EFFECTS OF FEEDING *LACTOBACILLUS REUTERI* X-18 ON BLOOD CHEMISTRY AND IMMUNE PARAMETERS IN BEAGLE (*CANIS FAMILIARIS*) PUPPIES

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

INTRODUCTION

The term "probiotic" or direct-fed microbial (DFM) was originally defined by Roy Fuller as "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance" (Fuller 1992). Since then, Guarner and Schaafsma have expanded that definition to "living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition" (1998). Probiotic therapy has been recommended for the treatment or prevention of a variety of conditions in different species. The advantages of probiotic consumption in humans have been recognized for centuries. However, their application and efficacy in domestic animals have only recently been pursued.

Properties of probiotics that one may find appealing include the ability to reduce antibiotic use and the positive perception about natural or alternative therapies. While antibiotic therapy is effective at reducing or eliminating pathogenic microorganisms, it is equally as effective at eliminating the host's gut microflora, upsetting the natural microbial balance essential to a functional gastrointestinal tract and immune system. By introducing probiotics into the gastrointestinal tract of humans and animals, many health benefits can be seen such as improved nutritional value of food, control of intestinal infections,

improvement of lactose utilization, control of some types of cancer, and control of serum cholesterol levels (Gilliland 1990).

A potential probiotic strain is expected to have the following desirable properties. The species-specific microorganism should be a normal inhabitant of the gastrointestinal tract of healthy hosts (Biourge and others 1998; Dunne and others 1999). To elicit health-promoting effects, the bacteria must survive the harsh environment of the gastrointestinal tract. The stomach's natural defense against microorganisms is the gastric acid having an extremely low pH (Suscovic and others 1997). The mean intestinal bile acids concentration in the human gastrointestinal tract is about 0.3% w/v (Gilliland and others 1984). Therefore, bile tolerance is important if the microorganism is expected to grow in the gastrointestinal tract. Finally, adhesion to the intestinal mucosa is considered a prerequisite for successful colonization and is important for immune modulation (Ouwehand and others 1999).

A number of direct-fed microbials are available commercially for use in dogs, mainly in the form of capsules, tablets, pastes, and liquid. However, none of these products are of canine origin. In addition, many of these products contain *Enterococcus faecium* (Benyacoub and others 2003; Rinkinen and others 2003) and therefore, raise questions of safety due to antibiotic resistance genes and the bacterium's pathogenic nature (Rinkinen and other 2003; Franz and others 1999). The addition of probiotics into diets may have the advantage of easy, daily administration. Therefore, the aim of this study was to determine the effects of a potential probiotic, *Lactobacillus reuteri* X-18, on several biochemical and

immunological parameters in healthy beagles. Twenty beagle puppies were used in this feeding trial. Of the twenty, ten were fed an experimental diet of 10% non-fat dry milk containing the probiotic while the remaining ten were fed a control diet of 10 % non-fat dry milk without the probiotic. Positive effects on biochemical and immunological parameters by the direct-fed microbial would indicate the culture could be used as a potential probiotic in dogs.

CHAPTER II

REVIEW OF LITERATURE

LACTIC ACID BACTERIA (LAB)

Fermentation as a food preservation method can be traced back thousands of years. It is only in the more recent past that microorganisms were found to be responsible for the fermentation process. Due to the industrial revolution, there was a shift from small-scale production to large-scale production of fermented foods and alcoholic beverages. The use of starter cultures, primarily lactic acid bacteria (LAB), contributed to extending shelf-life and quality of the food product. The group, lactic acid bacteria, is composed of a number of genera including *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus*. These bacteria generally produce lactic acid as their major end product. Lactic acid bacteria lack a terminal electron transport chain, therefore are strictly fermentative, and are catalase negative (Condon 1987). Lactic acid bacteria can be subdivided into two groups based on carbohydrate metabolism. Homofermentative LAB use the Embden-Meyerhof-Parnas (EMP) pathway to convert 1 mole of glucose into 2 moles of lactate. Heterofermentative bacteria use the hexose monophosphate or pentose pathway to produce equal molar amounts of

lactate, CO_2, and ethanol from glucose. Many organic acids such as lactic, acetic, and propionic acids produced as end products of fermentation provide an environment unfavorable for the growth of many pathogenic and spoilage microorganisms. Such acids interfere with the cell membrane potential, inhibit active transport, reduce intracellular pH, and inhibit various metabolic functions (Doores 1993) and can inhibit both Gram-positive and Gram-negative bacteria and yeasts and molds (Blom and Mortvedt 1991). Antimicrobial metabolites such ethanol and hydrogen peroxide can have oxidizing effects on membrane lipids and cellular proteins (Ray and Daeschel 1992; Condon 1987). Another example of an antimicrobial secondary metabolite produced is the compound reuterin, by the LAB, *Lactobacillus reuteri.* Reuterin consists of monomeric, hydrated monomeric, and cyclic dimeric forms of β-hydroxypropionaldehyde. It is considered to have broad spectrum activity that inhibits the growth of protozoa, fungi, and various Gram-positive and Gram-negative bacteria (Axelsson and other 1989; Chung and others 1989; Talarico and Dobrogosz 1989).

Almost all of the different species of LAB have been reported to produce antimicrobial compounds collectively called bacteriocins. Bacteriocins are proteinaceous substances which can inhibit or reduce undesirable microflora in food products (Klaenhammer 1988; Holzapfel and others 1995) by depolarizing target cell membrane or by inhibiting cell wall synthesis (Abee and others 1995).

PROBIOTICS

In the early 1900's, Metchnikoff proposed that the lactic acid bacteria were antagonistic to the spoilage microbes of the gut and, once introduced into the gastrointestinal tract, would prevent the growth of harmful microorganisms that required an alkaline environment (1921). By experimenting on himself, he noted his poor health improving by the regular ingestion of a sour milk prepared by cultures of lactic acid-producing bacteria. This discovery led to physicians recommending the use of such products as yogurt or sour milk to those who were generally regarded as suffering from poor health. Crediting his relative long life in part to the lactic bacilli, he hypothesized that "once people learned how to cultivate a suitable flora in the intestines of children as soon as they are weaned from the breast, the normal life may extend to twice my 70 years" (Metchnikoff 1921). Studies since then have shown health benefits ranging from improved lactose utilization in those otherwise considered lactose intolerant to increased immune response in an individual. In 1998, Guarner and Schaafsma expanded Fuller's traditional definition of a probiotic or direct-fed microbial (DFM) to "living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition" (Guraner and Schaafsma 1998).

In a study done by DeSimone and others, results showed that ingesting yogurt may enhance the immune response by increasing the percentage of B lymphocytes, phytohemagglutamin (PHA), and lipopolysaccharide(LPS)-induced proliferative responses of Peyer's patches in the intestine (1987). In 1999, Van de Water and others noted a decreased incidence of allergies in the live-active yogurt

group compared with the heat-killed and no-yogurt groups. The mechanisms by which probiotics stimulate the immune system have not all been elucidated but studies have been done that have allowed us to better understand the immune response.

Lactic acid bacteria constitute a major group of bacteria used as probiotics (Table 1).

Table 1. Species of bacteria used as probiotics[1,2]

Lactobacillus sps.	*Bifidobacterium* sps	*Streptococcus* sps.
L. acidophilus	*B. bifidum*	*S. thermophilus*
L. casei (rhamnosus)	*B. breve*	*S. salivarius ssp thermophilus*
L. fermentum	*B. lactis*	
L. gasseri	*B. longum*	
L. johnsonii	*B. infantis*	
L. lactis	*B. adolescentis*	
L. paracasei		
L. plantarum		
L. reuteri		
L. salivarius		
L. bulgaricus		

[1]Givson and Roberfroid 1995.

[2]Alvarez-Olmos and Oberhelman 2001.

Selection criteria for potential probiotic cultures consist of: 1) acid and bile tolerance, 2) persistence in the gut even if it cannot colonize the gut, 3) ability to adhere to the gut epithelium to withstand the flushing effects of peristalsis, 4) ability to interact or send signals to the immune cells associated with the gut, 5) species-specificity, 6) generally recognized as safe (GRAS), 7) having the capacity to influence local metabolic activity, and 8) withstanding processing conditions

(Harmsen and others 2000). The claimed benefits of probiotics include improved nutritional value of food, increased absorption of calcium (Hamilton-Miller 2004), lowered activity of β-glucuronidase, β-glycosidase, azoreductase, and nitroreductase which are known to convert pre-carcinogens to carcinogens (Burns and Rowland 2000), control of intestinal infections, improvement of lactose utilization in persons labeled as lactose intolerant, and control of serum cholesterol levels (Gilliland 1990). In humans, the dose of 5×10^9 CFU/day for at least five days is recommended to obtain an adequate amount of health benefits conferred by the probiotic (Gronlund and others 1999). Factors such as antagonism and competition help regulate the composition of the microflora as well as help to suppress the establishment of pathogenic bacteria in the intestinal tract (Tannock and Archibald1984). This process involves cooperation between the intestinal microflora and the immune system of the host (Tannock and Savage 1976). Immune effects can be influenced by the intestinal microflora, since the antigenic load provided by the intestinal microbes serves to stimulate the immune system (Gordon and Pesti 1971).

LACTOBACILLUS REUTERI

Among the numerous species of *Lactobacillus* found in the gastrointestinal tract of humans, pigs, chickens, and other animals, *Lactobacillus reuteri* is considered a prominent member of the gastrointestinal complex (Kandler and others 1980). Little is known about *L. reuteri* and less about reuterin, a newly discovered broad-spectrum antimicrobial substance produced by this species

(Talarico and others 1988). Studies have indicated that reuterin or 3-hydroxyproprionaldehyde (3-HPA) is a low molecular weight, neutral, water-soluble, non-protein substance, produced specifically from glycerol fermentation by anaerobic resting cells, that has anti-bacterial, anti-mycotic, and anti-protozoal activity (Luthi-Peng and others 2002). It has been shown that reuterin is an intermediary metabolite by which glycerol is first dehydrated to form reuterin, some of which is then reduced to 1,3-propanediol (Chung and others 1989; Axelsson and others 1989). Compositionally, reuterin is an equal mixture of monomeric, hydrated monomeric and cyclic dimeric forms of 3-HPA (Talarico and Dobrogosz 1989).

GASTROINTESTINAL IMMUNE SYSTEM

The intestinal tract is the largest mass of lymphoid tissue and is generally referred to as the gut-associated lymphoid tissue (GALT). This is the location where ingested bacteria first make contact with the host's mucosal immune system (Brandtzaeg and others 1989). Structural and functional components of the gastrointestinal tract are part of the mechanisms that provide protection from potential pathogens and excessive antigenic exposure. Such structural components consist of the epithelial tight junctions, the microvillus membrane, and a surface mucous layer. Examples of functional components include peristalsis, gastric acid, and proteolytic enzymes (Guilford and Strombeck 1996). Beneath the mucosal epithelium is the lamina propria. Within the lamina propria is the GALT. Gut-associated lymphoid tissue can be found along the entire length of the

gastrointestinal tract and is composed of Peyer's patches. The most immune activity occurs within the GALT (Ettinger and Feldman 2005). A single unit of GALT consists of the dome, follicle, and parafollicular region. The dome is comprised of specialized enterocytes, called M cells, that act as antigen-presenting cells (APC). Example of APC's are macrophages or dendritic cells. The follicle contains B lymphocytes while the parafollicular region contains mostly naive T lymphocytes (Banks 1992). Antigens are presented to lymphocytes by APC's. The lymphocytes becomes activated after receiving activation signals from APC's and helper T cells. These activated lymphocytes move through mesenteric lymph nodes into circulation and return to the lamina propria. T cells promote tolerance to food antigens and normal gastrointestinal microflora and play a key role in the development of intestinal inflammation. Therefore, treatments often target modulating T cells. B lymphocytes produce plasma cells. Plasma cells aggregate in the lamina propria and mainly produce immunoglobulin A (IgA). IgA bind antigens on mucosal surfaces such as the gastrointestinal epithelium, and are more resistant to proteolytic enzymes produced by enteric microflora than other types of immunoglobulins (Janeway and others 2005). The role of IgA is very important in gastrointestinal immune reponses. Secretory IgA is formed when a part of the immunoglobulin (Ig) receptor remains bound to the dimeric IgA, following transport of IgA across the epithelial barrier (Sampson 1991). Secretory IgA inhibits the attachment and penetration of bacteria and toxins in the lumen, increases time for digestive enzymes to function, binds and prevents absorption of undigested protein, increases mucous secretion and prevents inflammatory reactions (Russell and others 1989;

McKay and Perdue 1993). Secretory immunoglobulin A (IgA) plays an important role in mucosal immunity, acting as an added barrier against pathogenic bacteria. Probiotic bacteria increase the number of IgA-producing cells (Szajewska and others 2001) and certain cytokines that up or down regulate the immune responses (Arvola and others 1999). Unfortunately, interactions between probiotic microorganisms and GALT, mechanisms of immunomodulation and anti-inflammatory properties are not fully understood.

Recent research interests have focused on the effect of LAB on specific and non-specific immune functions. Strains of LAB have been found to secrete lysosomal enzymes and enhance phagocytosis (Gill 1998). Probiotic bacteria have been shown to enhance humoral immune responses, promoting the intestine's immunologic barrier (Isolauri and others 1993; Kaila and others 1992). Probiotics can also stimulate non-specific host resistance to microbial pathogens (Perdigon and others 1986) and modulate the host's immune responses to potentially harmful antigens (Sutas and others 1996a,b).

Stimulation of non-specific immunity can be observed by the release of the proinflammatory cytokines tumor necrosis factor α, interleukin-6 (IL-6), and interleukin-10 (IL-10) (Miettinen and others 1996). Probiotics can activate the production of macrophages (Perdigon and others 1986) and phagocytosis (Perdigon and others 1998). Phagocytosis is responsible for early activation of the inflammatory response before antibody production, the recruitment of immunocompetent cells, and the generation of the inflammatory response (Isolauri and others 1997). In healthy subjects, modulation of phaygocytosis resulted in an

immunostimulatory effect, while in subjects suffering from food allergies, down-regulation of the inflammatory response was observed (Pelto and others 1998). Since intestinal inflammation is accompanied with an imbalance in the gut microflora, probiotics may help stabilize the gut microflora (Salminen and others 1998) and prevent the production of inflammatory mediators (Brandtzaeg 1995).

Certain species of bacteria isolated from the gastrointestinal tract can produce low-molecular weight peptides that trigger immune responses. Proteases utilized by probiotics can degrade the milk protein casein generating peptides that suppress the proliferation of lymphocytes (Sutas and others 1996b).

DOGS AS COMPANION ANIMALS

For decades, dogs (*Canis familiaris*) have been used to aid humans in tasks such as hunting, herding and guarding. Dogs have also been used to enhance the quality of life of individuals with disabilities. However, as society has moved from small rural communities to increasingly large urban and suburban centers, the role of dogs as companions has increased (Table 2). Dogs decrease loneliness and provide opportunities for exercise and social interactions with other people. Furthermore, medical research has shown that contact with dogs can decrease feelings of anxiety and stress, and lower blood pressure in humans (Friedmann and others 1983). Since dogs live 10 to 20 years, people must be ready to provide them a home for that duration. Dogs must be properly nourished for good health.

Table 2. U.S. Pet Ownership and Demograhics.

	Dogs	**Cats**	**Birds**	**Horses**
Percent of households owning	37.2%	32.4%	3.9%	1.8%
Number of households owning	43,021,000	37,460,000	4,453,000	2,087,000
Average number owned per household	1.7	2.2	2.5	3.5
Total number in United States	72,114,000	81,721,000	11,199,000	7,295,000
Veterinary visits per household per year (mean)	2.6	1.7	0.3	2.2
Veterinary expenditure per household per year (mean)	$356	$190	$25	$360
Veterinary expenditure per animal (mean)	$200	$81	$9	$92

(American Veterinary Medical Association. 2007)

GASTROINTESTINAL MICROFLORA OF DOGS

Knowledge of the structure of the bacterial community in the gastrointestinal tract of the dog is essential to understand the interactive processes and the impact on the health status of the animal. The gut microflora of dogs consists of over four hundred various bacterial species with members of the genus *Bifidobacterium* making up the dominant microflora (Chien-Chang and Walker 2005). Traditionally, cultural and phenotypic approaches have been taken to quantify predominant fecal microflora of the canine gut. Previous studies have presumptively identified lactobacilli and bifidobacteria isolated from dog feces by Gram reaction and gas

liquid chromatography analyses of metabolic end products (Hartemink and Rombouts 1999; Martineau 1999). The use of molecular-based methods in combination with traditional methods will allow more accurate analyses of the diversity of the gastrointestinal tract (Drancourt and others 2000). More work must be directed on the derivation and testing of probiotics for dogs. Benno and others concluded that microbial populations in the canine gut change with time suggesting that bacterial populations may vary independently from the diet (1992). No detectable differences in gut microflora of the stomach, duodenum, jejunum and ilieum were seen but in the large bowel, differences in microbial populations were observed. Larger populations of lactobacilli were seen in the large bowel but decrease with age (Benno and others 1992). The large amount of the bacterial diversity remains undetermined emphasizing the need to develop more efficient recovery and identification procedures to determine future probiotic and prebiotic products.

PROBIOTICS FOR DOGS

Lactic acid bacteria (LAB) constitute a major group of bacteria to be used as probiotics for animals (Biourge and others 1998). The criteria that must be met for a given microorganism to be termed probiotic are the same for dogs as they are for humans. Strains of *Lactobacillus* and *Bifidobacterium* are most frequently used as probiotics in humans and large animals. Species of *Lactobacillus* are Gram-positive, non-spore forming, facultative anaerobic rods. They are capable of rapidly colonizing the intestinal epithelium and inhibiting the growth of enteric pathogens.

In addition, their immunomodulatory functions increase the activity of macrophages and the concentration levels of immunoglobulins (Chrzastowska and others 2009). Classical traits for the evaluation of probiotics such as higher feed conversion and weight gain are not relevant for companion animals (Lahrssen and Zentek 2002). However, modification of the immune function in dogs (Benyacoub and others 2003; Baillon and other 2004) and the composition and metabolic activity of canine intestinal microflora (Rinkinen and others 2000; Weese and Anderson 2002; Rastall 2004) are key characteristics of probiotics that are useful in determining their potential use in dogs. Unlike the effects of probiotics seen in dogs suffering from gastrointestinal conditions, modifications in the intestinal microbiology are not necessarily accompanied by obvious clinical benefits in healthy dogs. *Lactobacillus acidophilus* DSM 13241 has been found to survive the incorporation into dry dog food and transiently colonizes the colon of dogs (Baillon and others 2004). The desired outcome would be that it reduces or at least antagonizes undesirable, potentially harmful, or pathogenic microorganisms. It was observed that fecal concentrations of lactobacilli and bifidobacteria increased, while not significantly, during supplementation, indicating a small shift in the composition of the intestinal microflora. Research on probiotics for dogs has focused mainly on alleviating lactose intolerance in dogs. Lactose intolerance can be defined as the inability to metabolize lactose. Probiotics can convert lactose to lactic acid, enhancing lactose fermentation, thus alleviating symptoms of lactose intolerance (Lewis and Freedman 1998; Sullivan and Nord 2005). Probiotic bacteria have been proven effective in the treatment of small intestinal bacterial overgrowth (SIBO) in dogs.

SIBO is characterized by an uncontrolled increase in microbial populations in the small intestine (Lewis and Freedman 1998). This results in the alteration in the composition of intestinal microflora causing the accumulation of *E. coli, Salmonella*, *Campylobacter* or *Clostridium*, causing luminal fluid loss and subsequently leading to diarrhea (Delles and other 1994; Rutgers and others 1995). It has also been shown that probiotics can alleviate SIBO through competitive inhibition and by the production of bacteriocins such as reuterin (Marteau and others 2001; Barefoot and Nettles 1993). The addition of probiotics to dog food is thought to promote overall health over the dog's lifespan and particularly the development of its immune system (Benyacoub and others 2003; Biourge and others 1998). Clinical indications for using probiotics in dogs are not well defined and have been adapted from concepts developed for humans. However, the treatment and prevention of common digestive disorders and unfavorable changes in the gastrointestinal microflora is a relevant application for the use of probiotics in dogs. In 2007, McCoy and Gilliland published a study in which they isolated and characterized species of *Lactobacillus* having potential for use as probiotics for dogs. Based on their results, *Lactobacillus reuteri* was the predominant species isolated from dogs and the best prospective culture (X-27 and X-18) to use for potential probiotics for dogs based on bile tolerance, inhibitory action, and the production of reuterin.

IMMUNOMODULATORY EFFECTS OF PROBIOTICS

The role of immunoglobulin A (IgA) is very important in gastrointestinal immune reponses. Secretory IgA is formed when a part of the immunoglobulin (Ig)

receptor remains bound to the dimeric IgA, following transport of IgA across the epithelial barrier (Sampson 1991). Secretory IgA inhibits the attachment and penetration of bacteria and toxins in the lumen, increases time for digestive enzymes to function, binds and prevents absorption of undigested protein, increases mucous secretion and prevents inflammatory reactions (Russell and others 1989; McKay and Perdue 1993).

Four classes of immunoglobulins have been identified and characterized in dogs and appear to be similar to those of other species. The measurement of serum immunoglobulin concentrations is one of the most commonly used methods of assessing the immune competence in dogs. Dogs having IgA deficiency may suffer from diseases such as inflammatory bowel disease (IBD) or small intestinal bacterial overgrowth (SIBO) (Batt and others 1991). Past studies have utilized single radial immunodiffusion (SRID) to quantify canine immunoglobulins. However, the sensitivity of enzyme-linked immunosorbent assay (ELISA) renders this method more desirable (Ginel and others 1996). Findings from a study done by German and others (1998) show large variation in the concentrations of secreted immunoglobulins within individual dogs.

COMPLETE BLOOD COUNT

A complete blood count, commonly referred to as a CBC, is a count of the total number of cells in a given amount of blood. A second type of test is a blood chemistry panel, which measures the quantities of various electrolytes, enzymes, or

chemical compounds in the liquid portion of a blood sample. The CBC is concerned with the quantities and types of red blood cells, white blood cells, and platelets.

Red Blood Cells

Red blood cells (RBCs) are responsible for carrying oxygen throughout the body. Oxygen taken into the body attaches to the hemoglobin contained in the RBCs and taken to the lungs. The RBCs then deliver oxygen to the body and take the carbon dioxide back to the lungs. Red blood cells are formed in the bone marrow which constantly produce new RBCs, since the life span of an RBC is only about 120 days (NIH 1993).

There are several different ways one can determine the number of RBCs in the CBC. The quickest and easiest method is called the hematocrit, also referred to as the packed cell volume (PCV). A blood sample is centrifuged to allow heavier cells to collect allowing the PCV to be determined as the percentage of the cellular portion relative to the total amount of blood (the plasma)(Willard and Tvedten 2004). Anything that decreases the amount of oxygen reaching tissues in the body will cause higher numbers of RBCs in the CBC. Another method of determining the number of RBCs in the CBC is by the Red Blood Cell Count or "red count". An actual number of RBCs is counted in one microliter of blood. A final method to determine RBCs is by quantifying the amount of hemoglobin present (Willard and Tvedten 2004). Other indices of red blood cells include Mean Corpuscular Volume (MCV), which is the average volume of the red cells, Mean Corpuscular hemoglobin (MCH), the average amount of hemoglobin per red blood cell, and Mean Corpuscular

Hemoglobin Concentration (MCHC), which is the average concentration of hemoglobin in the cells (NIH 1993).

White Blood Cells

White blood cells (WBCs), also called leukocytes, are the other major type of blood cells. For every leukocyte, there are normally 600-700 RBCs. The role of WBCs is to defend the body against invading microorganisms. Elevated WBCs is typically indicative of infection or stress by metabolic toxins. White blood cells are divided into two groups depending on how they react to the stains that are used to observe them under a microscope. There are granulocytes which include neutrophils, eosinophils, and basophils and agranulocytes which include lymphocytes and monocytes (NIH 1993).

Neutrophils

Neutrophils are formed in the bone marrow. Segmented cells or segs are mature cells that have a multi-lobed nucleus while immature cells have a single-lobed nucleus and are called bands. Neutrophils engulf disease-causing microorganism. Elevated numbers of neutrophils is usually a sign of bacterial infection or extreme stress (Willard and Tvedten 2004).

Eosinophils

Eosinophils are also formed in the bone marrow. These cells can engulf foreign particles. Levels of eosinophils may be elevated due infection with parasites, or allergies (Willard and Tvedten 2004).

Basophils

Basophils are formed in the bone marrow but are the least common of the WBCs and usually not present in samples. Their function is unknown.

Lymphocytes

Lymphocytes are the most abundant of the agranulocytes. They are formed and released from lymphoid tissue such as lymph nodes and spleen. There are two major types of lymphocytes, B cells and T cells. B cells produce antibodies. T cells activate and help other cells destroy foreign invaders (Willard and Tvedten 2004).

Monocytes

Monocytes develop and are stored in the spleen and bone marrow and have the ability to engulf foreign invaders. In addition, they have the ability to secrete protein molecules that help clean up inflamed tissue (Willard and Tvedten 2004).

PLATELETS

Platelets are responsible for the formation of clots. Platelets and the protein, fibrinogen, repair damaged blood vessels (NIH 1993).

BLOOD CHEMISTRY PANEL

The blood chemistry panel or biochemical profile includes tests for multiple chemical constituents within a blood sample. Most chemistry panels check blood electrolytes and for diseases of the liver, kidney, and pancreas. While the CBC deals with the cell portion of blood, the chemistry panel deals with the liquid (serum)

portion of blood after the cells have been removed. A typical chemistry panel will measure the following:

- Blood Glucose
- Blood Urea Nitrogen (BUN)
- Creatinine
- Calcium
- Total Protein
- Albumin
- Globulin
- Total Bilirubin
- Alkaline Phosphatase
- Alanine Amino Transferase (ALT)
- Cholesterol
- Sodium
- Potassium.
- Aspartate Aminotransferase (AST)
- Gamma Glutamyl Transferase (GGT)
- Phosphorous
- Amylase
- Lipase
- Chloride
- Creatine Phosphokinase (CPK)
- Triglycerides
- Magnesium

Blood Glucose

Carbohydrates ingested are converted to glycogen and stored in the liver. When energy is needed, the glycogen is converted to glucose. Blood glucose can then be used to monitor the animal's metabolism and physiology (Loeb 1999).

Blood Urea Nitrogen

Proteins ingested are broken down by the body. The by-product of metabolism is nitrogen-containing urea compounds. Since these compounds are of no use to the body, they are excreted by the kidneys. If the kidneys are not

functioning properly, excessively high levels of those compounds accumulate and are not removed from the body (Feldman and others 1982).

Creatinine

The kidneys excrete creatinine. If creatinine accumulates, it is indicative of decreased filtration rate of the kidneys (Loeb 1999).

Calcium

Calcium is a mineral that is found fairly consistently within the bloodstream. It is linked to osseous and muscle tissues, and as an enzyme activator in all tissues. Lowered calcium levels can lead to serious heart and muscle disorders (Loeb 1999).

Total Protein

Total protein is a measurement of both albumin and globulin. Albumin is produced in the liver while globulins are produced by the body's immune system. Elevated protein level is usually a sign of dehydration.

Bilirubin

Bilirubin is the by-product of the breakdown of hemoglobin. When blood cells die or are destroyed, hemoglobin is released and quickly broken down and excreted by the liver as bilirubin (Loeb 1999).

Alkaline Phosphatase

Alkaline phosphatase is an enzyme that functions to assist chemical reactions, specifically to hydrolyze a wide range of monophosphates at an alkaline pH. In dogs, higher levels of this enzyme may be seen in certain forms of cancer and some muscle and liver diseases (Loeb 1999).

Alanine Aminotransferase

Alanine aminotransferase (ALT) is an enzyme important in liver function. It catabolyzes amino acids and is involved in inter-organ nitrogen transport. Elevated ALT usually indicates that liver cells are breaking down for some reason and that waste products and toxins, it normally filters from the blood, cannot be removed via the bile duct (Loeb 1999).

Cholesterol

Cholesterol is synthesized de novo from acetate by the liver. Before excretion, cholesterol is esterified in the liver. Unlike the connotation seen in humans, cholesterol fluctuations in dogs are generally secondary signs of other diseases. Inadequately functioning thyroid glands often cause elevated cholesterol levels while animals with poor nourishment may have lower cholesterol (Loeb 1999).

Potassium

Potassium is the main intracellular cation. Intracellularly, it regulates osmotic pressure. In plasma, it influences acid-base relationships. With sodium, it maintains membrane potentials (Lodish and others 1995).

Sodium

Sodium and potassium levels are usually compared concurrently. Diseases of the adrenal glands, heart, and kidneys can severely affect their levels resulting in secondary problems such as preventing the heart, nerves, and kidneys from functioning correctly.

Aspartate Aminotransferase

Aspartate Aminotransferase (AST) is an enzyme similar to ALT in that it is associated with liver function. It is involved in amino acid synthesis and degradation, gluconeogenesis, and serves as a link between the urea and TCA cycles (Loeb 1999).

Gamma Glutamyltranspeptidase

Gamma Glutamyl Transpeptidase (GGT) is a liver enzyme that is involved in the transfer of amino acids across the cellular membrane and the regulation of intracellular growth stimulating hormone (GSH) (Loeb 1999).

Phosphorous

Along with forming the structure of DNA and RNA, cells also use phosphorous to transport cellular energy in the form of adenosine triphosphate (ATP) (Loeb 1999).

Amylase

Amylase is an enzyme, made by the pancreas, that breaks down starch into sugar. Elevated levels of amylase may be caused by acute pacreatitis (Loeb 1999).

Lipase

Lipase has the important functions in digestion, transportation, and processing of dietary lipids. It catalyzes the transfer of fatty acids to peripheral cells leading to the release of glycerol into circulation (Olivecrona and Bengtsson-Olivecrona 1993).

Chloride

Chloride is an electrolyte that is necessary for metabolism and helps maintain an acid-base balance in the body. The amount of chloride in the blood is

closely regulated by the kidneys. It exists in combination with sodium and functions in the maintenance of osmotic pressure (Loeb 1999).

Creatine Phosphokinase

Creatine Phosphokinase (CPK) is an enzyme associated with acute renal injury. It catalyzes the exchange of a phosphate moiety between creatine phosphate and ATP (Loeb 1999).

Triglycerides

Triglycerides are energy sources for metabolism and transporters of dietary fat. They aid in the hydrolysis of phospholipids from lipoproteins, specifically HDL (Olivecrona and Bengtsson-Olivecrona 1993).

Magnesium

Magnesium is an important enzyme activator. It regulates functions similarly to calcium but its potency is only one-half to one-third that of calcium (Loeb 1999).

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CHAPTER III

EFFECTS OF FEEDING *LACTOBACILLUS REUTERI* X-18 ON BLOOD CHEMISTRY AND IMMUNE PARAMETERS IN BEAGLE (*CANIS FAMILIARIS*) PUPPIES

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ABSTRACT

Lactobacillus reuteri is a probiotic culture that has been shown to increase the overall general well-being of animals and humans (Gilliland 1990) as well as help prevent infections of the gastrointestinal tract. For successful use as a probiotic in dogs, the bacterial species should be of canine intestinal origin since these species exhibit host specificity. In a previous study done in our laboratory (McCoy and Gilliland 2007), lactobacilli were isolated from dogs and characterized for potential use as a probiotic for dogs. The majority of isolates were identified as *L. reuteri.* Of these isolates, *L. reuteri* X-18, which was one of the most bile resistant strains, appeared to be the best prospective probiotic that could be used to supplement dog food.

The main objective of the present study was to evaluate the effects of feeding *L. reuteri* on various immune parameters in two-month old beagle puppies. Twenty beagle puppies were assigned to one of two treatments. Ten puppies received the probiotic *L. reuteri* X-18 in ~5-10 mL of 10% nonfat dry milk (1.0×10^9 CFU each) while the control group received only 10% nonfat dry milk. Treatments were fed twice daily in addition to dry dog food for eight weeks. At the end of each week, blood and fecal samples were collected and body weights were measured in order to adjust dry food rations for performance measures. Serum and fecal immunoglobulin (IgA, IgE, IgM and IgG) levels, complete blood counts, and biochemistry panels were monitored on a weekly basis over the period of two

months. All data collected was analyzed using repeated measures ANOVA. A p-value <0.05 was considered significant. The immunological results of the experiment showed a trend of higher levels of serum IgA and IgM for the treatment group, although differences were not significant. There were week effects (P<0.05) for serum IgA and IgG. Three blood chemistry analytes had significant treatment x week interactions: Alkaline Phosphatase (P=0.0243), Albumin (P=0.0060), and Creatinine (P=0.0047). Calcium had treatment effects (P=0.0180).

INTRODUCTION

The emphasis of past research on the use of probiotics for dogs has been mainly on alleviating lactose intolerance in dogs (Lewis and Freedman 1998). More recently, probiotic bacteria have been proven effective in the treatment of small intestinal bacterial overgrowth (SIBO) in dogs. This condition is described as an uncontrollable increase in gut bacterial population that results in the disruption of the intestinal microflora, causing the accumulation of *Escherichia coli*, *Salmonella*, *Campylobacter*, or *Clostridium* in the gut (Lewis and Freedman 1998). This ultimately leads to diarrhea and the shedding of potential pathogens into the animal's surroundings (Rutgers and others 1995). The addition of probiotics into dog food is thought to promote overall health over a dog's lifespan, particularly, the development of its immune system (Biourge and others 1998). A number of probiotic supplements are available commercially for use in dogs. However, none of those products are of canine origin. McCoy and Gilliland isolated and characterized species of *Lactobacillus* having potential for use as probiotics for dogs. Their results indicated that *Lactobacillus reuteri* was the predominant species isolated from dogs and the prospective species to use for potential probiotics based on bile tolerance, inhibitory action, and the production of the antimicrobial substance reuterin (2007). While the classical means of evaluating the efficacy of probiotics has been to look at feed conversion rates and weight gain, these traits are not relevant for companion animals. However, the treatment and prevention of common digestive disorders

and unfavorable changes in the gut microflora, as seen in SIBO, is a relevant application.

The aim of this study was to determine the effects of feeding a potential probiotic of canine origin, *Lactobacillus reuteri* X-18, on several biochemical analytes and immunological parameters in healthy beagle puppies.

MATERIALS AND METHODS

Source and Maintenance of Cultures

Lactobacillus reuteri X-18 was isolated in a previous study done in the same laboratory as the current study. The culture was obtained from the food microbiology culture collection at the Robert M. Kerr Food and Agricultural Products Center (FAPC) at Oklahoma State University. It was subcultured weekly using a 1% inoculum into 10 mL of sterile Lactobacilli deMan Rogosa and Sharpe (MRS) broth (Difco Laboratories, Detroit, MI) and incubated at 37°C for 18 hours. In between subcultures, the isolate was stored at 4°C and subcultured at least three consecutive times before use in the feeding trial.

Milk Preparations

A set of 100 mL aliquots of heat treated, 10% nonfat dry milk was prepared every three to four days and stored at 4° until use to feed the control group. Frozen concentrated *L. reuteri* X-18 was prepared as described by Gilliland and Rich (1990) except that the culture was grown statically in 500 mL of sterile MRS broth for 18 hours at 37°. Concentrated cultures were stored in liquid nitrogen until needed. Frozen cultures used were prepared one week prior to the start of the feeding trial. The population of viable cells per vial was determined by pour plating serially diluted concentrated culture in 0.1% peptone (Difco Laboratories, Detroit, MI) with 0.001% Antifoam A (Sigma-Aldrich, St. Louis, MO) using MRS agar. Plates were incubated 48 hours at 37°C. Based on the population, the appropriate number of

vials were thawed in cold water then added to the prepared milk to obtain a final population of 1.0 x 10^9 CFU/mL for the treatment group. Frequency and storage of milk preparations for the treatment group were the same as the control group. On both day 0 and day 3 of each set of milk preparations, confirmation of the dose of *L. reuteri* X-18 administered was done by pour plate method using MRS agar, with incubation at 37°C for 48 hours.

Feeding Trial

From one group of ten female dogs and another group of ten male dogs, five 2-month old beagle puppies of each sex were randomly allocated to one of two treatment groups. Thus, each treatment group consisted of 5 female and 5 male puppies; average body weights of each group were very similar. Each puppy was individually housed in a separate run and allowed water ad libidum. The puppies were cared for and handled in accordance with Oklahoma State University Institutional Animal Care and Use Committee guidelines. All necessary vaccinations were administered during the 8-week period and the general overall well-being of the animals monitored by an in-house veterinarian. The control group was fed only 5-10 mL of 10% nonfat dry milk along with a measured dry dog food ration twice daily. The treatment group was fed 5-10 mL of 10% nonfat dry milk containing 1.0 x 10^9 CFU/mL *L. reuteri* X-18 along with a measured dry dog food ration twice daily. A day 0 baseline samples were obtained from each animal prior to administration of the treatments.

Sample Collection

At the conclusion of each week, body weights, blood samples, and fecal samples were collected. Based on the recorded body weight of each animal, the following week's dry food ration was calculated and pre-weighed for each individual. After obtaining his or her body weight, each puppy was restrained to draw approximately 5 mL of blood from the jugular vein which was dispensed into individual blood collection vials to: 1) be analyzed by Antech Diagnostics (Irvine, CA) for a complete blood count and biochemical panel, and 2) be transported to 306 FAPC where immunoglobulins would be analyzed. Fecal samples were also collected at this time from the animal into sterile disposable centrifuge tubes (VWR, Suwanee, GA) and placed in ice in order to determine fecal immunoglobulin levels.

Processing of Fecal Samples

A portion of the fecal sample was used to make a fecal extract to determine fecal immunoglobulin levels. The fecal extraction method was performed as described by Peters and others (2004). The following immunoglobulins were measured: IgA, IgG, IgE, and IgM. An enzyme linked Immunosorbent assay (ELISA) quantitation kit for each canine immunoglobulin was used (Bethyl Laboratories, Inc., Montogomery, TX). The appropriate dilutions were determined and assays performed according to the manufacturer's instructions.

Processing of Blood Samples

Approximately 5 mL of blood was drawn from the jugular vein of each animal once every week for 8 weeks. Aliquots of one mL were dispensed into appropriate tubes (tubes containing EDTA) designated by Antech Diagnostics to be used for a complete blood count and biochemical panel. Blood samples were analyzed using an Olympus Model AU 5400 System (Center Valley, PA). The biochemical panel tested for: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, gamma glutamyl transferase (GGT), total protein, albumin, globulin, cholesterol, blood urea nitrogen (BUN), creatinine, phosphorous, calcium, glucose, amylase, lipase, sodium, potassium, chloride, creatine phosphokinase (CPK), triglycerides, and magnesium. The remaining portion of blood was transferred to sterile red top vacutainers (BD vacutainer, Franklin Lakes, NJ) and placed in ice then at 4°C overnight. The blood was centrifuged at 3,000 x g for 10 minutes to separate the serum. The serum was then transferred to properly labeled cyrovials and stored at -20°C until analysis. The blood serum was analyzed for IgA, IgG, IgE, and IgM. An ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX) for each canine immunoglobulin was used and analyses performed according to the manufacturer's recommendations.

Statistical Analysis

Serum and fecal immunoglobulin data along with the CBC and biochemistry panel data were analyzed using a repeated measures analysis of variance (ANOVA) using SAS v9.1 (SAS 2003). The first-order autoregressive covariance structure was deemed appropriate. The slice effect was used to test for any differences between treatments at various time points. Differences were considered significant at the $P<0.05$ level. Pair-wise differences were not needed to separate Least Squares Means because there were only two treatments.

RESULTS

Serum Immunoglobulins

Blood collection was carried out on a weekly basis over a period of eight weeks with the Day 0 collection time serving as the baseline since treatments were given following sample collection. Results for serum immunoglobulins A, M, E, and G are in Table 3.

Table 3. Effect of *L. reuteri* X-18 on Serum and Fecal IgA, IgM, IgE[1], and IgG in Beagles

Immunoglobulin	Treatment		SEM[3]	P-values		
	Control[2]	*L. reuteri* X-18[2]		Trt	Week	Trt x Week
Serum IgA (ng/mL)	5.3E+05	4.9E+05	4.1E+04	0.4506	0.0004	0.8407
Serum IgM (ng/mL)	1.7E+06	1.7E+06	1.4E+05	0.846	0.1292	0.4984
Serum IgE (ng/mL)	1.7E+04	1.4E+04	1.9E+03	0.2028	0.0551	0.3637
Serum IgG (ng/mL)	8.2E+06	9.3E+06	1.6E+06	0.649	0.0305	0.9115
Fecal IgA (ng/mL)	3.7E+05	3.1E+05	3.8E+04	0.2864	0.0269	0.2382
Fecal IgM (ng/mL)	6.9E+04	7.5E+04	1.5E+04	0.7297	0.0119	0.6764
Fecal IgG (ng/mL)	5.7E+06	5.6E+06	6.1E+05	0.9001	0.0174	0.8009

[1]Fecal IgE levels were not within the detectable range

[2]Least Squared Means

[3]Standard Error of the Mean

Serum IgA

While the overall serum IgA levels were not significantly different between the treatments (P=0.45), there was a significant difference between weeks (P=0.0004). There was no significant treatment x week interaction (P=0.84). However, the treatment group produced lower levels of serum IgA for all sample collection times than the control group except during week 7 (Figure 1).

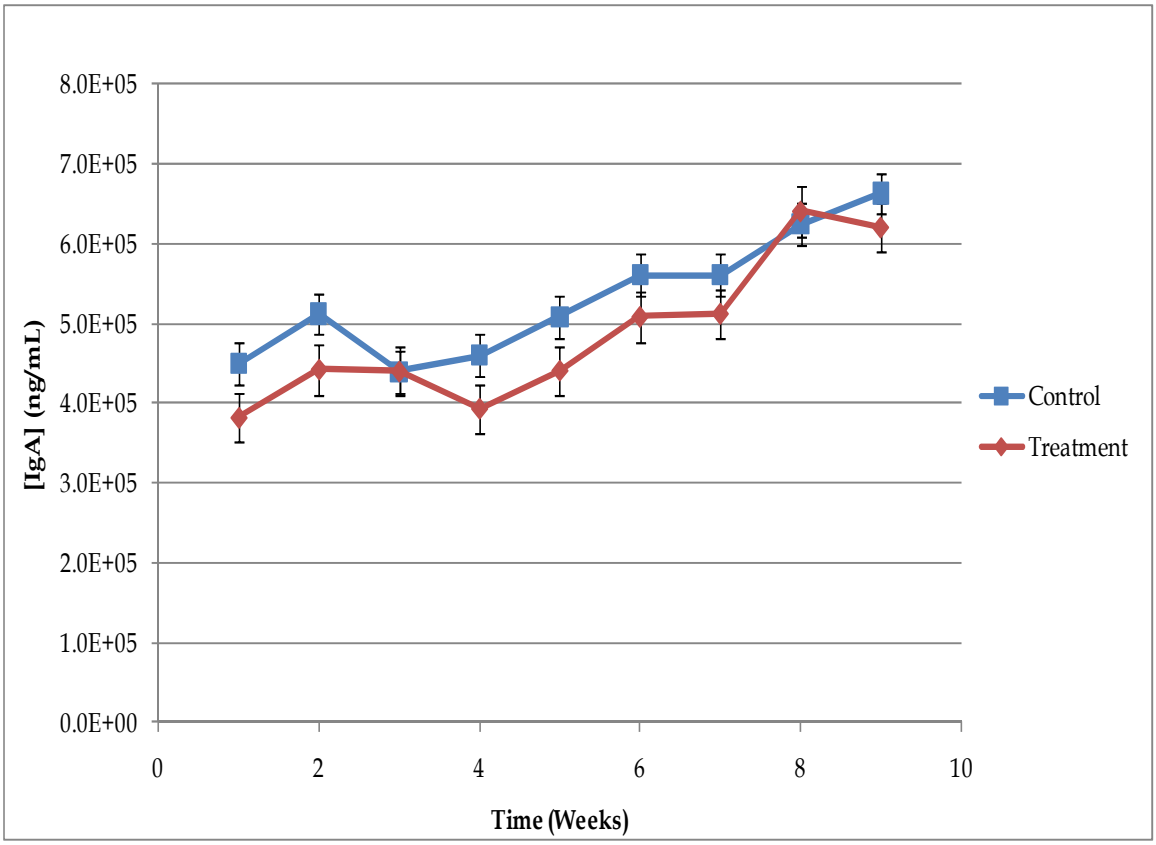

Figure 1. Comparison of serum IgA levels between the control group and the treatment group over a period of 8 weeks.

Serum IgM

There were no significant differences between treatments (P=0.85), by week (P=0.13), or treatment x week interaction (P=0.50) for serum IgM. The least square means of both groups were the same (Figure 2).

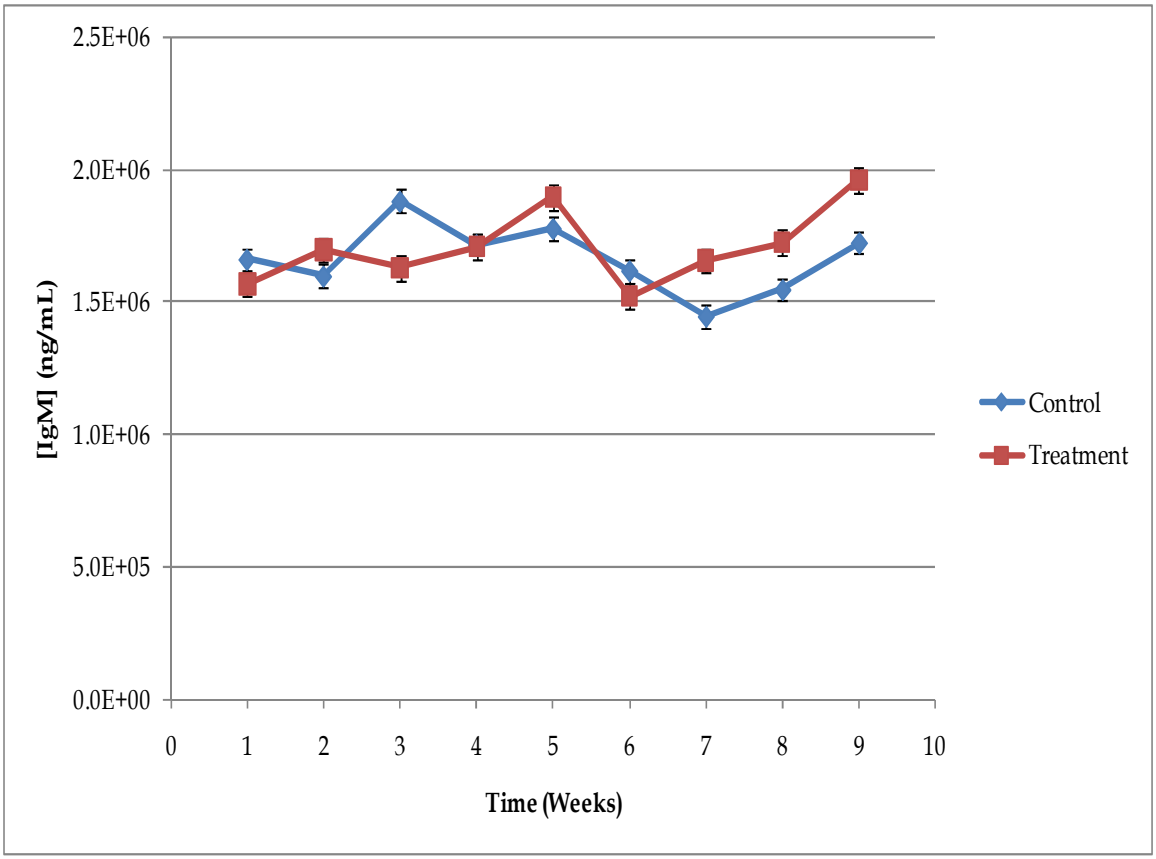

Figure 2. Comparison of serum IgM levels between the control group and the treatment group over a period of 8 weeks.

Serum IgE

The overall serum IgE levels were not significantly different between the treatments (P=0.20), and between weeks (P=0.06). There was no significant treatment x week interaction (P=0.36). Although not significant, the treatment group showed levels of serum IgE to be lower than the control group until week 8, while the control group experiences a decrease in levels starting at week 4 and continuing for the remainder of the trial (Figure 3).

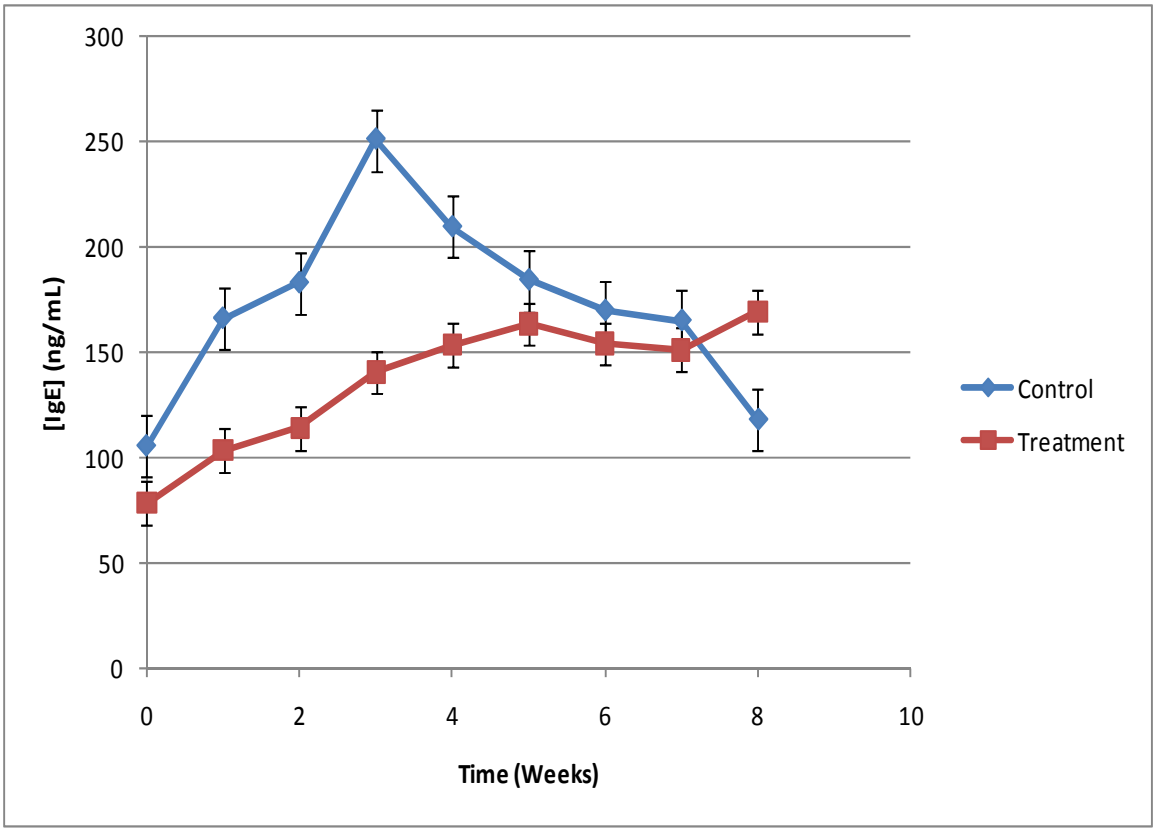

Figure 3. Comparison of serum IgE levels between the control group and the treatment group over a period of 8 weeks.

Serum IgG

The overall serum IgG levels were not significantly different between the treatments (P=0.65) but there was a significant difference between weeks (P=0.03). There was no significant treatment x week interaction (P=0.91). The least squared mean of the treatment group was higher than that of the control group (Figure 4).

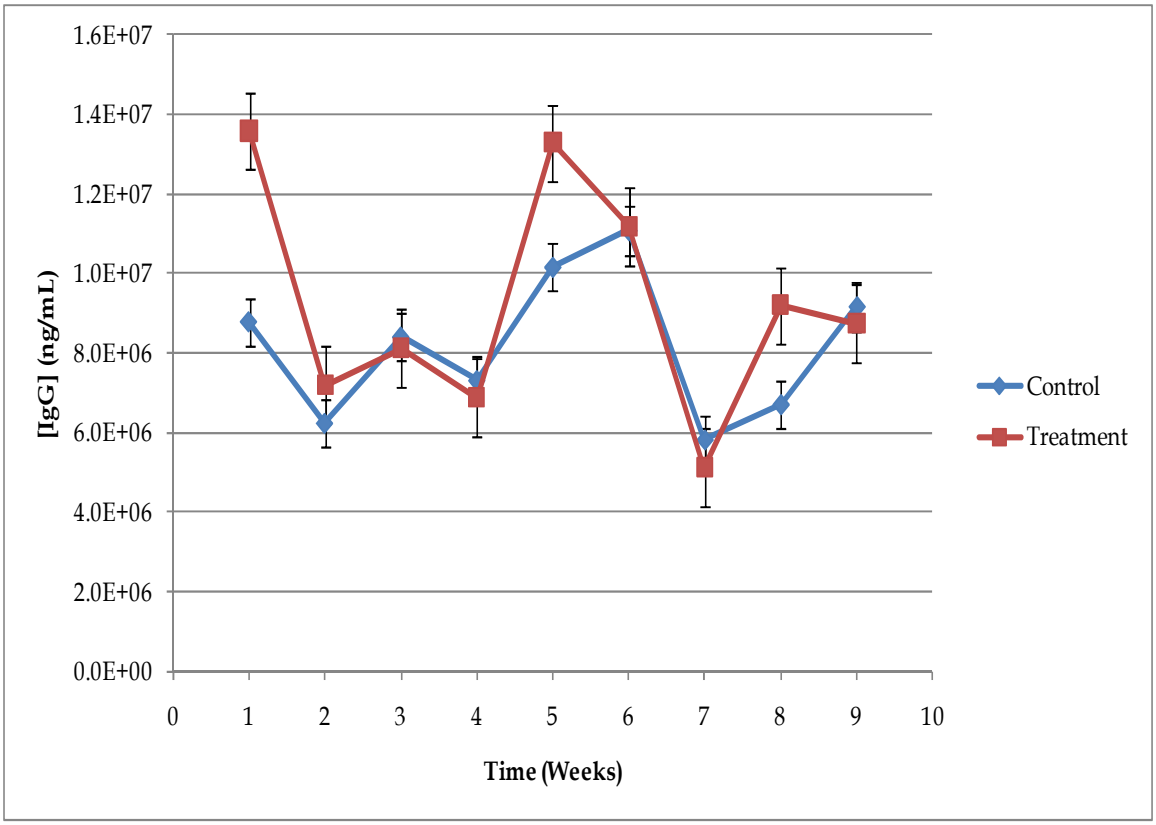

Figure 4. Comparison of serum IgG levels between the control group and the treatment group over a period of 8 weeks.

Fecal Immunoglobulins

Results for fecal immunoglobulins A, M, and G are given in Table 3. Fecal IgA had a significant week effect (P=0.03) with no significant treatment (P=0.29) or treatment x week (P=0.24) effect (Figure A1 Appendix A). Fecal IgM had a significant week effect (P=0.01) with no significant treatment (P=0.73) or treatment x week (P=0.68) effect (Figure A3 Appendix A). Fecal IgG had a significant week effect (P=0.02) with no significant treatment (P=0.90) or treatment x week (P=0.80) effect (Figure A2 Appendix A). Fecal IgE levels were not within the detectable range of the ELISA quantitation kits used.

Complete Blood Count

The complete blood count results are found in Table 4. Significant treatment effects could be seen for red blood cells (P=0.0247), mean cell volume (P=0.0056), mean cell hemoglobin concentration (P=0.0411), eosinophils (P=0.0355), and basophils (P=0.0500). Significant week effects could be seen in all measurements except for neutrophils (P=0.1733), eosinophils (P=0.3921), and basophils (P=0.1356). There were no significant (P>0.05) overall treatment x week effects seen.

Table 4. Effects of feeding *L. reuteri* X-18 on Complete Blood Count in Beagles.

Measurement	Treatment		SEM[2]	P-values		
	Control[1]	*L. reuteri* X-18[1]		Trt	Week	Trt x Week
White Blood Cells (WBC) (x 10^3/µL)	12.77	12.28	0.38	0.3712	0.0184	0.5132
Red Blood Cells (RBC) (x 10^6/µL)	5.96	6.18	0.06	0.0247	<0.0001	0.3113
Hemoglobin (HGB) (g/dL)	12.98	13.32	0.20	0.2358	<0.0001	0.1341
Hematocrit (HCT) (%)	39.76	40.73	0.48	0.1551	<0.0001	0.1635
Mean Cell Volume (MCV) (fL)	67.30	65.82	0.35	0.0056	<0.0001	0.7578
Mean Corpuscular Hemoglobin (MCH) (pg)	21.77	21.54	0.18	0.3838	<0.0001	0.3590
Mean Cell Hemoglobin Concentration (MCHC) (%)	32.48	32.80	0.11	0.0411	<0.0001	0.3014
Neutrophils (/µL)	8140.36	7387.97	366.13	0.1574	0.1733	0.2237
Lymphocytes (/µL)	3523.81	3796.36	154.67	0.2203	0.0017	0.4310
Monocytes (/µL)	813.56	754.54	33.77	0.2246	0.0132	0.0902
Eosinophils (/µL)	224.92	165.32	19.07	0.0355	0.3921	0.7593
Basophils (/µL)	73.56	97.95	8.61	0.0500	0.1356	0.9060
Platelet Count (x 103/µL)	518.62	484.66	18.24	0.1982	<0.0001	0.6636

[1]Least Squared Means;

[2]Standard Error of the Mean

Although no significant differences could be seen in treatment x week effects, the following observations were made from the data collected. The numbers of white blood cells were lower in the treatment group than the control group except for a slight spike during week 6 (Figure 5). The red blood cell counts were higher in the treatment group than the control group throughout the feeding trial (Figure 6). Levels of hemoglobin for the treatment group were higher than the control group except during week 4 where they were slightly lower (Figure 7). Mean cell volume (MCV) had a treatment ($P=0.0056$) and week effect ($P<0.0001$) and the MCV of the treatment group was lower than the control throughout the feeding trial (Figure 12). Lymphocyte counts for the treatment group were higher than those of the control group with the exception of week 5, where they were only slightly lower (Figure 8). Basophil counts were higher in the treatment group than the control group for the duration of the trial except for week 7 (Figure 9). Platelet counts for the treatment group were lower than the control group except for day 0 and week 8 (Figure 10). Eosinophil levels were consistently lower in the treatment group throughout the feeding trial (Figure 11). There were significant differences between control and treatment groups during various weeks. During week 1, the RBC and hematocrit levels in the treatment group were significantly higher in the treatment groups. During week 3, RBC levels were significantly higher in the treatment group than the control group. During week 4, the lymphocyte level was significantly higher in the treatment than the control group where as the neutrophil and monocyte levels in the treatment group were significantly lower than the control group. For week 5, the treatment group had significantly lower levels of

MCV than the control group and the treatment group had significantly higher levels of MCHC than the control group. During week 7, the same could be seen for MCV and MCHC. During week 8, the MCV levels were significantly higher in the treatment than control group.

Additional complete blood count parameters that were not statistically different between groups are in Figures C1 to C5 in Appendix C. Reference values for canine CBC parameters are included in Table C1 in Appendix C.

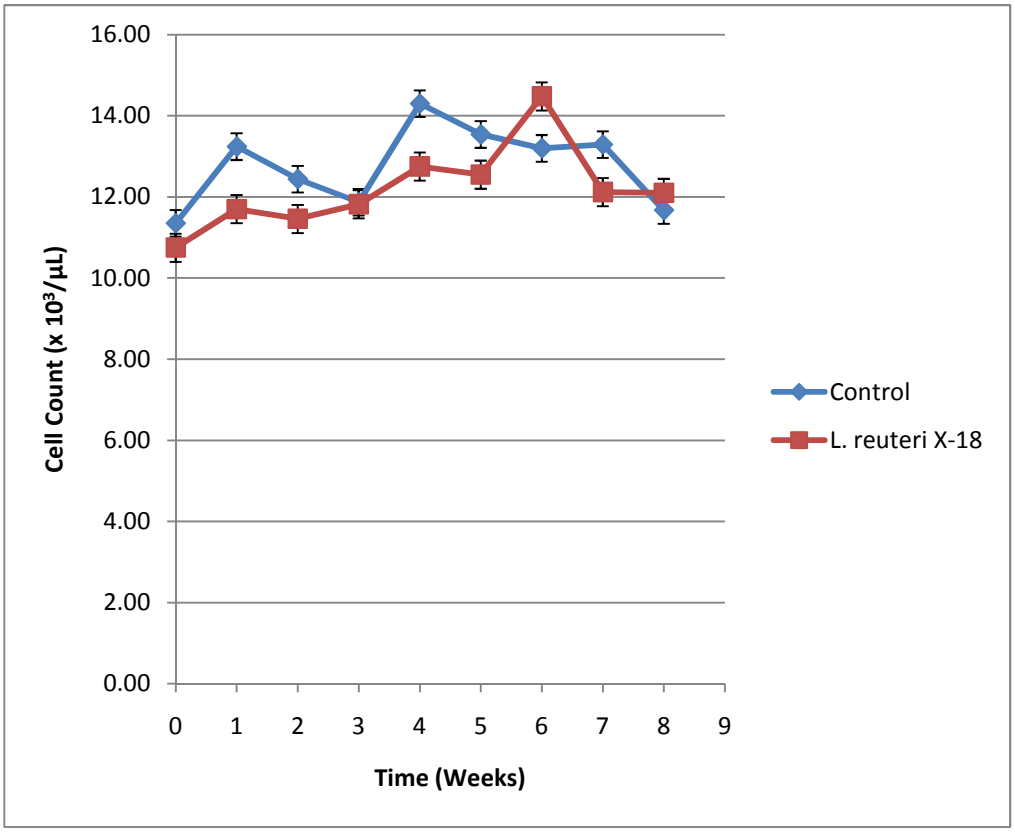

Figure 5. Comparison of white blood cell counts between the control group and the treatment group over a period of 8 weeks.

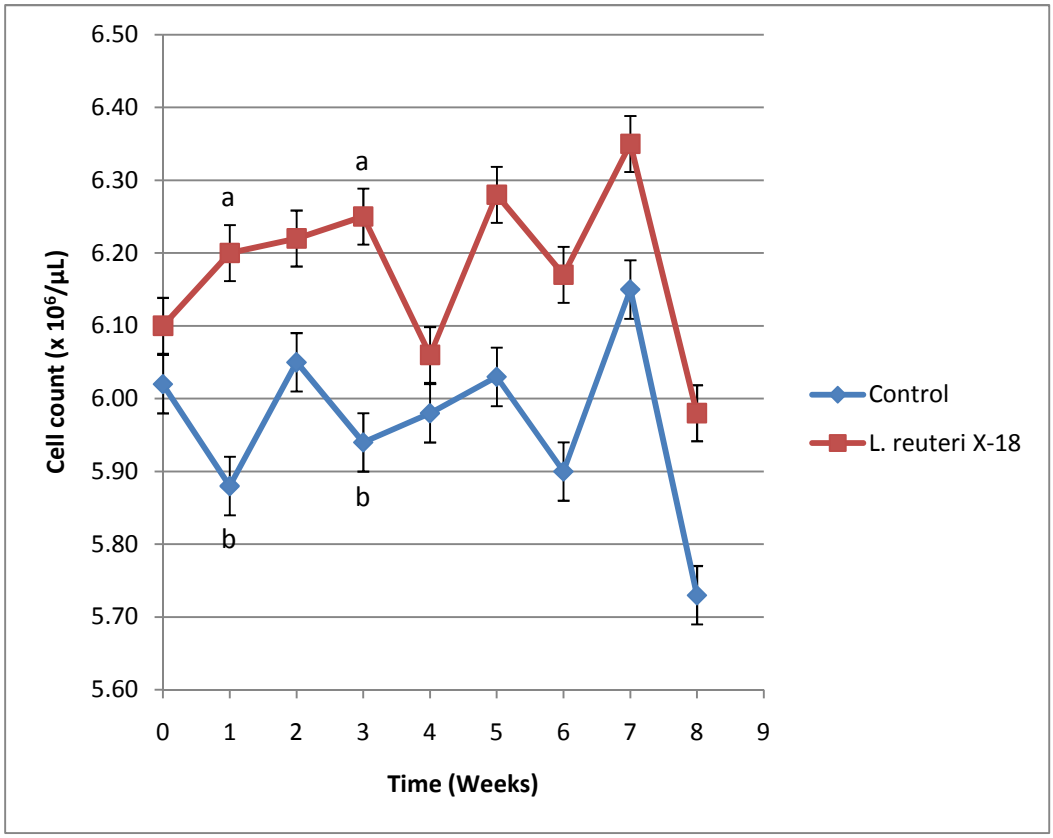

Figure 6. Comparison of red blood cell counts between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$).

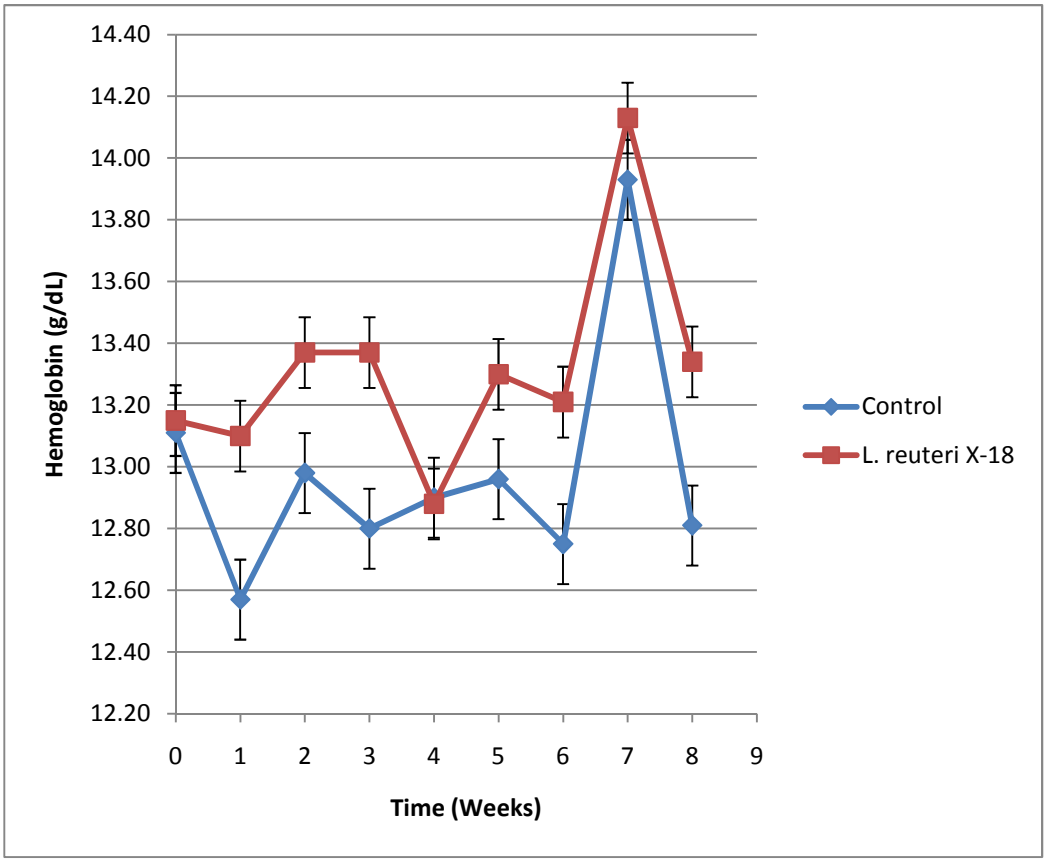

Figure 7. Comparison of levels of hemoglobin between the control group and the treatment group over a period of 8 weeks.

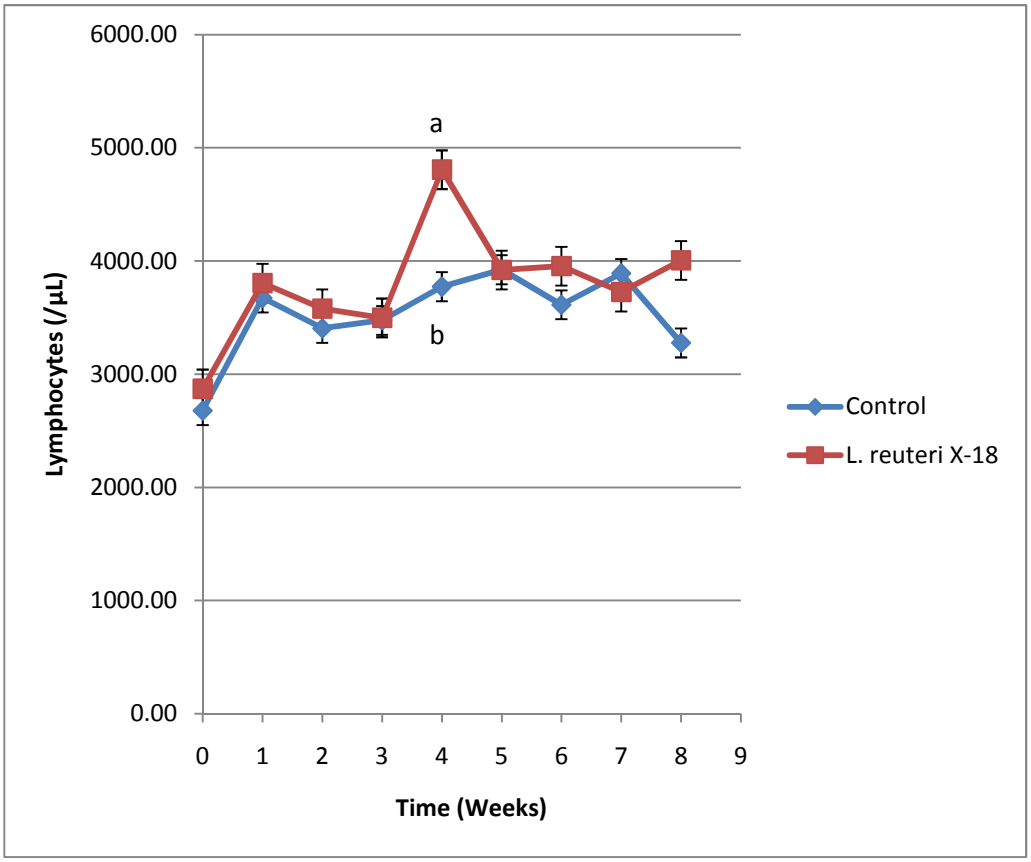

Figure 8. Comparison of lymphocyte counts between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$).

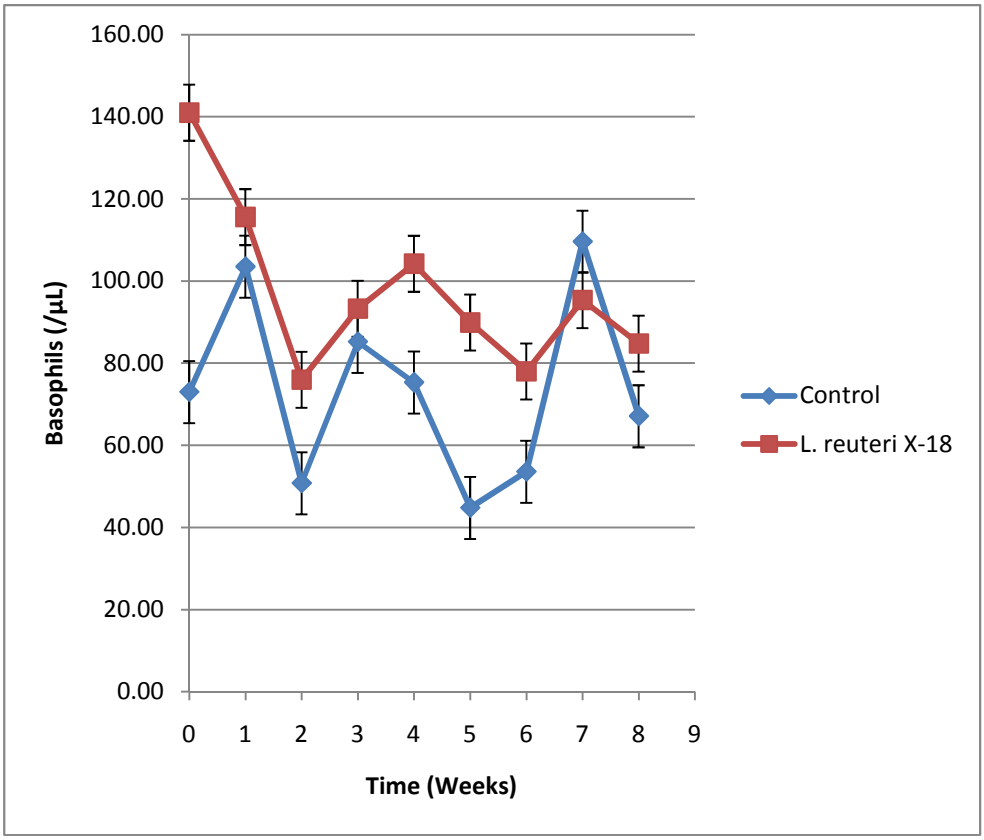

Figure 9. Comparison of basophil counts between the control group and the treatment group over a period of 8 weeks.

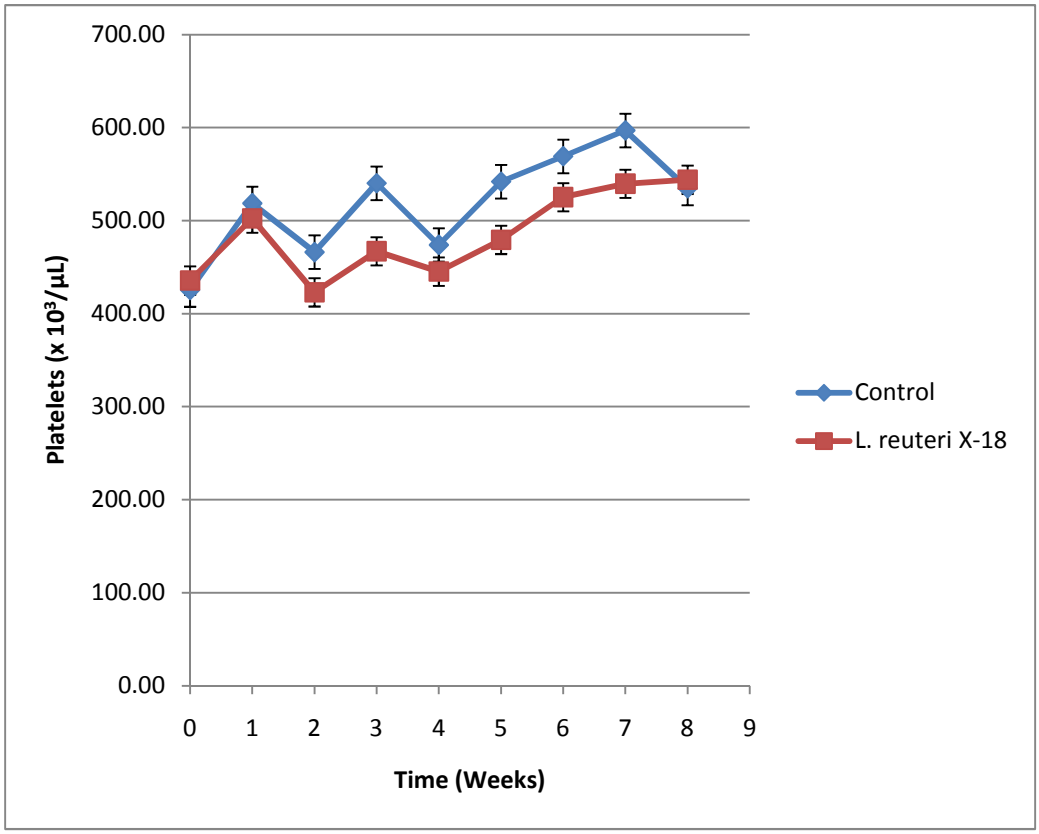

Figure 10. Comparison of platelet counts between the control group and the treatment group over a period of 8 weeks.

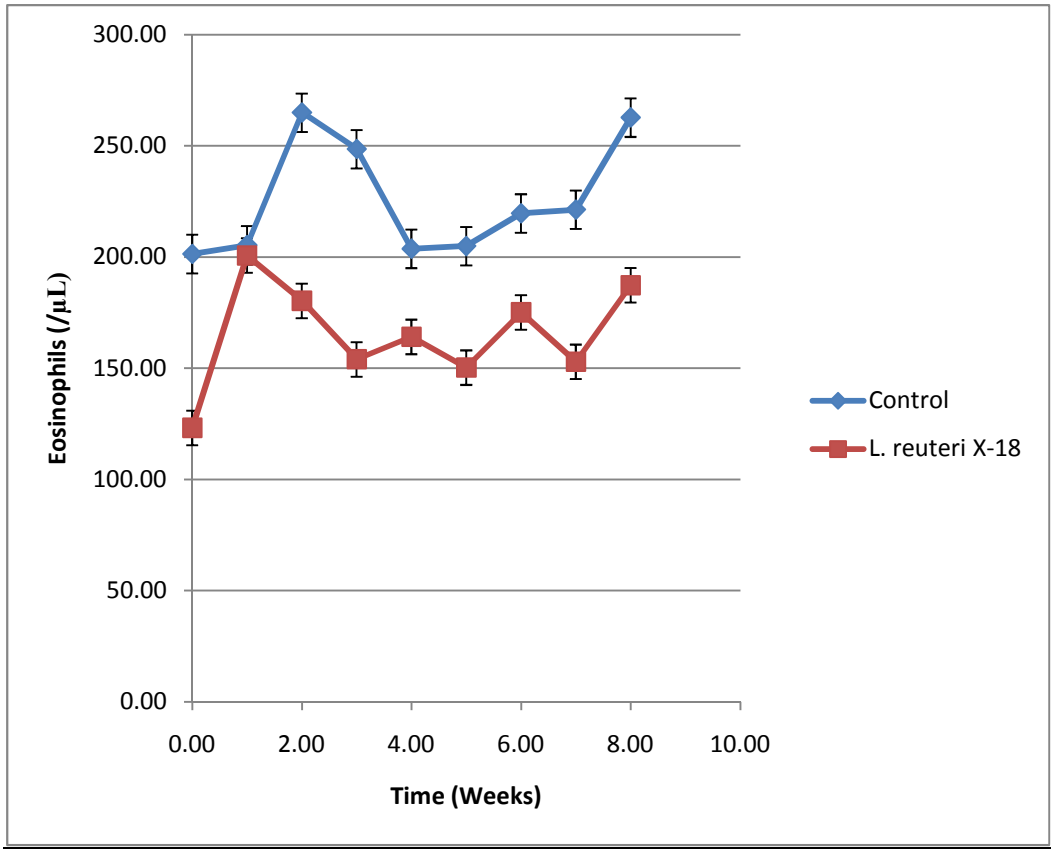

Figure 11. Comparison of eosinophil counts between the control group and the treatment group over a period of 8 weeks.

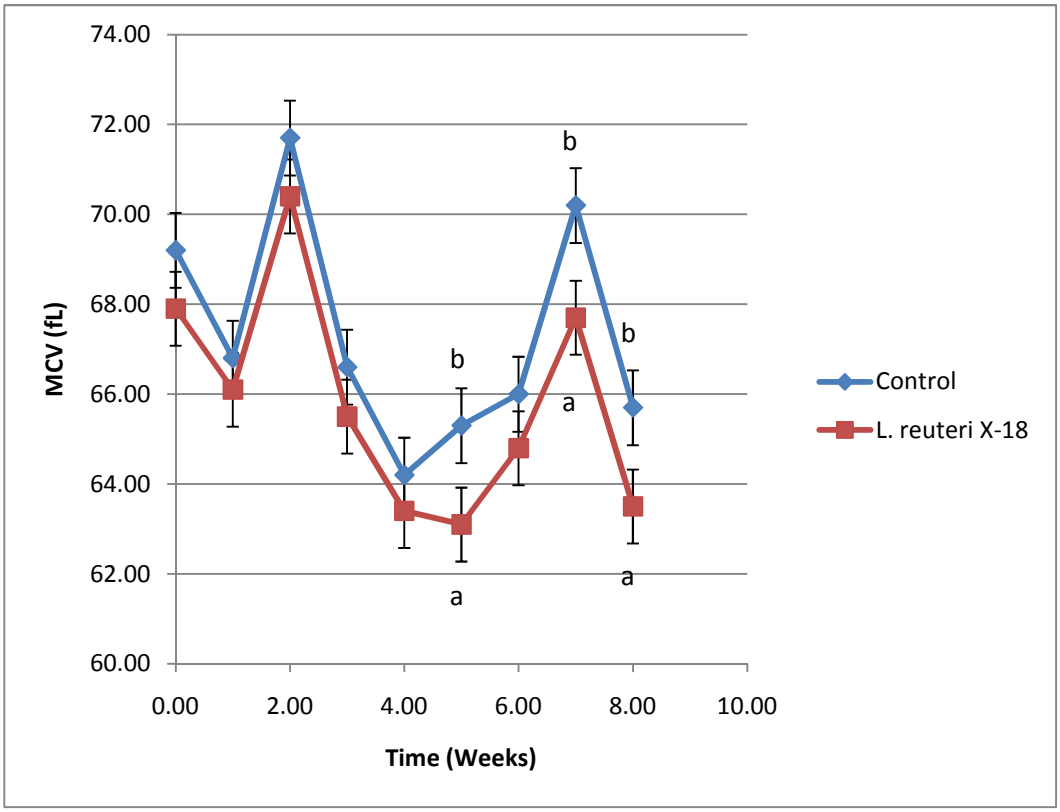

Figure 12. Comparison of mean cell volumes between the control group and the treatment group over a period of 8 weeks. [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

Blood Chemistry Panel

Overall statistical results for the blood chemistry panel are included in Table 5. All tested analytes showed a week effect ($P<0.05$). Calcium significantly differed by treatments ($P=0.0180$) and by week ($P<0.0001$) but not with a treatment x week effect ($P=0.48$). Calcium levels were higher in the treatment group than the control group except for weeks 6 and 7 (Figure 11). During weeks 1 and 3, calcium levels of the treatment group were significantly higher than the control groups ($P=0.0210$ and $P=0.0370$, respectively).

Total protein values showed significant difference between treatments ($P=0.0207$) and between weeks ($P<0.0001$) but not a significant treatment x week effect ($P=0.0803$). Throughout the feeding trial, the treatment group had higher total protein levels than the control group. During weeks 1, 3, and 7, total protein values were significantly higher in the treatment group than the control group ($P=0.0025$, $P=0.0329$, and $P=0.0397$, respectively) (Figure 13).

Aspartate aminotransferase (AST) showed a treatment effect ($P=0.0183$) and week effect ($P<0.0001$) but no treatment x week effect was observed ($P=0.7852$) (Figure 14). For the duration of the study, the treatment group had higher levels of AST than the control group. During week 1, AST values were significantly higher in the treatment group than the control group ($P=0.0110$).

Gamma glutamyl transferase (GGT) displayed significant treatment ($P=0.0211$), week ($P<0.0001$), and treatment x week ($P=0.0196$) effects (Figure 15). Values were generally higher in the treatment group than the control with the

exceptions of week 1 and week 8. GGT values were significantly higher in the treatment group than the control group during week 2 ($P<0.0001$) (Figure 15).

Week and treatment x week effects were observed with creatinine levels ($P<0.0001$ and $P=0.0047$, respectively). Significant differences could be seen between treatments on day 0 and week 1 ($P=0.0114$ and $P<0.0001$, respectively) (Figure 16).

Blood chemistry panel analytes that were not statistically different between treatments are in Figures B1 to B18 in Appendix B. Reference values for the canine blood biochemistry panel are included in Table B1 in Appendix B.

Table 5. Effects of *L. reuteri* X-18 on Blood Chemistry in Beagles

Measurement	Treatment		SEM[2]	P-values		
	Control[1]	*L. reuteri* X-18[1]		Trt	Week	Trt x Week
Aspartate Aminotransferase (IU/L)	31.88	33.90	0.59	0.0183	<0.0001	0.7852
Alanine Aminotransferase (IU/L)	35.64	37.61	2.47	0.5798	0.0004	0.9976
Total Bilirubin (mg/dL)	0.23	0.22	0.02	0.6101	0.0043	0.9514
Alkaline Phosphatase (IU/L)	135.11	136.69	5.86	0.8506	<0.0001	0.8671
Gamma Glutamyl Transferase (IU/L)	1.24	1.43	0.06	0.0211	<0.0001	0.0196
Total Protein (g/dL)	5.10	5.30	0.06	0.0207	<0.0001	0.0803
Albumin (g/dL)	3.01	3.14	0.05	0.0981	<0.0001	0.1266
Globulin (g/dL)	2.08	2.17	0.03	0.0629	0.0100	0.7343
Cholesterol (mg/dL)	133.97	138.74	4.94	0.5001	<0.0001	0.4794
BUN (mg/dL)	11.82	12.03	0.34	0.6598	<0.0001	0.8920
Creatinine (mg/dL)	0.56	0.57	0.02	0.5347	<0.0001	0.0047
Phosphorous (mg/dL)	7.69	7.58	0.08	0.3488	<0.0001	0.6443
Calcium (mg/dL)	10.86	11.05	0.05	0.0180	<0.0001	0.4838
Glucose (mg/dL)	37.80	42.04	2.27	0.1148	<0.0001	0.7383
Amylase (IU/L)	496.13	509.37	26.77	0.7300	<0.0001	0.6118
Lipase (IU/L)	192.29	185.46	24.56	0.8460	<0.0001	0.8897
Sodium (mEq/L)	145.16	145.48	0.21	0.2924	<0.0001	0.3549
Potassium (mEq/L)	4.87	4.93	0.04	0.2831	<0.0001	0.7241
Chloride (mEq/L)	107.47	107.69	0.26	0.5487	<0.0001	0.8034
Creatine Phosphokinase (IU/L)	370.73	346.06	15.68	0.2708	0.0006	0.6271
Triglycerides (mg/dL)	18.50	19.51	1.34	0.5989	<0.0001	0.9420
Magnesium (mEq/L)	1.54	1.55	0.04	0.9854	<0.0001	0.3664

[1]Least Squared Means

[2]Standard Error of the Means

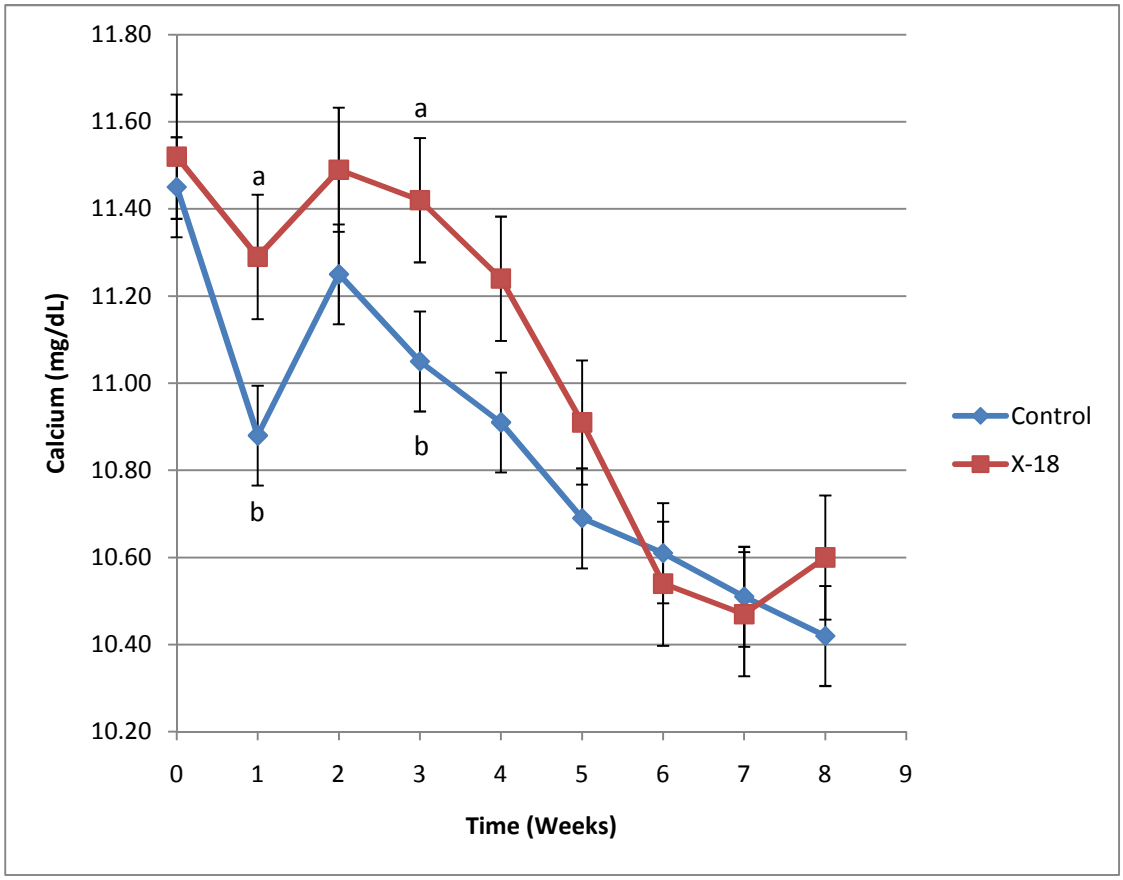

Figure 13. Comparison of levels of calcium between the control group and treatment group over the period of eight weeks; [a,b]Superscripts between trts on the same day are significantly different (P<0.05)

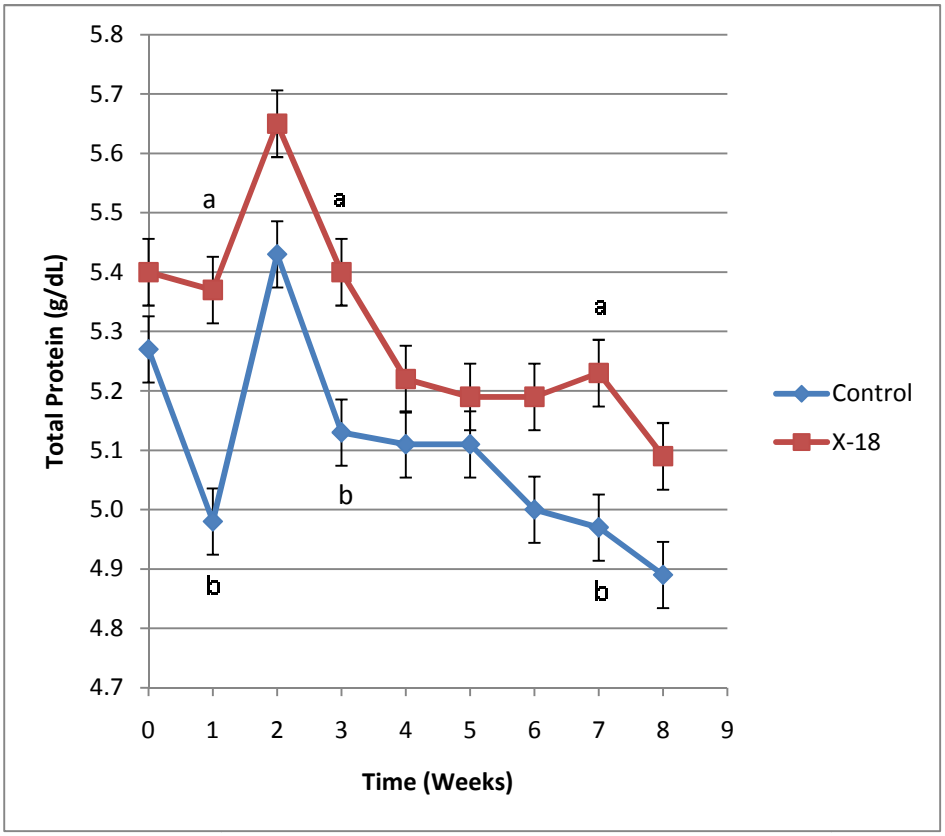

Figure 14. Comparison of levels of total protein between the control group and treatment group over the period of eight weeks; [a,b]Superscripts between trts on the same day are significantly different (P<0.05)

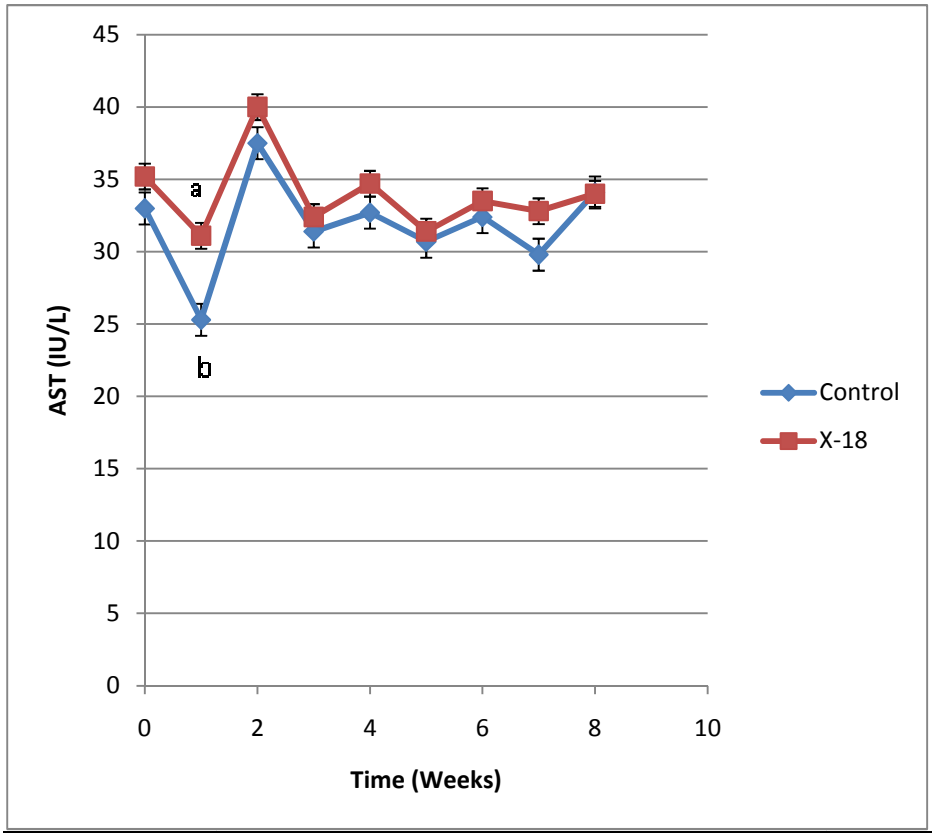

Figure 15. Comparison of levels of AST between the control group and treatment group over the period of eight weeks; [a,b]Superscripts between trts on the same day are significantly different (P<0.05)

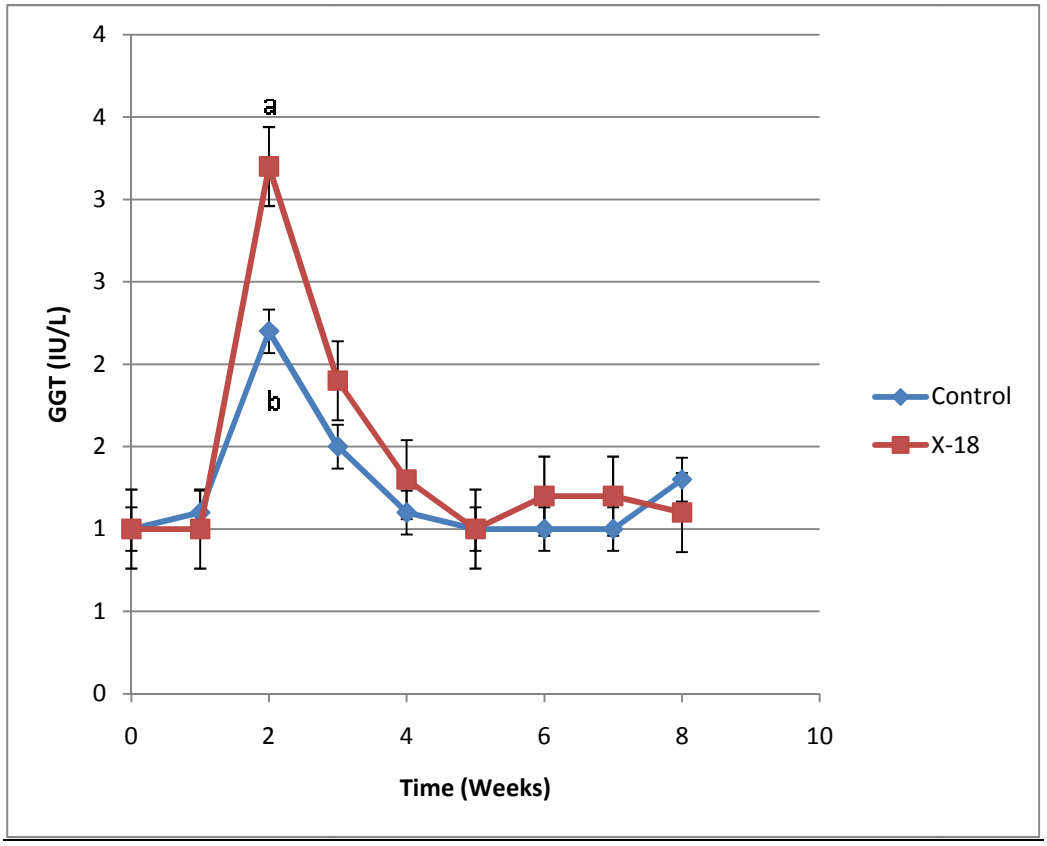

Figure 16. Comparison of levels of GGT between the control group and treatment group over the period of eight weeks; [a,b]Superscripts between trts on the same day are significantly different (P<0.05)

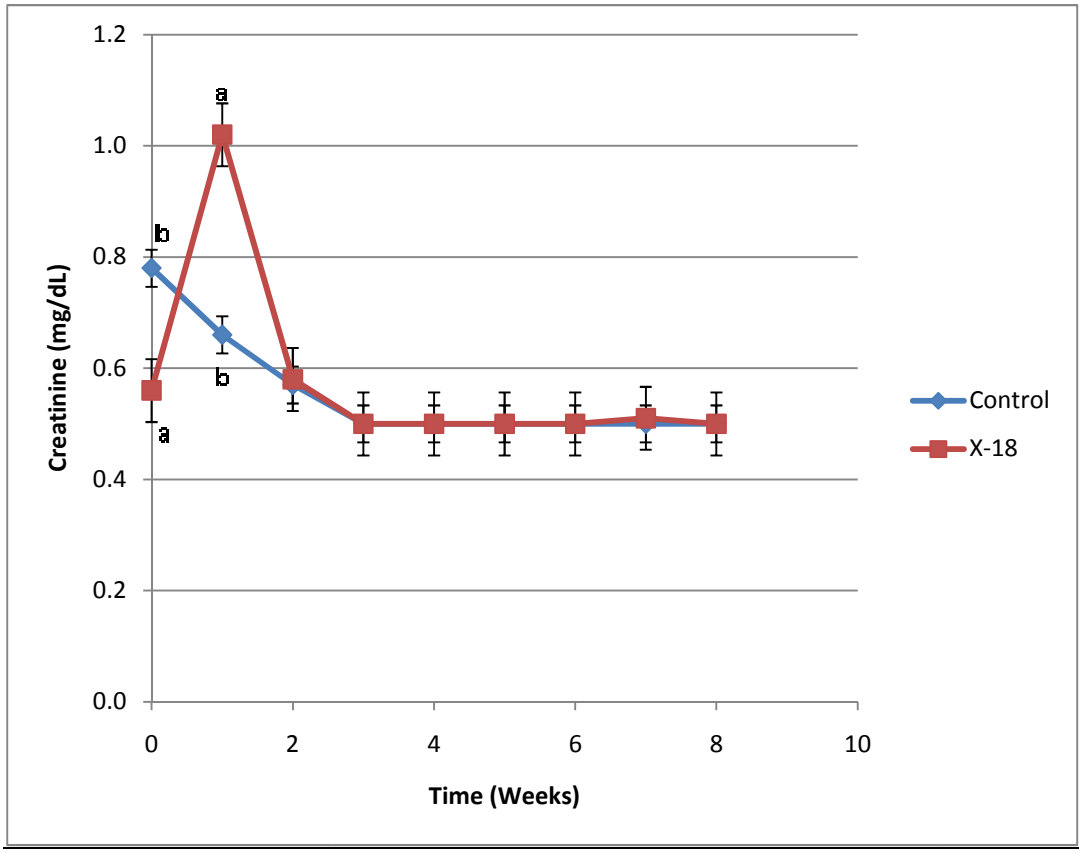

Figure 17. Comparison of levels of creatinine between the control group and treatment group over the period of eight weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

Body Weight

The dogs were randomly allocated to each treatment group based on body weight and sex (Table 6). The overall statistical results of body weights are included in Table 5. While there was no treatment (P=0.88) or treatment x week (P=0.82) effect, there was a significant difference by week (P<0.0001). Although not significantly different, the body weights of the treatment group were slightly higher than the control group except for week 8 (Figure 17 and Table 7).

Table 6. Dog Assignments for treatment groups.

Treatment[1]	ID	Gender	Weight (kg)
Control[2]	AIJ-7	M	4.48
	YTJ-7	M	3.82
	XYJ-7	M	3.69
	XEI-7	F	3.56
	XOJ-7	M	3.52
	YIJ-7	M	3.33
	YSI-7	F	3.16
	YKI-7	F	3.12
	ZVI-7	F	3.09
	YQI-7	F	2.76
	Mean		3.45
L. reuteri X-18[3]	XCJ-7	M	3.90
	ZTJ-7	M	3.81
	XZI-7	F	3.64
	AJJ-7	M	3.54
	YOJ-7	M	3.44
	YAI-7	F	3.35
	ZPI-7	F	3.23
	ZKI-7	F	3.17
	XQI-7	F	3.12
	YZJ-7	M	2.92
	Mean		3.41

[1]All treatments were fed 5 -10mL twice a day; Lactobacilli concentrations fed were ~1.0 x 10^9 CFU/mL

[2]Control group (n=10)

[3]*L. reuteri* X-18 (n=10)

Table 7. Effect of feeding *L. reuteri* X-18 on overall body weight of beagles

Measurement	Treatment		SEM[2]	P-value		
	Control[1]	Treatment[1]		Trt	Week	Trt x Week
Body Weight (kg)	4.59	4.63	0.20	0.8843	<0.0001	0.8193

[1]Least Squared Means

[2]Standard Error of the Mean

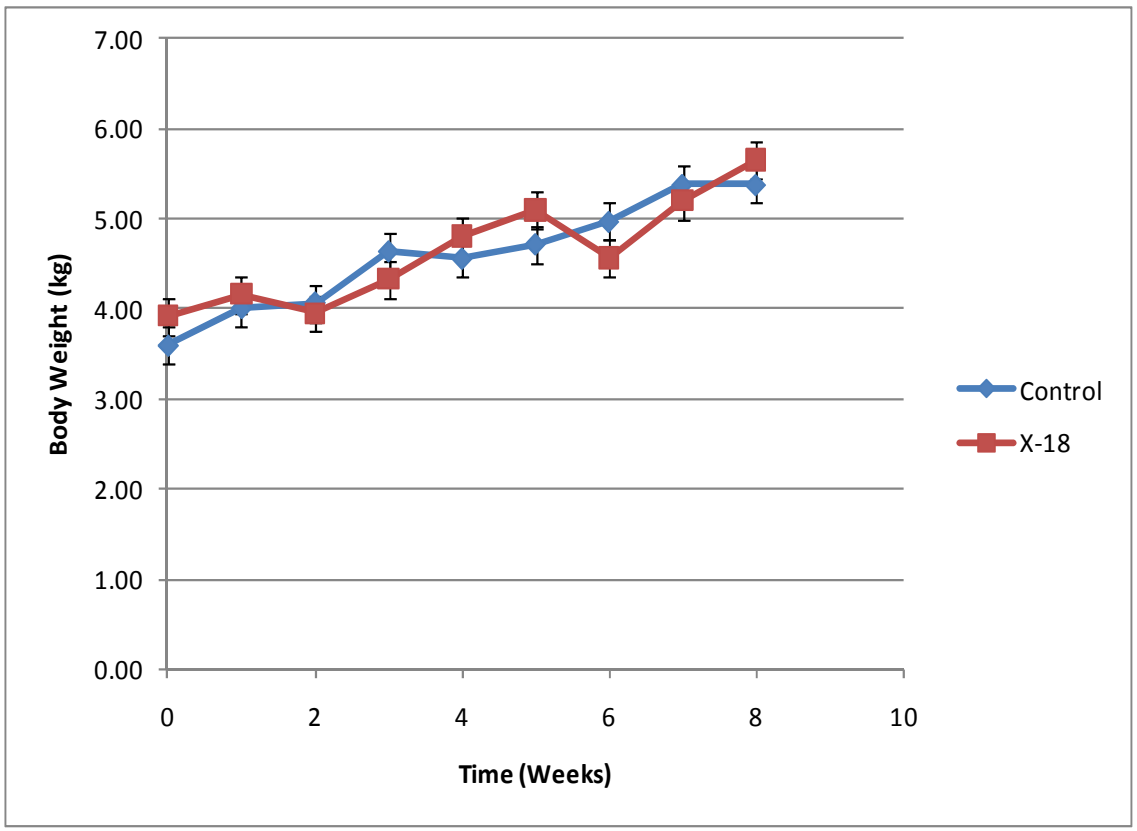

Figure 18. Comparison of body weights between the control group and treatment group over the period of eight weeks. Mean values of each treatment group were used.

Table 8. Weekly Dog Weights (kg)

Treatment[1]	Dog[1]	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Overall
Control	XOJ-7	3.90	4.14	4.41	4.71	4.89	5.25	5.18	5.65	5.78	
	XYJ-7	3.94	4.28	4.36	4.62	4.68	4.99	5.07	5.09	5.29	
	AIJ-7	4.75	4.94	5.25	5.48	4.76	5.92	6.26	6.35	6.65	
	YIJ-7	3.59	3.84	3.99	4.22	4.47	4.66	4.94	5.08	5.35	
	YQI-7	3.03	3.37	3.42	3.67	3.92	4.19	4.35	4.59	4.75	
	YSI-7	3.43	3.74	4.01	4.14	4.31	4.44	4.66	4.77	4.84	
	YKI-7	3.36	3.50	3.62	3.88	4.1	4.24	4.37	4.62	4.80	
	ZVI-7	3.29	3.43	3.56	3.72	3.87	3.96	4.14	4.14	4.34	
	YTJ-7	4.14	4.94	4.80	5.03	5.24	5.43	5.61	5.82	6.07	
	XEI-7	3.82	4.10	4.47	4.73	5.02	5.23	5.51	5.78	5.95	
	Mean	3.73	4.03	4.19	4.42	4.53	4.83	5.01	5.19	5.38	4.59
L. reuteri X-18	YOJ-7	3.70	4.07	4.28	4.72	4.97	5.24	5.46	5.64	5.89	
	AJJ-7	3.70	3.95	4.22	4.36	4.51	4.76	4.89	5.02	5.25	
	ZKI-7	3.34	3.45	3.67	3.82	3.99	4.12	4.24	4.26	4.25	
	YAI-7	3.55	3.67	3.94	4.19	4.26	4.44	4.68	4.77	5.02	
	XQI-7	3.43	3.78	4.02	4.2	4.45	4.64	4.86	5.02	5.21	
	ZTJ-7	4.12	4.41	4.73	5.03	5.35	5.57	5.83	6.01	6.27	
	YZJ-7	4.10	4.50	4.61	4.74	4.83	4.95	5.10	5.25	5.25	
	XZI-7	4.22	4.26	4.61	4.42	4.86	5.08	5.36	5.47	5.66	
	XCJ-7	4.31	4.62	4.68	4.12	5.17	5.41	5.51	5.62	5.65	
	ZPI-7	3.50	3.74	4.05	4.24	4.48	4.64	4.76	4.93	5.18	
	Mean	3.80	4.05	4.28	4.38	4.69	4.89	5.07	5.20	5.36	4.63

[1]Description of treatments and dogs - see Table 6.

DISCUSSION

Immunoglobulins

Immunoglobulins are gamma globulin proteins that are used by the immune system to identify and neutralize foreign particles such as bacteria or viruses. They are produced by plasma B cells. There are five kinds of antibodies grouped into different isotypes, IgG, IgA, IgM, and IgE usually observed in dogs. The antibody isotype of a B cell changes during cell development and activation. Immature B cells, which have never been exposed to an antigen, are known as naïve B cells and express only the IgM isotype in a cell surface bound form. B cells begin to express IgM when they reach maturity and are ready to react to antigens. After a response to an antigen, the cell divides and differentiates into an antibody producing cell called a plasma cell. In this stage, the B cell starts to produce antibody in a secreted form rather than a membrane-bound form. Some daughter cells of the activated B cells undergo isotype switching which causes the production of antibodies to change from IgM to the other isotypes, IgA, IgG, or IgE, which all have defined roles in the immune system. The measurement of serum immunoglobulin concentrations is one of the most commonly used methods of assessing immune competence in dogs.

Immunoglobulin G (IgG) is the major immunoglobulin produced by plasma cells (~70-75% of total immunoglobulins). It is the only antibody capable of crossing the placenta to give passive immunity. IgG is involved in secondary antibody response and is activated by proinflammatory cytokines. The mode protection against microorganisms is by agglutination, complement fixation,

opsonization, and neutralization (Burtis and others 2006). In this study, there was no significant difference between the control and treatment group. However, the mean concentration of serum IgG in the treatment group was higher than that of the control group with the value of the correlation coefficient being 0.75. We can deduce from this value that both groups had similar trends. Interestingly, both groups had elevated serum IgG concentrations compared to published reference values of $3.0 \times 10^6 - 6.0 \times 10^6$ ng/mL (Loeb 1999). This could be due to the fact that the parasitic infections that occurred prior to the feeding trial could have caused increases in IgG. While treatments clear feces of oocysts and classify animals as healthy, it is known that such infections can take months for the animals to produce enough antibodies to completely clear the parasite naturally.

Immunoglobulin A makes up 10-15% of total immunoglobulins. It is synthesized mainly by plasma cells in the mucous membranes of the gut, respiratory tract, and urogenital tract and can also be found in saliva and, tears, and breast milk. The mode of action of IgA is to prevent colonization by pathogens and initiate inflammatory reactions (Burtis and others 2006). One might expect that the probiotic treatment would enhance concentrations of IgA. However, there was no significant difference between the groups with concentrations of IgA in the treatment group being slightly lower than those in the control group. Similarly to serum IgG, the value of the correlation coefficient was 0.95 indicating a very strong correlation to each other. Compared to the reference value, 8.0×10^5 ng/mL (Loeb 1999), concentrations of serum IgA in this study were found to be slightly lower.

Immunoglobulin M (IgM) plays the role of complement activator and makes up 5-10% of total immunoglobulins. The role this immunoglobulin plays is in eliminating pathogens in the early stages of humoral immunity before there is sufficient IgG therefore appearing early in the course of an infection (Burtis and others 2006). There was not a significant difference in IgM concentrations between the two groups with the mean of the concentrations being the same for both groups. In comparison to the reference value of 1.5×10^6 ng/mL (Loeb 1999), the concentration of both groups were slightly higher. As in the case of IgG, the natural clearing of the parasite by innate antibodies can take several months. This elevated level of IgM correlates to the elevated IgG concentrations also observed.

Immunoglobulin E (IgE) is usually found in trace amounts since it tightly binds to mast cells. Allergens bind IgE, stimulating mast cells to release histamine, cytokines, and other inflammatory mediators. IgE has also been shown to protect against parasites (Burtis and others 2006). No significant difference was seen between the groups. To aid in decreasing hypersensitivity to allergens, one would expect enhanced concentrations of serum IgE by ingesting a probiotic culture. Unfortunately, levels of serum IgE in the treatment group were found to be slightly lower than those in the control group.

Only significant week effects could be seen when comparing fecal immunoglobulin concentrations of both control and treatment groups. Extremely high levels of variation were expected (Loeb 1999) and data collected supported this. It has been the center of much debate on whether or not fecal immunoglobulin concentrations are an accurate depiction of the immune system. As seen in previous

studies and the current study, variability in concentrations render these results inconsequential.

In a similar study using *Lactobacillus acidophilus*, the findings showed an increase in serum IgG (Baillon and others 2004) and the findings in this study are comparable.

Complete Blood Count and Blood Chemistry Panel

The standard research dog is the beagle. Their disposition, temperament, and size make this breed a suitable choice for scientific studies. Stress alters the results of various clinical chemistry determinations so the most uniform data is obtained by acclimating the dogs, housing them in a stress-free environment, and handling them gently but firmly when drawing blood samples (Loeb 1999).

The complete blood count (CBC) is a profile of tests used to describe the quantity and quality of the cellular components in blood and some substances in plasma. The CBC should be evaluated systematically and results outside reference values for that species are usually considered abnormal. However, reference values are often not optimal since they are derived from limited numbers of adult animals not segregated by age, sex, or breed (Willard and Tvedten 2004). Prominent age-related changes occur in dogs. At birth, red blood cells (RBCs) are very large with a mean corpuscular volume (MCV) about 95 fl. The MCV decreases to adult values by 2 to 3 months of age. At 5 to 6 weeks of age, puppies normally have a packed cell volume (PCV) or hematocrit % around 30%, and total protein around 5.3 g/dL.

These values could be confused with anemia if compared with adult reference ranges (Willard and Tvedten 2004).

A complete CBC was evaluated for each dog in this study from blood collections done weekly throughout the duration of the feeding trial. Following are the conclusions that were made based on those results.

White blood cells (WBC) or leukocytes are the cells of the immune system. They can be divided into two major groups: 1) Granulocytes and 2) Agranulocytes. Granulocytes have membrane-bound enzymes that aid in endocytosis. Cells included in this group are neutrophils, basophils, and eosinophils. Agranulocytes, contrary to their name have granules which are lysosomes. Cells included in this group are lymphocytes and monocytes. Neutrophils are the frontline attack cell in acute inflammation and the most abundant leukocyte. They phagocytize and kill microorganisms and can release antibacterial substances such as defensin and lysozyme (Gaunt 2004). Basophils have receptors for IgE and augments hypersensitivity reactions to allergens, leading to vascular permeability, induction of bronchoconstriction, and inhibition of coagulation. Basophils are present in relatively low numbers in dogs (Gaunt 2004). Eosinophils initiate the inflammation response and can suppress immediate-type hypersensitivity. If eosinophils are present in high numbers, cytotoxic mediators can injure host tissues. Therefore, they are usually present in very low numbers (Gaunt 2004). Monocytes develop into macrophages which have immunoglobulin and complement receptors to help enhance phagocytosis. They essentially function as antigen-presenting cells (APC) for humoral and cell-mediated immune responses (Gaunt 2004). Lymphocytes are

involved in immune function. Lymphocytes consist of B lymphocytes and plasma cells that produce antibody to specific antigens, providing a humoral response, and T lymphocytes that are involved in immunoregulation of antibody-mediated and cell-mediated immune responses. Lymphocytes are the second most abundant leukocyte (Gaunt 2004).

Red blood cells (RBC) or erythrocytes are the principal transporters of oxygen to from lungs to body tissues and carbon dioxide in the opposite direction. RBC's transport function is possible through the use of the iron-containing molecule, hemoglobin, present in the cell's cytoplasm (Meuten and Thrall 2002). In a CBC, erythrocytes are evaluated by looking at a panel of test parameters. This panel consisted of hemoglobin concentration, % hematocrit or packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and platelets. Hematocrit determines the proportion of blood volume occupied by RBCs, MCV is the average volume of a RBC, MCH is the amount of hemoglobin, by weight, in the average RBC, MCHC is the concentration of hemoglobin in the average RBC, and platelets function in hemostasis (Meuten and Thrall 2002). The results of the CBC done in this study showed that all measurements were, except for platelets and eosinophils , within the reference range determined in-house by Antech Diagnostics in Irvine, CA. Both control and treatment groups showed elevated levels of platelets and eosinophils compared to their respective reference ranges. However, the values were not so extreme to cause alarm especially knowing that reference ranges are not optimal values due to variations in breed, age, and sex. To note, when comparing least

square means of these “abnormal” measurements, the treatment groups had lower numbers of eosinophils and platelets compared to the control groups.

Studies on the effect of age on numerous clinical chemistry and hematology parameters in healthy dogs have found that even when statistically significant, the changes tended to be fairly small. Albumin and serum iron decreased with age, while potassium, globulin, and total protein increased. Age-related increases in GGT and amylase were also observed (Lowseth and others 1990; Strasser and others 1993; Vajdovich and others 1997). In this study, the following conclusions could be made.

When focusing on the least square means of both the control and treatment groups, all values were within the reference range and considered normal. However, there were three exceptions. Phosphorous and alkaline phosphatase levels of both the control and treatment groups were elevated compared to the reference values while triglyceride levels of both the control and treatment groups were below the reference range. The elevated phosphorous and alkaline phosphatase levels are not necessarily abnormal since this is commonly seen in young, growing animals. As in the case with similar results seen in the CBC, these values are not extreme enough to cause concern and the animals would still be considered healthy rather than diseased. Interestingly, significant differences between the control group and treatment group could be seen during various weeks. On day 0, creatinine levels in the treatment group were significantly lower than the control group. On the same day, magnesium levels in the treatment group were significantly higher than the control group. During week 1, the treatment

groups were significantly higher than the control group for the following analytes: AST, total protein, albumin, creatinine, calcium, and potassium. During week 2, levels of GGT in the treatment group were significantly higher than those in the control group. During week 3, levels of total protein, albulmin, and calcium were significantly higher in the treatment group than the control group. During week 7, levels of globulin were significantly higher in the treatment group than those in the control group.

Overall, no significant differences could be seen between the control and treatment groups with respect to immunoglobulins. For the CBC, the least square means of both the control and treatment groups were within the reference range with only two parameters (eosinophils and platelets) being slightly elevated. Those elevated values could be due to typical variations as a result of the testing profiles that were used to determine the reference ranges (sex, breed, and age). Based on these results, the animals were still considered healthy with no extreme effects seen. For the biochemistry panel, only GGT and creatinine show a significant treatment x week effect. The least square means of both the control and treatment groups were within the reference range, which are consistent with the healthy status of the dogs.

Possible confounding factors

There were a number of factors observed during the course of the study apart from the probiotic treatment that may have contributed to the results observed. These included routine vaccinations, parasitic infections, dehydration, and nictitans gland prolapsed. These factors and their possible influences are discussed below.

Vaccinations

Vaccinations work by presenting a foreign antigen to the immune system in order to evoke an immune response, essentially an artificially induced immune response. Vaccines are classified as live (attenuated) or killed (inactivated). As in Jenner's discovery of the smallpox vaccine, a live vaccine stimulates immunity via a transient infection produced by a live, replicating organism (Jenner 1798). Inactivated vaccines consist of killed organisms or non-replicating sub-cellular components that stimulate a lower level and shorter duration of immunity that that of live vaccines. Therefore, adjuvants are used in combination with killed vaccines to enhance antibody response (Kwak and Longo 1996). Adjuvants can stimulate innate immunity or adaptive immunity. Innate immunity adjuvants such as lipopolysaccharide (LPS), bind antigen-presenting cells to produce inflammatory cytokines and activate adaptive immunity while adaptive immunity adjuvants such as cytokines, bind and activate highly specific receptors on T cells (Marciani 2003). The widespread use of vaccines in dogs became possible with the development and commercial availability of egg-adapted and tissue culture-derived modified live distemper and adenovirus vaccines (Piercy 1961). As vaccination became widespread and less clinical disease occurred among domestic dogs, it was suggested that the natural re-exposure of vaccinated dogs would decrease, leading to a reduction in natural boosting. It was believed that this natural boosting was necessary for the maintenance of long-term population immunity (Piercy 1961) and, as a result, recommendations were made for vaccines to be administered annually. Since the 1960s, the annual vaccination of animals has become established as a

standard of practice and has reduced the incidence of some diseases (McCaw and others 1998). Vaccines can be grouped into 3 categories. Core vaccines include those against canine distemper, canine parvovirus (cpv), canine adenovirus (cAV), and rabies virus. Non-core vaccines are those against parainfluenza virus, *Bordetella* species, and *Leptospira* species. Vaccines against *Borrelia* species and coronavirus are considered non-core or not recommended, depending on the source (Schultz 1998). The time vaccinations are administered range from 5 weeks to twelve and fifteen weeks of age (AMVA 2002). According to records of the animals used in this feeding trial, the following vaccinations were given accordingly:

Table 9. Vaccinations administered to beagle puppies

Date	CPI[a]	DHL[b]	CPV[c]	BOR[d]	C. PAP[e]	DUV[f]
06/07/07				X		
06/14/07			X			
06/28/07			X			
07/02/07					X	
07/03/07	X	X	X			
07/06/07						X

[a]Canine Parainfluenza
[b]Canine distemper, Adenovirus Type 2, Leptospirosis
[c]Canine parvovirus
[d]Bordetella
[e]Canine papilloma
[f]Duramune

The vaccinations were administered before delivery of the animals to OSU Veterinary Medicine/Animal Resources with the last vaccination, given at OSU, one month prior to the start of the feeding trial. This is important to note since the

major goal of the trial was to study immune effects of dogs upon ingestion of a probiotic. Because of the immunostimulatory effects vaccinations confer, vaccinations administered during the feeding trial would alter immune parameters, preventing one from differentiating between the effects of the probiotic with the effects of the vaccine.

Parasitic Infections

Giardia has a broad host range, although the adverse consequences of *Giardia* infection and its pathogenic potential are best recognized in humans. Its simple life cycle provides ample opportunities for the parasite to be transmitted directly from one infected individual to another, or indirectly through contamination of the environment or food. In this respect, water is an important vehicle for the transmission of *Giardia*. The pathogenesis of *Giardia* has not been elucidated and symptoms, which include acute or chronic diarrhea, dehydration, abdominal pain and weight loss, are highly variable (Thompson and others 1993), and may not be evident in a significant proportion of infected individuals (Rodriguez-Hernandez and others 1996). Pathophysiological processes upon infection include altered, increased epithelial permeability that results in an inflammatory response and both digestive and absorptive changes (Scott and others 2002). *Giardia* also induces apoptosis that correlates with the loss of epithelial barrier function and the subsequent increase in permeability (Chin and others 2002). However, apoptosis and the severity of disease are determined by strain-dependent virulence factors of the parasite, as well as by the developmental, nutritional, and immunological status of the host (Chin and others 2002; Scott and

others 2002). Increased intestinal permeability may result in the uptake of luminal antigens which may exacerbate the occurrence of allergic disorders, a complication often reported in humans infected with *Giardia* (Scott and others 2002) and a factor that may confound the etiology of nutritional disorders. Of particular note in reference to the possible increase in allergic disorders, the levels of eosinophils found in this study were elevated. As you may recall, eosinophils are the immune cells responsible for allergic, hypersensitivity reactions. This increase could possibly be related to the concurrent parasitic infection. Although *Giardia* is common in dogs, it is rarely associated with clinical disease in these animals. However, if clinical giardiasis is reported, it is usually associated with kennel situations where the effects of overcrowding may cause stress and exacerbate the effects of an infection (Robertson and others 2000). Infections with *Giardia* stimulate humoral immunity that results in a self-limiting infection in many animal species (Olson and others 2000). Unfortunately, it may take several months for the host to produce protective antibodies that can eliminate the parasite. Treatment of *Giardia*-infected dogs is usually recommended whether or not they are clinically ill because of the perceived potential for zoonotic transmission and treatment generally consists of oral suspensions of benzimidazoles.

Isospora species are protozoan parasites that are members of the group of organisms referred to as coccidia. Coccidia are identified based on the structure of their sporulated oocyst stage. The oocyst stage is an environmentally resistant stage which is excreted in the host feces (Lindsay and others 1997). The presence of oocysts in the feces is not always indicative of clinical infection (Lindsay and

Blagburn 1991). *Isospora* species can cause serious disease in humans and nursing pigs. However, in dogs, clinical disease is seldom seen (Lindsay and others 1997). The diagnosis of coccidiosis in animals is based on clinical signs such as diarrhea, history, evaluation for potential co-pathogens, and demonstration of coccidial oocysts of a pathogenic species in the animal's feces (Lindsay and others 1997). *Isospora* develops in cells in the lamina propria of the posterior small intestine and it has been suggested that the stress of weaning and shipping may enhance *Isospora* infections (Lepp and Todd 1974). The significance of diarrhea caused by coccidia in dogs is unclear therefore the treatment of the condition is also unclear. Suspected clinical cases can be treated with a variety of drugs used alone or in combination. Sulfadimethoxine given at 50 mg/kg orally once a day for 10 to 14 days will eliminate oocyst excretion in most dogs (Lindsay and Blagburn 1995) or the combination of ormetoprim (11 mg/kg) and sulfadimethoxine (55 mg/kg) given orally for up to 23 days has also been effective in dogs (Dunbar and Foreyt 1985).

During the week following delivery of the beagles to OSU Veterinary Medicine/Animal Resources, blood and mucous was observed in the feces of almost all dogs. Upon testing, it was determined that 7of the control dogs tested positive for *Giardia* and 3 for *Isospora*. Of the treatment group, 9 tested positive for *Giardia* and 3 for *Isospora*. Infected animals were treated with oral suspensions of Panacur© (fenbendazole) for giardiasis and Albon© (sulfadimethoxine) for coccidiosis according to the manufacturer's recommended doses. A follow-up diagnosis was made 2 ½ weeks later with both control and treatment dogs testing negative for *Giardia* but 4 control dogs were positive for *Isospora* as well as 5 treatment dogs.

Treatments were administered as deemed appropriate. The week following diagnosis and treatment, the feeding trial began with no other documented cases of either giardiasis or coccidiosis occurring during the trial.

Dehydration

Dehydration indicates lack of water and fluids that the body needs to maintain normal functions. Dehydration could be a result of loss of fluids due to vomiting, diarrhea, and fever or the lack of fluid intake due to nausea, loss of appetite, or inaccessibility. Tests such as blood chemistry (specifically electrolytes), complete blood count, BUN, and creatinine are useful indicators of dehydration. Elevated electrolytes, red blood cells, hematocrit, and elevated BUN and creatinine levels could be the result of dehydration (Dugdale 2008). Elevated levels of the following analytes included in a CBC and blood chemistry panel are generally considered indicative of dehydration:

- Red blood cell
- Hemoglobin
- Hematocrit
- BUN
- Creatinine
- Sodium
- Potassium
- Chloride
- Total protein
- Globulin
- Albumin
- Phosphorous
- Calcium

(Loeb 1999).

As mentioned in the previous section, the dogs used in this study had experienced bouts of diarrhea, presumably, due to parasitic infections and stress

prior to the start of the feeding trial. In addition, individual animals were housed in separate runs with the water supply being freely available to each animal by a pressure trigger spout mechanism. This mechanism opens a valve releasing water upon the biting or tongue pressure of the animal. Once the animals were placed in individual runs, it was observed that some animals had difficulty utilizing this mechanism. The combination of infection, stress, and inaccessibility to water could have resulted in the animals suffering from mild dehydration. The overall results of the CBC we normal and within the published reference ranges and did not indicate that dehydration was an issue. In reference to blood chemistry, all analytes but two were also normal. Elevated levels of alkaline phosphatase could be contributed to skeletal growth and not dehydration. Elevated levels of phosphorous, as seen in our findings, are usually inversely related to calcium levels or seen in combination with elevated BUN and creatinine levels (Loeb 1999). However, calcium, BUN and creatinine levels were normal. Therefore, this phenomenon cannot be explained. Lowered triglycerides can not be the result of dehydration but possibly due to the effects of the probiotic (Taylor and Williams 1998)

Nictitans Gland Prolapse

Nictitans gland prolapse, otherwise known as cherry eye, is the prolapsed of the tear gland of the third eyelid. It is the result of weakened connective tissue that attaches the gland to the surrounding structures of the eye. This weakness of the connective tissue allows the gland to prolapse. Once the gland prolapses and is exposed to air and irritants and can become infected and/or begin to swell. The gland often becomes irritated, red, and swollen. A number of breeds of dogs are

pre-disposed to having this condition with beagles being one of them (Turner 2008). One dog from the treatment group and one from the control group were diagnosed with this condition midway through the trial. Inflammation and stress from cherry eye could cause variations in immune parameters and immune cells.

CONCLUSIONS

Consumption of the probiotic was not associated with any significant changes in the clinical status of the dogs as determined by the use of a standard physical examination, CBC, and biochemical profiles. Since healthy animals were used, one would not expect to see any differences between the treatment groups. The lack of differences could be considered advantageous, as there were no adverse side effects observed due to the consumption of the probiotic.

Future Research

A lipopolysaccharide (LPS) challenge done in a similar manner as those previously done in other animal and human trials (Gallay and others 1993; Altenburg and others 2002) would be a possible research project for the future that could allow one to discern differences expected between the control group and those ingesting the probiotic. The primary role of the immune system is to detect and recognize foreign invaders. Bacteria have developed mechanisms to promote their adhesion onto host cells and colonization. On the other hand, the host cells have developed mechanisms to detect bacterial cell surface components to promote phagocytosis and killing of the microorganisms. The concept of innate immunity is

derived from pattern-recognition systems which make innate immunity very specific, allowing differentiation between host and pathogens (Heumann and Roger 2002). The molecules and pathways involved in the recognition of LPS by the immune system have been studied quite extensively. The use of LPS allows scientists to simulate an infection resulting in a typical immune response one would see if actually dosed with a pathogen without actually having to handle the pathogenic microorganism. The use of challenged animals may accentuate differences in the tested parameters proving the efficacy of using probiotics in enhancing the overall well-being of the animals.

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APPPENDIX A

Additional Immunoglobulin Figures and Tables

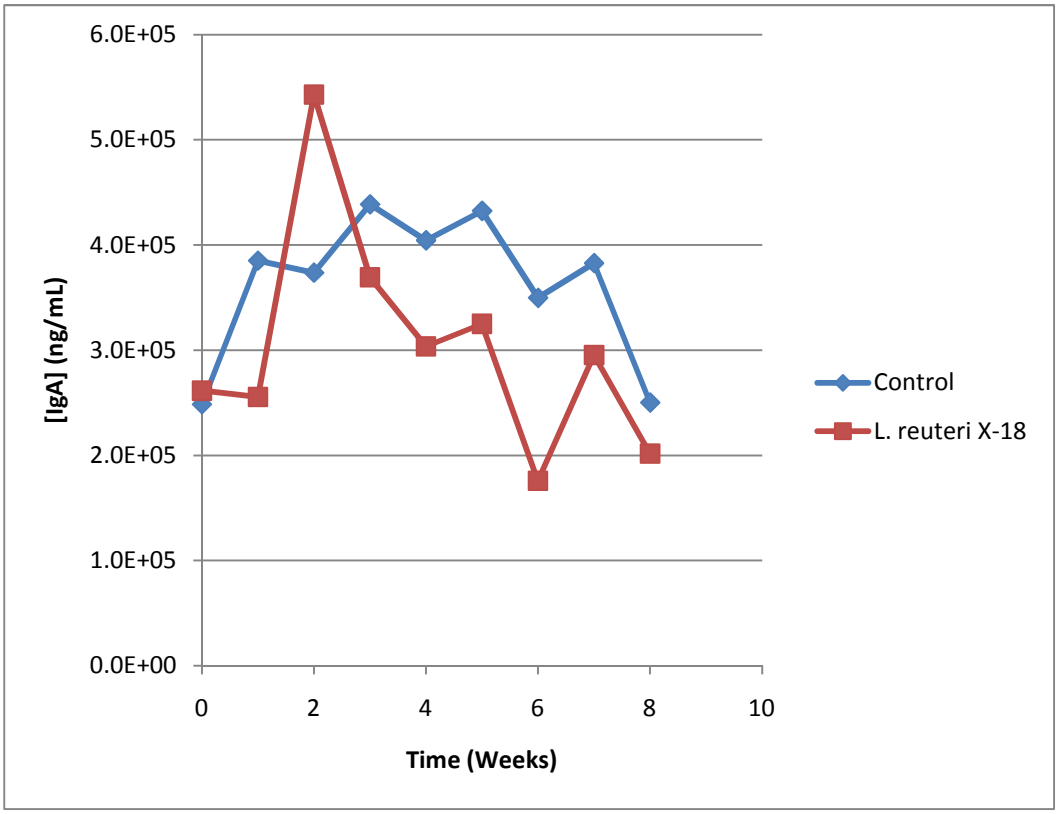

Figure A1. Comparison of fecal IgA levels between the control group and the treatment group over a period of 8 weeks.

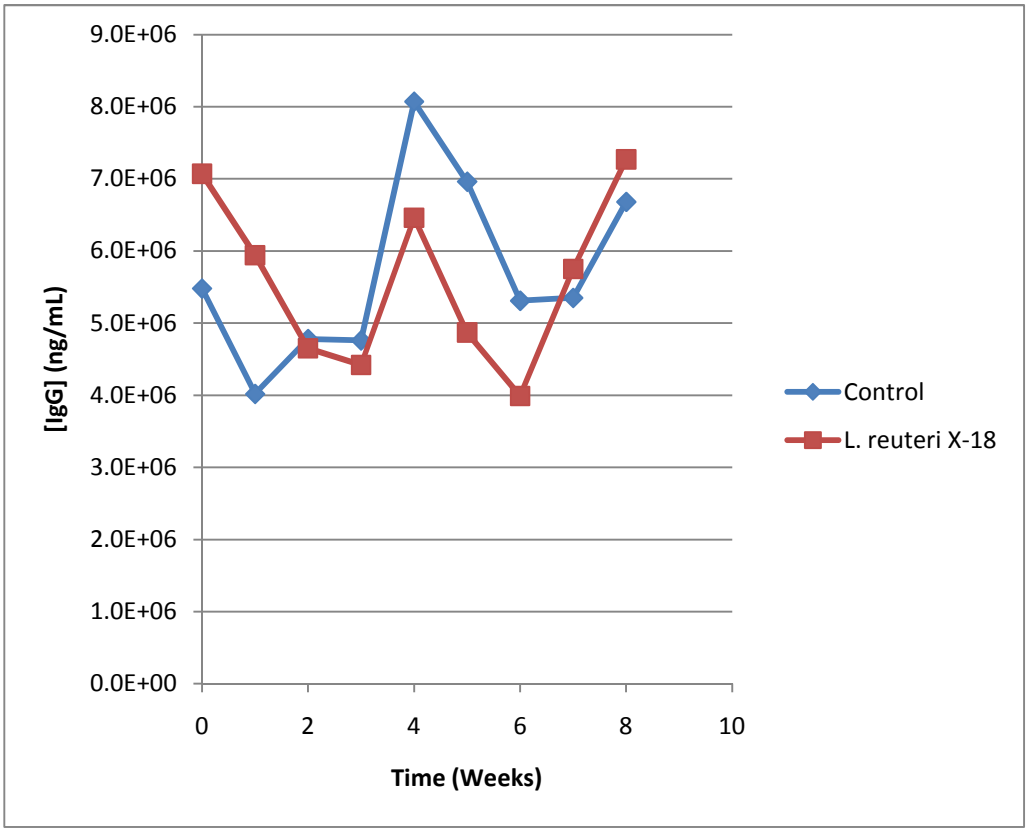

Figure A2. Comparison of fecal IgG levels between the control group and the treatment group over a period of 8 weeks.

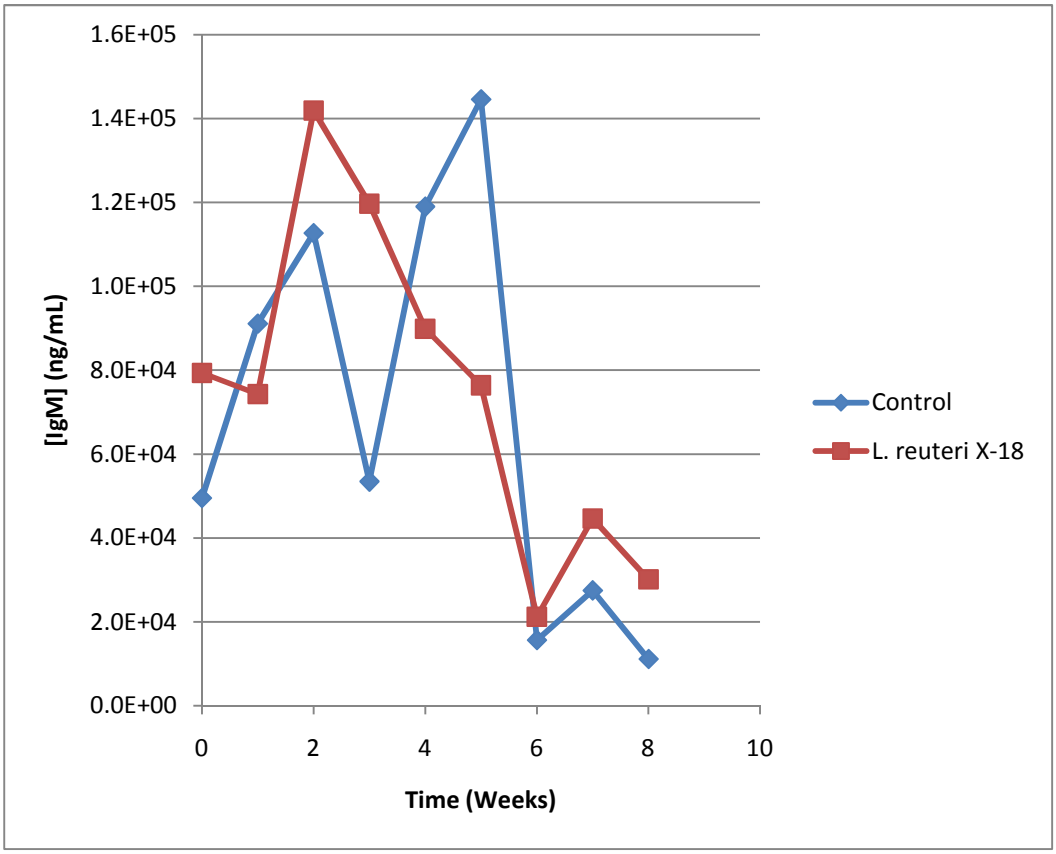

Figure A3. Comparison of fecal IgM levels between the control group and the treatment group over a period of 8 weeks.

Table A1. Weekly Serum Immunoglobulin A (IgA) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	2.8E+05	3.8E+05	3.6E+05	3.6E+05	5.2E+05	4.3E+05	4.7E+05	5.8E+05	6.7E+05
	AIJ-7	4.3E+05	4.8E+05	3.4E+05	3.8E+05	5.4E+05	3.9E+05	4.7E+05	4.0E+05	5.8E+05
	YIJ-7	6.1E+05	7.0E+05	4.8E+05	6.8E+05	5.4E+05	5.6E+05	6.9E+05	7.9E+05	8.7E+05
	YQI-7	6.6E+05	9.0E+05	7.1E+05	5.8E+05	7.9E+05	9.1E+05	6.7E+05	8.6E+05	8.5E+05
	YSI-7	6.2E+05	6.5E+05	8.9E+05	9.0E+05	5.0E+05	8.6E+05	9.0E+05	9.4E+05	8.9E+05
	YKI-7	4.9E+05	6.9E+05	5.0E+05	5.1E+05	8.7E+05	7.1E+05	7.2E+05	9.1E+05	9.0E+05
	XEI-7	4.6E+05	3.3E+05	3.5E+05	2.6E+05	3.5E+05	3.8E+05	3.5E+05	4.6E+05	3.7E+05
	XOJ-7	2.9E+05	3.1E+05	1.9E+05	2.9E+05	2.2E+05	3.3E+05	3.9E+05	3.7E+05	3.5E+05
	XYJ-7	2.9E+05	3.4E+05	3.5E+05	3.0E+05	4.0E+05	5.3E+05	4.6E+05	5.0E+05	5.6E+05
	ZVI-7	3.6E+05	3.3E+05	2.2E+05	3.4E+05	3.6E+05	5.0E+05	4.9E+05	4.4E+05	5.9E+05
	Mean	4.5E+05	5.1E+05	4.4E+05	4.6E+05	5.1E+05	5.6E+05	5.6E+05	6.2E+05	6.6E+05
L. reuteri X-18	ZKI-7	4.5E+05	4.9E+05	4.5E+05	5.8E+05	5.7E+05	5.1E+05	5.0E+05	7.7E+05	6.9E+05
	YOJ-7	6.8E+05	7.2E+05	6.5E+05	4.4E+05	7.0E+05	9.6E+05	8.3E+05	8.0E+05	8.2E+05
	YAI-7	2.0E+05	2.8E+05	5.3E+05	3.1E+05	2.9E+05	3.5E+05	5.0E+05	4.8E+05	8.5E+05
	AJJ-7	3.0E+05	2.9E+05	3.1E+05	4.7E+05	4.9E+05	5.6E+05	5.1E+05	7.2E+05	7.3E+05
	YZJ-7	3.1E+05	3.3E+05	4.5E+05	4.2E+05	3.5E+05	6.0E+05	6.8E+05	7.0E+05	5.8E+05
	XCJ-7	3.8E+05	4.7E+05	5.1E+05	4.3E+05	4.1E+05	4.0E+05	4.6E+05	6.3E+05	4.9E+05
	ZPI-7	3.8E+05	4.1E+05	4.3E+05	4.6E+05	5.2E+05	4.5E+05	6.0E+05	9.7E+05	6.8E+05
	XZI-7	2.7E+05	1.9E+05	1.9E+05	1.5E+05	1.7E+05	2.1E+05	1.9E+05	2.8E+05	2.3E+05
	XQI-7	3.3E+05	3.6E+05	3.1E+05	2.7E+05	3.4E+05	5.1E+05	4.2E+05	5.2E+05	3.7E+05
	ZTJ-7	5.3E+05	8.8E+05	5.6E+05	4.0E+05	5.6E+05	5.3E+05	4.5E+05	5.2E+05	7.7E+05
	Mean	3.8E+05	4.4E+05	4.4E+05	3.9E+05	4.4E+05	5.1E+05	5.1E+05	6.4E+05	6.2E+05

[1]Description of treatments and dogs - see Table 6.

Table A2. Weekly Fecal Immunoglobulin A (IgA) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	4.3E+05	7.4E+05	5.5E+05	9.1E+05	9.1E+05	8.1E+05	7.4E+05	6.0E+05	4.5E+05
	AIJ-7	2.7E+05	2.2E+05	5.2E+05	2.8E+05	6.1E+05	2.8E+05	2.2E+05	2.5E+04	
	YIJ-7	4.3E+05	7.4E+05	5.5E+05	9.1E+05	9.1E+05	8.1E+05	7.4E+05	6.0E+05	4.5E+05
	YQI-7	3.7E+05	3.0E+05	3.5E+05	9.0E+05	3.8E+05	1.7E+05	5.1E+05	3.8E+05	1.4E+05
	YSI-7	1.2E+05	4.8E+05		7.1E+05	5.3E+05	9.9E+05	2.8E+05	3.0E+05	
	YKI-7	2.9E+05	2.0E+05	5.2E+05	3.4E+05	1.9E+05	3.9E+05	3.7E+05	4.3E+05	4.0E+05
	XEI-7	1.1E+05	7.7E+04	2.1E+05	6.6E+04	2.6E+05	4.8E+05	2.2E+05	5.3E+05	4.1E+05
	XOJ-7		1.2E+05	6.9E+05	5.6E+04	1.3E+05	2.0E+05	3.3E+05	9.2E+04	1.5E+05
	XYJ-7	1.8E+04	9.1E+05	1.7E+05	4.3E+04	4.4E+04	6.3E+04	5.1E+04	1.4E+05	9.1E+04
	ZVI-7	2.0E+05	6.4E+04	1.6E+05	1.7E+05	8.0E+04	1.3E+05	3.6E+04	7.3E+05	3.8E+05
	Mean	2.5E+05	3.8E+05	4.2E+05	4.4E+05	4.0E+05	4.3E+05	3.5E+05	3.8E+05	3.1E+05
L. reuteri X-18	ZKI-7	5.9E+04	2.1E+05	4.5E+05	5.6E+05	9.4E+04	1.8E+05	7.7E+04	5.2E+04	1.0E+05
	YOJ-7	3.8E+05	2.4E+05	7.6E+05			5.5E+05	3.0E+05	3.8E+05	1.9E+05
	YAI-7	2.5E+05	3.0E+05	5.7E+05	4.9E+04	6.0E+04	1.2E+05	5.1E+04	1.0E+05	1.8E+05
	AJJ-7	3.8E+05	3.5E+05	7.8E+05	6.2E+05	7.3E+05	7.5E+05	4.3E+05	8.8E+05	5.0E+05
	YZJ-7	2.6E+04	1.6E+05	1.1E+05	2.7E+05	1.4E+05	1.5E+05	2.8E+04	1.7E+05	6.0E+04
	XCJ-7	5.4E+05	2.3E+05	8.0E+05	2.2E+05	4.9E+05	5.5E+05	3.5E+05	5.1E+04	8.6E+04
	ZPI-7	4.1E+05	4.2E+05	8.9E+05	6.3E+05	9.0E+05	3.3E+05	6.5E+04	2.6E+05	4.0E+05
	XZI-7	2.3E+05	2.9E+05	4.9E+05	8.7E+04	4.6E+04	1.4E+05	2.3E+05	1.6E+05	1.4E+05
	XQI-7	1.8E+05	2.7E+05	3.9E+05	5.9E+05	1.5E+05	2.8E+05	1.8E+05	6.6E+05	1.9E+05
	ZTJ-7	1.6E+05	8.6E+04	1.9E+05	6.5E+05	4.1E+05	2.0E+05	4.7E+04	2.4E+05	1.7E+05
	Mean	2.6E+05	2.6E+05	5.4E+05	4.1E+05	3.3E+05	3.3E+05	1.8E+05	3.0E+05	2.0E+05

[1]Description of treatments and dogs - see Table 6.

Table A3. Weekly Log_{10} Serum Immunoglobulin A (IgA) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	5.45	5.58	5.56	5.56	5.72	5.63	5.68	5.76	5.82
	AIJ-7	5.63	5.68	5.53	5.57	5.73	5.59	5.67	5.60	5.76
	YIJ-7	5.79	5.84	5.68	5.83	5.73	5.75	5.84	5.90	5.94
	YQI-7	5.82	5.95	5.85	5.76	5.90	5.96	5.83	5.93	5.93
	YSI-7	5.79	5.81	5.95	5.95	5.70	5.93	5.95	5.97	5.95
	YKI-7	5.69	5.84	5.70	5.71	5.94	5.85	5.86	5.96	5.95
	XEI-7	5.66	5.52	5.54	5.42	5.54	5.58	5.55	5.66	5.57
	XOJ-7	5.47	5.49	5.29	5.46	5.35	5.52	5.59	5.57	5.55
	XYJ-7	5.46	5.53	5.54	5.48	5.60	5.73	5.66	5.70	5.75
	ZVI-7	5.56	5.52	5.35	5.53	5.56	5.70	5.69	5.64	5.77
	Mean	5.65	5.71	5.64	5.66	5.71	5.75	5.75	5.80	5.82
L. reuteri X-18	ZKI-7	5.65	5.69	5.65	5.76	5.76	5.70	5.70	5.89	5.84
	YOJ-7	5.83	5.86	5.81	5.64	5.84	5.98	5.92	5.90	5.91
	YAI-7	5.31	5.45	5.72	5.49	5.47	5.55	5.70	5.68	5.93
	AJJ-7	5.47	5.46	5.49	5.67	5.69	5.75	5.71	5.86	5.86
	YZJ-7	5.49	5.52	5.66	5.62	5.54	5.78	5.83	5.85	5.76
	XCJ-7	5.58	5.67	5.71	5.63	5.62	5.60	5.66	5.80	5.69
	ZPI-7	5.58	5.61	5.63	5.66	5.71	5.65	5.78	5.99	5.83
	XZI-7	5.43	5.28	5.28	5.19	5.24	5.32	5.27	5.45	5.37
	XQI-7	5.51	5.56	5.49	5.42	5.53	5.71	5.62	5.71	5.57
	ZTJ-7	5.72	5.95	5.75	5.60	5.75	5.73	5.66	5.72	5.89
	Mean	5.58	5.65	5.64	5.59	5.64	5.71	5.71	5.81	5.79

[1]Description of treatments and dogs - see Table 6.

Table A4. Weekly Log_{10} Fecal Immunoglobulin A (IgA) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	5.63	5.87	5.74	5.96	5.96	5.91	5.87	5.78	5.66
	AIJ-7	5.44	5.34	5.72	5.45	5.78	5.45	5.35	4.39	
	YIJ-7	5.63	5.87	5.74	5.96	5.96	5.91	5.87	5.78	5.66
	YQI-7	5.57	5.47	5.55	5.95	5.58	5.24	5.71	5.58	5.16
	YSI-7	5.07	5.68		5.85	5.72	5.99	5.44	5.48	
	YKI-7	5.46	5.30	5.71	5.53	5.28	5.59	5.56	5.63	5.60
	XEI-7	5.02	4.89	5.33	4.82	5.42	5.68	5.34	5.73	5.61
	XOJ-7		5.06	5.84	4.75	5.10	5.31	5.51	4.96	5.17
	XYJ-7	4.25	5.96	5.23	4.64	4.65	4.80	4.71	5.14	4.96
	ZVI-7	5.30	4.80	5.21	5.24	4.90	5.11	4.55	5.87	5.58
	Mean	5.26	5.42	5.56	5.42	5.44	5.50	5.39	5.43	5.42
L. reuteri X-18	ZKI-7	4.77	5.32	5.66	5.75	4.97	5.26	4.89	4.72	5.00
	YOJ-7	5.58	5.39	5.88			5.74	5.48	5.58	5.29
	YAI-7	5.39	5.48	5.76	4.69	4.78	5.06	4.71	5.00	5.27
	AJJ-7	5.58	5.54	5.89	5.79	5.86	5.87	5.64	5.94	5.70
	YZJ-7	4.42	5.20	5.03	5.42	5.13	5.18	4.45	5.23	4.78
	XCJ-7	5.73	5.37	5.90	5.35	5.69	5.74	5.54	4.71	4.93
	ZPI-7	5.61	5.62	5.95	5.80	5.95	5.52	4.81	5.41	5.60
	XZI-7	5.35	5.46	5.69	4.94	4.67	5.14	5.36	5.20	5.15
	XQI-7	5.25	5.43	5.59	5.77	5.17	5.45	5.25	5.82	5.27
	ZTJ-7	5.20	4.93	5.27	5.82	5.61	5.30	4.67	5.38	5.23
	Mean	5.29	5.37	5.66	5.48	5.32	5.43	5.08	5.30	5.22

[1]Description of treatments and dogs - see Table 6.

Table A5. Weekly Serum Immunoglobulin G (IgG) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	1.0E+07	2.0E+07	7.9E+06	5.4E+06	5.7E+06	5.1E+06	4.0E+06	6.8E+06	1.2E+07
	AIJ-7	2.1E+07	4.1E+06	6.9E+06	5.5E+06	6.0E+06	1.2E+07	4.1E+06	6.3E+06	6.2E+06
	YIJ-7	3.5E+06	3.2E+06	4.1E+06	4.6E+06	3.6E+06	5.5E+06	7.3E+06	6.4E+06	1.2E+07
	YQI-7	5.0E+06	3.7E+06	3.3E+06	3.6E+06	3.9E+06	3.9E+06	6.9E+06	4.4E+06	1.1E+07
	YSI-7	6.5E+06	5.9E+06	4.1E+06	6.9E+06	4.0E+06	9.6E+06	7.0E+06	4.4E+06	6.4E+06
	YKI-7	3.6E+06	8.7E+06	7.0E+06	3.5E+06	2.0E+06	2.9E+06	2.2E+06	2.5E+06	6.9E+06
	XEI-7	5.8E+06	4.4E+06	5.0E+06	7.9E+06	1.2E+07	1.1E+07	8.9E+06	6.4E+06	5.8E+06
	XOJ-7	9.9E+06	7.8E+06	3.5E+07	1.5E+07	4.2E+07	4.5E+07	5.7E+06	1.1E+07	1.1E+07
	XYJ-7	2.0E+07	2.3E+06	9.0E+06	1.9E+07	2.1E+07	1.4E+07	1.1E+07	1.7E+07	1.1E+07
	ZVI-7	2.4E+06	2.2E+06	1.7E+06	2.4E+06	1.7E+06	1.6E+06	1.3E+06	1.6E+06	8.2E+06
	Mean	8.8E+06	6.2E+06	8.4E+06	7.3E+06	1.0E+07	1.1E+07	5.8E+06	6.7E+06	9.2E+06
L. reuteri X-18	ZKI-7	7.4E+06	9.7E+06	1.7E+07	7.4E+06	8.6E+06	5.2E+06	9.2E+06	2.3E+07	9.1E+06
	YOJ-7	4.4E+07	1.1E+07	1.4E+07	8.8E+06	6.4E+06	1.4E+07	3.9E+06	1.9E+07	1.0E+07
	YAI-7	9.4E+06	8.4E+06	6.2E+06	4.7E+06	3.7E+06	7.7E+06	4.8E+06	7.6E+06	9.7E+06
	AJJ-7	1.7E+07	5.9E+06	6.6E+06	4.0E+06	3.1E+06	3.6E+06	3.3E+06	4.7E+06	6.5E+06
	YZJ-7	6.5E+06	7.3E+06	3.3E+06	3.6E+06	8.8E+06	2.6E+06	2.1E+06	2.5E+06	8.6E+06
	XCJ-7	1.9E+07	1.2E+07	1.2E+07	2.2E+07	8.0E+07	5.2E+07	1.3E+07	1.3E+07	7.8E+06
	ZPI-7	7.1E+06	5.6E+06	1.1E+07	9.1E+06	1.2E+07	1.9E+07	8.1E+06	1.1E+07	9.9E+06
	XZI-7	7.2E+06	4.7E+06	6.1E+06	3.4E+06	3.5E+06	3.1E+06	2.3E+06	4.4E+06	9.3E+06
	XQI-7	1.5E+07	5.3E+06	4.1E+06	3.5E+06	4.2E+06	2.2E+06	2.5E+06	4.2E+06	3.9E+06
	ZTJ-7	2.8E+06	2.1E+06	1.9E+06	2.1E+06	2.7E+06	1.7E+06	1.5E+06	1.8E+06	1.2E+07
	Mean	1.4E+07	7.2E+06	8.1E+06	6.9E+06	1.3E+07	1.1E+07	5.1E+06	9.2E+06	8.7E+06

[1]Description of treatments and dogs - see Table 6.

Table A6. Weekly Fecal Immunoglobulin G (IgG) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	9.0E+06	6.6E+06	6.9E+06	4.9E+06	4.7E+06	4.5E+06	3.0E+06	5.8E+06	8.9E+06
	AIJ-7	9.0E+06	3.0E+06	5.4E+06	4.5E+06	4.9E+06	9.9E+06	3.5E+06	5.8E+06	7.5E+06
	YIJ-7	2.8E+06	2.4E+06	3.1E+06	3.6E+06	3.0E+06	4.9E+06	8.8E+06	5.5E+06	4.5E+06
	YQI-7	3.2E+06	3.0E+06	2.9E+06	2.4E+06	3.2E+06	3.0E+06	7.5E+06	3.5E+06	6.0E+06
	YSI-7	5.3E+06	4.2E+06	3.6E+06	5.1E+06	3.0E+06	6.4E+06	5.4E+06	4.0E+06	5.0E+06
	YKI-7	2.8E+06	7.5E+06	6.3E+06	2.2E+06	2.0E+06	2.0E+06	2.0E+06	2.0E+06	5.4E+06
	XEI-7	5.0E+06	3.7E+06	4.5E+06	6.5E+06	9.9E+06	7.0E+06	7.2E+06	5.5E+06	4.4E+06
	XOJ-7	8.8E+06	7.0E+06	6.6E+06	7.0E+06	3.0E+07	2.1E+07	6.1E+06	9.0E+06	8.5E+06
	XYJ-7	7.8E+06	9.9E+05	7.5E+06	9.6E+06	1.9E+07	9.8E+06	8.6E+06	9.9E+06	9.2E+06
	ZVI-7	1.1E+06	1.8E+06	9.9E+05	1.8E+06	9.9E+05	1.1E+06	1.0E+06	2.5E+06	7.4E+06
	Mean	5.5E+06	4.0E+06	4.8E+06	4.8E+06	8.1E+06	6.9E+06	5.3E+06	5.3E+06	6.7E+06
L. reuteri X-18	ZKI-7	6.5E+06	7.7E+06	5.8E+06	6.7E+06	7.5E+06	5.1E+06	9.0E+06	9.8E+06	8.8E+06
	YOJ-7	8.7E+06	8.6E+06	3.2E+06	7.0E+06	5.8E+06	8.9E+06	2.2E+06	8.6E+06	8.8E+06
	YAI-7	9.0E+06	6.9E+06	4.5E+06	3.6E+06	3.0E+06	6.5E+06	3.9E+06	6.2E+06	8.9E+06
	AJJ-7	9.2E+06	4.8E+06	6.6E+06	2.7E+06	2.0E+06	3.0E+06	2.3E+06	3.8E+06	5.8E+06
	YZJ-7	4.9E+06	7.0E+06	2.0E+06	3.2E+06	7.0E+06	1.0E+06	2.0E+06	2.0E+06	7.2E+06
	XCJ-7	9.2E+06	1.0E+07	5.4E+06	6.6E+06	2.1E+07	8.0E+06	9.9E+06	9.8E+06	6.2E+06
	ZPI-7	5.5E+06	4.7E+06	8.6E+06	7.1E+06	1.0E+07	9.9E+06	7.0E+06	8.8E+06	7.7E+06
	XZI-7	6.6E+06	3.7E+06	5.7E+06	2.8E+06	3.0E+06	2.8E+06	1.1E+06	3.7E+06	8.8E+06
	XQI-7	9.2E+06	4.9E+06	3.7E+06	3.0E+06	4.0E+06	1.9E+06	1.5E+06	3.2E+06	2.8E+06
	ZTJ-7	1.9E+06	1.1E+06	1.0E+06	1.5E+06	1.3E+06	1.6E+06	1.0E+06	1.6E+06	7.7E+06
	Mean	7.1E+06	5.9E+06	4.7E+06	4.4E+06	6.4E+06	4.9E+06	4.0E+06	5.7E+06	7.3E+06

[1] Description of treatments and dogs - see Table 6.

Table A7. Weekly Log_{10} Serum Immunoglobulin G (IgG) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	7.01	7.30	6.89	6.73	6.75	6.71	6.60	6.83	7.10
	AIJ-7	7.33	6.61	6.84	6.74	6.78	7.09	6.62	6.80	6.79
	YIJ-7	6.54	6.51	6.62	6.66	6.55	6.74	6.86	6.81	7.10
	YQI-7	6.69	6.57	6.52	6.56	6.59	6.60	6.84	6.64	7.03
	YSI-7	6.81	6.77	6.61	6.84	6.60	6.98	6.84	6.64	6.80
	YKI-7	6.56	6.94	6.85	6.54	6.31	6.46	6.34	6.40	6.84
	XEI-7	6.76	6.65	6.70	6.89	7.08	7.05	6.95	6.81	6.77
	XOJ-7	7.00	6.89	7.54	7.17	7.62	7.65	6.75	7.05	7.04
	XYJ-7	7.30	6.36	6.95	7.27	7.31	7.13	7.04	7.23	7.06
	ZVI-7	6.39	6.35	6.23	6.37	6.23	6.21	6.11	6.20	6.91
	Mean									
L. reuteri X-18	ZKI-7	6.87	6.99	7.23	6.87	6.93	6.71	6.97	7.36	6.96
	YOJ-7	7.65	7.04	7.14	6.94	6.81	7.16	6.59	7.29	7.00
	YAI-7	6.97	6.93	6.79	6.67	6.57	6.89	6.68	6.88	6.99
	AJJ-7	7.23	6.77	6.82	6.60	6.49	6.55	6.52	6.67	6.81
	YZJ-7	6.81	6.86	6.51	6.55	6.94	6.41	6.33	6.40	6.94
	XCJ-7	7.28	7.08	7.08	7.35	7.90	7.72	7.13	7.11	6.89
	ZPI-7	6.85	6.75	7.02	6.96	7.09	7.29	6.91	7.05	7.00
	XZI-7	6.86	6.67	6.78	6.53	6.55	6.49	6.37	6.65	6.97
	XQI-7	7.19	6.73	6.61	6.54	6.62	6.35	6.39	6.62	6.59
	ZTJ-7	6.44	6.32	6.28	6.32	6.43	6.22	6.18	6.25	7.10
	Mean	7.01	6.81	6.83	6.73	6.83	6.78	6.61	6.83	6.92

[1]Description of treatments and dogs - see Table 6.

Table A8. Weekly Log_{10} Fecal Immunoglobulin G (IgG) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	6.95	6.82	6.84	6.69	6.67	6.65	6.47	6.76	6.95
	AIJ-7	6.95	6.48	6.73	6.65	6.69	6.99	6.55	6.76	6.88
	YIJ-7	6.44	6.39	6.49	6.56	6.47	6.69	6.95	6.74	6.65
	YQI-7	6.51	6.47	6.46	6.37	6.51	6.48	6.88	6.54	6.78
	YSI-7	6.72	6.63	6.55	6.71	6.48	6.80	6.74	6.60	6.70
	YKI-7	6.44	6.88	6.80	6.35	6.30	6.30	6.30	6.30	6.73
	XEI-7	6.70	6.57	6.65	6.81	6.99	6.84	6.86	6.74	6.64
	XOJ-7	6.94	6.84	6.82	6.84	7.48	7.32	6.78	6.96	6.93
	XYJ-7	6.89	6.00	6.88	6.98	7.28	6.99	6.94	6.99	6.96
	ZVI-7	6.04	6.24	6.00	6.25	6.00	6.04	6.00	6.40	6.87
	Mean	6.66	6.53	6.62	6.62	6.69	6.71	6.64	6.68	6.81
L. reuteri X-18	ZKI-7	6.81	6.88	6.77	6.83	6.88	6.71	6.95	6.99	6.94
	YOJ-7	6.94	6.93	6.51	6.85	6.76	6.95	6.35	6.94	6.94
	YAI-7	6.95	6.84	6.65	6.56	6.48	6.82	6.60	6.79	6.95
	AJJ-7	6.96	6.68	6.82	6.43	6.30	6.47	6.36	6.58	6.76
	YZJ-7	6.69	6.84	6.31	6.51	6.85	6.01	6.30	6.30	6.86
	XCJ-7	6.96	7.00	6.73	6.82	7.32	6.90	6.99	6.99	6.79
	ZPI-7	6.74	6.67	6.94	6.85	7.00	7.00	6.85	6.94	6.89
	XZI-7	6.82	6.56	6.76	6.44	6.47	6.45	6.04	6.57	6.94
	XQI-7	6.96	6.69	6.57	6.47	6.60	6.29	6.19	6.50	6.44
	ZTJ-7	6.28	6.05	6.01	6.19	6.10	6.20	6.01	6.21	6.89
	Mean	6.81	6.71	6.61	6.59	6.68	6.58	6.46	6.68	6.84

[1]Description of treatments and dogs - see Table 6.

Table A9. Weekly Serum Immunoglobulin M (IgM) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	1.6E+06	1.5E+06	1.5E+06	1.4E+06	1.6E+06	1.5E+06	1.7E+06	1.6E+06	1.3E+06
	AIJ-7	1.9E+06	1.7E+06	2.2E+06	2.3E+06	2.3E+06	1.9E+06	2.1E+06	2.0E+06	1.8E+06
	YIJ-7	2.4E+06	2.2E+06	2.2E+06	2.4E+06	2.6E+06	2.1E+06	1.9E+06	2.0E+06	1.6E+06
	YQI-7	3.1E+06	2.3E+06	2.7E+06	2.4E+06	2.6E+06	2.0E+06	1.5E+06	1.6E+06	2.5E+06
	YSI-7	1.8E+06	1.5E+06	2.3E+06	1.7E+06	2.1E+06	1.7E+06	1.3E+06	1.2E+06	1.1E+06
	YKI-7	1.8E+06	1.5E+06	1.8E+06	1.6E+06	1.4E+06	1.6E+06	1.6E+06	2.0E+06	1.9E+06
	XEI-7	4.1E+05	4.1E+05	5.9E+05	4.9E+05	5.6E+05	6.7E+05	6.6E+05	5.3E+05	8.1E+05
	XOJ-7	1.3E+06	1.3E+06	1.5E+06	1.6E+06	1.5E+06	1.1E+06	1.1E+06	1.1E+06	2.3E+06
	XYJ-7	1.2E+06	1.5E+06	1.7E+06	1.5E+06	1.3E+06	2.1E+06	1.1E+06	1.8E+06	1.9E+06
	ZVI-7	1.2E+06	2.2E+06	2.2E+06	1.8E+06	1.9E+06	1.5E+06	1.6E+06	1.6E+06	2.0E+06
	Mean	1.7E+06	1.6E+06	1.9E+06	1.7E+06	1.8E+06	1.6E+06	1.4E+06	1.5E+06	1.7E+06
L. reuteri X-18	ZKI-7	1.1E+06	1.1E+06	1.8E+06	1.3E+06	1.1E+06	9.7E+05	1.3E+06	1.3E+06	1.2E+06
	YOJ-7	2.6E+06	2.5E+06	2.5E+06	2.7E+06	1.9E+06	2.3E+06	2.2E+06	2.1E+06	2.6E+06
	YAI-7	1.6E+06	1.7E+06	1.3E+06	1.7E+06	1.4E+06	9.4E+05	1.4E+06	1.3E+06	1.8E+06
	AJJ-7	1.8E+06	1.9E+06	1.7E+06	2.0E+06	2.2E+06	1.6E+06	1.7E+06	2.0E+06	2.7E+06
	YZJ-7	2.8E+06	2.5E+06	2.8E+06	2.8E+06	2.7E+06	2.3E+06	3.0E+06	2.5E+06	2.9E+06
	XCJ-7	8.5E+05	9.5E+05	9.0E+05	1.0E+06	9.8E+05	1.0E+06	6.1E+05	7.7E+05	1.2E+06
	ZPI-7	7.5E+05	7.7E+05	9.0E+05	9.0E+05	1.1E+06	1.0E+06	1.2E+06	1.4E+06	1.9E+06
	XZI-7	8.9E+05	1.2E+06	9.5E+05	1.3E+06	1.7E+06	1.8E+06	1.9E+06	1.5E+06	1.3E+06
	XQI-7	2.0E+06	2.0E+06	1.9E+06	2.2E+06	1.9E+06	1.7E+06	1.3E+06	1.9E+06	1.8E+06
	ZTJ-7	1.2E+06	2.3E+06	1.6E+06	1.2E+06	3.9E+06	1.5E+06	2.0E+06	2.5E+06	2.1E+06
	Mean	1.6E+06	1.7E+06	1.6E+06	1.7E+06	1.9E+06	1.5E+06	1.7E+06	1.7E+06	2.0E+06

[1]Description of treatments and dogs - see Table 6.

Table A10. Weekly Fecal Immunoglobulin M (IgM) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	1.9E+03	1.9E+05	1.9E+04	3.6E+03	2.6E+03	3.4E+04	6.3E+03	3.7E+04	1.7E+04
	AIJ-7	8.9E+04	1.5E+05	3.4E+05	5.0E+04	4.4E+05	1.9E+05	4.3E+04	2.0E+03	8.6E+03
	YIJ-7	1.0E+05	2.8E+05	6.8E+04	2.6E+05	4.3E+05	3.4E+05	3.1E+04	6.4E+03	1.3E+04
	YQI-7	8.7E+03	1.2E+04	8.9E+04	1.6E+05	1.4E+04	3.1E+03	2.5E+04	2.7E+04	2.4E+03
	YSI-7	4.8E+03	1.3E+05	5.3E+04	9.3E+03	1.3E+04	1.2E+05	4.0E+03	6.7E+03	
	YKI-7	1.5E+05	3.7E+04	3.2E+05	1.2E+04	9.2E+04	5.5E+05	2.3E+04	8.1E+04	7.0E+03
	XEI-7	3.8E+03	3.1E+03	6.6E+03	1.5E+03	2.2E+04	1.2E+05	4.7E+03	1.8E+04	3.2E+04
	XOJ-7		1.6E+04	2.0E+05	5.1E+03	4.8E+04	7.7E+04	1.6E+04	4.0E+03	7.6E+03
	XYJ-7	8.4E+03		1.2E+04	5.1E+03		2.5E+03	1.8E+03	1.9E+03	1.9E+03
	ZVI-7	7.9E+04	1.7E+03	1.9E+04	2.8E+04	9.2E+03	8.8E+03	2.0E+03	9.1E+04	1.1E+04
	Mean	4.9E+04	9.0E+04	1.1E+05	5.3E+04	1.2E+05	1.5E+05	1.6E+04	2.7E+04	1.1E+04
L. reuteri X-18	ZKI-7	5.7E+03	9.5E+04	2.6E+05	1.3E+05	2.5E+03	1.4E+04	2.7E+03		2.2E+03
	YOJ-7	3.3E+05	2.6E+04	5.5E+04	3.4E+05	5.1E+05	1.8E+05	1.7E+04	3.2E+04	9.7E+03
	YAI-7	6.4E+04	1.0E+05	5.5E+04	2.8E+03	2.5E+03	8.4E+03	2.0E+03	2.3E+03	3.8E+03
	AJJ-7	8.7E+04	6.8E+04	4.9E+05	2.6E+04	1.0E+05	2.4E+05	3.2E+04	1.6E+05	2.4E+05
	YZJ-7	2.7E+03	2.6E+04	5.8E+03	1.6E+05	1.9E+04	2.5E+04	1.7E+03	1.4E+04	1.7E+03
	XCJ-7	2.7E+04	1.1E+04	4.6E+04	2.7E+03	3.0E+04	3.2E+04	8.1E+03	7.6E+03	2.0E+03
	ZPI-7	1.7E+04	4.7E+04	2.2E+04	1.5E+05	9.8E+04	2.6E+04	1.9E+03	1.0E+04	1.8E+04
	XZI-7	2.2E+04	4.7E+04	1.0E+05	3.2E+03	2.4E+03	5.5E+03	7.0E+04	6.3E+03	2.7E+03
	XQI-7	2.0E+05	3.1E+05	3.2E+05	3.0E+05	6.7E+04	1.7E+05	7.3E+04	1.6E+05	1.3E+04
	ZTJ-7	3.8E+04	1.3E+04	6.5E+04	8.2E+04	6.7E+04	6.3E+04	3.5E+03	9.3E+03	8.4E+03
	Mean	8.0E+04	7.4E+04	1.4E+05	1.2E+05	9.0E+04	7.5E+04	2.1E+04	4.5E+04	3.0E+04

[1]Description of treatments and dogs - see Table 6.

Table A11. Weekly Log_{10} Serum Immunoglobulin M (IgM) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	6.19	6.18	6.18	6.13	6.20	6.17	6.22	6.22	6.10
	AIJ-7	6.28	6.23	6.35	6.37	6.36	6.29	6.32	6.29	6.26
	YIJ-7	6.39	6.33	6.35	6.38	6.42	6.32	6.27	6.31	6.20
	YQI-7	6.49	6.35	6.43	6.38	6.42	6.30	6.18	6.21	6.40
	YSI-7	6.24	6.17	6.37	6.22	6.33	6.22	6.11	6.07	6.04
	YKI-7	6.26	6.18	6.26	6.21	6.15	6.21	6.20	6.29	6.28
	XEI-7	5.61	5.61	5.77	5.69	5.75	5.83	5.82	5.72	5.91
	XOJ-7	6.11	6.10	6.18	6.19	6.16	6.04	6.05	6.03	6.36
	XYJ-7	6.09	6.17	6.24	6.18	6.11	6.31	6.03	6.26	6.29
	ZVI-7	6.07	6.34	6.34	6.26	6.27	6.18	6.21	6.21	6.31
	Mean	6.17	6.17	6.25	6.20	6.22	6.19	6.14	6.16	6.21
L. reuteri X-18	ZKI-7	6.05	6.02	6.25	6.12	6.05	5.99	6.11	6.11	6.08
	YOJ-7	6.41	6.40	6.40	6.43	6.28	6.36	6.34	6.32	6.42
	YAI-7	6.22	6.24	6.10	6.23	6.16	5.97	6.15	6.11	6.26
	AJJ-7	6.26	6.28	6.23	6.30	6.35	6.21	6.23	6.31	6.44
	YZJ-7	6.45	6.40	6.45	6.44	6.44	6.36	6.48	6.40	6.46
	XCJ-7	5.93	5.98	5.96	6.02	5.99	6.00	5.79	5.89	6.08
	ZPI-7	5.88	5.89	5.96	5.96	6.04	6.01	6.07	6.13	6.27
	XZI-7	5.95	6.09	5.98	6.12	6.22	6.26	6.27	6.18	6.13
	XQI-7	6.29	6.30	6.28	6.34	6.27	6.24	6.12	6.28	6.26
	ZTJ-7	6.09	6.36	6.19	6.09	6.59	6.19	6.29	6.39	6.33
	Mean	6.15	6.20	6.18	6.20	6.24	6.16	6.19	6.21	6.27

[1]Description of treatments and dogs - see Table 6.

Table A12. Weekly Log_{10} Fecal Immunoglobulin M (IgM) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	3.27	5.28	4.29	3.56	3.42	4.53	3.80	4.56	4.22
	AIJ-7	4.95	5.17	5.53	4.70	5.65	5.28	4.63	3.30	3.93
	YIJ-7	5.00	5.44	4.83	5.42	5.63	5.53	4.50	3.81	4.11
	YQI-7	3.94	4.07	4.95	5.20	4.16	3.49	4.39	4.43	3.37
	YSI-7	3.68	5.10	4.72	3.97	4.12	5.08	3.60	3.83	
	YKI-7	5.17	4.57	5.51	4.06	4.96	5.74	4.36	4.91	3.84
	XEI-7	3.58	3.49	3.82	3.18	4.35	5.08	3.67	4.26	4.50
	XOJ-7		4.22	5.29	3.71	4.68	4.88	4.19	3.60	3.88
	XYJ-7	3.93		4.08	3.71		3.39	3.26	3.28	3.28
	ZVI-7	4.90	3.23	4.28	4.44	3.97	3.94	3.30	4.96	4.03
	Mean	4.27	4.51	4.73	4.20	4.55	4.70	3.97	4.09	3.91
L. reuteri X-18	ZKI-7	3.75	4.98	5.41	5.10	3.39	4.15	3.44		3.35
	YOJ-7	5.52	4.42	4.74	5.53	5.71	5.25	4.24	4.51	3.99
	YAI-7	4.80	5.01	4.74	3.45	3.39	3.93	3.30	3.36	3.58
	AJJ-7	4.94	4.83	5.69	4.41	5.01	5.37	4.51	5.21	5.38
	YZJ-7	3.43	4.42	3.76	5.20	4.29	4.40	3.24	4.15	3.23
	XCJ-7	4.43	4.04	4.66	3.44	4.48	4.51	3.91	3.88	3.31
	ZPI-7	4.22	4.68	4.34	5.16	4.99	4.41	3.27	4.01	4.25
	XZI-7	4.34	4.67	5.01	3.50	3.37	3.74	4.85	3.80	3.44
	XQI-7	5.31	5.49	5.51	5.48	4.83	5.22	4.86	5.20	4.12
	ZTJ-7	4.58	4.11	4.82	4.91	4.82	4.80	3.54	3.97	3.93
	Mean	4.53	4.66	4.87	4.62	4.43	4.58	3.91	4.23	3.86

[1]Description of treatments and dogs - see Table 6.

Table A13. Weekly Serum Immunoglobulin E (IgE) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	1.6E+04	1.3E+04	1.3E+04	1.8E+04	3.9E+04	3.3E+04	2.3E+04	2.5E+04	1.6E+04
	AIJ-7	8.9E+03	9.7E+03	5.7E+03	4.0E+04	1.8E+04	8.8E+03	7.7E+03	5.6E+03	4.1E+03
	YIJ-7	1.4E+04	3.3E+04	3.3E+04	2.8E+04	2.9E+04	2.7E+04	2.8E+04	2.5E+04	2.1E+04
	YQI-7	4.4E+03	2.6E+03	1.9E+03	3.4E+03	9.6E+03	4.4E+03	4.3E+03	3.7E+03	4.6E+03
	YSI-7	2.2E+03	8.9E+03	3.6E+03	1.4E+04	8.0E+03	6.9E+03	5.6E+03	8.2E+03	5.7E+03
	YKI-7	1.1E+04	1.3E+04	3.3E+04	3.0E+04	2.3E+04	3.8E+04	2.1E+04	2.4E+04	1.6E+04
	XEI-7	7.8E+03	1.9E+04	2.0E+04	1.3E+04	1.2E+04	1.5E+04	1.6E+04	1.9E+04	1.4E+04
	XOJ-7	4.9E+03	1.2E+04	8.9E+03	2.9E+04	2.1E+04	1.2E+04	8.4E+03	6.7E+03	4.7E+03
	XYJ-7	7.2E+03	4.4E+03	1.2E+04	4.2E+04	1.7E+04	1.0E+04	9.1E+03	6.3E+03	6.4E+03
	ZVI-7	2.9E+04	5.1E+04	5.2E+04	3.4E+04	3.3E+04	2.8E+04	4.6E+04	4.1E+04	2.6E+04
	Mean	1.1E+04	1.7E+04	1.8E+04	2.5E+04	2.1E+04	1.8E+04	1.7E+04	1.6E+04	1.2E+04
L. reuteri X-18	ZKI-7	3.1E+03	3.5E+03	4.3E+03	1.4E+04	9.8E+03	6.7E+03	1.2E+04	1.2E+04	3.6E+04
	YOJ-7	3.9E+03	3.5E+03	3.6E+03	6.6E+03	9.0E+03	7.5E+03	6.2E+03	6.1E+03	4.7E+03
	YAI-7	3.7E+03	1.6E+04	1.7E+04	2.4E+04	1.1E+04	7.2E+03	7.9E+03	9.0E+03	8.9E+03
	AJJ-7	5.9E+03	5.0E+03	6.0E+03	1.9E+04	4.3E+04	2.9E+04	1.8E+04	3.2E+04	2.8E+04
	YZJ-7	7.3E+03	6.8E+03	1.0E+04	2.0E+04	1.2E+04	9.6E+03	9.3E+03	1.2E+04	1.7E+04
	XCJ-7	9.6E+03	6.2E+03	8.5E+03	7.1E+03	6.9E+03	2.2E+04	2.7E+04	2.6E+04	1.5E+04
	ZPI-7	3.5E+03	7.1E+03	2.4E+04	2.3E+04	1.9E+04	2.8E+04	2.2E+04	2.0E+04	3.1E+04
	XZI-7	1.9E+04	2.3E+04	1.4E+04	6.0E+03	1.2E+04	1.8E+04	1.8E+04	1.1E+04	5.8E+03
	XQI-7	8.4E+03	1.8E+04	9.9E+03	8.3E+03	9.5E+03	1.8E+04	2.0E+04	1.2E+04	6.1E+03
	ZTJ-7	1.4E+04	1.5E+04	1.7E+04	1.3E+04	2.1E+04	1.7E+04	1.3E+04	1.1E+04	1.7E+04
	Mean	7.8E+03	1.0E+04	1.1E+04	1.4E+04	1.5E+04	1.6E+04	1.5E+04	1.5E+04	1.7E+04

[1]Description of treatments and dogs - see Table 6.

Table A14. Weekly Log_{10} Serum Immunoglobulin E (IgE) Levels (ng/mL)

Treatment[1]	Dog	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	YTJ-7	4.20	4.11	4.11	4.25	4.59	4.52	4.35	4.40	4.21
	AIJ-7	3.95	3.99	3.76	4.60	4.25	3.94	3.89	3.75	3.61
	YIJ-7	4.15	4.52	4.52	4.45	4.46	4.43	4.45	4.41	4.32
	YQI-7	3.65	3.42	3.28	3.53	3.98	3.64	3.63	3.57	3.66
	YSI-7	3.35	3.95	3.56	4.14	3.90	3.84	3.75	3.91	3.76
	YKI-7	4.03	4.10	4.52	4.47	4.37	4.58	4.32	4.39	4.21
	XEI-7	3.89	4.27	4.29	4.10	4.07	4.16	4.21	4.28	4.13
	XOJ-7	3.69	4.08	3.95	4.46	4.32	4.09	3.92	3.82	3.67
	XYJ-7	3.86	3.64	4.06	4.63	4.23	4.01	3.96	3.80	3.81
	ZVI-7	4.47	4.71	4.72	4.53	4.52	4.45	4.67	4.61	4.41
	Mean	3.92	4.08	4.08	4.32	4.27	4.17	4.12	4.09	3.98
L. reuteri X-18	ZKI-7	3.49	3.54	3.63	4.16	3.99	3.83	4.08	4.08	4.56
	YOJ-7	3.59	3.55	3.56	3.82	3.96	3.87	3.79	3.79	3.67
	YAI-7	3.56	4.20	4.22	4.38	4.04	3.86	3.90	3.96	3.95
	AJJ-7	3.77	3.70	3.78	4.27	4.63	4.46	4.27	4.51	4.44
	YZJ-7	3.86	3.83	4.00	4.30	4.09	3.98	3.97	4.06	4.22
	XCJ-7	3.98	3.79	3.93	3.85	3.84	4.34	4.44	4.42	4.17
	ZPI-7	3.54	3.85	4.38	4.35	4.29	4.45	4.34	4.31	4.49
	XZI-7	4.27	4.36	4.14	3.78	4.08	4.27	4.27	4.04	3.76
	XQI-7	3.93	4.24	4.00	3.92	3.98	4.26	4.29	4.08	3.78
	ZTJ-7	4.16	4.17	4.24	4.10	4.32	4.24	4.11	4.04	4.24
	Mean	3.82	3.92	3.99	4.09	4.12	4.15	4.14	4.13	4.13

[1]Description of treatments and dogs - see Table 6.

APPENDIX B

Additional Blood Chemistry Panel Figures and Tables

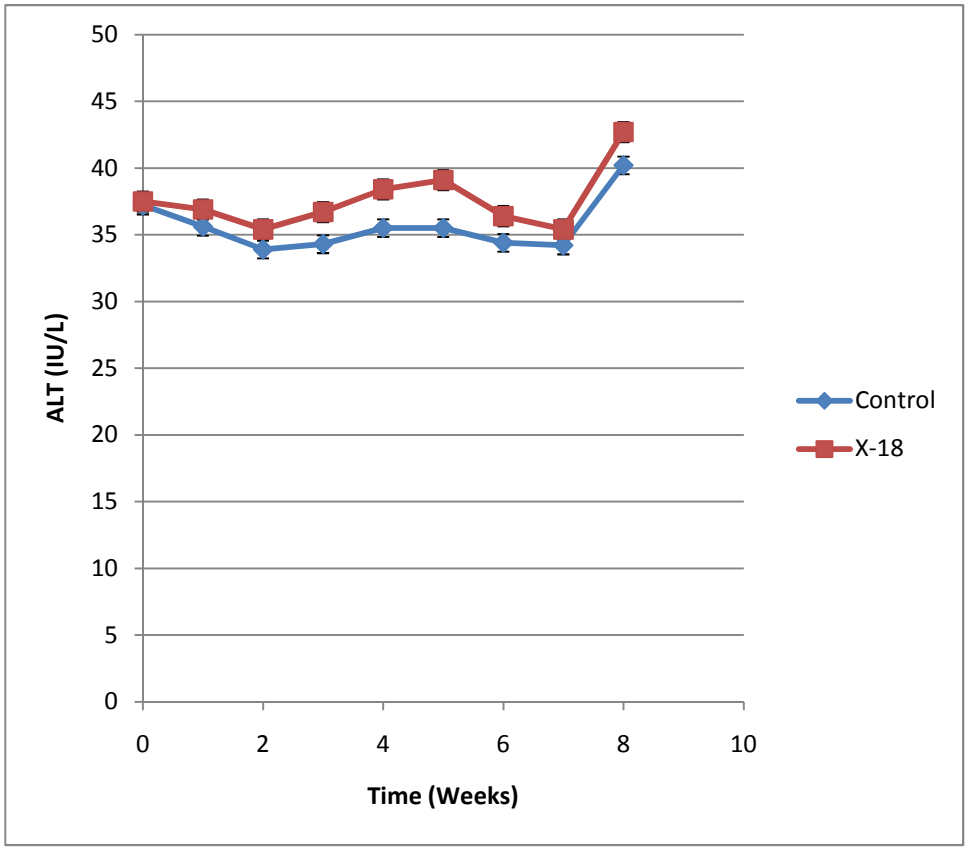

Figure B1. Comparison of ALT between the control group and the treatment group over a period of 8 weeks

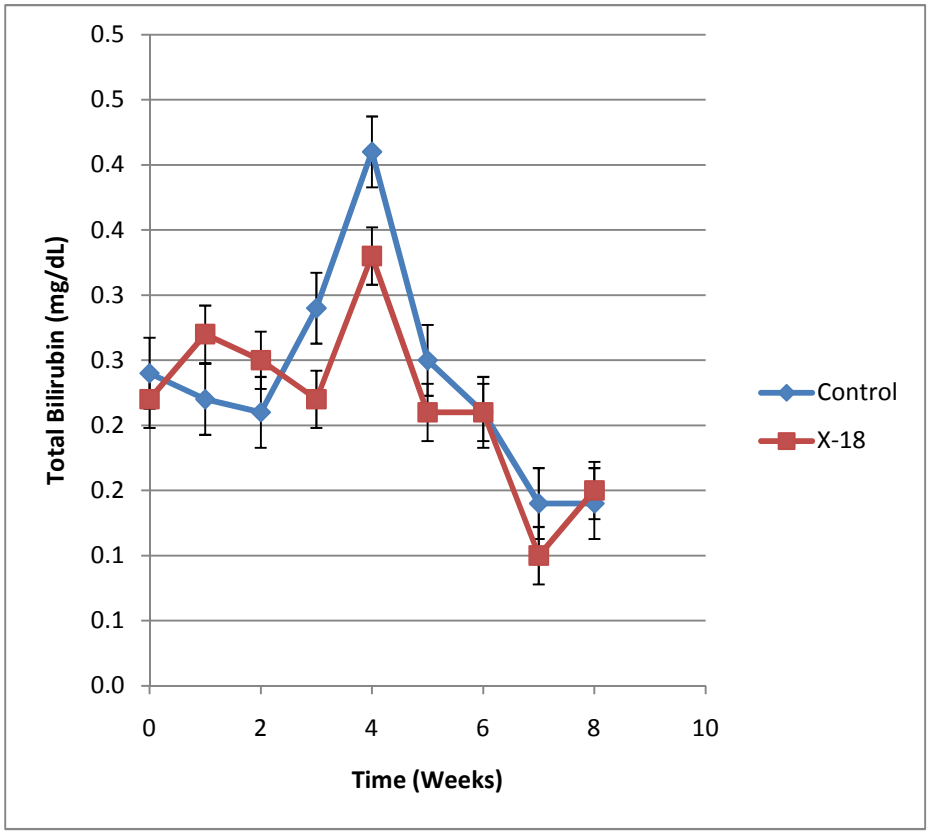

Figure B2. Comparison of total bilirubin between the control group and the treatment group over a period of 8 weeks

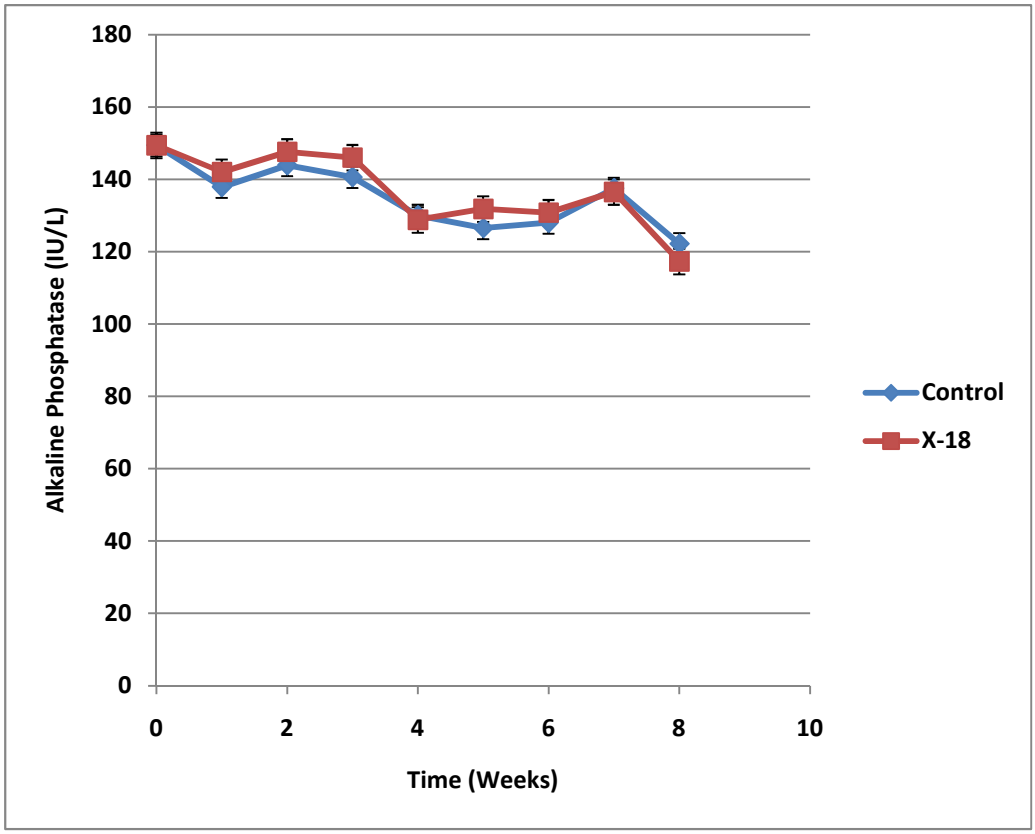

Figure B3. Comparison of levels of alkaline phosphatase between the control group and the treatment group over a period of 8 weeks.

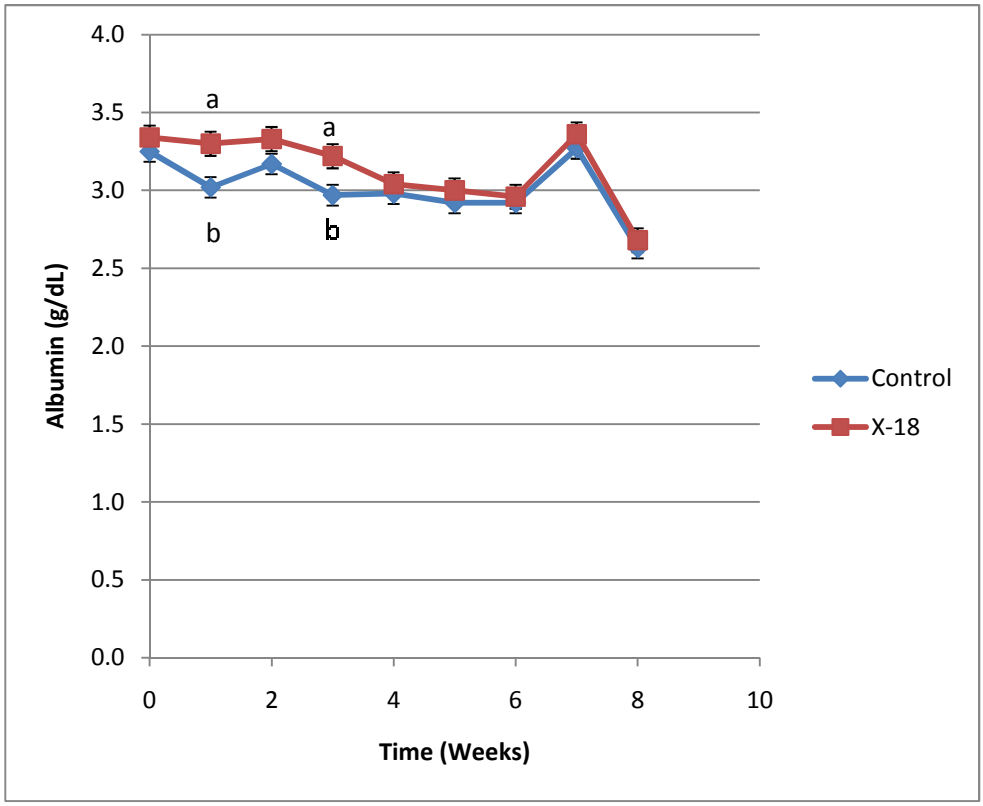

Figure B4. Comparison of levels of albumin between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

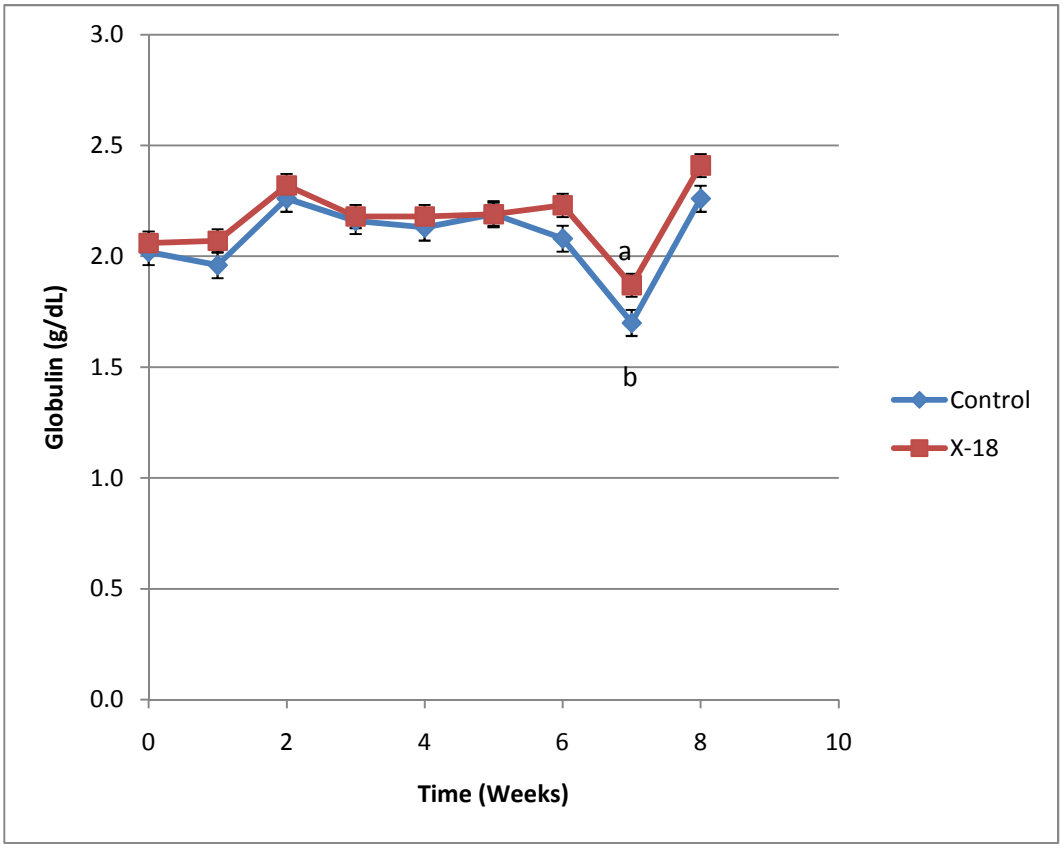

Figure B5. Comparison of levels of globulin between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$).

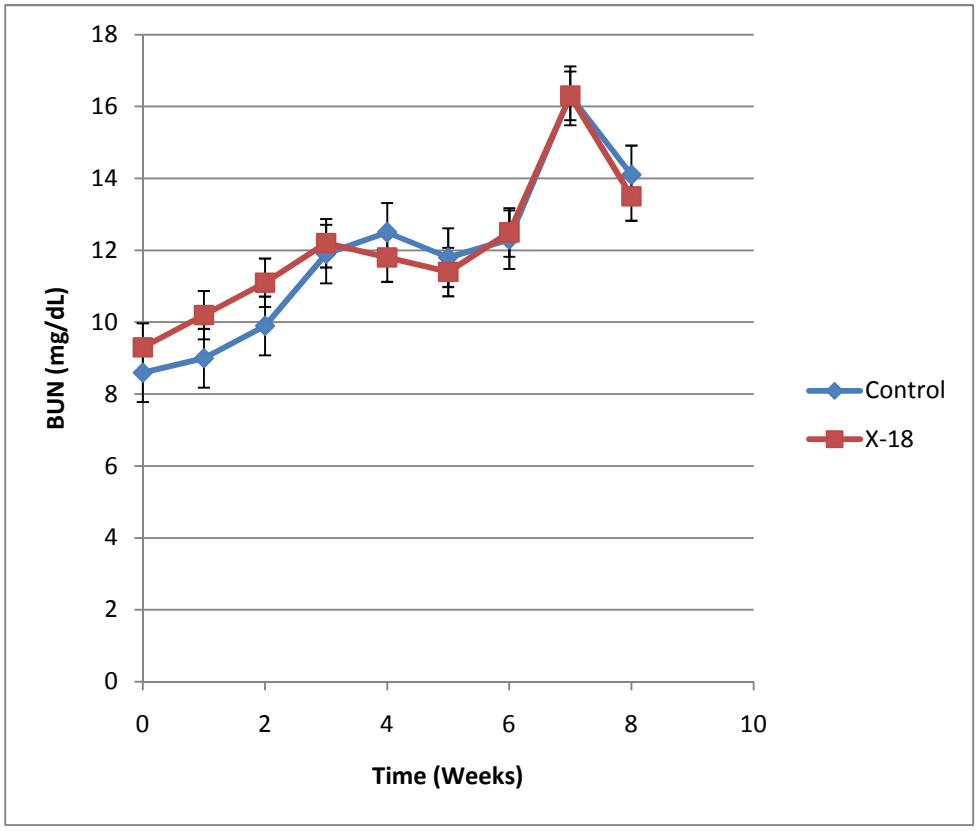

Figure B6. Comparison of levels of BUN between the control group and the treatment group over a period of 8 weeks.

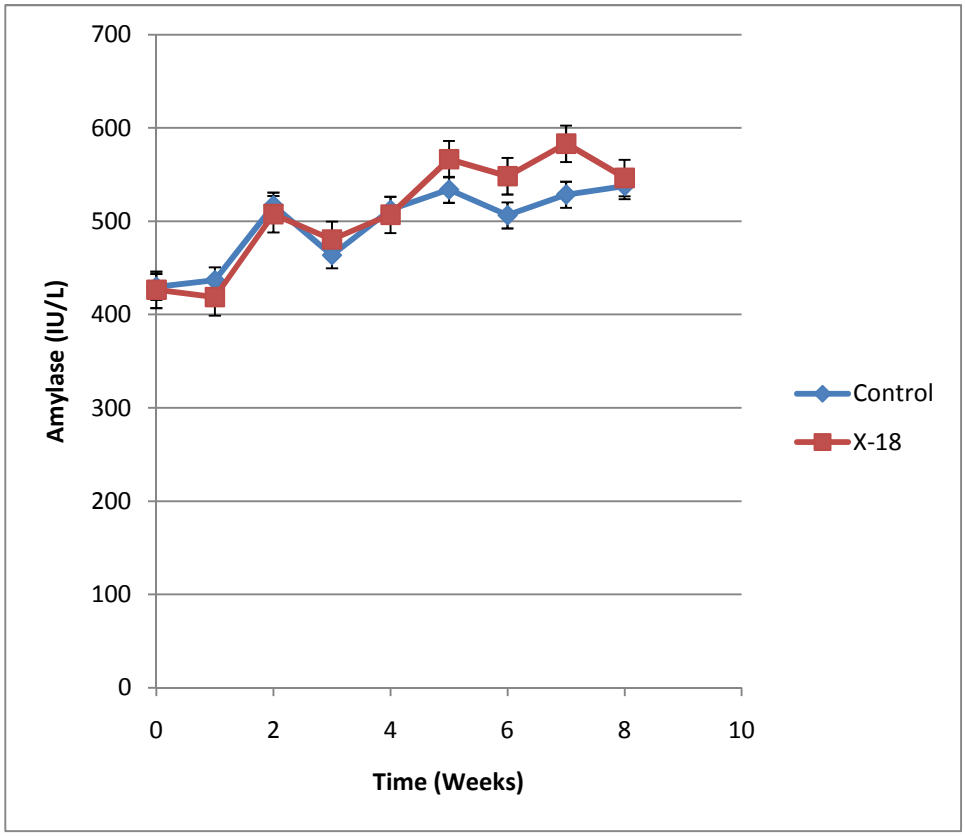

Figure B7. Comparison of levels of amylase between the control group and the treatment group over a period of 8 weeks.

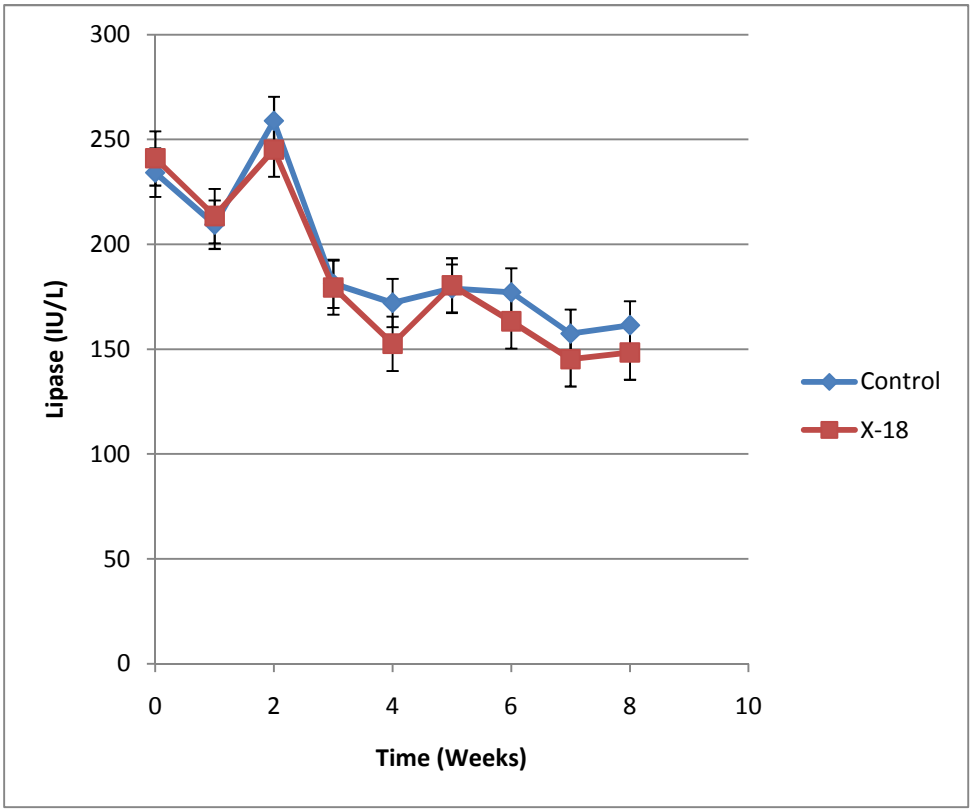

Figure B8. Comparison of levels of lipase between the control group and the treatment group over a period of 8 weeks.

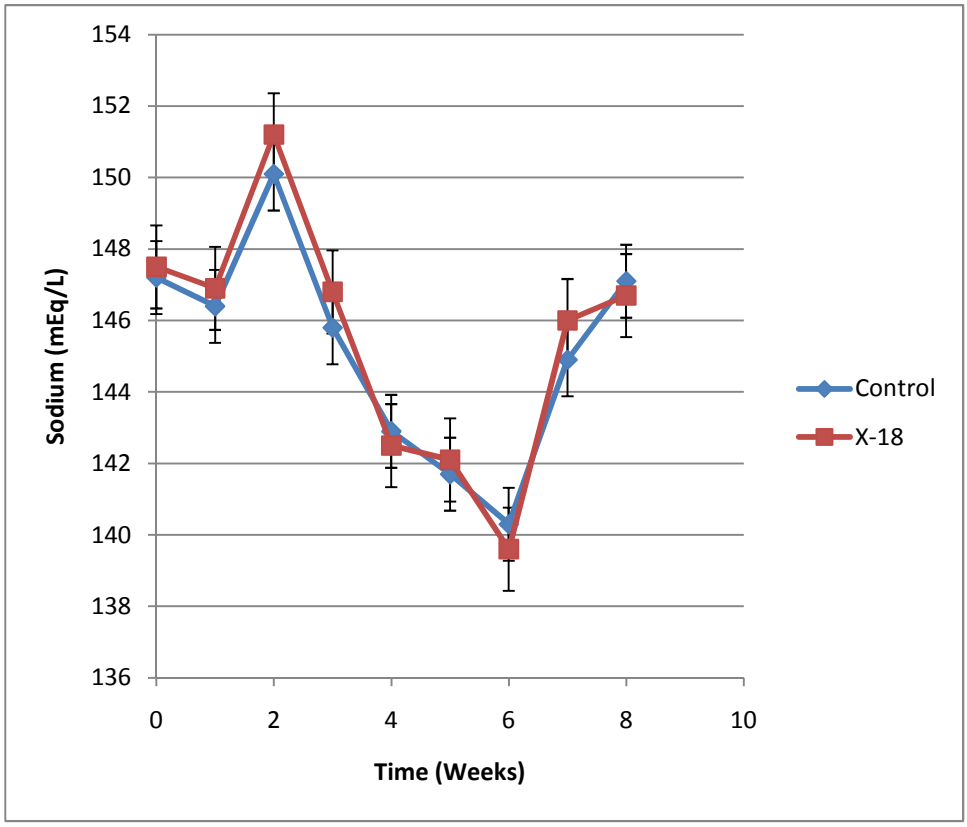

Figure B9. Comparison of sodium levels between the control group and the treatment group over a period of 8 weeks.

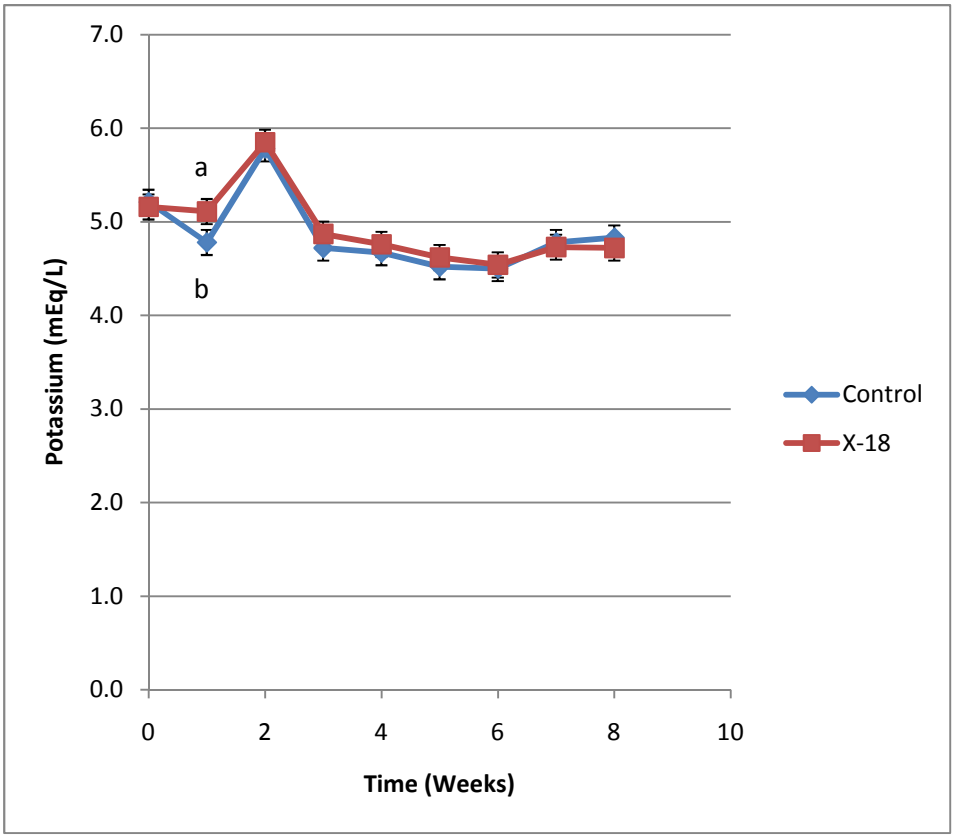

Figure B10. Comparison of potassium levels between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

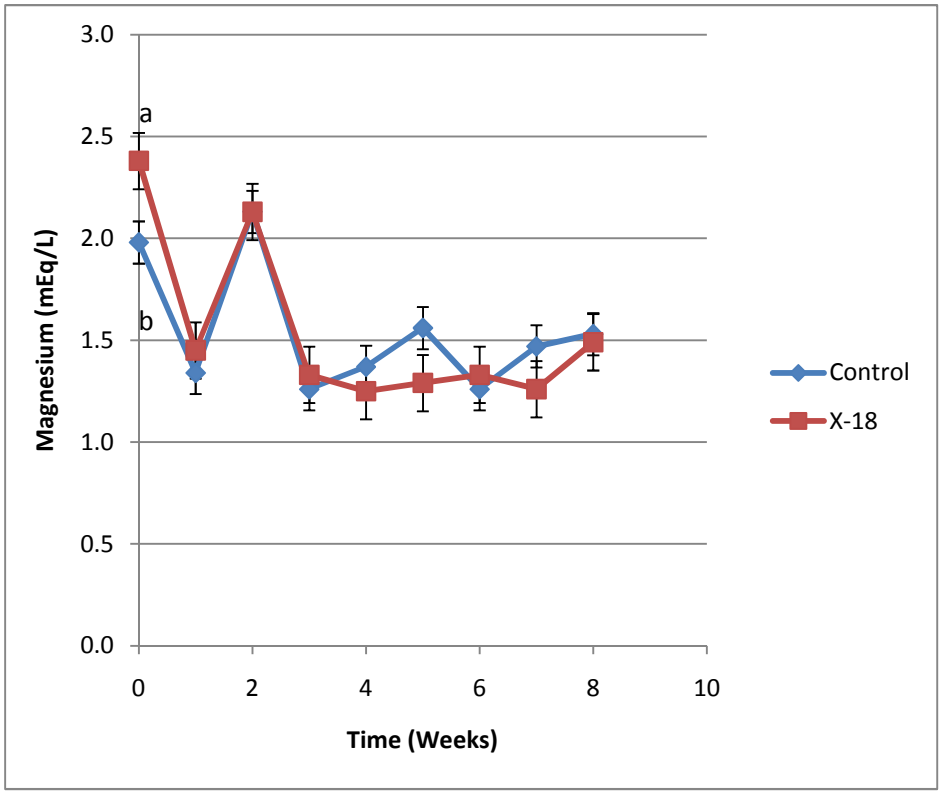

Figure B11. Comparison of magnesium levels between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

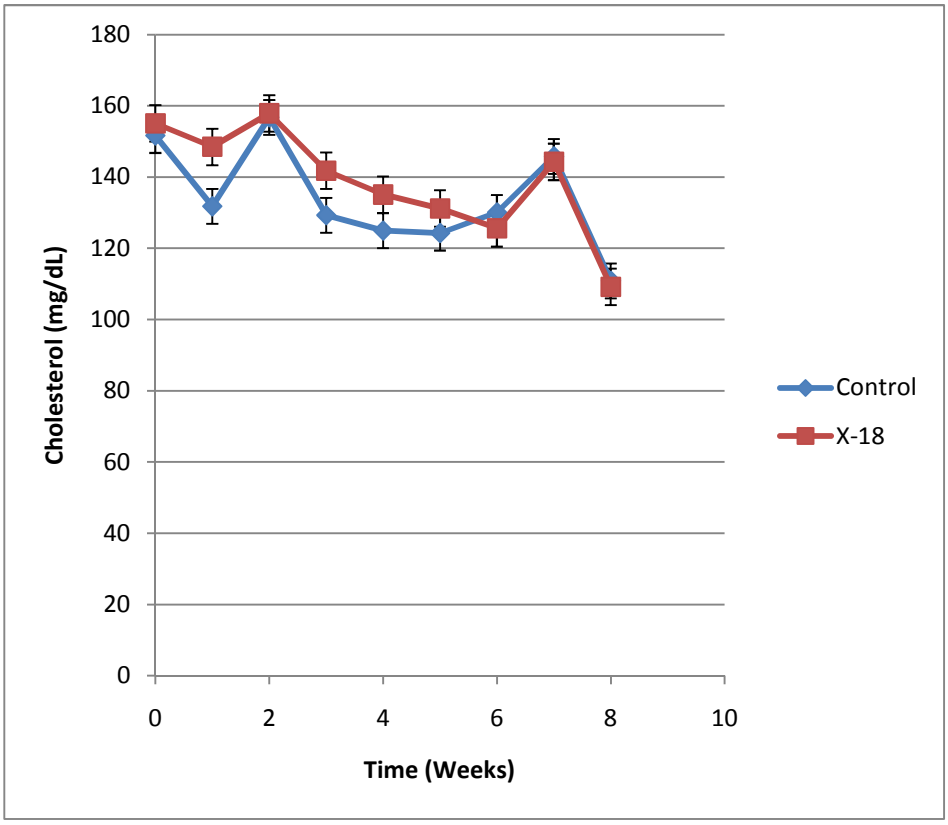

Figure B12. Comparison of cholesterol levels between the control group and the treatment group over a period of 8 weeks.

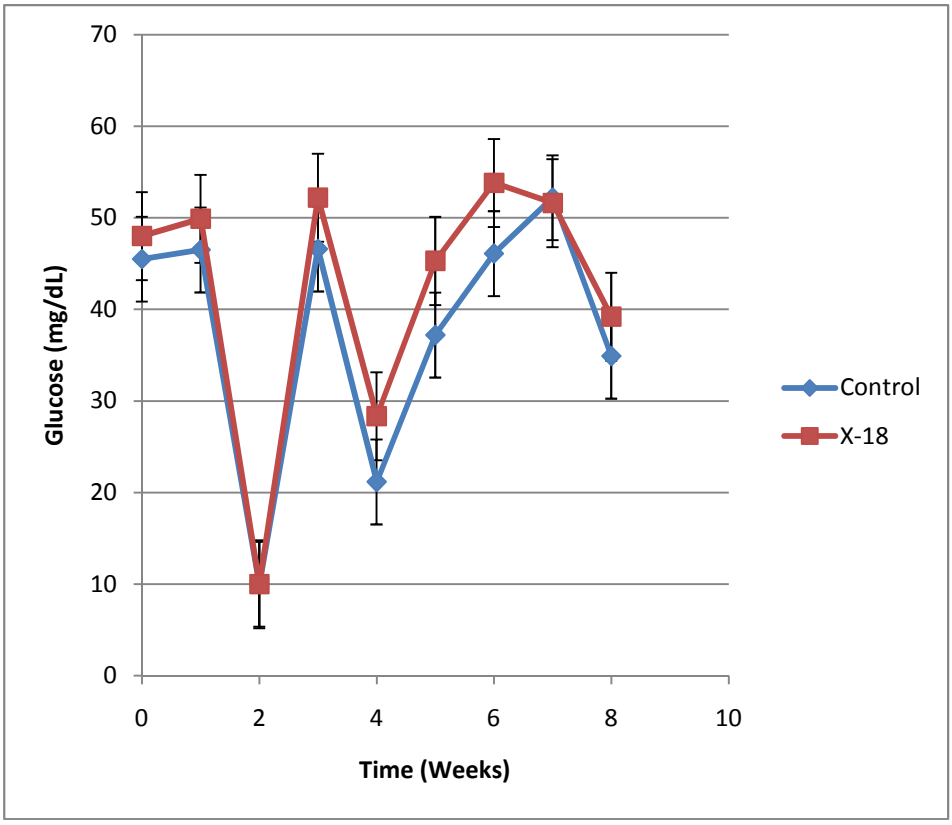

Figure B13. Comparison of glucose levels between the control group and the treatment group over a period of 8 weeks.

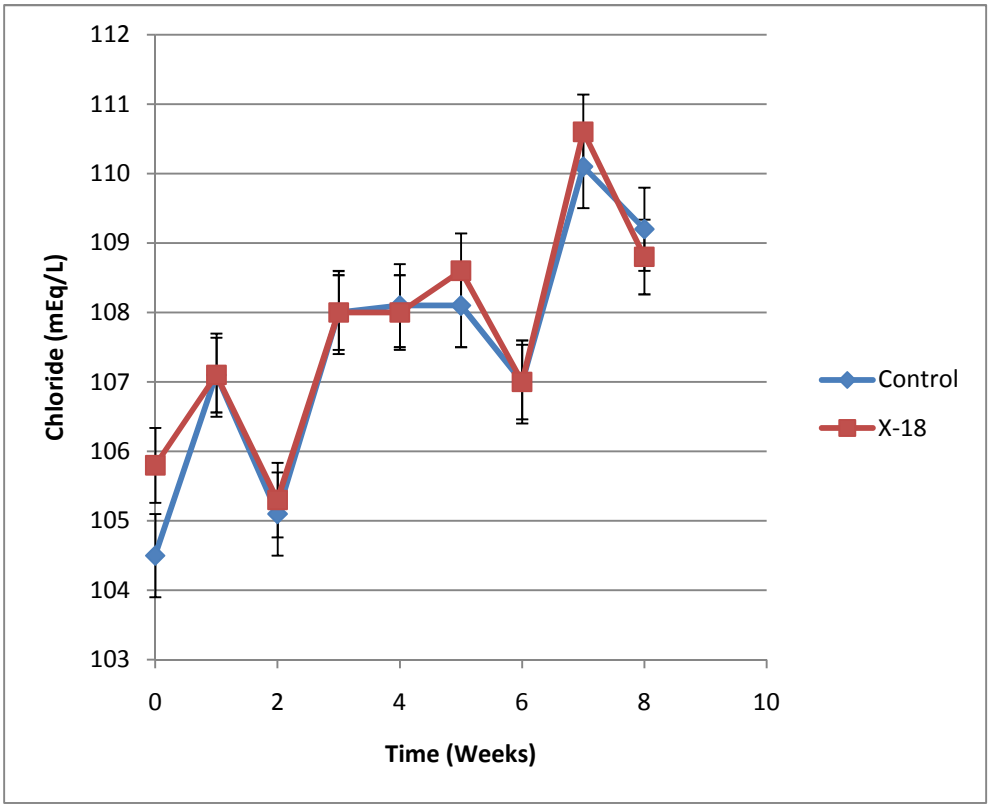

Figure B14. Comparison of chloride levels between the control group and the treatment group over a period of 8 weeks.

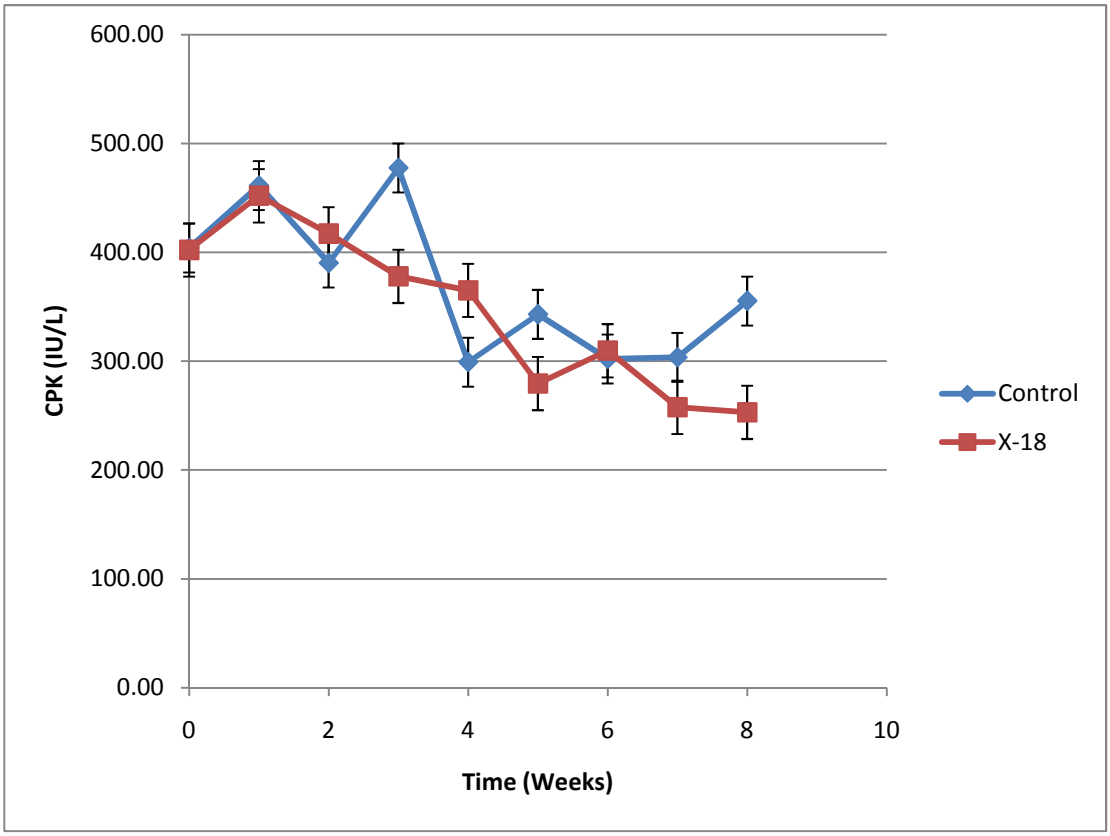

Figure B15. Comparison of levels of creatine phosphokinase between the control group and the treatment group over a period of 8 weeks.

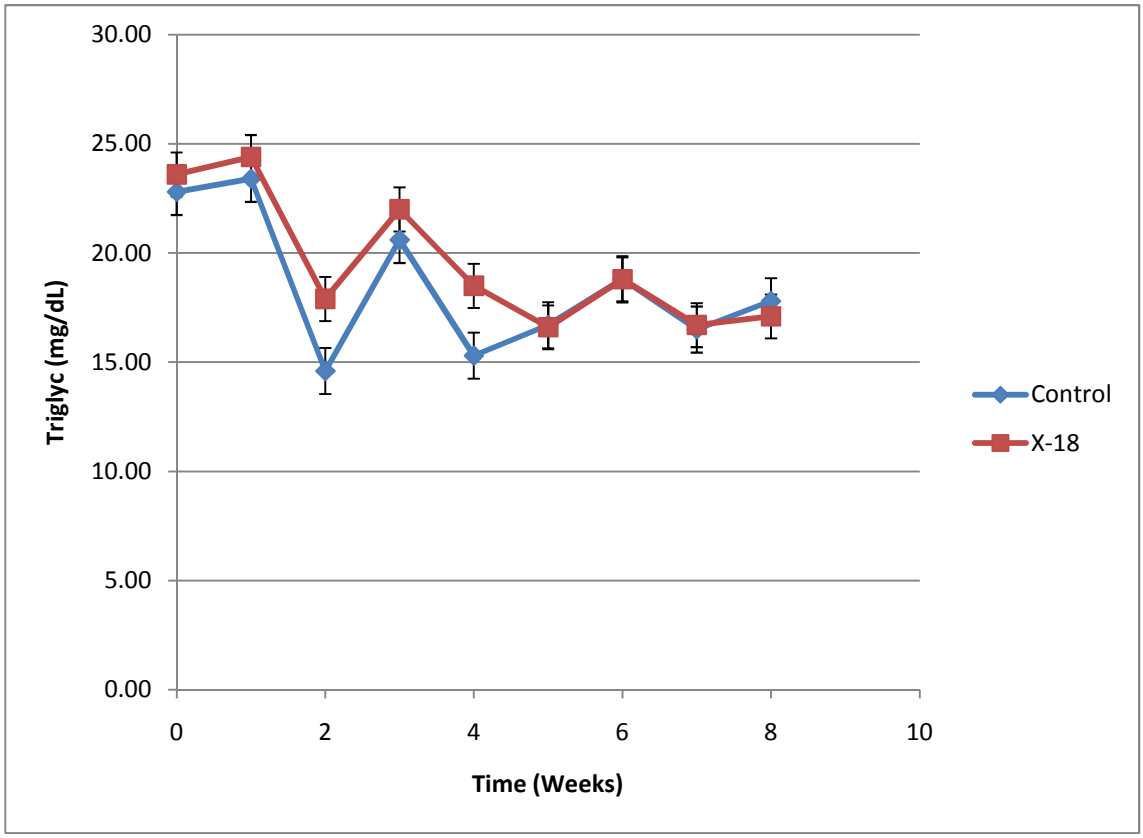

Figure B16. Comparison of levels of triglycerides between the control group and the treatment group over a period of 8 weeks.

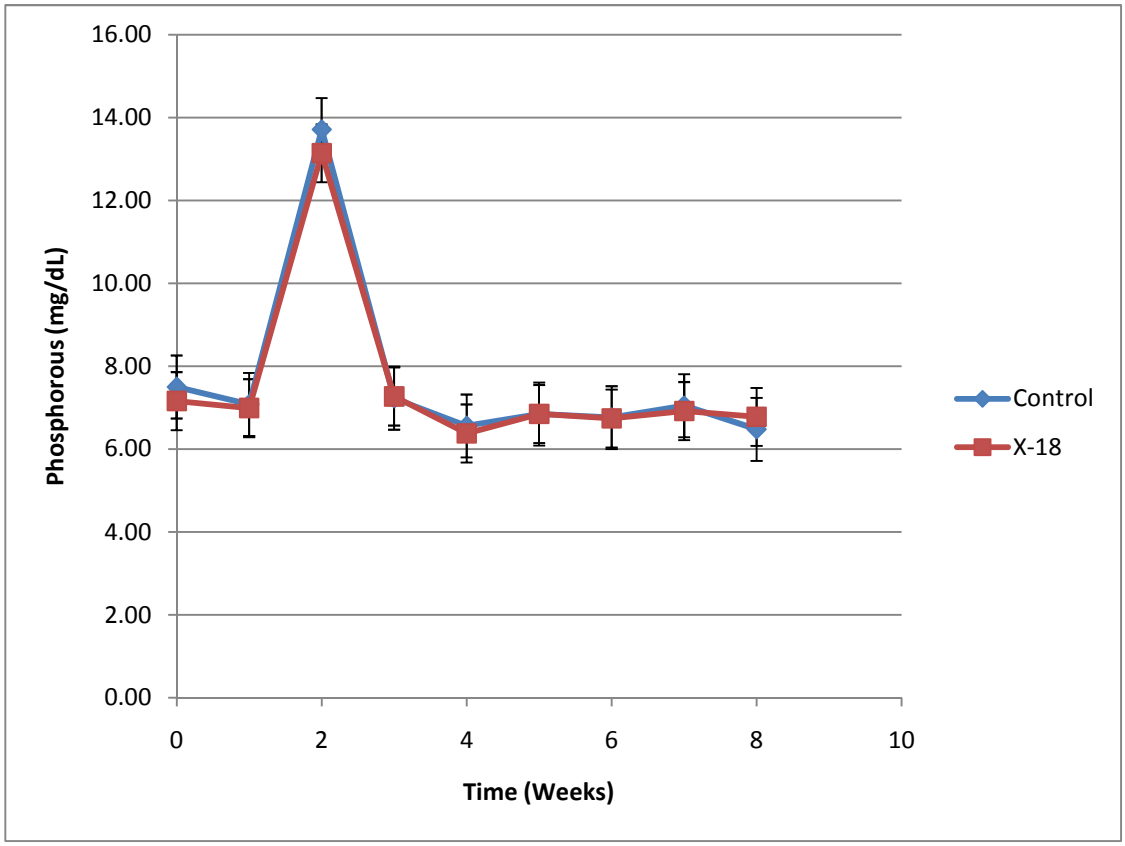

Figure B17. Comparison of levels of triglycerides between the control group and the treatment group over a period of 8 weeks.

Table B1. Blood Chemistry Panel[a]

Analytes Tested	Reference Values[b]	Units
AST[1]	15-66	IU/L
ALT[2]	12-118	IU/L
Total Bilirubin	0.1-0.3	mg/dL
AlkPhos[3]	5-131	IU/L
GGT[4]	1-12	IU/L
Total Protein	5.0-7.4	g/dL
Albumin	2.7-4.4	g/dL
Globulin	1.6-3.6	g/dL
Cholesterol	92-324	mg/dL
BUN[5]	6-25	mg/dL
Creatinine	0.5-1.6	mg/dL
Phosphorous	2.5-6.0	mg/dL
Calcium	8.9-11.4	mg/dL
Glucose	70-138	mg/dL
Amylase	290-1125	IU/L
Lipase	77-695	IU/L
Sodium	139-154	mEq/L
Potassium	3.6-5.5	mEq/L
Chloride	102-120	mEq/L
CPK[6]	59-895	IU/L
Triglycerides	29-291	mg/dL
Magnesium	1.5-2.5	mEq/L

[a]Performed using an Olympus Model AU 5400 System (ANTECH Diagnostics, Irvine, CA)

[b]Reference values established in-house using clinically normal dogs of all breeds and ages

[1]AST - Aspartate Aminotransferase

[2]ALT - Alanine Aminotransferase

[3]AlkPhos - Alkaline Phosphatase

[4]GGT - Gamma Glutamyl Transferase

[5]BUN - Blood Urea Nitrogen

[6]CPK - Creatine Phosphokinase

Table B2. Weekly Blood Chemistry Panel in Beagles

Treatment	Measurement	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	AST (IU/L)	33.00	29.50	40.67	29.33	31.00	29.50	31.67	30.00	32.50
	ALT (IU/L)	37.00	32.00	37.33	33.33	34.50	29.50	38.00	36.67	40.00
	Total Bilirubin (mg/dL)	0.20	0.30	0.10	0.17	0.20	0.30	0.13	0.10	0.15
	AlkPhos(IU/L)	143.50	115.50	141.67	169.33	125.50	100.50	117.33	172.67	105.00
	GGT (IU/L)	1.00	1.00	2.00	1.00	1.00	1.00	1.00	1.00	2.00
	Total Protein (g/dL)	5.15	5.20	5.27	5.20	5.15	5.05	4.83	5.17	5.20
	Albumin (g/dL)	3.35	3.15	3.07	3.07	3.15	2.65	2.73	3.50	2.85
	Globulin (g/dL)	1.80	2.05	2.20	2.13	2.00	2.40	2.10	1.67	2.35
	Cholesterol (mg/dL)	151.70	131.80	156.80	129.30	125.00	124.30	130.10	145.80	110.90
	BUN (mg/dL)	3.80	4.19	4.31	4.51	4.74	4.87	5.16	5.59	3.60
	Creatinine (mg/dL)	0.78	0.66	0.57	0.50	0.50	0.50	0.50	0.50	0.50
	Phosphorous (mg/dL)	7.50	7.08	13.71	7.23	6.56	6.85	6.76	7.05	6.48
	Calcium (mg/dL)	11.45	10.88	11.25	11.05	10.91	10.69	10.61	10.51	10.42
	Glucose (mg/dL)	45.50	46.50	10.00	46.60	21.17	37.20	46.10	52.20	34.90
	Amylase (IU/L)	4.05	4.38	4.54	5.04	4.94	4.93	5.39	3.59	3.79
	Lipase (IU/L)	34.50	31.50	35.00	30.33	33.50	29.50	35.67	34.67	23.50
	Sodium (mEq/L)	37.50	30.00	39.67	34.67	35.50	26.50	52.33	39.67	42.00
	Potassium (mEq/L)	0.20	0.20	0.77	0.23	0.40	0.10	0.20	0.17	0.20
	Chloride (mEq/L)	104.50	107.10	105.10	108.00	108.10	108.10	107.00	110.10	109.20
	CPK (IU/L)	404.00	461.40	390.20	477.50	299.20	343.10	302.20	303.60	355.40
	Triglycerides (mg/dL)	22.80	23.40	14.60	20.60	15.30	16.70	18.80	16.50	17.80
	Magnesium (mEq/L)	30.50	31.00	32.67	32.33	28.50	28.50	31.67	25.67	34.00

Table B3. Weekly Blood Chemistry Panel in Beagles

Treatment	Measurement	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
L. reuteri X-18	AST (IU/L)	35.00	35.00	45.00	33.00	38.00	31.67	32.50	34.50	33.50
	ALT (IU/L)	42.67	32.00	36.50	36.50	38.67	37.33	38.00	41.50	37.50
	Total Bilirubin (mg/dL)	0.20	0.33	0.30	0.15	0.40	0.23	0.25	0.10	0.35
	AlkPhos (IU/L)	142.67	150.33	151.50	118.00	127.33	139.00	136.00	105.50	111.00
	GGT (IU/L)	1.00	1.00	2.50	1.00	1.00	1.00	1.00	1.00	1.00
	Total Protein (g/dL)	5.50	5.27	6.10	5.30	5.17	5.17	5.40	5.20	5.00
	Albumin (g/dL)	3.37	3.13	3.65	3.15	2.93	3.00	3.15	3.35	2.60
	Globulin (g/dL)	2.13	2.13	2.45	2.15	2.23	2.17	2.25	1.85	2.40
	Cholesterol (mg/dL)	155.10	148.50	157.90	141.80	135.10	131.20	125.60	144.30	109.20
	BUN (mg/dL)	4.19	4.33	4.09	4.67	4.99	5.28	4.64	5.42	3.86
	Creatinine (mg/dL)	0.56	1.02	0.58	0.50	0.50	0.50	0.50	0.51	0.50
	Phosphorous (mg/dL)	7.16	6.99	13.14	7.27	6.38	6.85	6.74	6.92	6.78
	Calcium (mg/dL)	11.52	11.29	11.49	11.42	11.24	10.91	10.54	10.47	10.60
	Glucose (mg/dL)	48.00	49.90	10.00	52.20	28.33	45.30	53.80	51.60	39.20
	Amylase (IU/L)	4.43	4.35	4.25	4.86	5.20	5.43	4.75	3.52	4.00
	Lipase (IU/L)	40.00	30.67	35.00	32.50	32.33	30.00	37.50	29.50	27.50
	Sodium (mEq/L)	40.67	36.33	40.50	43.50	35.33	28.00	44.50	39.00	34.50
	Potassium (mEq/L)	0.20	0.27	0.30	0.20	0.17	0.10	0.10	0.20	0.40
	Chloride (mEq/L)	105.80	107.10	105.30	108.00	108.00	108.60	107.00	110.60	108.80
	CPK (IU/L)	402.20	452.00	417.20	378.00	365.10	279.50	309.70	257.70	253.10
	Triglycerides (mg/dL)	23.60	24.40	17.90	22.00	18.50	16.60	18.80	16.70	17.10
	Magnesium (mEq/L)	32.67	32.67	30.00	34.00	35.33	31.00	44.00	25.50	42.50

APPENDIX C

Additional Complete Blood Count Figures and Tables

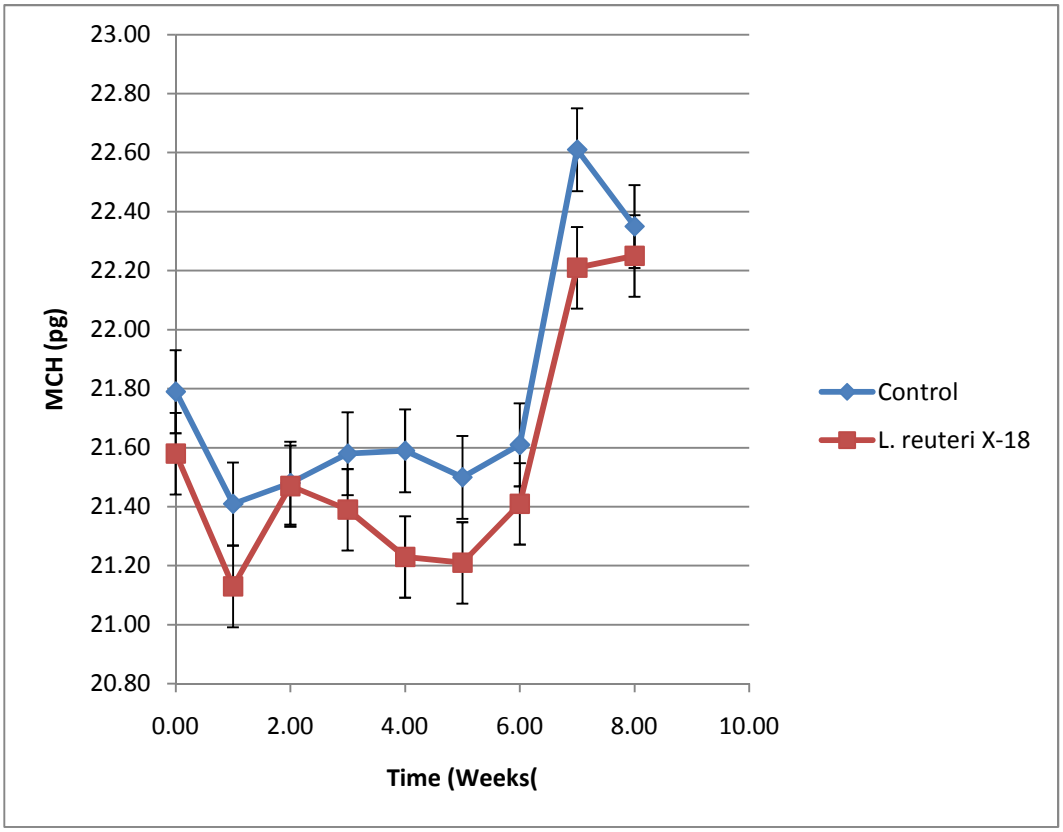

Figure C1. Comparison of MCH between the control group and the treatment group over a period of 8 weeks

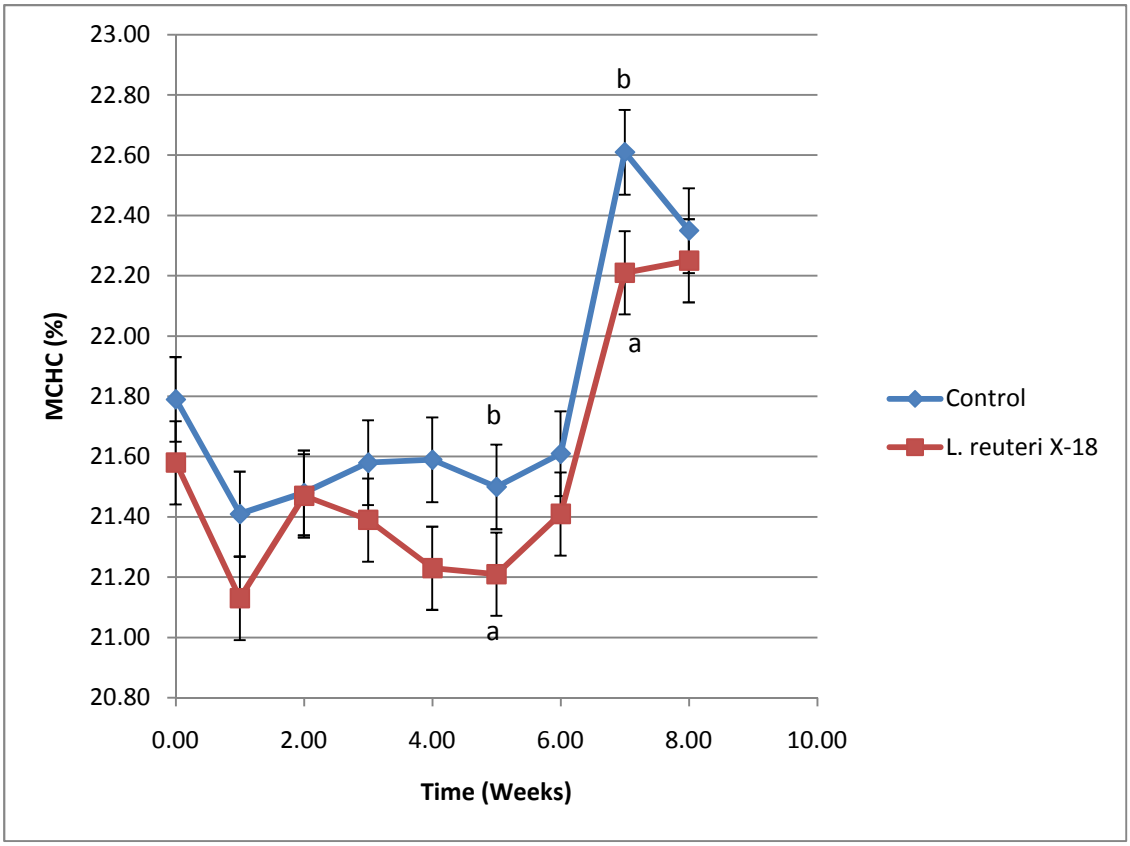

Figure C2. Comparison of MCHC between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

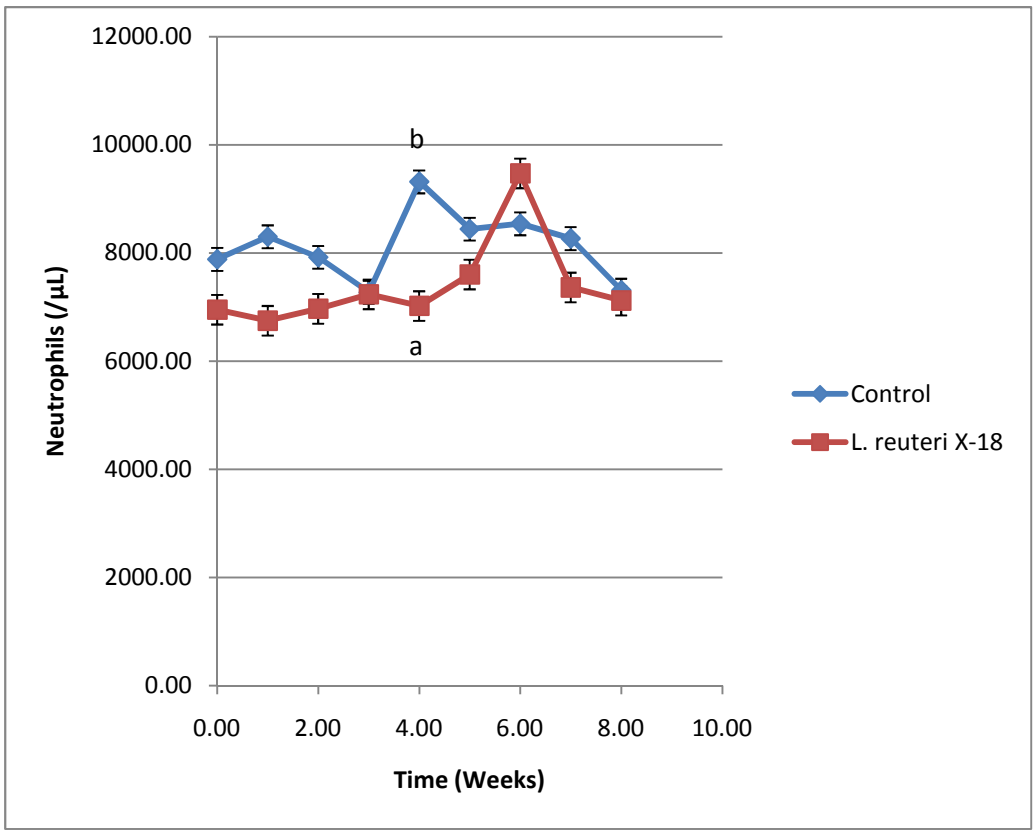

Figure C3. Comparison of neutrophils between the control group and the treatment group over a period of 8 weeks: [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

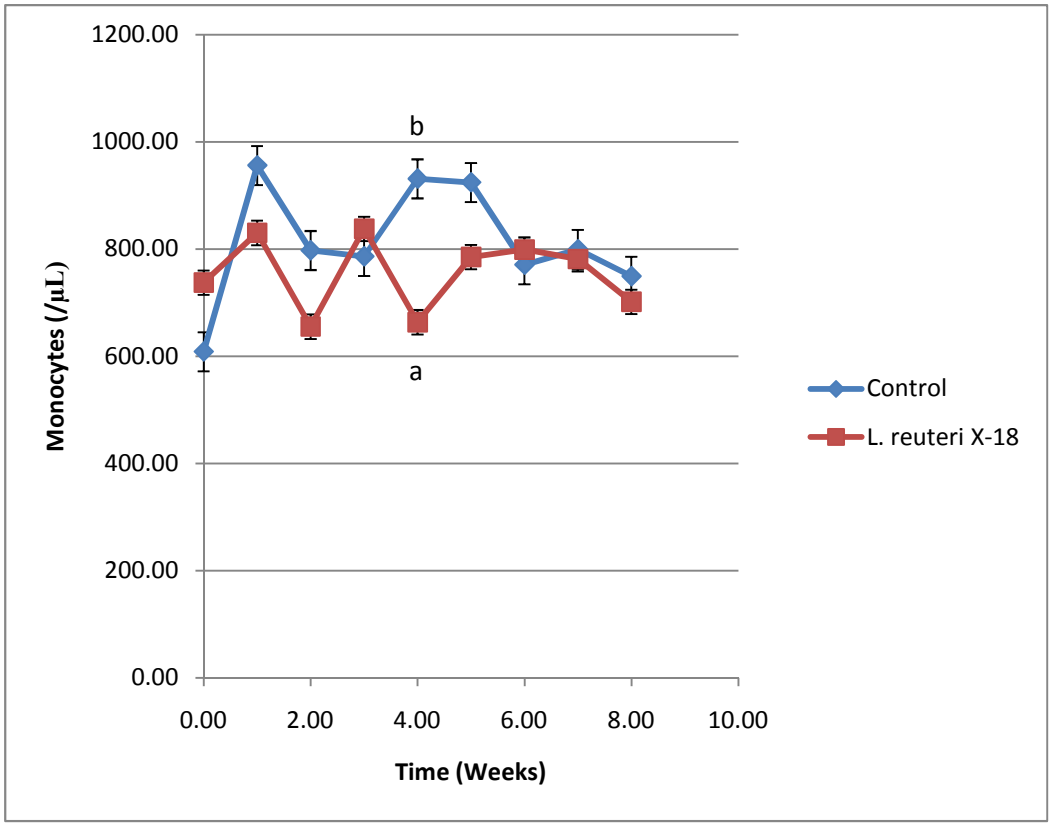

Figure C4. Comparison of monocyte counts between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$)

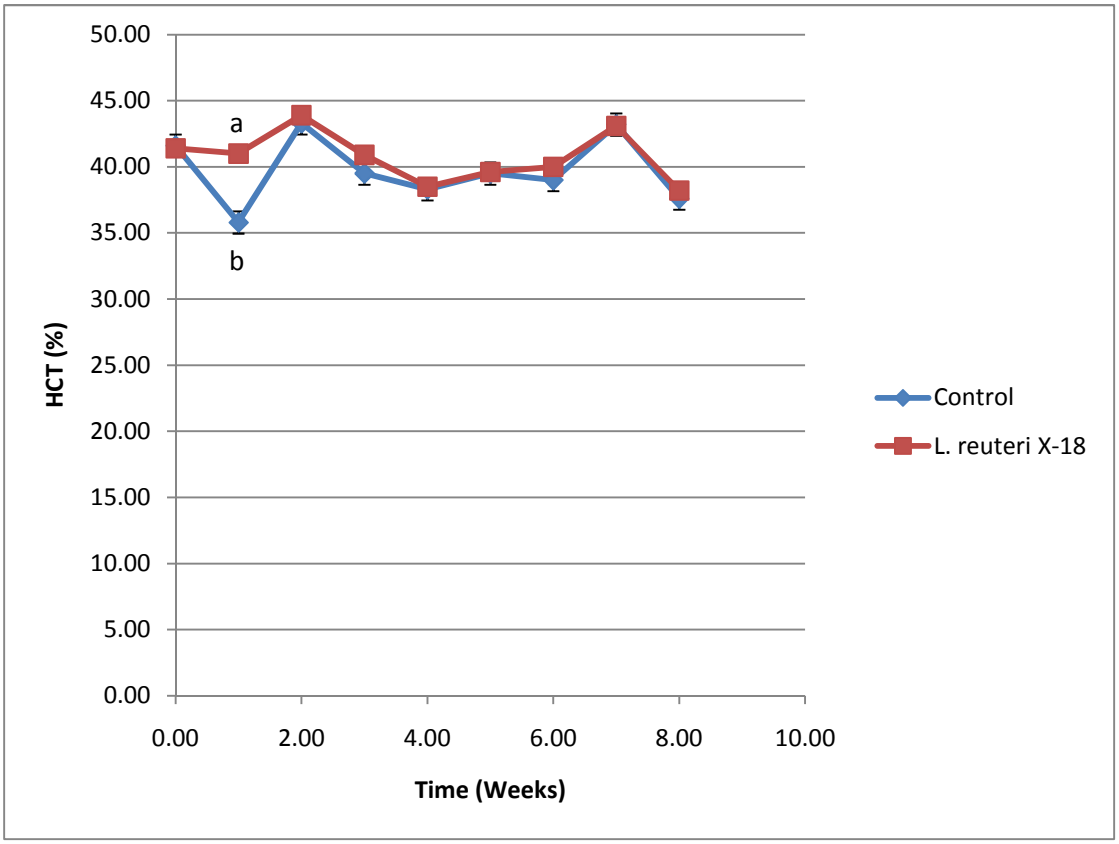

Figure C5. Comparison of levels of hematocrit between the control group and the treatment group over a period of 8 weeks; [a,b]Superscripts between trts on the same day are significantly different ($P<0.05$).

Table C1. Complete Blood Count[a]

Analytes Tested	Reference Values[b]	Units
White Blood Cell (WBC)	4.0-15.5	$10^3/\mu L$
Red Blood Cell (RBC)	4.8-9.3	$10^6/\mu L$
Hemaglobin (HGB)	12.1-20.3	g/dL
Hematocrit (HCT)	36-60	%
Mean Cell Volume (MCV)	58-79	fL
Mean Corpuscular Hemoglobin (MCH)	19-28	pg
Mean Cell Hemoglobin Concentration (MCHC)	30-38	%
Neutrophils	2,060-10,600	/μL
Lymphocytes	690-4,500	/μL
Monocytes	0-840	/μL
Eosinophils	0-1,200	/μL
Basophils	0-150	/μL
Platelet Count	170-400	$10^3/\mu L$

[a]Performed using an Olympus Model AU 5400 System (ANTECH Diagnostics, Irvine, CA)

[b]Reference values established in-house using clinically normal dogs of all breeds and ages

Table C2. Weekly Complete Blood Count in Beagles

Treatment	Measurement	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	White Blood Cells (WBC) (x 10^3/µL)	11.35	13.24	12.44	11.87	14.30	13.54	13.20	13.29	11.67
	Red Blood Cells (RBC) (x 10^6/µL)	6.02	5.90	6.05	5.94	5.98	5.70	5.90	6.15	5.73
	Hemoglobin (HGB) (g/dL)	13.11	12.57	12.98	12.80	12.90	12.96	12.75	13.93	12.81
	Hematocrit (HCT) (%)	41.60	35.77	43.30	39.50	38.30	39.50	39.00	43.20	37.60
	Mean Cell Volume (MCV) (fL)	69.20	66.80	71.70	66.60	64.20	65.30	66.00	70.20	65.70
	Mean Corpuscular Hemoglobin (MCH) (pg)	21.79	21.41	21.48	21.58	21.59	21.50	21.61	22.61	22.35
	Mean Cell Hemoglobin Concentration (MCHC) (%)	31.70	32.10	30.20	32.50	33.80	32.90	32.80	32.30	34.00
	Neutrophils (/µL)	7883.80	8301.70	7921.60	7274.30	9315.90	8442.10	8542.00	8268.60	7314.10
	Lymphocytes (/µL)	2678.80	3673.70	3405.40	3475.90	3774.10	3924.00	3614.20	3891.20	3277.00
	Monocytes (/µL)	608.60	955.80	797.30	786.10	931.00	924.20	770.60	799.30	749.10
	Eosinophils (/µL)	201.38	205.30	264.90	248.50	203.70	204.90	219.60	221.30	262.70
	Basophils (/µL)	73.00	103.50	50.80	85.20	75.30	44.80	53.60	109.60	67.10
	Platelet Count (x 103/µL)	425.50	518.70	466.20	540.30	474.00	542.00	569.20	596.90	534.80
L. reuteri X-18	White Blood Cells (WBC) (x 10^3/µL)	10.75	11.70	11.46	11.82	12.75	13.40	14.48	12.12	12.10
	Red Blood Cells (RBC) (x 10^6/µL)	6.1	6.20	6.22	6.25	6.06	6.28	6.17	6.35	5.98
	Hemoglobin (HGB) (g/dL)	13.2	13.10	13.37	13.37	12.88	13.30	13.21	14.13	13.34
	Hematocrit (HCT) (%)	41.4	41.00	43.90	40.90	38.50	39.60	40.00	43.10	38.20
	Mean Cell Volume (MCV) (fL)	67.9	66.10	70.40	65.50	63.40	63.10	64.80	67.70	63.50
	Mean Corpuscular Hemoglobin (MCH) (pg)	21.6	21.13	21.47	21.39	21.23	21.21	21.41	22.21	22.25
	Mean Cell Hemoglobin Concentration (MCHC) (%)	32.0	32.00	30.70	32.60	33.50	33.70	33.00	32.80	34.90
	Neutrophils (/µL)	6953.4	6748.50	6969.70	7236.90	7021.80	7604.40	9472.70	7363.70	7120.60
	Lymphocytes (/µL)	2870.6	3805.00	3578.70	3498.10	4806.90	3920.30	3955.30	3726.60	4005.70
	Monocytes (/µL)	737.4	830.20	655.30	837.70	663.40	785.10	798.90	781.40	701.50
	Eosinophils (/µL)	123.2	200.70	180.30	154.00	164.10	150.30	175.10	152.90	187.30
	Basophils (/µL)	141.0	115.60	76.00	93.30	104.22	89.90	78.00	95.40	84.80
	Platelet Count (x 103/µL)	435.7	502.30	423.00	467.10	445.30	479.40	525.30	539.70	544.10

VITA

Sandra McCoy

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF FEEDING *LACTOBACILLUS REUTERI* X-18 ON BLOOD CHEMISTRY AND IMMUNE PARAMETERS IN BEAGLE (*CANIS FAMILIARIS*) PUPPIES

Major Field: Food Science

Biographical:

Personal Data: Born in Singapore, Republic of Singapore, September 12, 1976. The daughter of Clifford and Suong Ngoc McCoy.

Education: Graduated from Neosho High School, Neosho, Missouri in May of 1994; Received a Bachelors of Science degree in Biology/Pre-Medicine/Chemistry minor at Missouri Southern State University, Joplin, Missouri in May of 1998; Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in December of 2005; Completed the requirements for the Doctorate of Philosophy in Food Science at Oklahoma State University, Stillwater, Oklahoma in December of 2009.

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ADVISER'S APPROVAL: Dr. William McGlynn for Dr. Stanley E. Gilliland

Name: Sandra McCoy Date of Degree: May, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECTS OF FEEDING *LACTOBACILLUS REUTERI* X-18 ON BLOOD CHEMISTRY AND IMMUNE PARAMETERS IN BEAGLE (*CANIS FAMILIARIS*) PUPPIES

Pages in Study: 147 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study: The main objective of the present study was to see the effects of feeding *L. reuteri* on various immune parameters in two-month old beagle puppies. Twenty beagle puppies were assigned to one of two treatments. Ten puppies received the probiotic *L. reuteri* X-18 in ~5-10 mL of 10% nonfat dry milk (1.0×10^9 CFU each) while the control group received only 10% nonfat dry milk. Treatments were fed twice daily in addition to dry dog food for eight weeks. At the end of each week, blood and fecal samples were collected as well as body weights to adjust dry food rations for performance measures. Serum and fecal immunoglobulin (IgA, IgE, IgM and IgG) levels, complete blood counts, and biochemistry panels were monitored on a weekly basis over the period of two months.

Findings and Conclusions: Overall, no significant differences could be seen between the control and treatment groups with respect to immunoglobulins. For the CBC, the least square means of both the control and treatment groups were within the reference range with only two parameters (eosinophils and platelets) being slightly elevated. Those elevated values could be due to variations as a result of the testing profiles that were used to determine the reference ranges (sex, breed, and age). Based on these results, the animals were still considered healthy with no extreme effects seen. For the biochemistry panel, only GGT and creatinine showed a significant treatment x week effect. The least square means of both the control and treatment groups were within the reference range, which are consistent with the healthy status of the dogs. Consumption of the probiotic was not associated with any significant changes in the clinical status as determined by the use of a standard physical examination, CBC, biochemical profiles, and immunoglobulin concentrations. The lack of differences could be considered advantageous, as there were no adverse side effects observed due to the consumption of the probiotic.

ADVISER'S APPROVAL: Dr. William McGlynn for Dr. Stanley E. Gilliland