.2% fiber). All puppies and their mother received corresponding vaccines as part of normal veterinary care. Puppies in the control group continued to be fed the 'control diet', while puppies in the test group were fed the 'control diet' supplemented with 5g of Colostrum). The puppies were fed their respective diets until the end of the study. Food intake was measured weekly per group control and treated group.

The chemical composition on the label of the basal diet was represented by 8 %, protein 30 %, crude fat 22 %, crude fiber 1.8 % and ash 6.9 %; metabolisable energy: 17,786 kJ/kg (4251 kcal/kg), compliant with FEDIAF 2011 recommendations.
The basal diet was analysed to measure the principal components: dry matter (AOAC method 930.15); crude protein (CP, AOAC method 2001.11); ether extract (EE, DM 21/12/1998); crude fiber, (CF, AOCS method Ba 6a-05); neutral detergent fiber, (NDF, AOAC method 2002.04) and ash (AOAC method 942.05).

From the 4th week of age the amount of dry diet was increased gradually. The daily ration was calculated based on the number of puppies in the litter and it was administered 3 times a day. One third of the quantity was distributed early in the day, the second, third at midday and the last third at the end of the day. Feeding amounts were calculated from amounts consumed during the previous week and adjusted weekly for the remainder of the study with the aim of maintaining puppies on standard growth curves with ideal body condition scores.
For young puppies 4–6 weeks of age, the dry diet was rehydrated with water just before distribution, and they also received 15g of the formula baby milk (Table 4). Babydog milk was reconstituted in water, creating a totally homogenous formula, according to the instruction of the producer.

4.2.2 Treatment

Each puppy of TG was treated with a single dose per day of bovine colostrum (Volostrum) individually from 4 to 8 weeks. The treatments were administered one a day through oral route (5g per animal) through a specific oral syringe for 5 weeks. The principal component of bovine colostrum is reported in following table 5.

Component (as fed)	Amount
Crude protein (%)	76
Crude fat (%)	8
Cellulose (%)	3
Sodium (%)	0.17

Table 5: Nutritional composition of bovine colostrum (Treatment).

4.2.3 Health status and body condition evaluation

During the entire experimental trial different individual parameter was evaluated in order to establish the health status, the grow curves and the body condition.
Each puppy was summited to a clinical evaluation for determinate their health status. We wanted to discard an altered gastrointestinal function:
- Vomiting.
- Diarrhea.
- Nausea.
- Flatulence.
- Constipation.
- Inadequate or inappropriate feeding management.

Body condition score.
The puppies were assessed scoring scale from 9 levels (of which levels 4 and 5 represent an ideal body mass. Use a consistent method and scale to measure body weight (BW), body condition score (BCS) to assess current status and changes over time. These BCS goals are based on a limited number of studies in dogs and cats as well as those from other species (Lund EM et al., 2005; Kealy RDet al., 1992; Scarlett JM and Donoghue S., 1998).

Food intake was recorded immediately following each meal as the difference between the mass of food presented and the mass of food returned.

4.2.4 Fecal evaluation

Fecal score

Fecal examination included observation of fecal consistency and color.
Fecal score and fecal color were individually recorded from 4 to 8 weeks by a single operator. Between 4 and 8 weeks of age, fecal samples were collected individually from rectum weekly. The fecal samples were transported to the laboratory in agar medium at 4°C. A scale of four levels was used to score fecal consistency: 0 = normal (feces firm and well formed), 1= soft consistency (feces soft and formed), 2= mild diarrhea (fluid feces, usually yellowish), 3= severe diarrhea (feces watery and projectile). A fecal consistency score ≤1 (0, 1) was considered normal, whereas a fecal score >1 (2, 3) was defined as a clinical sign of diarrhea. Fecal color was evaluated using a three point scale: 1= yellow, 2= green, 3= brown. A fecal color ≥2 (green-brown) was considered normal, while a fecal color <2 (yellow) was considered pathological (Rossi et al., 2012).

Microbiological analysis

Fecal samples were performed by the Department of Animal Pathology, Hygiene and Veterinary Public Health, Università di Milano. In particular the amount of *E. coli, Streptococcus fecalis* and *Lactobacillus* strains were evaluated on selective agar medium each strain was cultured on MacConkey agar (OXOID) and Blood Agar incubated at 37°C for 3-4 days. The colony forming units (CFU) of each standard curve serial dilution were determined by plating the *E. coli* and *Streptococcus fecalis* grown in Luria-Bertani Medium [10 g/l tryptose, 5 g/l yeast extract, 5 g/l NaCl (ph=7)], *Lactobacillus* genus on *Difco Lactobacillus* MRS broth (Becton Dickenson Company, Sparks, MD, USA).

4.2.5 Statistical analysis

The dates collected during the trial were analysed using the SAS (Statistical analysis system) program. The effects of colostrum supplementation on recently weaning puppies were evaluated by analysis of variance (ANOVA). This statistical model permitted analysed body weight, food intake and microbiologist count by two way procedure interacting treatment and treatment day. This study was considered a significance level of P< 0.05.

4.3 Results and discussion

The weaning period is critical for puppies. The maternally derived antibodies (MDA), acquired by ingestion of immunoglobulin-rich colostrum, are essential for the protection of puppies against neonatal infection and death. Colostrum, the secretion produced by the mammary gland for the first few days after parturition, provides the neonate with both nourishment and passive protection from disease. The nutrients and growth factors in colostrum also have local effects on the gut epithelium, helping to ensure proper gut development, nutritional uptake, and growth in the neonate.

Diarrhea is a common problem in recently weaned puppies. Changes in diet and separation from the dam occur at a time when the immune system is not fully competent, and passive immunity is waning. This combination of events increases the susceptibility of the puppy to a variety of infections and gastrointestinal disturbances.

Bovine colostrum is rich in bioactive compounds and peptide-based nutrients, including growth factors, immunoglobulins, and other immune factors that can neutralize viruses, and inhibit the colonization of the gut and production of biological toxins by harmful microorganisms. Moreover, substantial amounts of orally ingested bovine colostrum concentrate survive their passage through the stomach to remain intact and active in the lower parts of the bowel.

A number of studies showed bovine colostrum powder is beneficial in the prevention and treatment of gastroenteritis in young mice, piglets, human infants, however, little is known about the potential benefits of bovine colostrum in companion animals.

Therefore the principal aim of this trial was to evaluate the effect of a bovine colostrum protein concentrate on health status and fecal quality in puppies during a period of environmental change.

All puppies included in this study were born and grown in the same kennel. They were thus exposed to the same management, food distribution and environmental pathogens.

4.3.1 Animals and diet

The results of the analysis of the diet confirmed the values indicated on the label, thereby ensuring the proper nutritional profile for puppies (Table 7).

Analisys	Results
Humidity	8.10%
Protein	33.43%
Fatty acids and oil	17.17%
Fiber	2.96%
Ash	7.68%

Table 7: Results of the basal diet.

4.3.2 Health status and body condition evaluation

There was no significant trend in energy intake in kilograms of body weight between the control and the treated groups.
Over time the body weight of puppies significantly increased in all experimental groups ($P < 0.05$) (Table 6), and all puppies maintained an ideal body condition score of 5-6 throughout the trial. There were neither significant differences in body weight between the groups at any time nor any significant differences in the growth rate. Individual body weights were not influenced by the treatment.

	CTR	Treated	s.e.m	***P*-value**
BW, kg				
4 wks	2.59	2.57	0.07	0.84
6 wks	4.66	4.80	0.08	0.24
8 wks	6.76	6.87	0.14	0.59
ADG, g/d				
4-6 wks	147^{b}	159^{a}	3	0.02
6-8 wks	151	148	7	0.81
4-8 wks	149	154	3	0.35

^{a,b} Means listed in the same row with different superscripts are significant ($P < 0.05$).

Table 6: Effect of Colostrum supplementation on body weight and average daily gain of puppies.

The effect of weekly oral supplementation with 5g of bovine colostrum powder (n=7) from 4th week to 8th week in weaned puppies did not show any effect on body weight, as it is shown in the table 6 at 4th week when the treated subjects weighed less than control subjects but without any statistical differences. From week 6 to 8 the treated subjects showed a higher body weight compared to the controls however this was not statistically significant.
In reference to the average daily gain of puppies, between 4 to 6 week the difference shown in the treated group was statistically significant ($P < 0.05$).

In the 8th weeks of experiment we did not find that colostrum had any influence on the daily weight gain of the pupies, unlike many studies carried out in weaned piglets (Boudry et al., 2008).

4.3.3 Fecal evaluation

Fecal score

Since weaning is a high risk period for puppies, a tool for precise follow-up of digestive health would be more useful. Fecal quality tended to improve in both groups during the course of the trial but the colostrum-supplemented group showed a greater improvement in fecal quality. The puppies fed with bovine colostrum supplement showed a lower average fecal scores for d 5-6 when compared with the control group.
Considering the trend of the feacal score, from the 5th to 8th week the puppies showed liquid feces but during the following weeks they produced feces with shape, consistence and normal colour.
However young puppies had higher fecal moisture content and a lower fecal quality (Giffard et al., 2004) which may be related to a relatively low total mucosal surface (Paulsen et al., 2003) or to faster gastric emptying. Digestive disorders may therefore be overestimated in studies considering all soft feces as abnormal. Scoring systems used in adult dogs cannot adapt to young puppies because of a physiological lower fecal quality prior to the weaning period. So the pathological fecal score for threshold was defined as the highest score associated with a significant reduction in ADG. For these reasons the obtained results of average fecal score cannot be considered as pathological for the specific weaning period.

In a previous publication by Giffard et al., 2004, they reported on gut health benefits in dogs who were fed with bovine colostrum supplemented diets. They showed that colostrum supplemented diet fed puppies improve their fecal score when exhibiting stress-related diarrhea, such as during the weaning period. However neither immune benefits nor any other gut health benefits were documented.

Microbiological evaluation of feces

The domestic dog plays several important roles in modern human society and it is commonly used as a model species for biomedical research as well as being commonly considered as a pet. The normal canine large intestine microbiota, like most mammals, contains a complex microbiota in which *Streptococcus*, *Bifidobacteria*, *Lactobacillus*, *Bacteroides spp.* and *Clostridium spp.* constitute the main predominant bacteria.

The microbiota provides both nutritional and protective functions to the animal, in the form of fermentation and other secreted products, stimulation of host immunity and prevention of colonization by pathogens. Prior to hatch or birth, the GI tract of an animal is sterile. The animal's microbiota is established via a series of colonization that is remarkably similar in the mammalian GI tracts.

It is interesting to note that while the environment plays a significant role in the order of species colonization, animals appear to have powerful selection mechanisms to ensure a proper sequence of succession, e.g., in a study comparing sterile piglets (obtained using caesarian section and raised in sterile cages) to conventionally born and raised piglets, both sets of piglets exhibited a similar progression of microbial colonization. *E. coli* and *Streptococcus* populations established rapidly, followed later by *Lactobacillus* and *Clostridium*, in both sets of piglets. Regardless of the mechanisms providing for this orderly succession, the end result generally is the establishment of microbiota composed of a stable and diverse population of bacteria that serve to control enteric bacterial pathogens.

Although significant species-to-species and even animal-to animal variations do occur. Previous studies suggest that the succession of intestinal microbiota and its underlying principles can be similar across different animal species (Gong et al. 2002a; Zhu et al. 2002).

The weaning is a challenging period during which animals are separated from their mothers and switched from a highly digestible milk diet to a less digestible solid diet. Weaning is often associated with decreased intake of feed and water; decreased growth; dramatic structural, functional, and barrier function in the intestines; diarrhea; and increased mortality. The gut microbiota, which is still developing and fragile, plays an important role during this period. During this time, animals are susceptible to pathogen overgrowth (e.g. Pathogenic *E. coli* strains).

With respect to animal health, an important goal is to manipulate the microbiota through different strategies, such as diet and supplements, to achieve an optimal microbiota that yields maximum benefits and represents a low cost to the host. Many products, including antibiotics, organic acids, probiotics, prebiotics, trace

minerals, enzymes, herbs and spices, and others are sold with the goal of altering the microbiota for the benefit of animal health.
Weaning, a new environment and dietary changes are all conditions that are known to affect the intestinal microbiota of dogs, for which the supplementation with bovine colostrum might be beneficial.

Colostrum is the specific first diet of mammalian neonates and is rich in immunoglobulins, antimicrobial peptides, and growth factors. Recent studies suggest that colostral fractions, or individual peptides present in colostrum, might be useful for the treatment of a wide variety of gastrointestinal conditions, including inflammatory bowel disease and nonsteroidal anti-inflammatory drug–induced gut injury. Colostrum and milk are important secretions with nutritional and immunological properties.
Intestinal microbiota plays an important role in the health of all mammals, but species differences do exist. In ruminants and large herbivores, for example, microbes are crucial in extracting energy from fibrous substrates through anaerobic fermentation. Without it, these animals would be unable to meet their energy requirements. In most non-ruminant omnivores and carnivores, the amount of energy derived from microbial fermentation is low because of the low dietary fiber concentrations and (or) variations in gastrointestinal anatomy and physiology. Despite these differences, microbial populations play an important role in several gastrointestinal functions, including pathogen resistance and the immune system. Because dogs and humans are omnivores consuming plant and animal ingredients, numerous studies have been performed to test the effects of diet on gut microbial populations. Being true carnivores, consuming little vegetable matter, the feline microbiota has often been ignored. Thus, it is known in regards to feline microbiota, the changes that occur from dietary manipulation, and any health-related implications related to diet composition.

The immune system plays an important role in maintaining healthy intestinal microbiota (DAS UN, 2010).
In a study involving 24 bovine colostrum fed Alaska dogs, the treated group showed a notoriously high rate of antibodies (IgA in feces) versus the control group. This indicated an improvement in local immune age (Purina 2006).

In our experiment, referring to *E. coli, Streptorococcus fecalis* and *Lactobacillus spp.* concentrations were not different for treated and control groups. The values expressed in colony forming units (Table 8) were maintained throughout the trial.

					P-value	
	CTR	**Treat**	**Pooled s.e.m.**	**Diet**	**Time**	**Diet × Time**
***S. faecalis*, log$_{10}$ cfu/g**						
4 wks	3.80	3.85	0.19	0.98	0.46	0.83
6 wks	3.66	3.73				
8 wks	4.01	3.87				
***E. coli*, log$_{10}$ cfu/g**						
4 wks	2.73	2.89	0.30	0.79	0.25	0.75
6 wks	2.81	2.74				
8 wks	2.49	2.20				
***Lactobacillus*, log$_{10}$ cfu/g**						
4 wks	4.27	3.71	0.36	0.87	0.37	0.47
6 wks	3.54	3.71				
8 wks	4.00	4.24				

Table 8: Effect of colostrum on bacterial counts of puppies.

In the figure 9 shows that *Streptococcus fecalis* does not change, it maintained the same level at 4th, 6th and 8th week. Colostrum supplementation did not have any effect on *Streptococcus fecalis* growth. *Streptococcu fecalis* concentrations were not affected by diet in this study.

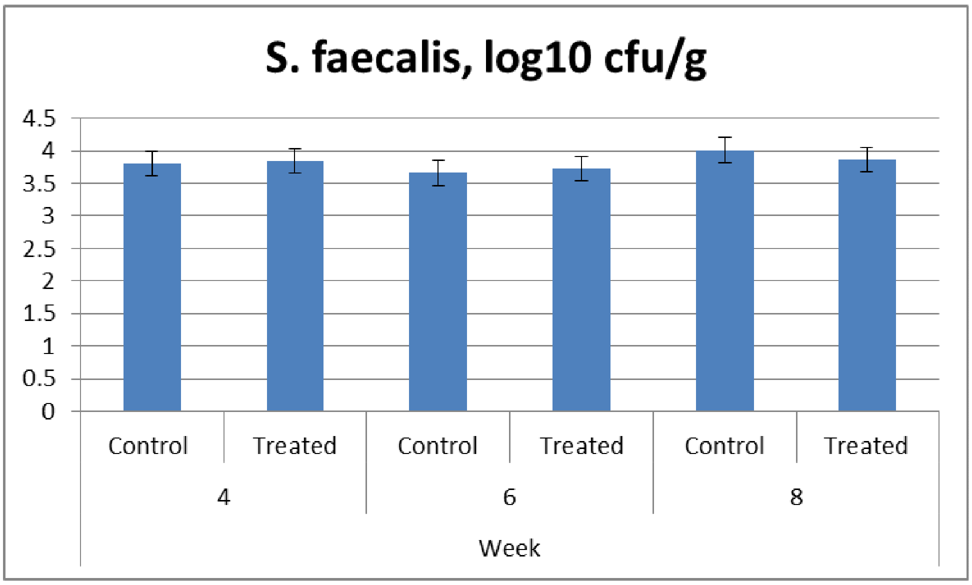


Figure 5: Microbiological count of S. Fecalis.

The *E. coli* concentrations were not significantly correlated. At the same time the treated group showed a lower *E. coli* concentration than the control group at 6th and 8th week of the trial.

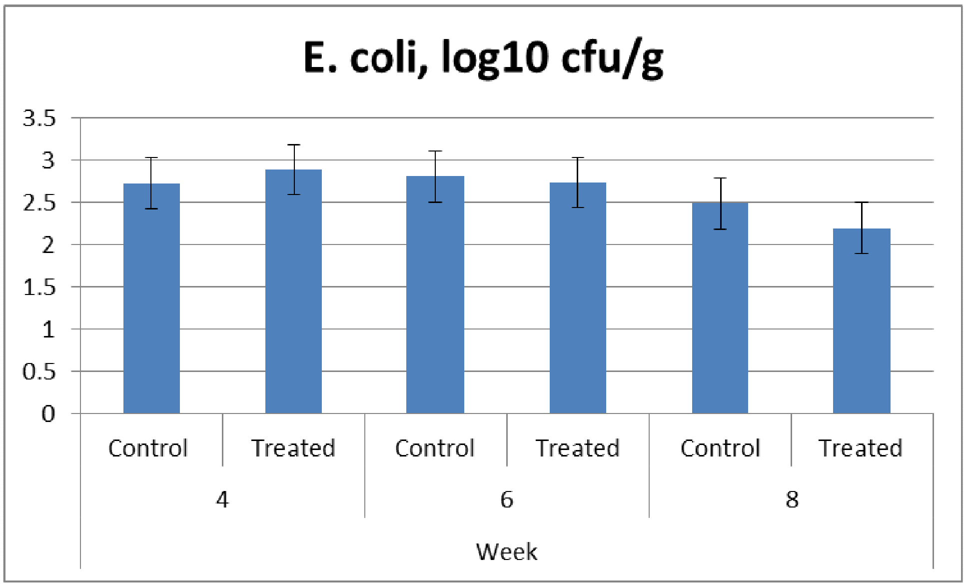


Figure 6: Microbiological count of *E. coli.*

After 8th week of the treatment period, *Lactobacillus* population increased but without any statistical significance in the bovine colostrum fed treated puppies vs controls. *Lactobacillus* species are generally considered to be beneficial to the host as it may reduce harmful bacteria by producting antibacterial compounds, thus reducing the competition for nutrients and competition for colonization spots.

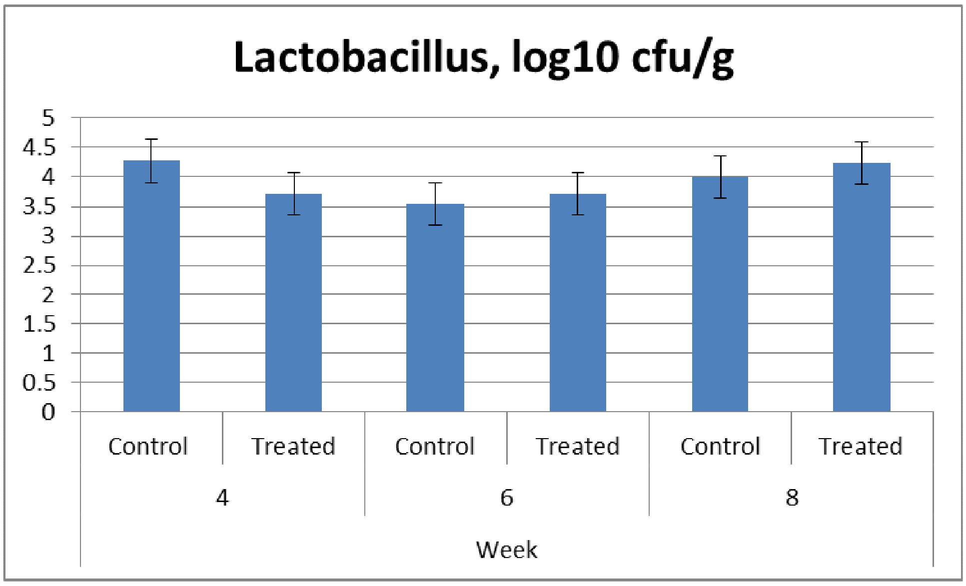


Figure 7: Microbiological count of *Lactobacillus*

In the figure 7 we see that during the 6th and 8th week the colostrum supplementation had an effect on *Lactobacillus* by making it increase.

Ebenezer et al., 2013 carried out a study with 24 Husky adults dogs, supplementing their diet with 0.1% bovine Colostrum and reported a positive response in the intestinal microbiota, as increased beneficial bacteria even in dogs were subjected to some kind of stress. This suggests that colostrum supplemented diets in dogs may potentially be more resistant to bacterial colonization or infection by pathogens.

Probiotics are living microorganisms which may confer a health benefit to the host when consumed in adequate amounts. Prebiotics are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus also conferring health benefits to the host (Gibson et al., 2010).
The treatment with bovine colostrum showed a prebiotic effect through the ability of modulating the constituents of the intestinal microbiota. This is a mechanism by which probiotic may affect the immune system.

The gastrointestinal microbiota is an incompletely defined, dynamic community of several hundred species of primarily anaerobic bacteria. Species composition and bacterial numbers vary depending on animal age, the gastrointestinal location and a variety of nutritional and environmental factors. The microbiota positively and negatively impacts host physiology and performance in many important ways.
The role of *Escherichia coli* in the intestine is complex. The natural niche for *E. coli* is the large bowel of man and animals. Some strains may persist in the bowel microbiota of an individual for months or years (so-called resident strains), while others disappear within a few weeks (so-called transient strains).
Some studies demonstrated that differences in gut microbiota composition in early infancy are associated with the subsequent development of atopic disease manifestation and sensitization.

Prebiotics like colostrum remain an option in the prevention and treatment of gastrointestinal diseases or antibiotic therapy. They also cause frequently alterations in the intestinal microflora, have immunomodulatory effects and favour the growth of improving animal digestive tract function.

Obtained data confirmed the ability of bovine colostrum supplementation in the modulation of the gastrointestinal microbial population. This study suggests that bovine colostrum administration leads to quantitative changes in the GI microbiota of healthy weaned puppies.

4.4 Conclusions

This trial was not indicative about the fact that oral supplementation with bovine colostrum has any beneficial effects on intestinal health of the puppy. A slight increase of *Lactobacillus spp. vs E. coli* was noticed in the treated weaning pups, however, this was not statistically significant. Bacteria such as *Escherichia coli* pathogens are commonly associated with diarrhea in weaned puppies, it would be appropriate to elaborate on the growth of beneficial bacteria and see if the supplementation of bovine colostrum can be a real aid in reducing rates of diarrhea recurrent.

Although the main growth performances were not incluenced, we noticed an augmentation of *Lactobacillus spp.* This aspect is very important as regards the gut flora balance. Colostrum is probably able to condition in a positive way the gut micro-flora during a critical period as weaning.

An additional test would require a greater number of puppies to confirm the effect of bovine colostrum on these zootechnical parameters.
More research may be also required to identify which components of colostrum are effective in improving bowel health as well as to explore their mechanisms of action on gastrointestinal tract.

4.5 References

Adkins, Y., Lepine, A. J.; Lonnerdal, B. (*2001).* Changes in protein and nutrient composition of milk throughout lactation in dogs. *American journal of veterinary research, v. 62, n. 8, p. 1266-1272, aug. Issn 0002-9645.*

Boudry C., J. Dehoux P., Wavreille J., Portetelle D., The´wis A. and Buldgen A. (2008). Effect of a bovine colostrum whey supplementation on growth performance, faecal Escherichia coli population and systemic immune response of piglets at weaning. *Animal 2:5, pp 730–737 & The Animal Consortium.*

Case, l. P., Daristotle, I., Hayek, M.G., Raasch, MF. (2001) chapter 21: pregnancy and lactation. In: (ed.). *Canine and feline nutrition 3. P.199-207.*

Costachescu, E.; Hoha, G.; Fotea, I. (2011) Research regarding the lactating period of the bitch. Lucrari stiintifice - universitatea de stiinte agricole si medicina veterinara, seria zootehnie, v. 55, p. 180-183. *Issn 1454-7368.*

Costachescu, E.; Hoha, G.; Fotea, L. (2011). Research regarding the lactating period of the bitch. Lucrari stiintifice - *universitatea de stiinte agricole si medicina veterinara, seria zootehnie, v. 55, p. 180-183. Issn 1454-7368.*

DAS UN (2010). Obesity: genes, brain, gut, and environment. *Nutrition 26, 459-473.*

Fieni, F. et al. (1999). Physiology of prolactin, the pharmacology of prolactin inhibiting drugs and applications for bitches. Pratique medicale et chirurgicale de l animal de compagnie, v. 34, n. 3, p. 187-198. Issn 0758-1882.

Hand, M. S.; Lewis, I. D. (2000) Small animal clinical nutrition. 4th. Topeka, kan.: mark morris institute. *Xxi, 1192 p. Isbn 0945837054*

Heayns, B. (2012). Immunology for veterinary nurses - humoral immunity. *Veterinary nursing journal*

Kealy RD, Olsson SE, Monti KL, et al.(1992). Effects of limited food consumption on the incidence of hip dysplasia in growing dogs. *J Am Vet Med Assoc 1992;201:857–863.*

Lund EM, Armstrong PJ, Kirk CA, et al. (2005). Prevalence and risk factors for obesity in adult dogs from private US veterinary practices. Intern *J Appl Res Vet Med; 4(2):177-186.*

Norcross, N. L. (1982). Secretion and composition of colostrum and milk. Journal of the american veterinary medical association, v. 181, n. 10, p. 1057-60, 1982-nov-15 . *Issn 0003-1488.*

Poffenbarger, E. M. Et al. (1991). Use of adult dog serum as a substitute for colostrum in the neonatal dog. *American journal of veterinary research, v. 52, n. 8, p. 1221-1224. Issn 0002-9645*

Satyaraj E (2011). Emerging paradigms in immunonutrition. *Top Companion Anim Med 26, 25-32*

Scarlett JM, Donoghue S. (1998). Associations between body condition and disease in cats. *J Am Vet Med Assoc ; 212:1725-1731*

Voldoire, E. (2002). Physiologie et pathologie néonatales du chiot de moins de quinze jours. *121 th. Med. Vet 1*

CHAPTER 5

Nutritional strategies to control diarrhea in adult dogs: clinical cases

5. Chapter 5 Nutritional strategies to control diarrhea in adult dogs: clinical cases

5.1 Introduction

Diarrhea is defined as frequent and excessive emission of liquid or soft feces due to a disturbance of the water exchange in the intestine and to an increased peristalsis resulting in loss of water in the stool.

Stool quality is objectified by using an index called fecal score. This is a score given to the saddle and translating its appearance and consistency. Referring to score fecal optimal, variable according the used fecal scoring scale, the saddle will be estimated diarrheal or not. The notion of diarrhea is a complex and multifactorial concept. In fact, diarrhea depends on several factors, including infectious factors (parasitic, viral or bacterial), environmental stress generators in animals, food or physiological.

Various studies were conducted in adult dogs, regarding factors which may impact on the quality of the stool. Some studies were also conducted on puppies, although few of them were conducted over the period of blood extraction, or on youngest puppies. Nevertheless they reveal certain factors and suggest some explanatory hypotheses.

Moreover Weber et al. (2003) report that enzymatic changes take place in the digestive system during growth, in particular between the weaning period and adulthood. The activities of amylase, sucrose and peptidase increase, while those of pancreatic enzymes such as trypsin, chymotrypsin and lipase do not seem affected by age (Elnif and Buddington (1998) cited by Weber et al., 2003). This fact together with changes in the absorption of nutrients in the small intestine and a greater transit time in young puppies affects the digestibility. The authors conclude by suggesting that a more digestible feed in puppies compensates for their relatively low digestive capacity.

Stress is defined as the set of responses from a body subject to environmental constraints. When these are too numerous or too frequent, excessive stress is often harmful to animals, whether being breeding, sport dogs or pets.
There are several studies in veterinary literature dealing with the effects of stress. In addition to the fears or uncertainties, it generates stress which can also affect the digestive functions, therefore influencing the behavior of the animal.

Simpson (1998) found that stress is a major cause of IBS (IBS) when manifesting as abdominal pain. He added that the treatment of this syndrome exclusively depends on identifying stressors, which is not always obvious.

Nutrients influence the gastrointestinal tract in a variety of ways. Then the type of diet may have an impact on the fecal score of dogs. This observation was carried out by several authors in the veterinary literature.
Meyer et al. (1999) studied the digestibility of dry food and wet food of 66 different dog breeds by examining the quality of their saddles through a fecal scoring scale of 0 to 10. Whatever the studied race, a wet type of diet induced stools whose fecal score is less than or equal to that obtained when using a dry diet.

Rolfe et al. (2002) also made the same observation. They studied stool quality of two groups of dogs (12 digestive sensitive dogs, dogs and 8 controls) who were fed with dry and wet regimes. For this purpose, they used a fecal score scale from 1 to 5, with 1 representing very dry stool, friable, and 5 the diarrheal stools. Each level was then divided into 4 classes so as to obtain a 17-point scale, enabling greater accuracy. No significant differences were observed regarding the fecal score of the controls whether the dogs were fed with a dry or a wet regime. However, this was not the case for sensitive dogs, in which the feces were significantly softer when they were fed with a wet diet.

The GI mucosa represents the largest surface area in contact with the external environment; it is also responsible for preventing the entry of harmful pathogens into the body. The GI barrier consists of several anatomic, Physiologic and immunologic components. It is covered by a thick mucus layer that is able to trap particles and microorganism (Chehade M et al., 2005). Changes in luminal pH across the different parts of the digestive tract, luminal and brush border digestive enzymes, and bile salts all contribute to destroy any potential pathogens, breaking down the ingested food and therefore rendering dietary antigens less immunogenic. Cells and factors from the innate and adaptive immune system are additional obstacles to foreign antigens and significantly contribute to the mucosal barrier (Sicher SH et al., 2010).

The gut-associated lymphoid tissue (GALT) is the largest and most complex part of the immune system. This system can be divided into organized tissues and effector sites (Mowat AM, 2003).

Alimentation

Food allergy (FA) is characterized by an abnormal or exaggerated immunologic response to the ingestion of a specific food allergen. On the other hand, food intolerance is caused by an abnormal physiological response (Shicher et al., 2010). A new term of diet-responsive chronic enteropathy (CE) was coined and it encompasses AFR as well as mild intestinal inflammatory. Diet elimination trials are recommended in most dogs with chronic idiopathic GI signs of mild to moderate severity (Guilford WG et al., 2001).

Despite the large extent of exposure to dietary antigens, only a small percentage of people and dogs develope FA. Oral tolerance to dietary proteins and commensal microorganisms is an active immunologic process that results in inhibition of the immune response to an antigen after prior exposure through the oral route. Other factors that may influence oral tolerance include the normal intestinal microbiota and the genetics of the host. In FA subjects, this complex balance is disrupted. Oral tolerance may be breached directly by an inflammatory process that increases mucosal barrier permeability, causing malabsorption of allergenic antigens and sensitization (Chehade M et al., 2005).

	Beef	Daily	Wheat	Lamb & mutton	Egg	Chicken	Soy	Pork	Rabbit	Fish	Canned foods*	Dry foods*	Diverse	Number of animals
Denis & 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			4		1					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	

Table 1: Common food allergens in the dog.
*Canned foods and dry foods: Commercial foods in which the exact causative food allergen was not identified.
*Diverse: corn, rice, gluten.

Although all food proteins are antigenic only a small component of the total protein content of a food is allergenic; the capacity of a protein to induce an allergenic reacton is influenced by the immunogenicity and the permeability depends on stimulation of IgE production and histamine release of mast cells after bridging of the allergen between two IgE molecules on the surface of the

mast cell membrane (Verlinden et al., 2007). The maximum size limit is related to the absorption capacity of the enteric mucosa for the protein.
There are a lot of potential food allergens and because of the multiple ingredient content in commertial pet food, it is difficult to detect the specific causative food allergens. Several publications were analyzed in which the allergenic was identified by elimination (table 1).

Dogs with food allergy respond to an elimination trial but do not relapse after provocation with their original diet or components thereof and they do not have true AFR. Higher bioavailability of the nutrients decreases the amount of undigested/unabsorbed substances that are otherwise metabolized by the intestinal flora and may cause changes in its composition.

A diagnosis of food allergy or intolerance can only be made by prescription of a strict home or commercial elimination diet following by a test challenge to reduce clinical signs. The ideal elimination diet should respond to some criteria (Roudebush et al., 1994) and contain a limited number of new highly digestible proteins. The most recommended food components in dogs are lamb, chicken, fish, rabbit, venison, rice, potatoes and tofu (Verlinden et al., 2007).

5.2 Case 1. Diagnosis of recurrent diarrhea in a Flat Coated Retriever by dietary approach.

Recurrent diarrhea in a Flat Coated Retriever: a case report.

5.2.1 Indroduction

Food allergy occurs far more frequently in dogs than how it is habitually diagnosed in veterinary practice. The signs produced by food allergy can widely vary, though the most common problems usually concern the skin and the gastrointestinal and otic tracts. For this reason it is quite rare that a pet owner may think that e.g. diarrhea was due to the allergy, as we are going to see further. A phenomenon called *Oral Tolerance* begins to develop in young animals. Oral tolerance represents an essential aspect of their life and it keeps on developing thoughout life and thanks to the food the animals receives during its first months. The pet owner constantly changed his pet's diet: this may have modified its gasto-intestinal tract and therefore specifically the pet's oral tolerance. This means that the dog may have first developed a food allergy and later diarrhea and bad absorption.

5.2.2 Material and methods

Case presentation

A 2-year-old male Flat Coated Retriever was examined because since when it was 2 months old he has suffered of recurring diarrhea. He did not lose weight and appetite.

Clinical examination

The dietary history was complex because more of 12 different commercial diets were offered to this dog. The owner changed pet food frequently and suddenly, most of the time after the recurrence of episodic diarrhea, whose severity varied over the months (fluid or mucous feces). The vaccinations and deworm treatments were current.

Figure 1: Body condition score. The physical examination revealed a normal appearance.

The dog weighed 42 kg and had a BCS of 5/9 and a normal MCS. Ribs were palpable without any fat covering excess. No waist was observed behind ribs when viewed from above. Abdomen tucked up when viewed.

Diagnosis

The following conditions, antibiotic-responsive enteropathy, diet-responsive enteropathy, inflammatory bowel disease (IBD) (mild form), and chronic idiopathic large bowel diarrhea and other intestinal disorders were considered.

Serum biochemestry

Parameter	Quantity
AST	24 IU/L
ALT	31 IU/L
ALP	524 IU/L
GGT	3 IU/L
Tolal billirubin	0.26 mg/dL
Serium protein	7 g/dl
Albumine	3.69 g/dL
Globuline	3.31 g/dL
A/G	1.11
Cholesterol	236 mg/dL
Tryglicerides	55 mg/dL
CK	87 IU/dL
BUN	12.6 mg/dL
Urea	27 mg/dL
Creatinine	1.17 mg/
Glucose	90 mg/dL
Calcium	9.1 mg/dL
Phosphorus	4.4 mg/dL
Sodium	143 mEq/L
Potasium	04:03 mEq/L
Na:K	33.3
Chloro	97 Meq/L
Serium osmolarity	304 mOsm
Magnesium	3.1 mg/dL

Table 1: Serum chemestry. It can be seen that the ALP was high (524 IU / dL) as its ranges are between 24-147 mg / dL.

The fact that the ALP is up to 5 times indicates high intestinal inflammation. Similarly, we can see that the serum osmolality is low because its range is between 314-330 mOsm. This may be due to dehydration caused by constant diarrhea.

Blood cunt and abdominal ultrasounds

The complete blood count and abdominal ultrasounds were usual.

Abdominal ultrasonography is useful to examine the gastrointestinal tract of dogs with CE, the technique does not distinguish diet responsive animals from those requiring other treatments.

Elisa

Currently the definitive diagnosis of food allergy in dogs is based on results of an elimination diet followed by a food challenge test. The patient dog resulted allergic to beef, turkey, chicken, soy, lamb, rice and beans.

Cobalamin

Also analyzed cobalamin was highly related to malabsorption syndrome. The result was low (150 picog / mL). Vitamin B12 is released from food by the action of acids and pepsin of the stomach, here it gathers with haptocorrin secreted in saliva with an affinity that the constantly secreting acidic pH of the gastric juice.

Treatment

Exclusion homemade diet

We calculated the obtained energy requirements basing ourselves on the results from the nutritional assessment with the following formula:

$$132 \times \text{dog body weight (kg)} \hat{} \ 0.75$$

We also considered the presence of any factors, including physiological state and age, which may have influenced it.

The propietary pledged that food diet was the only food that the animal would consume at least during the last 8 weeks. In addition the level of vitamin B12 was analyzed for a more accurate diagnostic.

According to the data that were carried out from the clinical history as weight, age and physiological state, compared with laboratory tests on the patient, the following diet was formulated.

Its daily energy requirement was based on about 2, 200 kcal/day.

		Daily quantity	Meal (2)
Protein	Pork	475 g	237.5 g
Lipids	Linseed oil	25 g	12.5 g
Carbohidrates	Potato	500 g	250 g

Table 2: Exclusion diet. Pork meat should be lean, raw and then cooked (either boiled, roasted or baked) before giving it to the patient. Rolled oats were added to the pre-cooked meat.

The cobalamin supplementation (25 μg/kg once/week SC) was associated with an elimination trial.

5.2.3 Results and discussion

Analyzed cobalamin was highly related to malabsorption syndrome. The result was low (150 picog / mL).
Vitamin B12 works as a cofactor in the metabolism of proteins, fats and carbohydrates. It is assimilated in the small intestine in the presence of intrinsic factor secreted in the stomach.
Cobalamin deficiency in dogs is commonly associated with canine CE with a reported prevalence of 6–73% (Allenspach K et al., 2007; Kathrani A, Steiner JM, Suchodolski J, et al. 2009). Cobalamin deficiency induces various clinical and metabolic consequences, including anorexia, weight loss, failure to thrive, central and peripheral neuropathies, immunodeficiency and intestinal changes including villous atrophy and malabsorption of other vitamins and nutrients (Fordyce; 2000).

Hypocobalaminemia may result in severe metabolic consequences and is associated with a negative outcome (Allenspach etal., 2007; Battersby et al., 2005) Thus rapid identification and early intervention is recommended (Dossin, 2011). Some centers therefore recommend starting cobalamin supplementation when serum cobalamin concentration is in the low reference range. A recent study assessing MMA in dogs with different levels of serum cobalamin concentrations demonstrated that 19% of all dogs with serum cobalamin concentrations in the lower reference range had an increased MMA (Berghoff et al., 2012). This suggests that some of these dogs were cobalamin deficient on a serum level and supports the recommendation to start treatment in the low reference range.

In a recent study (Kawano et al., 2016) on CE with 32 dogs, 18 of these dogs (56.2%) responded to the elimination diet and hypoalbuminemia was not observed to have a higher probability of being diagnosed as food-responsive enteropathy, like in our case. It is therefore recommended the food allergens to be avoided in the elimination diet. The selected elimination diet therapy should be carried out in order to prevent any unnecessary use of immunosuppressants.

According to clinical data malabsorption associated with intestinal inflammation was present in the dog patient, which is conducted to not absorbing water and nutrients. Limiting the intestinal mucosa exposure to potential antigens was the adopted dietary strategy, following the immunodeficiency. In particular, a homemade exclusion diet consisting in a novel protein (pork meat) source and carbohydrate (rolled oats) source was designed in relation to the dog's body conditions.

5.2.4 Conclusions

The owner reported that diarrhea completely disappeared after one week, so a lipid source (fish oil) was introduced after 4 weeks.
Due to the owner attitude in changing pet food, the dog's oral tolerance may have been breached. An inflammatory process increasing the intestinal permeability was the main cause of the possible absorption of allergens and the following sensitization of the subject.
The basis for the above mentioned dietary recommendation is that the faster the intestinal inflammation is controlled, the faster the intestinal permeability barrier will be restored and the less the dog will be exposed to intestinal antigens.

Associated Cobalamin supplementation with the dietary elimination trial sustained the remission of the clinical signs. These suggest that oral cobalamin supplementation might be an alternative to administration in dogs with CE and cobalamin deficiency. The evolution of the dog's clinical conditions in the following weeks was good therefore determining the complete resolution of the food-responsive chronic enteropathy (CE).

5.3 Case 2. Diagnosis of recurrent diarrhea in an Great danes dog by dietary approach

Recurrent diarrhea in an Great danes dog: a case report.

5.3.1 Introduction

Diarrhea is one of the most frequent problems for dogs. Recent diet and food studies on dogs demonstrated that when occurring any drastic change in this field the animal runs the risk of bad digestion syndrome; in this case the enzymatic balance and the micro-flora lose their harmony with the inner digestive tract substratum. Loss of weight, matt fur, variable appetite and vomit almost always occur. Diarrhea may be continuous as well inermittent. The causes may be inflamatory bowel disease, malabsorption syndrome, gut funcional disorders or metabolic alterations. In certain cases, such as the following one, a simple exclusion diet was dignosed as a consequence of a food allergy. The food allergy prognosis is very good when succeeding in identifying the food allergens.

5.3.2 Material and methods

Case presentation
A 1-year and a half old male Geat Dane was examined for recurrent diarrhea.
He lived in an apartment; he had 3 daily activity hours. He lost weight and he was hungry.

Clinical examination
The physical examination revealed a normal appearance. Its weight was 65 kg, and had a BCS of 4/9 and a normal MCS. He presented diarrhea from 2 months of age and for that reason had taken probiotics for 8 months. The vaccinations and deworm treatments were current. Its last diet was a prescription diet, adult dry dog food sensitive digestion chicken, but he is allergic to chicken between other components.

Figure 2 Body condition score.

We observed a BCS 4/9; ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.

Diagnosis

Serum biochemestry

Parameter	Unity
GLU	104 mg/Dl
BUN	22 MG/Dl
CREA	1.1 mg/Dl
BUN/CREA	20
PHOS	5.3 mg/Dl
CA	9.6 mg/Dl
TP	5.9 mg/Dl
ALB	3.0 mg/Dl
GLOB	2.9 mg/Dl
ALB/GLOB	1.0
ALT	71 U/L
ALKP	43 U/L
GGT	0 U/L
TBIL	0.2 mg/Dl
CHOL	122 mg/Dl
AMYL	708 U/L
LIPA	227 U/L
Na	146 mmol/L
K	4.1 mmol/L
Na/K	36
Cl	113 mmol/L
Osm Calcium	293 mmol/kg

Table 1: Serum chemestry. We can see the parameters were normals.

Parameter	Unity
HGB	14.8 g/Dl
MCV	67,1 Fl
MCH	23.9 pg
RETIC	40.5 K/ µL
WBC	8.86 K/ µL
NEU	5.46 K/µL
LYM	2.40 K/ µL
MONO	0.86 K/ µL
EOS	0.09 K/ µL
BASO	0.06 K/ µL
PLT	426 K/ µL
MPV	5.4 Fl

Table 2: Blood count. We can see that eosinophyls were low in blood.

When the medical examination indicates that has a low level of eosinophils less than 50 ml, this means that the patient's immune system may be significantly compromised. This may be due to acute allergic reactions (table 2).

Elisa

Currently, the definitive diagnosis of food allergy in dogs is based on results of an elimination diet followed by a food challenge test. Pet Alimentary Elisa test (IgE + IgG) was performed.

Gastroendoscopy

Stomach: Moderate edema and moderate hyperemia of the body, antrum and pylorus, the presence of granulomas inflammatory disseminated in antral-pyloric region and the pylorus. The presence of abundant grass in the gastric lumen.

Duodenum: Moderate hyperemia and mild edema. Short villi at both the duodenum that the first part of the fast.

Colon: Mild hyperemia and moderate edema. Presence of numerous inflammatory granulomas. Presence of mild feces.

Gastro enteric biopsy

The framework is associated with severe irritation-hyperplasia of the mucosa in the antral prepiloric evident entero-gastric reflux chronic. The infection by GHLOs is quantifiable in >30 bacteria per microscopic field at 400 X (average count of 10 fields). Intestine with the framework of lymphoplasmacytic enteritis type moderate, at times with continuous infiltrated and partly compelling glandular mucosal structures, diffuse interstitial form. The mucosa shows a picture of inflammation with severe atrophy of Mucosal epithelium, associated with atrophy of the glandular component and mucinic mucosal hyperplasia, in the grip of strong coccobacilli colonization. The villi appear especially of decreased height. Biopsies colic feature interstitial colitis severe, type macrofollicular, with follicles that push from the glands. Some glandular crypts appear altered to a widespread mucoid metaplasia.

Treatment

Exclusion homemade diet

Based on the medical history and laboratory findings, it was decided to prove an exclusion diet. The following diet was formulated. For the dog of his race the daily calorie needs are 3600-3800 Kcal/day.

		Daily quantity	Meal (2)
Protein	Pork	900 g	450 g
Lipids	Fish oil	24 g	12 g
Carbohidrates	Rolled oats	340 g	170 g

Table 3: Exclusion homemade diet. Pork meat should be lean, raw and then cooked (either boiled, roasted or baked) before giving it to the patient. Rolled oats were added to the pre-cooked meat.

An approach diet requires complicity with the owner and aims to provide the animal for at least 8-12 weeks' food components which has not had, if possible previous contact.

5.3.3 Results and discussion

The patient results allergic to soy, corn, wheat, rice, potatoes and peas.
At the test the dog had diarrhea from 2 months of age and he consumed a prescription diet for dogs with allergies but without an exact diagnostic. There is literature that assess that the uptake of antigen by the enterocytes depends on the content of proteins and phospholipids of the cell membrane. A change in composition and function of the enterocytes occurs at a young age (Sampson., 1991). The larger neonatal permeability of enterocytes enhances the absorption of food molecules and colostral antibodies. During the development of the enterocyte along the crypt-villus axis, the composition of the cell membrane also changes: immature crypt cells have twice the endocytotic capacity (i.e. protein absorption) of mature crypt cells (Sanderson & Walker., 1993). Recent research in dogs concerning postnatal development of nutrient transport in the intestine of dogs, revealed a decreased absorption for most nutrients between birth and adulthood (Buddington & Malo, 2003).

The biopsy diagnosis was chronic-active superficial gastritis, associated with strong mucosal hyperemia and obvious signs of reflux entero-gastric chronic. Framework that is accompanied by chronic enteritis type is widespread that localized and high increase in mucinic production, severe, in combination with conglutinating villous and decrease of villus height to mononuclear infiltration. Colon framework of mixed interstitial colitis, both microfollicolar diffuse of severe grade.

Eosinophil accumulation in the gastrointestinal tract is a common feature of numerous gastrointestinal disorders, including classic IgE-mediated food allergy,1,2 eosinophilic gastroenteritis (Keshavarzian et al., 1985), allergic colitis (Odze et al., 1995), eosinophilic esophagitis (EE) (Rothenberg et al., 2001), inflammatory bowel disease (IBD) (Rothenberg & Marc, 2014).

Treatment of eosinophilic gastritis and gastroenteritis is commonly elimination diet of the foods implicated in allergy by Elisa test. Once disease remission has been obtained by means of dietary modification, the specific food groups are slowly reintroduced (at approximately 3-week intervals for each food group) and endoscopy is performed every 3 months to identify sustained remission or disease flare up.

It has been reported that allergic colitis of infancy, in this case after weaning (2 months) might be an early expression of protein-induced enteropathy or protein-induced enterocolitis syndrome. Soy proteins are the most frequently implicated foods in allergic colitis of infancy, but other food proteins can also provoke the disease (Guajardo & Rothenberg, 2003). When eosinophilic colitis shows in the first year of life, the prognosis is very good, with the vast majority of patientsbeing able to tolerate the culprit food or foods by 1 to 3 years of age.

Sometimes a concept of such a hypoallergenic diet is used but it is not entirely correct, because it does not really exist. Food itself is antigenic – foreign to the body, capable of binding to specific antibodies – and the treatment of an allergy for a certain component consists of switching it to an alternative with a different set of antigens (Brown et al., 1995).

An elimination diet typically contains a source of protein and can be a homemade diet or diet available commercial hypoallergenic or hydrolyzed.
-Hypoallergenic diet (30-50% of subjects cannot be controlled as well).
-Hydrolyzed diet (10-20% of the animals showing a recurrence versus hydrolyzed protein).

When we carry out an approach diet it is important to use only a source of protein and carbohydrates, no additives of any kind should be used.
The dog presented a good improvement in the following 3 weeks as it recovered weight.

5.3.4 Conclusions

The causes of recurrent diarrhea in young adult dogs like a result of food allergy may be due to a weakened immune system as a result of poor intake of colostrum or lower quality colostrum when they were puppies.

Recent studies indicated that the use of probiotics reduces acute diarrhea in infants; it was also proved the use of probiotics is useful in preventing gastrointestinal diseases. These help replenish the destroyed intestinal flora. They also tend to be well tolerated and have few contraindications. However, if supplied excessively it may slightly alter the function of the colon, resulting in unusual metabolic changes such as weight loss or absorption problems. The gastrointestinal tract does not absorb nutrients properly, resulting in fatigue and swelling, increasing permeability to large molecules not normally absorbed, bacteria can even proliferate and therefore provoke secondary infections. Absorption of large food particles creates an awareness of some food proteins by IgE antibody formation and/or IgG provoking a food allergy. We could notice that the homemade exclusion diet is a very a diagnostic tool for food allergies in young dogs with persistence diarrhea.

5.4 References

Allenspach K, Wieland B, Gröne A, Gaschen F. (2007). Chronic enteropathies in dogs: Evaluation of risk factors for negative outcome. *J Vet Intern Med ;21:700.*

Battersby IA, Giger U, Hall EJ. (2005). Hyperammonaemic encephalopathy secondary to selective cobalamin deficiency in a juvenile border collie. *J Small Anim Pract ;46:339.*

Berghoff N, Suchodolski JS, Steiner JM. (2012). Association between serum cobalamin and methylmalonic acid concentrations in dogs. *Vet J ;191:306.*

Brown, C.M., Armstrong, P.J., and Globus, H. (1995). Nutritional Management of Food Allergy in Dogs and Cats. Compendium on Continuing Education for the *Practicing Veterinaria, 17:637–658.*

Buddington, R. K. and Malo, C. (2003). Postnatal development of nutrient transport in the intestine of dogs. *American Journal of Veterinary Research, 64(5): 635–645.*

Dossin O. (2011) Laboratory tests for diagnosis of gastrointestinal and pancreatic diseases. *Top Companion Anim Med; 26:86.*

Cave N. (2012). Nutritional management of gastrointestinal diseases. In: Applied Veterinary Clinical Nutrition, First Edition. Edited by Andrea J. Fascetti, Sean J. Delaney. *Published 2012 by John Wiley & Sons, Inc.*

Chehade M, Mayer L. (2005) Oral tolerance and its relation to food hypersensitives. *J Allergy Clin Immunol ;115(1):3-12.*

Cianferoni A, Spergel JM. Food allergy. Review, classification and diagnosis. *Allergol Int 2009;58(4):457-66.*

Fordyce HH. (2000). Persistent cobalamin deficiency causing failure to thrive in a juvenile beagle. *J Small Anim Pract ;41:407.*

Gaschen F.P, Merchant S.R. (2011) Adverse food reaction in dogs and cats. *Vet Clin Small Anim 41, 361-379.*

Guajardo JR, Rothenberg ME. (2003). Eosinophilic esophagitis, gastroenteritis, gastroenterocolitis, and colitis. In: Metcalfe DD, Sampson HA, Simon RA, eds. Food allergy: adverse reactions to foods and additives. 3rd ed. *Malden (MA): Blackwell Publishing. p. 217-26.*

Guilford WG, Jones RB, Markwell PJ, et al. (2001) Food sensitivity in cats with chronic idiopathic gastrointestinal problems. *J Vet Intern Med ;15(1):7-14.*

Kathrani A, Steiner JM, Suchodolski J, et al. (2009). Elevated canine pancreatic lipase immunoreactivity concentration in dogs with inflammatory bowel disease is associated with a negative outcome. *J Small Anim Pract; 50:126.*

Kawano K1, Shimakura H, Nagata N, Masashi Y, Suto A, Suto Y, Uto S, Ueno H, Hasegawa T, Ushigusa T, Nagai T, Arawatari Y, Miyaji K, Ohmori K, Mizuno T. (2016). Prevalence of food-responsive enteropathy among dogs with chronic enteropathy in Japan. *J Vet Med Sci. 2016 Sep 1;78(8):1377-80. doi: 10.1292/jvms.15-0457. Epub 2016 May 5.*

Keshavarzian A, Saverymuttu SH, Tai PC, Thompson M, Barter S, Spry CJ, (1995) Activated eosinophils in familial eosinophilic gastroenteritis. *Gastroenterology ;88:1041-9.*

Roudebush P., Guilford W.G., Jackson H.A. (2010). Adverse reactions to food. In Small Animal Clinical Nutrition. 5th Edition Edited by Hand M.S., Thatcher C.D., Remillard R.L., Roudebush P., Novotny B.*J. 2010. Mark Morris Institute, Topeka, Kansas, US.*

Ortolani C, Pastorello EA. (2006). Food allergies and food intolerances. Best Pract Res Clin Gastroenterol ;20(3): 467-83.

Odze RD,Wershil BK, Leichtner AM, Antonioli DA. (1995). Allergic colitis in infants. *J Pediatr; 126:163-70.*

Mowat AM (2000). Anatomical basis of tolerance and immunity to intestinal antigens. *Nay Rev Immunol ;33(4):331-41.*

Sampson, H. A. (1991). Immunologic Mechanisms in Adverse reactions to *Foods. Immunology and Allergy Clinics of North America, vol. 11(nr. 4): 701–*716.

Sanderson, I. R. and Walker, W. A. (1993). Uptake and Transport of Macromolecules by the Intestine: Possible Role in Clinical Disorders (an Update). *Gastroenterology,, 104: 622–639.*

Sicher SH, Sampson HA. Food allergy. (2010) J Allergy Clin Immunol;*125(2 Suppl 2) :S116-25.*

Meyer H, Kienzle E, Zentek J. (1993) Body Size And Relative Weights Of Gastrointestinal Tract And Liver In Dogs, *J. Vet. Nutr., 2,31-35*

Meyer H, Zentek J, Habernoll H, Maskell I. (1999) Digestibility And Compatibility Of Mixed Diets And Fecal Consistency In Different Breeds Of *Dog, Zentralbl Veterinarmed A., 46 (3), 155-165*

Rothenberg & Marc E (2014). Eosinophilic gastrointestinal disorders (EGID) *Journal of Allergy and Clinical Immunology , Volume 113 , Issue 1 , 11 - 28*

Rothenberg ME, Mishra A, Collins MH, Putnam PE. (2001). Pathogenesis and clinical features of eosinophilic esophagitis. *J Allergy Clin Immunol. 1;108:891-4.*

Simpson JW. (1998) Diet And Large Intestinal Disease In Dogs And Cats, J. *Nutr., 128 (12 Suppl), 2717s-2722s*

Weber MP, Stambouli F, Martin LJ, Dumon HJ, Biourge VC, Nguyen PG. (2002), Influence Of Age And Body Size On Gastrointestinal Transit Time Of Radiopaque Markers In Healthy Dogs, Am. *J. Vet. Res., 63 (5), 677-682*

Weber M, Martin L, Biourge V, Nguyen P, Dumon H. (2003) Influence Of Age And Body Size On The Digestibility Of A Dry Expanded Diet In Dogs, J. Anim. Physiol. Anim. *Nutr., 87(1-2), 21-31*

Weber M. (2006) Influence Of Size On The Dog's Digestive Function, Bull. Acad. Vét. *France, 159 (4), 327-332*

CHAPTER 6

General Discussion

6. General discussion

Nutrition plays a fundamental role in immune function of dogs. Immunodeficiency may seriously undermine the health of an animal by causing debilitating problems such as infections, malignancies or autoimmune diseases. Nutritional Immunology is an emerging field of study dealing with the impact of dietary components in immune function. While dietary deficiencies may contribute to immunocompromise, diet can actively influence the immune system beyond providing essential nutrients. This is because over 65% of the immune cells in the body are found in the gut, causing the intestine is the largest immune organ. The gut innate immune system receptors represent the main objectives of immunomodulation through diet. Although malnutrition may cause immunodeficiencies, as most pets are fed with nutritionally complete and balanced diets, veterinarians are more likely to find immunodeficiencies related or caused by stress. The latter causes immune deficiency, which cannot be corrected simply by correcting nutritional deficiencies. They may be more difficult to assess, understand and manage, and are the focus of ongoing research in nutritional immunology.

In addition to being the gateway to the nutrient intake, the intestine is the largest immune organ with more than 60% of all immune cells in the body and more than 90% of all immunoglobulin-producing cells (Bengmark, 1999) in the intestine, they are crucial for the development of a healthy immune system (Cebra, 1999).

Colostrum is the first milk the mother produces. In dogs, a key role is represented by transferring colostrum immune protection to newborn animals. During their first weeks of life, the immune system of newborn animals depends mainly on passively transferred antibodies through colostrum from their mother.

In cases where the canine colostrum is not available for newborn puppies, bovine colostrum may provide a suitable alternative. On the other hand, it is well known that weaning is a critical period for the puppy, because while the immune system is not developed yet and therefore does not work optimally. This explains why the intestinal microflora remains unstable. It is suggested that bioactive compounds such as immunoglobulins and growth factors containing bovine colostrum may provide protection for the puppy as well as for adult dogs.

The aim of our study was to evaluate the benefical effects of bovine colostrum on zootechnical parameters as conditional body score, body weight gain, food intake, but also its effect on intestinal microflora in weaned puppies.

Numerous studies about colostrum supplementation powder demonstrate the importance of colostrum in the establishment of beneficial intestinal bacterial

population as well as prevention of gastroenteritis in other species such as pigs (Boudry et al., 2008), mice and human infants (Evina et al., 1992).
Human studies show that babies who were fed with colostrum show a predominance of beneficial bacteria (*Bifidobacteria* and *Lactobacillus*) and fecal pH less than babies who were fed with breast milk only.

We did not notice any effect on zootechnical parameters in treated dogs versus control dogs. Concerning weight gain, treated puppies did not gain more weight if compared to controls. Regarding the SBA there was a significant difference in treated puppies between 4th and 6th week of treatment.

Unlike our study, the École nationale de vétérinaire of Toulouse, France, carried out a study with 27 German shepherd and Newfoundland puppies; the group fed with bovine colostrum showed a greater weight gain (100 g) at 15 days compared to the group fed with breast milk only (Oliver, 2014). In addition to that, the mortality of the puppies dropped significantly with the administration of bovine colostrum during the first few days of life (5% of total mortality until weaning compared to 9% without bovine colostrum supplementation). This study indicates that it may begin to be supplied colostrum from the first days of birth to ensure a greater contribution of antibodies and active substances that naturally contains colostrum, also ensuring increased absorption of nutrients and better development of the immune system of the puppy.
In the study carried out by Giffard, it was found that the supply of colostrum to newly weaned pups improved the quality of the feces of puppies under stress by changing their diet or alter their environment.
Another study concerning 70 weaned puppies demonstrated that the puppies who were treated with 0.5g of colostrum had less diarrhea. Apparently the colostrum may play an important role in the prevention of gastroenteritis at early age.
In our study, we could only observe a statistically non-significant decreasing slight effect on *E. coli* and an increase of *Lactobacillus* to 8th week (last dose).
Lactobacilli are microorganisms maintaining the balance of intestinal flora. They are not pathogenic for the puppy, as they strengthen the immune system and are also known as prebiotics. These bacteria are lactic acid-producing, i.e. a substance that can alter the pH of the environment and is harmful to many pathogens such as enterobacteria. Our study had a slight effect on the growth of *Lactobacillus.*
On the other hand, the composition of the diet is important for the development of the puppy and for the good health of adult dogs as well. The fiber quantity should be appropriate, contain a minimum number of ingredients, be palatable and balanced.

The fiber has an effect on fecal volume and transit time, which can cause increased fecal output and flatulence. The installation of specific bacterial flora (*Bifidus* and *Lactobacillus*) there is not digestible fiber used to directly feed the cells of the large intestine, promoting, whose beneficial effects on the health of the digestive tract are well known:
- Inhibition of growth of pathogenic bacteria (*E. coli*).
- Improve the digestion and absorption of nutrients.
- Protection of the digestive tract: "barrier effect".

The purpose of the second study was to evaluate the role of integration of an alternative source of fiber, such as microcrystalline cellulose, relative to its potential action in the intestine, in the diet of dogs showing acute gastrointestinal symptoms. In the test we considered twenty subjects, ten of which received a simple industrial diet, while the remaining ten received the same diet but enriched with insoluble industry fiber (microcrystalline cellulose) in such an amount that the final ration presented a crude fiber content of 10% of the dry matter. The subjects featured the symptomatic gastrointestinal clinical manifestation characterized by diarrhea.
Throughout the duration of the study, measured parameters such as body weight, Body Condition (BCS) and Muscular Condition (MCS) remained unchanged in involved subjects except for two mongrel subjects, one Golden Retriever and one Corso puppies, whose conditions involved weight changes. In particular, it should be noted in the literature using a diet rich in fiber, such as cellulose, it has been given special attention in handling problems related to weight. During the study, this parameter remained unchanged and there were no significant differences in subjects receiving the control diet and those receiving microcrystalline cellulose. The only major changes were related to the physiological state. The presence of microcrystalline cellulose in the amounts we considered in this study also helped keep constant the other parameters included in the nutritional assessment of subjects, such as the BCS and MCS. With respect to the BCS, this parameter remained unchanged in the topics; these were recognized in young or senior dogs.
Many authors focused their attention on the effects of fiber on some fecal markers. Brigitta Wichert et al., 2002 recognized that as cellulose was equipped with a moderate water holding capacity, allocating to such source of fiber, a superior ability to bind water in the stool is consequently exploited to improve the quality of feces and in particular to increase the content of dry matter.
Burrows and colleagues in 2011 showed that this fiber source was equipped with more features such as the ability to increase the fecal mass and in particular the moisture content of the fecal addition to normalize the intestinal transit time.
Prola and colleagues (2010) have confirmed the action of cellulose on fecal consistency in cats. In particular in the study these authors carried out was put in

relation with the length of the fiber used to detect the fecal consistency by a scoring system.
The inclusion in the diet of cat's fiber length increased in a dose of 4% on the dry, it resulted in the formation of a more solid stool.
The fiber is now considered as important for the gastrointestinal well-being. Aslan and collaborators in 1992 also stressed the importance of dietary fiber in health of the intestinal tract as well as of appropriate ratios of polyunsaturated fatty acids (n-6) : (n-3) in reducing the severity of the pathology.

Dietary treatment is of great importance in the management of diarrheal disorders in dogs. Depending on the etiology and localization of the disease, varying dietetic measures may be necessary. Besides ensuring an adequate energy and nutrient supply, pathophysiological processes in the intestinal tract should be counteracted. In the case of diarrheal disorders of the small intestine, diets should be highly digestible to advance nutrient assimilation, and have a reduced fat and fiber content to facilitate the digestive processes. In contrast, disorders of the large intestine require increased levels of dietary fiber. In addition, insoluble fibers like cellulose can enhance the fecal bulk and consequently help to normalize the intestinal peristalsis. Moreover insoluble fibers can influence the intestinal water absorption and therefore improve the fecal consistency. Elimination diets are considered to be the method of choice for food allergies or intolerances. Diets for patients with chronic diarrhea should be highly digestible.
Nutrients can influence the gastrointestinal tract in several ways; if the diet is not properly balanced the animal suffers from deficiencies; the content of the diet (allergic or antigenic proteins); a diet can even alter motility, morphology, immune response, cells of the intestinal mucosa, absorption of fluids and electrolytes, mucus secretion, acids and enzymes, and balance bacterial flora composition, etc.
The wall of the gastrointestinal tract is the major surface of the body is exposed to the environment.
There are four mechanisms of the immune system that confer tolerance functions and antigen exclusion; 1) mucosal barrier; 2) regulation of the immune response; 3) elimination; 4) tolerance of antigens (Gilford, 1996).
The absorption of intact proteins will depend on the integrity of the intestinal barrier so that this integrity will remain unchanged depending on many factors, such as the morphology and function of enterocytes, the presence of IgA, an effective digestion, good quality food composition and the presence of inflammation.
Recent researches in dogs concerning postnatal development of nutrient transport in their intestine revealed a decreased absorption for most nutrients between birth and adulthood (Budington & Malo, 2003).

A state of malnutrition may increase intestinal permeability to macromolecules due to change in morphology of enterocytes (Sanderson & Walter, 1993).
Moreover penetration of the epithelium by antigen triggers an immune response. In the case of dogs suffering from food allergy a specific response for the antigen is triggered; formation of IgM, IgG or IgE (Sampson 1999).
The increased absorption of macromolecules is followed by a continuous gastrointestinal hypersensitivity with an allergic reaction. According to Jeffers et al., 1996), the average number of dog allergies is 2.4. From here we see the importance of introducing specific components to elimination diets to identify food allergies.
The elimination diet is the most important in dogs with suspected adverse reactions to food reactions diagnosis. The other test as vitro testing, biopsies, gastro endoscopies are not relevant for diagnosing food allergy. In our case studies the best diagnosis of food allergy was homemade elimination diet. To carry out such diet the most commonly used protein sources are pork or fish, horse or rabbit, depending on the eating habits of the animal. As a source of carbohydrates rice or potato are generally advised in our clinical cases but this was impossible because the subjects were allergic, less than 10% of cases in dogs with allergies are allergic to potato or grain.

For reasons of balanced diet and owner's convenience, after diagnosis a prescription hydrolyzed proteins containing diet was proved as effective and recommended for dogs with food allergy.
Although homemade diets are the best alternative for the diagnosis of long-term and adverse reactions, they may cause vitamin deficiencies in animals if not adapted. After diagnosis commercial diets, better known as hypo-allergenic, are recommended. These nutritionally balanced diets include a limited number of allergens; however, on many occasions contained additives and stabilizers can contribute to the maintenance of allergy. Therefore the usually recommended hypo-allergenic diets may contain additives that may contribute to keep the allergy. Such diets should be there used after diagnosis. They recently appeared on the market hypo-allergenic diets in which, instead of limiting the number of ingredients and selecting a single type of protein, a new technology was used that reduces the size of proteins (<12.000 daltons), therefore improving its absorption.

In this study, we conclude that dietary manipulation gives the clinician veterinarian a powerful therapeutic strategy for treating the patient suffering from a gastrointestinal disease such as diarrhea that may occur from an early age as the puppy is still weaning as it is subject to great stress when separated from their mother. Such diet may apply to young adult dogs as well when changing environment or food. Also including prebiotics or probiotics to serve as aids in maintaining the body's natural defenses.

In summary, the role played by the immune system and its interaction with diet takes a whole new meaning. As our understanding of the relationship between nutrition and the immune system deepens, a vast array of diet-based options focusing on immune needs will become available. The food we feed our pets with may clearly deliver several other benefits beyond basic nutrition and therein lays the promise of immune nutrition.

CHAPTER 7

Summary

7. Summary

Diarrhea is a common problem in recently weaned and young dogs. If the separation from the litter occurs at a time when the immune system is not fully competent or whether as the puppy had a lower colostrum consummation or a bad alimentation, this combination of events increases the susceptibility of the puppy or young dog to a variety of infections and gastrointestinal disturbances. Bacterial pathogens, such as enteropathogenic *Escherichia coli* and enterotoxigenic *E. coli* are also associated with enteric disease in young dogs.

Our study about bovine colostrum supplementation aimed to know its effects on recently weaned puppies at 32 days of age. The puppies were divided into 2 groups each composed of 3 and 4 animals, the control one (CT) and the treatment one (TG) randomly. The experimental groups belonged to the same litter. The involved puppies were fed with the same basal diet. A treatment was represented by administering bovine colostrum once a week through a specific oral syringe. Feed intake was registered daily. Individual body weight and average daily gain were measured weekly. We found that its principal benefit was an increased gut flora such as *Lactobacillus spp.* versus *E.coli.* in treated puppies.

The purpose of the second study was to evaluate the role of the integration of an alternative source of fiber, i.e. microcrystalline cellulose, in relation to its potential for action in the intestine, in the diet of dogs suffering from acute gastrointestinal symptoms. For the test we took into consideration twenty subjects, ten of which received a simple industrial diet, while the remaining ten were given the same industrial insoluble fiber (microcrystalline cellulose) enriched diet in such quantity that the final ration contained crude fiber of 10% dry matter. All subjects featured a symptomatic gastrointestinal clinical manifestation characterizing variable output of feces, soft feces, formed feces, the feces without definite form until liquid stools.

The third study was about chronic diarrhea as a consequence of food intolerance. The dietary management of these cases varies according to the underlying disease. Generally, the animal should receive a diet with moderate levels of fiber and fat divided in into small portions during the day. The objective of this diet was to facilitate intestinal regulation, beneficially modify the composition and metabolic activity of the intestinal microbiota and exclude antigens from the diet. In our cases the patents showed a good response to these elimination homemade diets.

CHAPTER 8

Acknowledgements

8. Acknowledgement

First of all I thank God for having given and for keeping giving me so many good things and for always putting some extraordinary people in my way.

I thank my family for always supporting my decisions.

My teacher Valentino Bontempo for allowing me to write this thesis.

Luciana Rossi, who has always been available not only for the professional aspect but also for listening to me when I needed it the most.

To my Mexican and international friends who spent with me so many good and complicated times as well all over these years.

Andrea, Sergio and Lorena, who were my angels when I first reached Milan.

To you my love Simone, because partly thanks to this project I met you, for always being there during my days under stress and my sleepless nights.

To my beloved doggies: Lucky, Sandy, Jully, Molly, Stella and Youlà who inspired me to devote myself to this beautiful profession.