EVALUATION OF A PROBIOTIC (LEVUCELL SB®) AND A PREBIOTIC (AGRIMOS®) ON PERFORMANCE, HEALTH AND FECAL MICROFLORA OF VEAL CALVES

By

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ABSTRACT

Master of Science Animal Science (Nutrition)

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EVALUATION OF A PROBIOTIC (LEVUCELL SB®) AND A PREBIOTIC (AGRIMOS®) ON PERFORMANCE, HEALTH AND FECAL MICROFLORA OF VEAL CALVES

Probiotics and prebiotics have been used in many areas of animal husbandry for their beneficial effects on health and productivity. The aim of the study was to determine the impact of a yeast probiotic and prebiotic on health, growth performance and fecal microflora of veal calves. Sixty-eight Holstein calves were fed for 8 weeks a control diet or diets in which a probiotic (Levucell SB®; 0.5g/d) or a prebiotic (AgriMOS®, a manno-oligosaccharide; 3g/d) was added to the milk replacer (MR). The only significant effect of the additives on calf performance was on MR intake; calves fed Levucell SB® (SB) consumed more (P<0.05) MR than those fed AgriMOS®. To assess the effect on the gut flora, fecal samples were collected on d0, 7, 13, 28, 41 and 57. On days7 and 28 the fecal population of lactic acid bacteria (LAB) was greater (P<0.05) with SB than with AgriMOS; however the average effect of the additives on fecal LAB was no different (P>0.05) in comparison with control. Animals fed the additives showed a reduction in *E. coli* over time, whereas *E. coli* counts in the control group remained static. Clostridial numbers were also reduced in the SB group. Results of PCR-TTGE on DNA extracted from feces revealed that fecal microbial population associated with SB was significant different from that associated with AgriMOS or control groups. Despite housing, environmental and management conditions which comprised calf health, neither a probiotic nor a prebiotic proved beneficial in veal production.

Résumé

Maîtrise en Science Sciences Animales (Nutrition)

Katherine Chong

EVALUATION D'UN PROBIOTIQUE (LEVUCELL SB®) ET D'UN PREBIOTIQUE (AGRIMOS®) SUR LA PERFORMANCE, LA SANTE ET LA MICROFLORE FECALE DE VEAUX DE BOUCHERIE

Les probiotiques et les prébiotiques sont utilisés dans l'élevage d'une variété de cheptels pour leurs effets bénéfiques sur la santé et la productivité des animaux. Le but de cette recherche était d'évaluer l'impact d'une levure probiotique et d'un prébiotique sur le taux de croissance, la santé et la microflore fécale de veaux de boucherie. Soixante-huit veaux Holstein ont été nourris, lors d'une étude menée sur une période de 8 semaines, de succédané de lait (SL) constitué soit d'un régime témoin (ne contenant aucun additif), soit d'un régime contenant le probiotique (Levucell SB®, 0.5g/j) ou d'un régime contenant le prébiotique (AgriMOS, un manno-oligosaccharides 3g/j). Le seul effet significatif des additifs sur la performance des veaux a été retrouvé sur la consommation du SL; les veaux nourris avec le Levucell SB® (SB) ayant consommé plus ($p<0.05$) de SL que ceux nourris avec AgriMOS®. Pour évaluer les effets des additifs sur la flore intestinale, des échantillons fécaux ont été recueillis aux jours 0, 7, 13, 28, 41 et 57. Aux jours 7 et 28, la population fécale de bactéries lactiques (BL) était supérieure ($p<0.05$) avec SB qu'avec AgriMOS; cependant l'effet moyen des additifs sur les BL fécales n'était pas significatif ($p>0.05$) lorsque comparé avec le groupe témoin. Une réduction de la population de *E.coli* été notée pour la durée de l'étude chez les animaux nourris des additifs alors que sa population est restée stationnaire dans le groupe témoin. La numération de Clostridium a aussi été retrouvée diminuée dans le groupe nourris avec le SB. Les résultats obtenus à l'aide de

la technique PCR-TTGE sur l'ADN extrait d'échantillons fécaux ont montré un effet significatif de l'additif SB sur la flore microbienne lorsque comparé aux groupes témoins et nourris avec AgriMOS ($p<0.05$). Lorsque de saines pratiques en matière de conditions environnementales et managériales ne sont pas respectées, ni probiotique ni prébiotique n'exercent un effet bénéfique sur la production des veaux de boucherie.

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1. INTRODUCTION

Veal is the meat from young male dairy calves and is considered as a speciality meat in the Canadian livestock industry. “Grain-fed veal”, the object of this study, is defined as calves fed milk-based feeds for the first 6 weeks and then grain corn and a protein supplement for a further 17 weeks until a slaughter live weight of about 295-320 kg (OMAFRA, 2006; Ngapo and Gariepy, 2006). “White veal” is differentiated from grain -fed veal in that the former are calves fed exclusively on milk based feeds and slaughtered at approximately 16-19 weeks (Ngapo and Gariepy, 2006). In Canada, calves with a carcass weight, hide off, of over 180 kg are not considered veal (OMAFRA, 2006).

Compared to poultry, beef, pork and lamb, veal is a minor commodity in the global meat market (Ngapo and Gariepy, 2006) but it is a significant meat source in European countries, especially in France and Italy (European Commission, 2007), in the United States (American Veal Association, 2009) and in Canada, especially in Quebec and Ontario (Ontario Veal, 2008). Quebec accounts for the majority of veal production in Canada and between 1991 and 2003 the number of calves contributing to veal production in Quebec increased from 40,000 head to 100,000 head. However, due to the occurrence, in 2003, of the Bovine Spongiform Encephalopathy (BSE) in Canada, the national output of veal in that year declined to 349 million pounds. A recovery was observed between 2006 and 2007, when the veal meat output in Quebec increased from 29.2 million pound to 32.3 million pound. The 2008 farm year was marked by relatively stable veal production throughout the economic recession is expected to cause a reduction in veal production in 2009 (FPBQ, 2008).

Due to the system of acquisition and management of veal calves, veal producers face major challenges in ensuring the health and productivity of these young animals. Under current

husbandry conditions, veal calves usually suffer from diarrhea and respiratory diseases which are the main causes of morbidity and mortality in the early life of veal calves (Drevjany et al., 1986; Kyriakis et al., 1999; Snowder et al., 2005). Several reasons are thought to be responsible for the high incidence of intestinal and respiratory disease in veal calves. The calves are often separated from their mothers just after birth, preventing them from obtaining sufficient colostrums. Inadequate passive transfer of colostrums and immunoglobulin increases the risk for disease (Tyler et al., 1998; Donovan et al., 1998), and may contribute to bacterial infection of the digestive tract and death during the first 3 months (Virtala et al., 1999).

In order to improve the productivity of veal calves, farmers have relied on antibiotics (Quigley et al., 1997; Berge et al., 2005) and growth promoting hormones but the latter agents has been banned for use in the Canadian veal industry. Given public heath concern about the spread of antibiotic resistant bacteria (Fuller, 1989; Phillips, 1999) veal producers' reliance on antibiotics is unsustainable. On the 1st January, 2006, the European Union initiated a ban of antibiotic growth promotants (AGP) (Union of Concerned Scientist, 2006) and a future global ban on AGP seems unavoidable.

Alternative agents have been investigated to improve health and performance of veal calves and among these are probiotics and prebiotics. According FAO/WHO (2001), probiotics are '*Live microorganisms which when administered in adequate amounts confer a health benefit on the host'*. Probiotics have been used for many years to improve the health of humans and the health and productivity of production animals, both ruminants and monogastrics. The microorganisms normally used as probiotics include the lactic acid bacteria (LAB), *Lactobacilli*, *Bifidobacteria* and *Enterococcus.* Yeasts can also be used as probiotics and research has indicates that these products may be effective in preventing diarrhea in calves (Abe et al., 1995;

Galvao et al., 2005; Timmerman et al., 2005). Prebiotics are defined as '*Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health'* (Gibson and Roberfroid, 1995). Some of the common prebiotics include fructooligosaccharide (FOS), mannan oligosaccharides (MOS), and polysaccharide-protein complexes derived from yeast, *S. cerevisiae* (Spring et al., 2000; Xu et al., 2003). AgriMOS® is a commercial prebiotic containing mannooligosaccharides and glucose (β-glucans) extracted from the cell wall of the yeast, *S. cerevisiae*. Very little research has been conducted on the effects of prebiotics on the growth performance and health of calves; for these reasons, and to discover alternatives to antibiotics, the present study was conceived.

1.1 Overall Research Objective

This research aims to evaluate the role of probiotics and prebiotics in improving the productivity and health of veal calves.

1.2 Specific research objective

- To determine whether the yeast probiotic, Levucell SB® and the prebiotic, Agrimos®, can improve growth performance and reduce diarrhea in veal calves.
- To assess the effects of Levucell SB® and Agrimos® on fecal microbiology with specific interest on *Lactic acid bacteria* (LAB*), E. coli*, *Clostridia*, *Campylobacter* and *Salmonella*.
- To determine whether Levucell SB® is more effective than Agrimos® or vice versa.

2. LITERATURE REVIEW

2.1 Nutrition and Performance of Veal Calves

Animals require nutrients to sustain life. Ruminants, such as cattle and sheep, have a relatively simple requirement for nutrients compared to non-ruminants and monogastrics such as swine, poultry and humans. The ruminant stomach is subdivided into four compartments: rumen, omasum, abomasum and reticulum and unlike monogastrics where digestive enzymes are typically produced by the host itself, the rumen microbes provide the enzymes that aid the digestion and break down cellulose, the principal carbohydrate in forage based diets for ruminants (Cheeke, 1999). The calf is classified as pre-ruminant because at this stage of calf development the rumen is non-functional. In pre-ruminants, milk is the principal source of nutrients and food bypasses the non-functional rumen as a result of the functioning of the reticular (esophageal) groove (Ortigues et al., 1995).

Nutrient supply and the performance of veal calves are closely correlated to each other. A good growth performance can be maintained only when the animal is supplied with sufficient macro and micronutrients. However, there are many other factors that can adversely influence growth performance; these include health, disease and management. Poor health management will lead to the occurrence of severe disease and even death. Therefore, health management plays a major role in the development of the young animal.

2.2 Health Management of Veal Calves

Immunoglobulins (Ig), which include IgG, IgA, IgM, IgE and IgD confer imunity to calves. A newly born calf has little or extremely low blood immunoglobulin (Ig) concentrations

and receives passive immunity through absorption of Igs during the first few days after birth (Roy, 1970; Davis and Drackely, 1998).The importance of ingestion and absorption of colostral immunoglobulins in minimizing calf morbidity and mortality has been described by both Robison et al. (1988) and Virtala et al. (1999). Virtala et al. (1999) found that low post-colostral IgG level was a significant risk factor in the development of pneumonia, a respiratory infection, which is one of the major diseases that leads to poor performance of calves. To prevent the problem caused by failure of passive transfer, Chigerwe et al. (2008) found that at least 150 to 200g of colostral IgG was required for adequate passive transfer of colostral immunoglobulins. It was recently revealed that the importance of colostrum has been recognized among farmers and there have been improvements in the management and feeding of colostrum; more farms have started to use the manual feeding colostrum as a strategy to enhance passive transfer of immunity (Kehoe et al., 2007).

Diarrhea, pneumonia and bloat are the three common diseases which affect the health of veal calves under the present husbandry conditions. Diarrhea is the main cause of both morbidity and mortality in veal calves, especially during early life. Furthermore, the major cause of neonatal calf diarrhea is infectious disease of the intestine. For example, bovine viral diarrhea virus (BVDV) is an important viral pathogen in cattle that causes many disease syndromes, including contributing to bovine respiratory disease (Fulton et al., 2000). To prevent diarrhea from happening, other than vaccination, farmers should focus on management and take the time to feed the calves with warm milk. Pneumonia often occurs during the first four weeks of age and *Mycoplasma spp.* has been established as being associated with pneumonia in cattle; *Mycoplasma bovis* is the most common pathogenic inhabitant of cattle's respiratory tract (Howard, 1983). This disease can cause poor performance and/or death of veal calves and is

often related to stress. Stress seems to be significantly related to an outbreak of pneumonia, with a high degree of stress causing an increase in the incidence of pneumonia. In addition, environment can play a part as well and suboptimal levels of relative high humidity within the building favor bacteria and viruses that cause disease (Caldow and Crawshaw, 2005). Thus, it is necessary to ensure adequate ventilation to reduce the incidence of the onset of pneumonia and reduce any stress factors such as large fluctuations and changes in temperature.

Bloat is an excessive production of gas in either the abomasum or rumen coupled with the inability to expel the gas by the calves themselves. To avoid bloat it is necessary to reduce any stress associated with feeding by ensuring an adequate food and water supply. Simply by doing this, it minimizes the occurrence of bloat (Drevjany, 1986). Based on the above reasons, it shows that proper farmer management plays an important role, especially in keeping the calves healthy.

Very young calves tend to experience several major stress events during their early life; such stresses include maternal separation, dietary changes and transportation. Overcrowding and poor air quality during transportation are considered to be some of the major reasons that cause stress to young calves. All these factors reduce milk consumption (Loerch and Fluharty, 1999) by calves, which in turn reduces nutrient intake from milk and reduces the barrier function in the gut (Soderholm and Perdue, 2001). As a result, the shipped calves can suffer from impaired immune function. The stress signals that pass from the central nervous system lead to an alteration of the response to neuroendocrin factors by the intestinal mucosa. The neuroendocrin factors act both directly and indirectly on the epithelium that induces barrier dysfunction and uptake of the pro-inflammatory material from the lumen of the gut. The inflammation will then cause disability and increase stress which further amplifies this effect. In a study of dietary stress

on fecal shedding of *Escherichia coli O157:H7* in calves, Cray et al. (1998) found that the normal protective microbial gut flora tended to decrease while there was significantly more *E. coil O157:H7* in feces of calves fed with a stressful diet than in the calves fed with a normal diet. It has been concluded in several studies that in the event of stress, protective *lactobacilli* bacteria tend to decline while coliform bacteria tend to increase (Fuller, 1989).

Vaccination is one of many options to control pathogenic infection in calves; however, results have been very variable. Some studies have shown a positive effect in either increasing the antibody titers to capsular polysaccharide of *Pasteurella haemolytica A1* or decreasing the shedding of *Escherichia coli* O157:H7, after vaccination (Hodgins et al. 1996, Potter et al. 2004). However, the contrary has been found and some studies indicated these responses did not protect calves against infection (Waltner-Toews et al., 1985, Dziva et al., 2007). In order to evaluate the feasibility of enhancing the passive immunity by vaccination, preparturient vaccination of the dams was investigated in the study of Hodgins et al. (1996). The authors found the antibody titers in colostral whey were significantly higher in the vaccinated dams; as a result, calves of vaccinated dams had significantly higher passive antibody titers than those of non-vaccinates. Also, a decrease in the shedding of *E. coli O157:H7* was observed in cattle that had been vaccinated with type III secreted proteins, proteins that play a role in colonization of non-bovine hosts by *E. coli O157:H7* (Potter et al., 2004). Dziva et al. (2007) vaccinated calves with EspA, a major colonization factor of *E. coli O157:H7*, and were able to induce antigen-specific humoral responses (both IgG1 and salivary IgA responses were induced). Unfortunately, the study did not prevent the intestinal colonization of *E. coli O157:H7* Similarly, it was found that a combination of vaccine, and a rotavirus-coronavirus/ *E. coli* vaccine, was not effective against disease, or preventing scours, nor did they lead to increased weight gain in calves from the dam received

either vaccination or no vaccination (Waltner-Toews et al., 1985). These studies highlight the need for further studies to develop and test novel vaccines to control this major foodborne pathogen.

While a vaccine may be used to prevent infection by pathogenic bacteria, antibiotics can be used as a therapy to treat the infection. In the dairy industry, antibiotic therapy is used especially for curing any intramamary infections during the dry-cow period. Oliver et al. (2004) and Oliver et al. (2003) studied the efficacy of lactating cow antibiotic therapy on heifer intramammary infections (IMI) and found it was effective at treating the prepartum IMI in heifers. Oliver et al. (2003) found the percentage of samples with mastitis pathogens was higher (P<0.001) in untreated controls than in antibiotic-treated heifers. If insufficient colostrum is transferred to the calf, there will be an inadequate titer of protective antibiodies and the calves will be more susceptible to disease problems in later life. Therefore, once again, it indicates the importance of good colostrum /health management (Berge et al., 2005).

2.3 Use of Antibiotics in Veal Production

Additives are non-nutritive substances applied to livestock feeds. The introduction of additives has been found to improve the efficiency of feed utilization and feed acceptance, allowing beneficial effects to the health of livestock (Cheeke, 1999). The long history in the evolution of ruminant animals along with ruminal microorganisms have contributed to a relatively unstable ruminal community which can be easily perturbed especially with any rapid transition in diet. Ruminant animals evolved to consume and utilise fresh grass and forages. However, humans have altered the diet that ruminants consume in the past 50 years by using grain-based diets. The advantages of using this type of diet are increased host productivity and

improved efficiency. However, a variety of disorders have occurred due to the use of grain-diets, primarily due to drastic changes in ruminal pH and perturbation of the ruminal microbial ecology. When rumen pH falls from normal levels (6.5 to 7.0) to less than 5.0, the fermentation product in the rumen (volatile fatty acids and lactic acid) will accumulate and be absorbed into the bloodstream. Too much lactic acid in the blood will lead to depression of feed intakes, or even death of the animal. In order to solve the problem, feed additives, for example antibiotic and buffers are added to offset the effects of increasing the amount of rapidly fermentable carbohydrate to the diet but these additives can also alter the composition of the ruminal ecosystem even further (Russell and Rychlik, 2001).

Antibiotics have been widely used as feed additives and reported to have positive effects in calves. Quigley et al. (1997) indicated that antibiotics (138 mg/kg of oxytetracycline and 276 mg/kg of neomycin) included in the milk replacer as feed additive to calves not only improved the performance but also reduced scours (diarrhea) in dairy calves. In addition, Berge et al. (2005) indicated that a high dose of feed-additive antibiotics (22 mg/kg per day of neomycin and 22 mg/kg per day of tetracycline hydrochloride) increased overall weight gain and decreased overall morbidity. The widespread use of antibiotics as feed additives for growth promotion and improved production is controversial at present due to the possibility of its adverse effect on human health.

The over-use of antibiotics in animal production has been associated with the development of antibiotic-resistant strains of bacteria, which do not respond to commonly prescribed antibiotics for humans; this renders the treatment of human diseases more challenging (Cheeke, 1999). If the incidence of antibiotic resistance continues to increase, a pandemic may occur for humans since bacteria may eventually be resistant to all available antibiotics. A study

by the Union of Concerned Scientists (2001) revealed an approximately two-fold increase in the non-therapeutic use of antibiotics in animal agriculture from the 1980s to 2001. Such findings have increased the pressure within the North American livestock industry to reduce the usage of antibiotics to protect human health.

2.4 Microbial Ecology of the Gastrointestinal Tract of Calves

The intestinal microflora of mammals is a complex ecosystem that comprises a diverse collection of microbial species which have an impact on the overall health of the host (Vlkova et al., 2006). The normal microflora refers to the microbial species commonly encountered in healthy individuals (Tannock, 2001); when this composition is perturbed, opportunistic pathogens may proliferate. The intestinal tract of the human is, perhaps the most studied of all mammals, and the sequences of events involved in the colonization of the intestinal tract are very similar to those observed in the animal (Tannock, 2001). The colonization of neonatal calves' gastrointestinal tracts starts at birth and is strongly influenced by the environment, the mother, diet and genetic background (Ozutsumi et al., 2005). The initial microbial population of the intestines of the new born calf is unstable but gradually stabilizes with age as the animal matures (Smith, 1965; Karney et al., 1986).The rumen is also not functional in the newborn calf and several sequential colonization events have to occur to ensure correct maturation of the rumen, with generally the last organisms to populate, the ciliate protozoa being taken as a signal of correct rumen maturation and development (Chaucheyras-Durand and Fonty, 2001). The gastrointestinal tract goes through anatomical and physiological changes during the development until the rumen is completely functional in an adult calf (Roy, 1970).

Not many studies have focused on the dynamics of colonization of the gut by the intestinal microflora of young calves. Normally, bacterial colonization starts with *E. coli* in the digestive tract 8 hours after birth, and *lactobacilli* and *streptococci* start to colonize after 1 day. *Lactobacilli* displace coliforms and colonize the gut rapidly in healthy animals (Smith, 1965; Karney et al., 1986). The complexity of the microbial ecosystem within the gastrointestinal tract suggest that further studies need to be undertaken to obtain a better knowledge in improving veal production.

2.4.1 *Diversity of Microbial Species in the G.I. Tract*

The rumen is the major site of microbial digestion and activity in the weaned calf the lower gut provides more valuable information about the health of the pre-ruminant calf. A large number of microbial species can be found in the gastrointestinal tract, and their distribution and diversity can act as an indicator of health in the claves. An increase in the number of pathogenic bacteria, such as Salmonella, in the gastrointestinal tract, will lead to several signs of sickness that includes fever and diarrhea (Smith, 2002). Fecal sampling is usually used as a method to determine gut health in these cases. Previous studies have also performed fecal collection, and enumeration of key bacteria in the sample to determine the health condition of these animals (Jenny et al., 1991; Timmerman et al., 2005; Rada et al., 2006). Other studies have used fecal score to assess consistency of the feces as an indicator of the severity and the presence of diarrhea (Cruywagen et al., 1996; Galvao et al., 2005). Fecal pH can also be used to assess gut health because it is linked to the activity of enteric pathogenic bacteria such as *E. coli* (Buchko et al., 2000; Berg et al., 2004). Lactic acid bacteria normally associated with a balanced normal flora and are viewed as beneficial gut bacteria will be enumerated in the fecal samples and their

numbers taken as an indicator of both pathogen load and good gut health respectively (Krehbiel et al. 2003).

2.4.1.1 *Escherichia coli*

Among the coliforms, *Escherichia coli* is perhaps the organism with the greatest notoriety, especially the strain *Escherichia coli O157*, a gram negative bacteria that is a normal component of the gut flora. This organism is a significant cause of food-borne illness in humans, and generally occurs with cattle implicated in the causal chain, and can be found in hamburger meat due to fecal material contamination of the ground beef (Stampia et al., 2004). *E. coli O157* was first recognized as a pathogen for humans after outbreaks of hemorrhagic colitis in 1982 (Wells et al., 1983). In the longitudinal study of *E. coli O157* in cattle herds, Hancock et al. (1997) suggested that cattle act as a reservoir of *E. coli O157*, especially in young animals. Although pathogenic to humans, *E. coli O157* does not appear to be pathogenic in weaned calves (Cray and Moon, 1995; Brown et al., 1997). The shedding of coliforms, including *E. coli* can be increased by management practices such as transportation stress (Loerch and Fluharty, 1999) and high proportional grain feeding (Diez-Gonzalez et al., 1998). It has been shown that the terminal rectum is the principal site of *E. coli* colonization in experimentally infected calves (Naylor et al., 2003, Low et al., 2005). Low et al., (2005) stated that ten cattle were found to have high levels of *E. coli O157* carriage. In 9 of these 10 cattles, $>10^3$ $CFUml^{-1}$ was detected in the terminal rectum.

2.4.1.2 *Campylobacter jejuni*

Campylobacter spp. has been recognized as a cause of diarrhea in neonatal calves where *Campylobacter jejuni* is generally the most common species of *Campylobacter* found. *C. jejuni* is readily isolated from the feces of beef cattle (Garcia et al., 1985) and dairy cows (Humphrey and Beckett, 1987). The growth of *C. jejuni* is limited within temperatures of 32-44 C^{o}. Therefore, living within the host is vital for the *C. jejuni* to survive and reproduce (Stanely et al., 1998). Fecal samples were taken in the study of Acha et al. (2004) to determine *C. jejuni*'s infection rate; the results showed that a high percentage (25%) of *Campylobacter* in one of two high prevalence farms. The authors suggested that calves could be a source of *C. jejuni* for food borne disease in humans. *C. jejuni* was also found to be associated with bovine fecal contamination of unpasterurized milk which led to an outbreak of enteritis in the human population (Robinson et al., 1979). Johnsen et al. (2006) indicated that *the Campylobacter* carriage rate was higher in calves (46%) than in adult cattle (29%). In addition, the authors investigated the carriage of *C. Jejuni* in cattle and compared with human isolates. It was found that among human isolates, 58% showed to have >90% similarity with bovine isolates. The results show that cattle are a significant reservoir for *C. jejuni*.

2.4.1.3 *Clostridium difficile*

Clostridium difficile is a gram-positve, anaerobic bacterium that has been suggested to be associated with diarrhea of calves and this can lead to lower average daily weight gain (ADG) or loss of weight. *C. difficile* is also considered as a potential bovine reservoir for infection in humans (Rodriguez-Palacios et al., 2006; Hammitt et al., 2008). Toxins A and toxins B are the main virulence factors of *C. difficile* found throughout the colon in calves (Hammitt et al., 2007).

Toxin A is a potent enterotoxin while toxin B is a potent cytotoxin *in vitro*. The toxins act synergistically; toxin A creates widespread damage to the mucosa which allows toxin B to affect epithelial cells (Lima et al., 1988; Kamaras and Murrell, 2001). A retrospective study on cattle performed in canadian farms, Rodriguez-Palacios et al. (2006) indicated a high relationship between calf diarrhea and the presence of *C. difficile* toxins in the fecal sample. Surprisingly, the authors found that more *C. difficile* was isolated from the control calves (14.9%) than from the diarrheic calves (7.6%). The authors claimed that the reason for this finding is unclear and methodological reasons should be considered and that the isolation method used in this particular study might have resulted in an identification bias in favor of one of the groups. Furthermore, the results indicated that calves were more likely to have detectable levels of *C. difficile* toxins in their feces early in life. Although the reason behind is still unclear, the author suggested that *C. difficile* colonized better in younger animals which had less developed intestinal microflora. Compared with the duodenum and ileum, the largest amount of *C. difficle* was isolated from the cecum of calves, which implies that the cecum could be the main site of colonisation of *C. difficle* in calves (Rodriguez-Palacios et al., 2007).

2.4.1.4 *Salmonella*

Salmonella is also pathogenic to humans and has been described as “a resistant flow from animal to human” (Bezanson et al., 1983). Humans become infected with *Salmonella* either by direct contact with the infected animal or feces or more commonly, through contaminated food products; 95% of the cases of infection are thought to be foodborne (Mead et al., 1999). Salmonellosis infections in calves can cause fever, diarrhea, or even lead to death (Smith, 2002). There are several factors which have led to an increase in Salmonella within calves (Fossler et

al., 2005b). The lack of providing a routine milk replacer with antimicrobials or infection from sick animals will lead to an increase in the amount of *Salmonella* shedding. Furthermore, Fossler et al. (2005a) showed that season can also affect Salmonella shedding in the cow as more *Salmonella*-positive animals were found in summer, spring and fall compared to winter. In the study of Acha et al. (2004), 63 out of 1,241 calves had signs of diarrhea. It was shown that *Salmonella* does not appear to correlate with diarrhoea, based on the result that only 2% of *Salmonella* was isolated in 393 fecal samples from both healthy and diarrheal claves.

2.4.1.5 *Lactic acid bacteria*

The pathogenic bacteria mentioned above are considered as 'bad bacteria', but lactic acid bacteria (LAB) are considered 'good bacteria'. Lactic acid bacteria can be found in the natural microbial population within animal's digestive tract and are considered as probiotics due to their ability to confer a health benefit on the host when administered (Cruywagen et al., 1996). LAB comprise various microbial groups, although they have a common trait which is the ability to convert fermentable carbohydrates into lactic acid (Leroy and De Vuyst, 2004). Most LABs are gram-positive and are classified under the following groups: *Lactobacillus*, *Leuconostoc*, *Pediococcus* (Carr et al., 2002). Generally, lactobacilli are present in feces of healthy animals in greater numbers than are coliforms; the converse is true in unhealthy animals (Sandine et al., 1979).

The LABs have routinely been added ruminant to decrease the prevalence of fecal shedding of *E. coli* O157. Studies (Elam et al., 2003; Younts-Dahl et al. 2005) have shown that dietary addition of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* or a mixture of NP45 and NP51 as direct-fed microbials decreased fecal shedding

of *E. coli* O157:H7. The beneficial effect of LAB as a probiotic on health and productivity of calves has studied and reviewed (Krehbiel et al. 2003) and their effects are discussed below.

2.4.2 *Technology for Analysis of Intestinal Microflora*

The application of molecular methodologies to analyze intestinal microflora has led to a more detailed knowledge of the microbial ecology of the gut (Tannock, 2001). The majority of molecular methods involve the Polymerase chain reaction (PCR) amplification of 16S ribosomal RNA genes from microbial DNA that have been extracted from samples from a wide range of ecosystems and habitats. 16S rDNA is found in every bacterial cell and is made up of nine different hypervariable regions, the sequences of which confer identity of each species of bacterium. Different species and strains have different sequence information. Flanking each hypervariable region are conserved regions which are found in all bacteria and which have been conserved through evolution. By using primers based on the conserved regions it is possible to amplify up the hypervariable regions in between. This can allow the amplification of all the different bacterial 16S rDNA present, even from a very complex sample that contains DNA that has been extracted from many thousands of different species.

DNA extraction is very important, and there needs to be a good representation of all the organisms present in the sample; in addition, sample contamination must be avoided. Some types of samples can prove problematic due to the presence of inhibitors. They can interfere with the PCR reaction at different levels, leading to different degrees of attenuation and even to complete inhibition. Most of the inhibitors, such as polysaccharides and humic acids, exhibit similar solubility to DNA. They are not completely removed by using classical extraction

protocols (such as detergent, protease and phenol–chloroform treatments); as a result, remaining as contaminants in the final DNA preparations and can affect the amplifiability of the target DNA (Moreira, 1998). Since the 16S rDNA is very important to the cell, little has been changed through the evolution. Extraction methods have been developed but it has taken time to get optimal conditions. Yu and Morrison (2004b) has found a novel method called RBB+C (repeated bead beating plus column) method to improve the extraction of PCR quality community DNA from digesta and fecal samples, which can act as starting material for a wide variety of different molecular techniques and acts as an indicator for gut microbiology.

Additional intestinal microflora analysis techniques are temperature-gradient gel electrophoresis and denaturing-gradient gel electrophoresis. These DNA fingerprinting techniques are able to separate the 16S rDNA from different bacterial species in a complex sample (Riesner et al., 1992) thereby generating a DNA fingerprint of the microbial population in a given sample. Each fingerprint contains a unique banding pattern with each band representing one species of organism. Therefore, different band patterns indicate different diversity of microflora in the sample. A DNA fingerprint of the total bacterial population can then be generated specific to a particular treatment and time.

Temporal temperature gradient gel electrophoresis (TTGE) differs from denaturing gradient gel electrophoresis (DGGE), the 'original' method. TTGE is a recently modified method from DGGE in using a temporal temperature gradient instead of using a chemical gradient in DGGE to denature the amplicons (Shaji et al., 2003). TTGE was used to successfully distinguish differences between closely-related species and demonstrated that it is a powerful method for revealing the heterogenic sequence in 16S rRNA genes (Vasquez et al., 2001) as well as being able to distinguish different bacterial species in both liquid and solid dairy products (Ogier et al.,

2002). In addition, TTGE was used to investigate the fecal microbial population in patients with Crohn's disease (CD) to compare with healthy volunteers and also to compare the fecal microbial community between CD patients with active disease and those in disease remission (Seksik et al., 2003). Novel methods have been discovered over time to obtain a better result. The knowledge of the application and moleculer analysis of the intestinal microflora is a very useful tool to advance knowledge of the microbiology of the gut.

2.4.3 *Interaction between the Host and Intestinal Microflora*

The intestinal microflora is very important in the development of the host's innate and adaptive immune response (Cebra, 1999). In humans, most bacteria in the colon obtain their energy through the fermentation of carbohydrates. Some indigenous bacteria are beneficial to the host's health, for example lactobacilli and bifidobacteria. These 'good bacteria' can competitively exclude pathogens, facilitate digestion through the production of enzymes, stimulate the growth of gut colonocytes and may demonstrate antitoxigenic activity and stimulation of immune system of the host (Guarner and Malagelada, 2003).

The composition of the intestinal microflora is regarded as being relatively stable in adulthood overtime (Palmer et al., 2007). However, a number of factors can influence the pattern and composition of the gut microbial population not only in human but in cattle as well. These factors include individual diet, stress and the environment (Finegold et al., 1974*;* Holdeman et al., 1976, Guarner and Malagelada, 2003; Ozutsumi et al., 2005). If any of these factors causes a perturbation of the normal flora, the conditions of digestion could be modulated and could result in an increase of pathogenic bacteria and a decline of the beneficial bacteria. This unbalanced bacterial ecosystem can lead to the onset of disease; one common disease caused by an

imbalance of the gut flora exemplified in humans is inflammatory bowel disease (IBD). The study of Duchmann et al. (1995) demonstrated that chronic Inflammatory Bowel Disease (IBD) may occur when the immune system fails to react against the commensal microflora and there is a breakdown of immunological tolerance to the normal native bacteria. Frank et al. (2007) showed that patients with more severe IBD appeared to lack some metabolically important organisms. Therefore, modulation of the bacterial flora of IBD patients seems to be a rationale to control this disease (Dotan and Rachmilewitz, 2005). The normal intestinal microflora plays a key role as a defender against invading microorganisms as well as toxic substances in the diet. It has been shown that the composition of the intestinal microflora is significant in both immune modulation and physiology of the gut (Schiffrin and Blum, 2002). An imbalance in the gut flora causes disease in humans.

As is the case with humans, an imbalance in the gut flora can cause problems in calves. Many of the micro-organisms are responsible intestinal disease in humans also cause disease in animals. For example Salmonella infection can cause of diarrhea in both humans and calves (Tsolis et al., 1999). It is generally accepted that in relatively recent evolutionary history *Salmonella* has gained large pieces of DNA by horizontal gene transfer that confer virulence-associated functions upon the host bacteria. These genetic loci have been termed pathogenicity islands. Bispham et al. (2001) found that *Salmonella* pathogenicity island 2 (SPI-2), a locus that contains genes with characteristic for cell invasion, is required for the induction of both systemic Salmonellosis and *Salmonella*-induced enteritis in calves. Other than diarrhea, acidosis is considered another problem which can be caused by an imbalance of the flora in the gut (Owens et al., 1998). It was found that acidosis was widespread among diarrheal suckler calves; however, no relationship between severity of dehydration and acidosis was observed (Grove-White and

White, 1993). Grove-White (1998) showed that calves less than 6 days old were less acidotic than older calves. The author suggested it might be due to the fact that the younger calves are more likely to be affected by pathogenic bacteria that cause rapid dehydration and thus, reduce production of lactic acid in the body.

2.5 The Role of Probiotics and Prebiotics in Gut Health and Performance of Calves

2.5.1 *Probiotics*

According to the definition by FAO/WHO (2001), probiotics are "*Live microorganisms which when administered in adequate amounts confer a health benefit on the host'*. Probiotics have been used for many years to improve the health of humans and the health and productivity of production animals, both ruminants and monogastrics. Probiotics can either be obtained from bacteria, yeast or fungi, but all must be on the generally regarded as safe (GRAS) list.

The most commonly used probiotic bacteria are the Lactic acid bacteria (LAB), for example, *Lactobacilli*, *Bifidobacteria* and *Enterococcus*. In the treatment of humans, probiotics that are administered to clinically affected patients should be of human orgin, nonpathogenic, resistant to gastric acid digestion and intestinal enzymes, be able to adhere to intestinal epithelium and modulate mucosal immune responses of the host (Dotan and Rachmilewitz, 2005).

With regard to animal health, probiotics have to be on the GRAS list and have generally already been used for human health or in the food and brewing industries; they must prove to be efficacious at either affecting ruminal fermentation or improving gut health in a similar manner as outlined for human health (FAO/WHO, 2001).

Several mechanisms have been proposed for the mode of action of probiotics. Firstly, LABs have an antimicrobial effect. By administering the probiotic, the LABs produce lactic acid which results in a drop in pH value in their surrounding environment. It suppresses the growth of pathogenic bacteria due to the intolerance of an acid environment (Bongaerts and Severijnen, 2001). LABs also promote bacterial interference by the formation of organic acids, free fatty acids, ammonia, diacetyl, hydrogen peroxide and bacteriocins (Vandenberg, 1993). A bacteriocin is a protein or protein complex that has antimicrobial activity and one example has been isolated and well characterized from Lactobacilli and has a bactericidal effect on a range of pathogenic bacteria (Klaenhammer, 1988). LABs produce many safe natural bacteriocins that inhibit pathogenic bacteria, for example nisin. Due to the ability of nisin to prevent clostridial spoilage, nisin is permitted to be used as a food additive to extend the shelf life of milk and canned foods (Vandenberg, 1993; DelvesBroughton et al., 1996).

Secondly, by ingesting probiotic, the host increases the amount of LAB in the gut which results in increased competitive exclusion of pathogenic bacteria. Thirdly, probiotics have been shown to enhance humoral immune responses (Kaila et al., 1992), and the use of probiotic can be beneficial in down-regulate hypersensitivity reactions to ingested proteins with food allergy (Sutas et al., 1996) and alleviate intestinal inflammation in infants with atopic eczema/dermatitis syndrome and food allergy (Viljanen et al., 2005). Last but not least, probiotics may affect the epithelial barrier and influence the production of mucus. After supplementing a *Lactobacilli* preparation to rats in the study of Zareie et al. (2006), it was demonstrated that the probiotic had the ability to prevent bacterial translocation to the mesenteric lymph nodes by maintaining the intestinal barrier function in the rat with chronic psychological stress. However, the exact mechanism behind it is still unknown.

Up until now, there have not been many studies on the use of probiotics in calves and the majority of these have been very variable in their outcomes (Krehbiel et al. 2003). In the study of Abe et al. (1995) body weight gain and feed conversion were improved when new born calves were given an oral administration of probiotics that contained *Lactobacillus* or *Bifidobacteria*. Additionally, probiotic can be seen as a treatment to reduce the problems of weight loss caused by diarrhea. It was shown that when 2g of a *Lactobacillus spp.* was used as probiotic in the morning milk, the claves fed with probiotic were healthier than the control ones, with improved health and a decrease in mortality and veterinary costs observed (Gorgulu et al., 2003). The results showed that the treatment costs for calves which suffered from diarrhea were higher in the control group than in the probiotic group. The medication cost of the control group was 2.75 folds more than the probiotic group. Similar results were reported in a study of the health and growth of veal calves fed milk replacers with or without probiotics by Timmerman et al. (2005). The authors indicated that the use of a probiotic treatment had the capability to reduce the number of calves that required therapy and reduced the amount and costs of antibiotic treatment against diseases. This study used two probiotics groups. One probiotic group was called multispecies probiotic (MSPB), which contained different probiotic species of human origin; while the other probiotic group was called calf-specific probiotic (CSPB), and contained 6 *Lactobacillus* species isolated from calf feces. The occurrence of diarrhea and the duration of diarrheic day were reduced by probiotics. As a result, a lower percentage in mortality was observed. Both treatment groups significantly reduced the percentage of animals in need of therapeutic treatment for any cause as well as reduced the total number of treatments needed; MSPB caused a 72% decrease and CSPB caused a 57% decrease. Furthermore, it was also found that CSPB treatment significantly reduced the fecal

coliform population. Several studies also found a reduction in the shedding of *E.coli O157* in cattle (Elam, 2003; Younts-Dahl et al., 2005). Furthermore, Abe et al. (1995) not only studied new born calves, but also studied the effect of the same two probiotics *Lactobacillus* or *Bifidobacteria* on the piglet. The authors found out that probiotic treatments increase the survival rate of the piglet, especially in the last 10 days of the experiment. The survival rate of the probiotic treatments was 95%, which is much higher than 75% in control group. In the study comparing bacterial flora in feces of infants and calves, Rada et al. (2006) observed that bifidobacteria were the dominant bacterial group in fecal flora and were the most preferable probiotic bacteria for both infants and calves.

While some studies show the beneficial effect of probiotics, other studies did not observe a positive significant effect on the performance in calves. For example, in their study, Cruywagen et al. (1996) reported no beneficial effects on general health by feeding probiotics that contain lactobacillus to dairy calves. Nevertheless, the authors did recommend the use of probiotics during the first two weeks of life since the result showed that the calves fed with *Lactobacillus* maintained initial body weight, while the calves fed in the control group lost 4% of body weight during the first two weeks. Furthermore, similar to Cruywagen et al. (1996), in the study of Holstein calves, Jenny et al. (1991) found no significant effect on the performance of animals supplemented with probiotics. The authors observed variability among calves in early growth rate as well as the acceptance of dry feeds that could have contributed to the difficulty of indicating the beneficial effect of probiotics. Three treatments were studied in the following research: a control with no additives; a mixed microbial concentrate that contains 10g of mixed *Lactobacillus acidophilus*, *Lactobacillus lactis* and *Bacillus subtilis;* 10g of *Bacillus subtilis* concentrate. To achieve a maximum effect of probiotics, the calves had to

contain an unbalanced gut microflora. The results indicated that the calves were in a healthy condition, and it is probable that significant effects of probiotics were not observed in the study due to the fact that probiotics are generally only significantly effective in the animals that were stressed or depressed (Fuller, 1989). This concept is also supported by the study of Krehbiel et al. (2003) where it was observed that the calves which did not respond to probiotic treatment were the healthy ones.

As mentioned earlier, yeasts rather than bacteria, can also be used as probiotics. *Saccharomyces cerevisiae* (SC) and a subspecies of *S. cerevisiae*, *S. cerevisiae boulardii* (SB) were used in the study of Galvao et al. (2005) to determine the effects of both of these live yeast products in calves with failure of passive transfer. The results indicated that the feeding of SC in the grain improved calf performance and provided a positive effect prior to weaning. A 1.72 % body weight increase due to an increase in DM intake was observed in the SC treatment group compared to a 1.53 % body weight increase in the control animals. The treated animals exhibited 464.7g/d in body weight gain with SC treatment compared to 298g/d in control. The addition of SB in the milk replacer had a tendency to lead to an improvement in dry matter intake before weaning but showed no significant beneficial effect to performance. The study concluded that a significant improvement in performance was observed only when live yeast (SC) was added in the grain. This shows that different strains of yeast can have different effects. Previous studies have shown that the inclusion of SC can improve the rate at which the rumen can mature in young animals (Chaucheyras-Durand and Fonty, 2001) which is why it probably had more of an effect during the grain feeding period compared to SB which is used more to reduce pathogenic infection and improve gut health. SB has also been shown to have an effect on the gut health of young pigs.

The inclusion of SB (2g/kg of diet) on piglet performance was studied in weanling piglets (Bontempo et al., 2006). The addition of SB resulted in a significantly higher ADG, 474±0.01, from weaning throughout 30 days post weaning than the control diet, 432±0.01. The yeast diet also offered the ability to modify morpho-functional aspects of ileum mucosa by altering villi height and crypt depth which were significantly greater than those observed in the piglet fed with the control diet. In terms of villous height, 243±3um was observed in yeast diet; while 195±3um was observed in control. For the crypt depth 177±2um and 130±2um were observed for yeast diet and control diet, respectively. The implementation of probiotics improved both the intestinal health and growth of piglets.

Recently, more evidence to support the beneficial effect of applying probiotics to animals, especially those under stress, has been presented. In adult rats, chronic stress was found to induce barrier dysfunction (Soderholm et al., 2002). Zareie et al. (2006) demonstrated that the administration of *Lactobacillus* probiotics to rats that underwent chronic psychological stress were able to increase their mucosal defense against luminal bacteria and eliminate bacterial translocation to the mesenteric lymph nodes. In addition, Gareau et al. (2007) suggested that feeding rats with a combination of 10^8 *Lactobacillus* species (*L. rhamnosus* and *L. helveticus)* as probiotics during their neonatal stress period prevented stress-induced effects on colonic physiology in both neonates and the adult rat. In that study, stress-induced bacterial adherence/penetration in the colon was reduced in maternal separation rats treated with probiotic compared with controls. The result demonstrated the significance of the neonatal period to the stress' response in later life.

The use of antibiotics is associated with the risk of causing antibiotic-associated diarrhea (AAD) (Bergogne-Berezin, 2000). Hatakka et al. (2001) reported that long term treatment with

the probiotics 5-10x10^5 cfu/ml of *Lactobacillus rhamnosus GG* (LGG) reduced the need for antibiotic treatments against respiratory infection in children. While the LGG was also successful in humans for diarrhea treatment, Ewaschuk et al. (2004) studied whether LGG was able to maintain viability in the gastrointestinal tract in calves, since the problem of diarrhea is very common in neonatal calves. 3 groups of LGG were administered orally with morning milk on 3 consecutive days at low (2x10^{10}cfu), medium (1x10^{11}cfu) or high dosage rates (2x10^{12}cfu). No LGG was recovered in feces on day 0, the control day. 24 hours after the first feeding, LGG was recovered in 1 out of 5 calves at low dose, 4 out of 5 in medium dose and 5 out of 5 in high dose. Overall, the LGG recovery in the high dose group was significantly higher (P<0.05) than the low dose group. The result indicated that the LGG successfully survived in the intestine of young calves and could be successfully administered in an oral rehydration solution. Several microorganisms have been studied as a potential solution to treat antibiotic-associated diarrhea.

In the meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease, McFarland (2006) indicated that two types of single strain probiotics: SB, *Lacatobacillus rhamnosus GG* and 7 different probiotic mixtures were found to have the capability to reduce the development of antibiotic-associated diarrhea. Most importantly, only SB was found to be effective for reducing *Clostridium difficile* disease. SB involves a neutralization of *Clostridium difficile* toxins in different ways: It produces a protease which destroys both toxins of *C. difficile* (Castagliuolo et al., 1996); maintains epithelial cell integrity (Czerucka et al., 1999); transforms toxinogenic *C. difficile* clone into non-toxinogenic *C. difficle* clone (Corthier et al., 1988) and increases indirect synthesis of IgA immunoglobulins (Qamar et al., 2001). Furthermore, Colombel et al. (1987) demonstrated that

when *Bifidobacterium longum* was administered in yogurt, there was a significant reduction in lessening the abdominal discomfort in patients treated with antibiotics.

Probiotics have been suggested to have beneficial effect on not only animals but also in humans; there are many probiotic products currently on the market for human applications; however, the number of different organism used is limited. It is difficult to culture commensal bacteria under laboratory conditions. It has been indicated that only 20%-50% of commensal bacteria have been successfully cultured in laboratory conditions (Patterson and Burkholder, 2003). Although probiotics have the potential to provide benefits on humans, the difficulty of bacteria culture, the limited knowledge of the normal gut ecosystem and also the wide variations of the result from different studies make the process of developing probiotic products a challenge and further research are required in the selection of novel probiotic organisms.

2.5.2 *Prebiotics*

Prebiotics like probiotics have a similar ability to improve gut health; however, instead of being live microbes like probiotics, prebiotics stimulate the growth of either commensal or beneficial bacteria of the host gut (Vijaya Kumar et al., 2005). Prebiotics are defined as '*Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health'* (Gibson and Roberfroid, 1995). Thus, the levels of probiotic organisms already inhabiting the intestine can be increased by consumption of these dietary substrates, prebiotics. Prebiotics consist mainly of carbohydrates such as oligosaccharides and also small amounts of non-

carbohydrate. Prebiotics can improve the host's ability to compete against pathogens, by providing compounds such as Fructooligosaccharide (FOS) which can be utilized by the normal indigenous probiotic flora (Xu et al., 2003), but can also induce removal of pathogens from the body by binding, through the action of Mannan oligosaccharides (MOS) (Spring et al., 2000).

The overall effects of prebiotics on gut health are similar to those observed with probiotics; however, the mechanisms behind each are totally different. FOS can act as a substrate for fermentation by the gastrointestinal microflora to produce more lactic acid and short chain fatty acid which reduces the pH value as short chain fatty acid is produced as a by-product of fermentation (Wynn, 2009). The addition of FOS in the human diet appears to selectively stimulate the growth of *bifidobacteria* in the colon and stimulates fecal bulking (Van Loo et al., 1999). Unlike FOS, MOS does not act as a substrate; it has a competitive binding site for pathogenic bacteria. When the pathogenic bacteria bind to the MOS, they will loss the ability to attach to the intestinal wall. As a result, it will be excreted in the feces (Ofek et al., 1977). Prebiotics can also increase the transfer of passive immunity as well as increasing the Ig concentration; this is very important, especially to the new born calves. Franklin et al. (2005) demonstrated that after vaccinating the cow against rotavirus twice before the expected parturition, higher serum rotavirus neutralization titers were observed in the calves from the cows fed MOS than the control cows, 3.236±0.07 and 3.013±0.08, respectively. The exact mechanism is still unknown, but it has been hypothesized that the potential mechanism may include the action of MOS. Collectins are the protein contain mannose-binding that maybe act as an opsonin that binds to the mannan-binding particle, the action of which is known as a complement system. It will then increase phagocytosis through the innate immune system. In

other words, MOS may acts as an adjuvant, an immunological agent, to stimulate the production of collectins and enhance the immunity of the host.

In studying the effect of dietary MOS on chicken caecal microflora, Fernandez et al. (2002) concluded that mash diets supplemented with 2.5% MOS achieve beneficial effects, a higher level of *bifidobacterium spp*. and *lactobacillus spp.* with a reduction in *enterobacteriaceae* groups. An experiment determining the effects of MOS and antibiotics in neonatal diets of dairy calves on health and growth carried out by Heinrichs et al. (2003) who suggested that MOS (4g of BioMos/d) could substitute antibiotics (400g/ton neomycin and 200g/ton oxytetracyclne) in milk replacer and still provide similar calf performances. The MOS also have the potential ability to amplify the passive immunity transfer to offspring, thereby decreasing the use of therapeutic antibiotics and morbidity in calves. There was an increase in normal fecal scores for antibiotic and MOS treatments compared to control. However, the study of Terre et al. (2007) showed that the supplementation of 4g/d of MOS in the milk replacer although initially stimulated starter intake right after weaning, 1.94±0.044 kg/d, compared to control, 7.71±0.044 kg/d,; did not have any further consequences in growth rate, resulting in a lower feed efficiency than the non-supplemented milk replacer. A significant reduction in fecal pathogenic bacteria counts, *Cryptosporidium spp*. was not observed in the supplemented dietary group either, indicating no effect on pathogen reduction.

Mannan oligosaccharides are obtained from the yeast cell wall (Spring et al., 2000). It is the primary antigenic components of yeast cells that can be found on the cell surface (Ballou, 1970). MOS provides competitive binding sites for the intestinal pathogens since these pathogenic bacteria use mannose-specific fimbriae to attach to the intestinal epithelium (Ofek et

al., 1977). In Spring et al. (2000), the authors observed that multiple strains of *E. coli* and *Salmonella* agglutinated MOS *in vitro*. Based on the fact that MOS can not be digested by the enzyme in the intestine, the bacteria that bind to MOS lose the ability to attach to the epithelium and as a result are removed from the intestine. In addition, the study of Baurhoo et al. (2007) indicated that adding MOS or low levels of lignin to broiler diets improved gut integrity by increasing the number of goblet cells significantly. On day 42, the MOS supplemented animals had a greater number of goblet cells/villus, 118.03, compared to any other treatment groups, especially to the positive control which contained antibiotics (11mg/kg of virginamycin) that had 35.31 goblet cells/villus. The positive control group (antibiotic growth promoter group) failed to improve growth performance and feed efficiency when compared to the MOS treatment group. Unfortunately, the MOS diet did not seem to have any effect on the growth performance of the chicken. Furthermore, the MOS was found not only to reduce the pathogenic bacteria*, E. coli* in the feces, but also increased the amount of LAB. Therefore the use of MOS and lignin as a replacement to antibiotics in poultry production is a viable option.

Solis de los Santos et al. (2007) used either 1 or 2 lb/ton of Alphamune, containing mannan-oligosaccharide yeast extract and β-glucans, to determine the effect of MOS on the maturation of the gastrointestinal tract of turkey poult. The result showed that Alphamune was capable of accelerating gastrointestinal maturation in turkey poults especially in the ileum. Differences in ileal morphology in the 2 lb/ton of Alphamune treatment group appeared to be always significantly different compared to control group in terms of ileal morphology. For example, in terms of villus height in the ileum, 453.3±33.6um and 310.6±22.8um were observed in 2 lb/ton of Alphamune treatment group and in control group, respectively, thus the addition of the MOS stimulated villi growth and development.

While MOS are polysaccharide-protein complexes derived from yeast that are indigestible as mentioned earlier; β-glucans are polymers of glucose that can also be derived from yeast cell walls and are considered as immunostimulators of macrophages and other immune effector cells. Prebiotics that contain both MOS and β- glucans have been used together and show that in combination it was possible to increase cytokine levels and the percentage of the lymphocyte subpopulation in calves (Szymanska-Czerwinska et al., *2009)*. In addition, Cary et al. (2005) found out that feeding glucan to dariy calves result in an up-regulation of innate cell surface proteins associated with immune activation, and is further modulated by ascorbic acid. This suggested that there is a potential of decreasing the mortality and morbidity in calves when supplementing with glucans.

2.5.3 *Future Prospects for Probiotics and Prebiotics*

There is a growing interest the use of probiotics and prebiotics as a safe way to alter and manipulate the intestinal microflora of animals and to reduce the use of antibiotics in animal agriculture. The main issues that need to be resolved in the development of new probiotics and prebiotics are: efficacy of these products, consistency in responses to these feed additives, and a better understanding of their mode of action. Some of these challenges would be addressed by the application of molecular biology techniques to the study of the role of probiotics and prebiotics in gut microbiology and animal heath.

3. MATERIALS AND METHODS

3.1 Animals, Housing and Experimental Design

The study was carried out on a commercial farm in La Chute, Southwest of Quebec, 50 km from McGill University's research farm on the Macdonald Campus, Ste. Anne de Bellevue. The initial study plan involved conducting the milk feeding phase (pre-weaning) on the commercial farm, then transporting the weaned calves to the Macdonald Campus research farm for the conduct of the grain feeding phase (finishing phase) of the experiment. The research was approved by McGill University Animal Care Committee, and all procedures involving animals followed the guidelines of the Canadian Council on Animal Care (1993). The experiment was conducted from August 6th to October 1st, 2008, and began with sixty-eight male Holstein veal calves; the animals were approximately 7days old, and were purchased at a commercial auction, Réseau Encan Québec (St-Isidore, QC, Canada) under the auspices of the Federation des Producteurs du Bovin du Quebec (FPBQ). The calves were regrouping at st-Hyacinthe at the grouping area of Délimax then transported 104km to the farm on July 30th, 2008 and were identified with Agri-Traceability Quebec (ATQ) ear tags which allowed for a permanent identification system to trace back to the farm of origin of the calf. On arrival at the farm, the calves were housed in a barn measuring 45.72 m in length, 6.10 m wide and 2.44 m high. The barn was ventilated by a set of eight fans (four 16 inches fans and four 12 inches fans), all mounted along one side of the long sides of the barn. The ventilation rate for the 16 inches fans and the 12 inches fans were 2474 cubic feet per minute (CFM) and 1163 CFM, respectively. In total, it provided a ventilation rate of 213.9 CFM/calf or 36.7 air changes per hour.

All calves were weighed on the day of arrival and the average weight was 45.0±6.03 kg. The calves were placed in individual wooden pens (1.2 meters wide, 1.8 meters long) that

provided 2.2 square meters per calf. Straw was used as bedding throughout the study and the fresh straw was added to the pen 3 times per week. Manure was removed whenever the calves had diarrhea. Daily measurement were made of barn temperature and relative humidity, as well as ambient temperature at the Environment Canada weather station at Mirabel Quebec (Environment Canada, 2008), (24 km from the site of the experiment). Throughout the study, the average maximum barn temperature was 22.3°C and the average minimum barn temperature was 16.4°C.

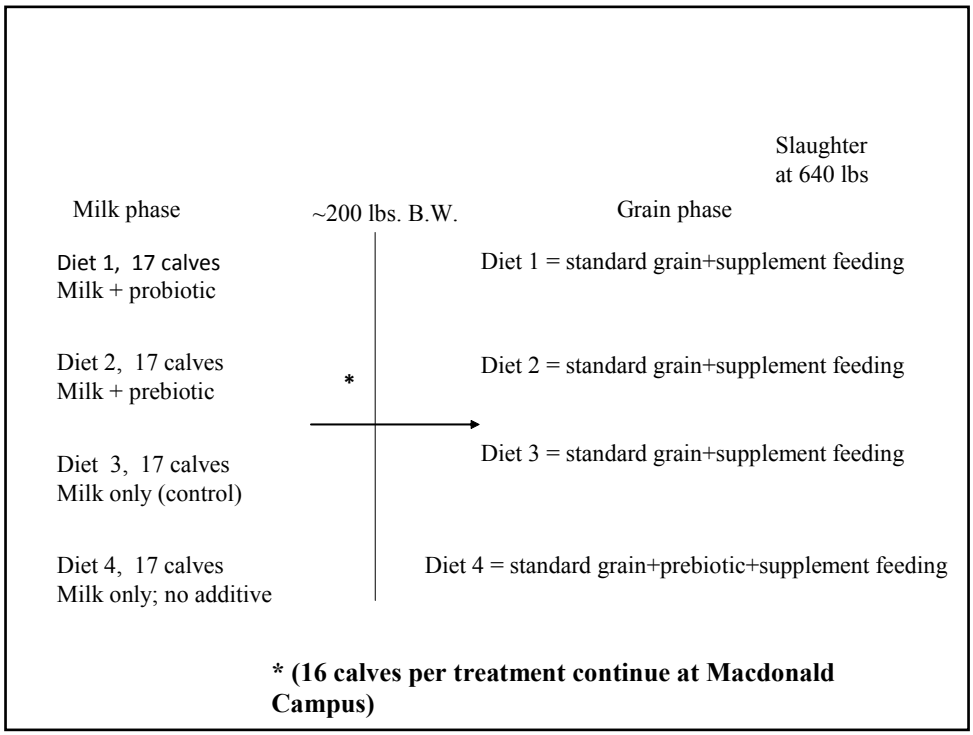

Figure 1. Experimental plan of the study

The study was initially designed to evaluate the impact of a prebiotic/ probiotic offered during the two phases of veal production: a) milk feeding; b) grain feeding from weaning to market weight of 290 kg (640 lb). Consequently, as shown in Figure 1, there were two control groups (no feed additive), one group fed a probiotic (Levucell SB®, a live yeast preparation of *S.*

cerevisiae. spp. boulardii (Lallemand Inc., QC, Canada) only during the preweaning phase, and one group fed prebiotic (AgriMOS ® a specific combination of manno-oligosaccharides and glucose extracted from the yeast cell walls of *S. cerevisiae;* Lallemand Inc., QC, Canada) during both the pre- weaning and finishing phases. This arrangement of treatments would have allowed for a comparison of the effects of a probiotic and a prebiotic during the pre-weaning phase, a determination of whether the prebiotic is more effective during the pre-weaning rather than finishing phase and finally, an assessment of the carry-over effects of the treatments imposed during the milk phase.

At the beginning of the experiment, the sixty-eight calves were randomly assigned to the four treatments: two control (no additive) groups (N=17 for each control group); probiotic group- 0.5g/d of Levucell SB® per calf ; prebiotic group- 3g / d of AgriMOS® (N=17). Both additives were incorporated into the milk replacer but the inclusion of additives (day 1) did not begin until five days after arrival date. By the end of the pre-weaning phase (day 56), a large number of calves (20 calves) had unfortunately died from respiratory disease; for reasons of biosecurity, a decision was made to terminate the study and avoid transporting the remaining calves to the Macdonald Campus research farm, the planned location for the conduct of the finishing phase of the experiment. For the purpose of data management, all results from the control groups were combined; this resulted in twice as many observations for the control diet. As discussed later, the mortality rate was unrelated to any dietary treatment effect.

3.2 Feeding Management

The pens were equipped to accommodate two plastic buckets, one for calf starter and the other for milk replacer or water. The feeding of milk replacer began one day after the calves

arrived at the farm. The commercial milk replacer contained 20% milk protein and 20% of crude fat, and was reconstituted with water at feeding time according to the instructions of the manufacturer (LA COOP FEDEREE, QC, Canada). The milk replacer was reconstituted by placing the weighed quantity of powder in a stainless steel container (175ml) and adding the desired amount and hot water (65ºC) up to 2/3 the capacity of the mixing container. The milk replacer powder was automatically mixed and allowed to dissolve in the hot water (it took approximately five minutes); cold water was added to lower the liquid temperature to 43°C, the appropriate milk replacer temperature for calves. The respective additives (Levuvell SB® and AgriMOS®) were added to the reconstituted milk replacer only after it had attained the temperature of 43°C; as soon as the reconstituted milk replacer (with or without additives) attained the target temperature, it was offered (by bucket) to the calves. From arrival day to day 2 of the experiment, the powder was reconstituted to 10% dry matter (DM); from day 3 and day 4, the powder was reconstituted to 11.25% DM; then from day 5 until day 53, the powder was reconstituted to 11.5% DM. Each calf was fed 1.75kg of MR per feeding on day 0 and day 1; on day 2 and day 3, the calves were offered 2 kg of MR per feeding; then from day 4 to day 9, the calves were offered 2.5kg of MR per feeding; on day 10 and day 11, the calves were offered 2.75kg of MR per feeding; then from day 12 to day 14, the calves were offered 3kg of MR per feeding; from day 15 to the morning feeding of day 49, the calves were offered 3.25kg of MR per feeding; 2.75kg of MR was offered to the calves in the afternoon feeding on day 49; on day 50 and day 51, the calves were offered 2.5kg of MR per feeding; then on day 52 and day 53, the calves were offered 2 kg pf MR per feeding.

The calves were offered the milk replacer twice daily (at 0630 and 1530h), in two equal portions. When animals were ready to be weaned, one of the daily offering of milk replacer was

discontinued for two days. From the beginning of the experiment, all calves were offered, *ad libitum*, a commercial calf starter (LA COOP FEDEREE, QC, Canada) that contained 19% CP; the animals also had free access to clean water. The calves were considered to be completely weaned once they had consumed 1kg of calf starter for 3 consecutive days. All calves (including the calves that were not completely weaned) were considered to be weaned on the same day (day 56).

3.3 Health Management and Fecal Scores

Throughout the study, the calves were subjected to health assessment by a licensed veterinarian. On the day of arrival and arrival, thirteen calves had to be replaced due to navel infection. In the afternoon of the day of arrival, all calves were injected in the neck muscle with 1ml of Dytosel® (Pfizer Canada Inc., QC, Canada) to supply 136 IU of vitamin E *dl* -α-tocopherol acetate and 3mg of selenium for prevention of white muscle disease; this was followed by subcutaneous injection of 1.25ml of the antibiotic, Draxin® (Pfizer Canada Inc., QC, Canada) as prophylactic treatment against bovine respiratory disease (BRD), then vaccination against infectious bovine rhinotracheitis by intranasal administration (2ml per calf) of Bovi-Shield Gold 5® (Pfizer Animal Health, Pfizer Inc., NY, U.S.A). On the day of arrival, a blood sample was also collected from each calf and submitted to Lachute Veterinary Hospital for BVD testing (Smith, 2008) and analyses of hematocrit (Feldman et al., 2000), gamma glutamyl transferase (GGT) activity and serum protein concentration (Jezek et al., 2008). 5 cc of Dexafer® injectable iron (Vetoquinol Canada Inc. QC, Canada) was administered to those calves exhibiting hematocrit values less than 27%, which was requested by the technical advisor of the veal calf industry in Quebec.

The commercial electrolyte, Calf lite® (Vetoquinol Canada Inc. QC, Canada) was provided to the calves to prevent dehydration if diarrhea was observed. The severity of diarrhea was based on the assessment of the fluidity of the feces, as determined by fecal score (code 1 to code 4) using the reference standard of Larson et al. (1977) as follows: code 1- normal, firm but not hard; code 2 - soft, does not hold form; code 3- runny, spread like pancake mix; code 4 – watery, liquid like orange juice. Throughout the study, daily records were maintained of the frequency of use and type of medication. Calf mortality and morbidity were also recorded.

3.4 Animal Performance and Diet Analysis

The body weight of the calves was recorded at the beginning and the end of the experiment; the animals were weighed on alternate days and the average of the two weights was taken as the initial weight; however, only one measurement was considered as final body weight. The calves were also weighed bi-weekly to monitor interim changes in growth performance. The animals were always weighed four hours after the morning feeding. The consumption of milk replacer and calf starter was recorded daily. Records of body weight and feed consumption were used to calculate average daily gain (ADG) and feed efficiency. The number of days to consume 1 kg of calf starter was recorded as an indicator of readiness for weaning. Samples of calf starter and milk replacer were collected daily and composited weekly, for determination of nutrient composition.

The feed samples were analyzed for DM, crude protein, fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, calcium, phosphorus, and magnesium. Milk replacer was analyzed for the same components as state above except NDF and ADF. DM content, ash and fat (ether extract using petroleum ether for distillation) were analyzed from the sample (AOAC

1990). Crude protein (CP = N x 6.25) was analyzed using a Leco Nitrogen Analyser (Truspec Nitrogen Determinator System, Leco Corporation, MI). Analyses of NDF and ADF were performed by using Ankom Fiber Analyser (Ankom Technology Corporation, Fairport, NY) according to the method of Van Soest et al. (1991). Samples were digested using wet oxidation method (Parkinson and Allen, 1975), calcium and magnesium were measured by direct flame atomic absorption spectrophotometer (Perkin-Elmer model 2380), and phosphorus was analyzed by colorimetry using a flow injection analyzer (Lachat QuickChem, Milliwaukee, WI, USA) according to the Lachat Instruments method 13-115-01-1-B.

3.5 Fecal pH and Fecal Microbiology

Using disposable plastic gloves, trans-rectal collection of fresh feces was performed on six randomly selected animals from each of the 3 treatments on day 0 (one day before the start date of the experiment) and on day 7, day 13, day 28, day 41 and day 57. The samples were collected at approximately 0700 h into sterile 50ml falcon tubes, placed immediately on ice, then transported to the laboratory. A subsample (1g) of the feces was placed in a 50ml falcon tube and mixed with 9 ml of distilled water. The mixture was vortexed until homogenous, the pH was determined with pH meter using standardizing buffers (Hanna instruments, RI, USA)

Another fecal subsample (1g) was placed into a sterile 25ml falcon tube for the enumeration of *E.coli, Salmonella, Campylobacter, Clostridia* and lactic acid bacteria (LAB). Bacterial enumeration was carried out using selective growth media and growth conditions specific for each organism. Each fecal subsample was serially diluted, 10-fold, with 9 mL of phosphate buffered saline (PBS) dilution medium up to 10^{-6} dilution. The mixtures were

vortexed until completely homogeneous, and then aliquots (100ul) were plated out, in triplicate, for each dilution and each of the selective growth media. The Hicrome agar (Sigma-Aldrich, Steinheim, Germany) and the MRS agar (Oxoid, Hampshere, U.K.) were used for *E.coli* and LAB, respectively. The Difco Differential Reinforced Clostridial agar (Becton, Dickinson and Company, MD, U.S.A.), BG Sulfa agar (Becton, Dickinson and Company, MD, U.S.A.) and Columbia Blood agar (Oxoid, Hampshere, U.K.) were used for *Clostridia*, *Salmonella* and *Campylobacter,* respectively. The Difco YPD agar (Becton, Dickinson and Company, MD, U.S.A.) was used to determine whether there were viable yeasts present in the animal. All the media were prepared under manufacturer's instructions (and sterilized at 121°C prior to use. The media was kept molten at 45ºC and poured over an aliquot of the sample. After plating each sample, the plates were inverted and incubated at the appropriate temperature for 48 h, then placed at 4°C until the colonies were counted. MRS and Differential Reinforced Clostridia agar plates were grown in an anaerobic environment, in Biomerieux anaerobic boxes with three packs of Gaspak EZ Anaerobe container system (Becton, Dickinson and Company, MD, U.S.A.) at 37°C. Columbia blood agar plates were incubated under anaerobic conditions, in a container with two packs of Campy Anaerobe GazPak in the anaerobic container system (Oxoid, Hampshere, U.K.) at 42°C. The rest of the media were incubated aerobically; HiCrome and BG Sulfa Agar plates were placed in the incubator at 37°C. The YPD Agar was placed directly in the incubator at 30°C. All media were incubated for two days before enumeration of the colonies formed.

3.5.2. *PCR and DNA Fingerprinting (TTGE)*

Subsamples of feces were also stored at -80°C for subsequent extraction of DNA, PCR amplification, and TTGE (temporal temperature gradient gel electrophoresis) fingerprint analysis

of the total bacterial population; PCR analysis was carried out to provide amplicons which were then separated by TTGE, thereby generating a microbial profile of the bacterial community at the various time intervals of fecal sampling.

Total DNA was extracted from 0.25g of each fecal sample using the method of Yu and Morrison (2004b); DNA was quantified on the nanodrop and diluted with MilliQ H_2O to provide a stock solution (10ng/ul) to standardize the quantity from each sample to the same amount in the pooled sample. The six fecal samples from each animal, within each treatment, were pooled to yield one sample for subsequent DNA analysis. The V6-V8 variable region of the 16S rDNA gene was amplified using primers based on conserved regions that flanked the V6-V8 region (Yu and Morrison 2004a). This region was chosen over the V3 region in order to obtain a longer sequence of amplicons whilst still giving good diversity (Yu and Morrison 2004a). All of the PCR amplification was performed using a PTC-100 thermocycler (Eppendorf, Germany) in 50 ul volumes containing 35.75ul of dH_2O, 5ul of 10x HotStar PCR buffer, 1ul of dNTP mix, 1ul of 25nM $MgCl_2$, 2.5ul of two 10uM primers (Table 1) and 0.25ul of Taq DNA polymerase (1000 unit) (Qiagen, MD, U.S.A); PCR amplification consisted of an initial hold for 15 min followed by 30 cycles of 95 °C for1 min and 58°C for 1min and finally 72°C for 90 s. There was a final extension step for 15 min at 72°C. A negative control, containing all the components but with sterile water instead of a DNA template, and a positive control of DNA extracted from pig feces from an earlier study , were included to ensure that the PCR reaction proceeded correctly.

The resulting amplicons were loaded into a 1% (w/v) agarose gel for electrophoresis to verify that the PCR was successful, and that amplicons of the correct size were obtained, by comparing with a known 1 kb sized DNA molecular ladder marker (New England Biosystems, U.S.A.). The amplicons were then separated by TTGE to generate a DNA fingerprint of the

bacterial flora present in the sample. The TTGE gel was made by mixing 16.8g of urea, 1ml of 50x TAE, 18mL of MilliQ H_2O, 8 ml of Acrylamide, 55ul of Temed and 550ul of 10% ammonium persulfate. The solution was degassed for 2 min before pouring the gel. Once the gel had polymerized, it was added to the TTGE DCode system (Bio-Rad Industries, Ontario, Canada), running buffer (1.25 x TAE) was added to the system, and the wells of the gel were washed with running buffer to remove any residual urea. The system was pre-equilibrated to the initial start temperature of 66ºC. PCR amplicons (12.5ul) were mixed with 12.5ul H_2O and 5ul of 6x loading dye and loaded into the gel. After all of the sample has been loaded into the wells of the TTGE gel, the samples were run into the gel by passing a voltage of 25V for 25 min through the gel. The voltage was then increased to 60V with a temperature ramp rate of 0.2ºC/ h for 16 hours in order for it to reach the final temperature of 69.2°C. After the final run temperature was reached, the TTGE gel was then removed from the machine and stained with 60ul of Gel stain (Cedarlane, Ontario, Canada) with 600ml of 1.25x TAE for 1 hour and de-stained with 600ml of 1.25x TAE for 10 minutes. Bands were visualized by examination under UV light in a GelDoc 2000 system (Bio-Rad Systems, Ontario, Canada).

A DNA fingerprint of the total bacterial population was generated specific to a particular treatment and time with each band on the gel representing a particular species of bacteria. The DNA fingerprint of the fecal bacterial community was analyzed using GelComparII (Applied Maths, TX, USA). Similarity indices were calculated using the Pearson coefficient, and dendograms were constructed by Unweighted Pair Group Method with Arithmetic averages (UPGMA). Different banding patterns were observed on different days and with different treatments, indicating differences in the composition of the gut flora.

Table 1. Sequences of PCR primers that were used in this study

Primer	Sequence	Company
GCclamp-U968GC	5’. GCclamp- GAA CGC GAA GAA CCT TAC)	Invitrogen
L1401	5’GCG TGT GTA CAA GAC CC	Invitrogen

3.6 Statistical Analysis

The data were analyzed using the mixed model procedure of SAS (SAS Institute, 2003. SAS® User’s Guide, Version 9.1, SAS Institute Inc., Cary, NC). The following statistical models were adopted:

Model I:

$Y_{ij} = \mu + T_i + e_{ij}$

where: Y_{ij} = dependent variable of the j^{th} calf on the i^{th} treatment

u = overall mean

T_i = the fixed effect of i^{th} treatment effect (i = 1,2,3)

e_{ij} = random residual (error) associated with the dependent variable from the j^{th} calf on the i^{th} treatment, $e_{ij} \sim N(0,\sigma^2_e)$

Model II:

$Y_{ijk} = u + T_i + calf_{ij} + time_k + T_i*time_k + e_{ijk}$

where: Y_{ijk} = dependent variable of j^{th} calf on the i^{th} treatment and by k^{th} time

u = overall mean

T_i = the fixed effect of i^{th} treatment effect (i= 1,2,3)

$calf_{ij}$ = random effect of j^{th} calf within the i^{th} treatment. $calf_{ij} \sim N(0, \sigma^2_{calf})$

$time_k$ = the fixed effect of k^{th} time

T_i*time_k = the fixed effect of the interaction of T_i and $time_k$ on dependent variable

e_{ijk} = random residual (error) associated with the dependent variable from the k^{th} time within j^{th} calf within the i^{th} treatment, $e_{ijk} \sim N(0,\sigma^2_e)$

The study had two main outcomes of interest: the calf performance traits and the fecal microbial population; concentrations of fecal microorganism (cfu/g) were transformed to logarithm for the purpose of SAS analysis. Two orthogonal contrasts were use to assess treatment effects: one contrast was used to compare the effects of probiotic and prebiotic; the other to compare the average effects of the additives with the control. Statistical significance was declared at a 5% level of probability.

Model I, a one-way ANOVA, was used to analyze data for: initial body weight, final body weight, total weight gain, overall ADG, average daily milk replacer intake, average daily calf starter intake, overall total milk replacer intake, overall total calf starter intake, overall feed consumption, overall feed efficiency, days to consume 1kg of calf starter, total medication cost, medication cost of Baytril®, Fluazine®, overall average fecal score, and overall average fecal pH. Model II, repeated measures analysis, was used for the following variables: bi-weekly body weight, bi-weekly ADG, weekly total milk replacer intake, weekly total calf starter intake, weekly total feed intake, bi-weekly feed efficiency, interval average fecal DM content, weekly average fecal score, interval average fecal pH and interval fecal bacterial populations of *E.coli*, *salmonella*, *campylobacter*, *clostridia* and LAB. The better fitting model was the model of autoregressive, AR(1), and compound symmetry (CS) covariance structure with the lower value of bayesian information criterion (BIC).

4. RESULTS

The nutrient composition of the experimental diets is shown in Table 2. Both crude protein (23.9±1.24%) and crude fat of the experimental milk replacer (24.0±0.77%) are higher than the manufacturer's estimated content (%) by approximately 3 to 4 percentage, respectively (Appendix Table 1). The experimental calf starter (CS) contain less crude protein (16.3±3.7%) than the manufacture's value (19%) and contain more crude fat (3.16±0.14%) compared to the manufacturer's value (2%). The NDF (20.7±1.27) and ADF (9.19±0.736) of the experimental CS contain higher than those values of the manufacturer (6% of crude fibre), especially NDF. The mineral (Ca, P and Mg) values of both MR and CS are similar to those from the manufacturer values.

Table 3 shows the statistical significance of the main effects and the interactions for all the variables in this study; estimates of error variance, as well as the covariance structure for those variables analyzed as repeated measures are presented in Appendix Table 2. With the exception of fecal LAB population, there was no significant interaction of treatment x day for any of the variables (Table 3).

4.1 Calf Health and Fecal Score

Results of barn temperature and relative humidity (RH) are shown in Figures 2 and 3. There were weekly fluctuations in barn temperature and humidity but the fluctuations seemed more pronounced in barn temperature in the last three weeks of the study. Barn temperature ranged from a night time low of 13.5ºC to a day time high of 27.3 ºC; the weekly maximum barn temperatures overlapped the maximum outside temperature (Mirabel weather station) but the

weekly minimum barn temperatures were consistently higher than the minimum outside temperatures. During the first five weeks, the difference between the maximum and minimum temperatures (differential) was 5.8ºC to 11.3ºC but during the last three weeks, the differential was 1ºC to 2ºC (Figure 4). The RH within the barn ranged from 50.0 % to 92.8 % (Figure 3); in contrast to barn temperature, RH, especially the maximum RH, remained stable throughout the eight weeks. However, the differential in RH humidity was also greater during the first five weeks than during the last three weeks. The weekly maximum RH was always greater than 75% (Figure 3).

There was an abnormally high mortality rate (29.4%) among the calves; 20 calves out of 68 died during the study. The number of deaths over time is shown (Figure 5a). There was a surge in mortality in the 3rd week of the study but most of the calves died in the last two weeks of the study (Figure 5a). As shown in Figure 5b, there were five deaths among the groups fed the probiotic or the prebiotic and 10 deaths (out of 34) among the control group; hence, the incidence of mortality was the same among treatment and control groups. Autopsy examination revealed that with the exception of one calf which died from a stomach ulcer, all the calves died from pneumonia (Appendix table 3). The following bacterial cultures were isolated from the lung tissue of the dead calves: *Mycoplasma sp., Pasteurella multocida, Mannheimia haemolytica, Histophilus somni, Serratia sp., Pseudomonas sp., Peoteus sp., Enteriobacteriacae, E. coli, Streptococcus sp. and Bacillus sp.;* in some calves, a combination of several of the above pathogens was identified.

Results of gamma glutamyl transferase (GGT) activity and concentration of total protein in the serum are presented in Appendix Table 3. Of 68 calves purchased at the auction only 15 had levels of GGT and serum protein greater than 324U/L and 52g/L, respectively. Among the

20 dead calves, 16 of them had levels of GGT and serum protein less than 324U/L and 52g/L, respectively. Values for hematocrit ranged from 17% to 36% (Appendix Table 3). Twenty four calves had hematocrit concentration less than 27%.

Fecal score was ranked from 1 to 4 according to the fluidity of the feces, where 1 indicates as normal and 3 indicates as diarrhea. Figure 6 shows the weekly fecal score across treatments. There was no significant treatment x day effect on fecal score; in addition, there were no differences between control and the groups fed additives or between the two additives. There were significant (P<0.01) differences in fecal score during the 8 weeks with higher values recorded in the first two weeks; thereafter, fecal score stabilized. Throughout the experiment, fecal scores never exceeded a value of 2 in average. Fecal dry matter content was only affected by the day of fecal collection (Figure 7).

The total cost of medication was $1997.48 for all calves throughout the study; with Baytril® ($1072.21) accounting for more than half of the medication costs (Appendix Table 4). The medication cost per calf throughout the study ranged from $26.7 for the prebiotic group to $30.7 for the control but the treatment differences were not significant (Table 4).

4.2 Growth Performance

The results of calf performance are shown in Table 5. Compared with control, incorporation of the additives in the diet had no significant effect on any of the performance parameters; there was a tendency (P<0.10) for milk replacer intake by the control group to be greater than the average of the groups fed additives. With the exception of milk replacer intake, there were no significant differences between the probiotic and the prebiotic with regard to

performance measurements. Calves fed the probiotic consumed more (P <0.05) MR than those fed the prebiotic (Table 5).

Even though the interaction of treatment x time (week) was not significant (P > 0.05), the weekly changes in intake of MR, CS and total feed are presented in Tables 6, 7 and 8. As shown in Table 6, the higher rates (P <0.05) of MR intake in the control group, compared to the groups fed the additives, occurred in the last two weeks of the study; however, from week 4 through the end of this study, the calves fed probiotic consistently (P <0.05) consumed more MR than those fed prebiotic (Table 6).

Table 7 shows that during the last two weeks calves fed the additives consumed more CS (P < 0.05) than those fed the control diet; however, no significant difference in CS intake was observed between the two additive groups at any period during the eight weeks. There was no significant effect on total feed intake (Table 8) but a tendency (P <0.10) occurred during the last week of the study when the calves fed prebiotic consumed more feed than those fed probiotic.

The weekly changes in body weight (BW), ADG and feed efficiency are presented in Figures 8a, 8b and 8c. At day 57, the day after batch weaning, the calves achieved a BW of approximately 80 kg (Figure 8a); estimates of ADG (Figure 8b) during the last four weeks ranged from 700g to 900g. There was no significant (P>0.05) effect of treatment on BW, ADG or feed efficiency. The effect of day (P <0.01) was significant for both body weight and ADG but not for feed efficiency.

4.3 Fecal pH and Fecal Microbiology

There was no significant interaction of treatment x day on fecal pH (Figure 9). The effect of treatment was also non significant but there were significant differences due to the day of fecal collection. With the exception of day 0, fecal pH was above 7.5.

The populations of lactic acid bacteria (LAB) are shown in Table 9 and the populations of pathogenic bacteria, *E. coli, Campylobacter* and *Clostridia* are shown in Figures 10, 11 and 12, respectively. Data for LAB are presented as a table because of the significant interaction of treatment x day, and the desire to present the interval estimates of least square means and the p-values associated with the contrasts. Data for *E. coli,* C*ampylobacter* and *Clostridia* are presented graphically because of the non-significant interaction of treatment x day and the desire to highlight the significant changes in response over time.

4.3.1 *Lactic acid bacteria (LAB)*

On the day prior to the study, d 0, there were unexpected differences in LAB counts between the probiotic group and the prebiotic and control groups, with a log less in numbers being observed between the probiotic and the prebiotic groups (log8.7 and log 9.8, respectively) and almost a log less between the probiotic and the control group (log8.7and log 9.6, respectively). For all the treatments, the number of LAB in the feces decreased as the study progressed, probably due to a reduction in milk intake. Generally compared to control, the addition of the probiotic did not significantly affect fecal LAB counts at anytime throughout the study (Table 9); however, on day7 and day 28, fecal LAB counts were 1 log greater with probiotic than prebiotic.

4.3.2 *E. coli, Campylobacter and Clostridia*

There were no detectable counts of *Campylobacter* in the prebiotic group on day 43 and day 57; nor were there counts in the probiotic group on day 0 and day7. *Clostridia* were not detected in the feces of any group on day 7 of fecal collection; on day 57, there were also no *Clostridia* counts in the probiotic group. No significant interactions of treatment x day were observed for *E. coli*, *Campylobacter* and *Clostridia,* and there were no significant differences among treatment for any of the pathogenic microorganisms. There were significant effects of day of collection on the fecal populations of these pathogenic bacteria. On day 0, one day before feeding the additives, the fecal populations of *E. coli* for calves fed probiotic or prebiotic were paradoxically significantly greater than control (log 8.0, log 8.3, log 7.1 respectively); however from day 0 to day 7, there was a marked decline in *E. coli* populations in the groups fed additives (decreased to log7.3 and log 7.5 for the probiotic and prebiotic respectively) but there was no such change in the control group which remained relatively constant throughout the trial at approximately log 7.0. From day 7 until the end of the study, the fecal populations of *E. coli* fluctuated in the treatment groups. The lowest number of *E. coli* was obtained in the prebiotic group on d41, with a log 6.

The number of *Campylobacter* and *Clostridia* also fluctuated throughout the study. On certain days, for some of the treatments, no *Campylobacter* were detectable. For the probiotic group, there were no detectable *Campylobacter* on d0 and d7; however after this point the numbers significantly increased to a maximum of log6 on d13 and then they declined to log4.5 by the end of the study. In the prebiotic group, initially the numbers of *Campylobacter* were at

the same level as found in the control animals (log 5.1) and although it increased over the next few weeks (maximum log 6.7 on d13), after day 28 there were no *Campylobacter* detectable in this group. In the control group, numbers of *Campylobacter* increased from log 5.1 on d0 to a maximum of log 6.3 on d7 after which they slowly declined until they reached a minimum of log 5 by the end of the study.

The number of *Clostridia* in the study also varied significantly with day. All the groups contained significantly high levels of *Clostridia* on the first day of the study. On d7, no *Clostridia* were detectable, however, after this day, *Clostridia* were recovered in all the treatment groups for the reminder of the study except for the probiotic group on the last day. On d13, the level of *Clostridia* in the probiotic group was noticeable less than the control group and the prebiotic group. On day 28 the fecal population of *Clostridia* was significant greater in the control group than in calves fed the additives (Figure 12). The feces were found to be almost free of *Salmonella* with only colonies being obtained from some of the calves. In the probiotic group, colonies were only observed on d0 and d7, in the prebiotic group, only on d0 and in the control group only on d7, d13, and d57 from an obvious *Salmonella* shedder which was observed in the control group; therefore, there were not enough observations for a valid statistical analysis of *Salmonella* populations (Figure 13).

4.4 TTGE DNA Fingerprinting

Figure 14 shows the TTGE fingerprint analysis of the microbial population. Some bands were conserved throughout the study no matter what the treatment or day of sampling, indicating that they were generally associated with the normal fecal flora of veal calves. However, some

samples contained different banding patterns, indicating differences in the composition of the flora, either by day or by treatment. Generally, more bands were present in the later sampling periods, indicating an increase in the diversity of the composition of the fecal flora. Analysis of the banding patterns by GelCompar II and UPMGA yielded three distinct clusters A, B, and C.

One cluster, A, was related to the probiotic group, where the addition of the probiotic to the feed had caused a significant change in the composition of the fecal flora when compared to the prebiotic and control groups. Within this probiotic cluster, early sampling days (d7, 13, 28) tended to group together as did the later sampling days (d41, 57). This effect is probably reflective of the change in the diet as more CS was consumed. Interestingly, on d0, before the calves were exposed to the dietary treatments, the bacterial DNA bands from calves in the probiotic and prebiotic groups clustered together. This implies at least initially, that the composition of the flora in these two groups was the same, but different from the contro, as was observed in the microbial counts on d0. It was only after supplementation with either the probiotic or the prebiotic that a significant change in the composition of the flora occurred.

The other two distinct clusters had been formed on the basis of day, rather than treatment, with later sampling days (d41, 57) clustering together in cluster B, and early sampling days (d7, 13, 28) clustering together in cluster C. Both prebiotic treatment and the control groups were interspersed within these two clusters, indicating that in this instance the effect of day was greater than the effect of treatment and that the prebiotic did not have as large an effect on the composition of the fecal flora as the probiotic.

Table 2. Chemical Composition of feed samples[1]

	(%)	
	Milk Replacer Average	Calf Starter Average
DM	85.9±0.74[2]	86.0±1.51
Crude protein	23.9±1.24	16.3±3.70
Crude fat	24.0±0.771	3.16±0.141
NDF	N/A	20.7±1.27
ADF	N/A	9.19±0.736
ash	7.29±0.327	7.51±0.687
Ca	0.763±0.0509	0.812±0.2454
P	0.831±0.0564	0.500±0.0917
Mg	0.134±0.01777	0.216±0.0511

[1] Values are the mean of 8 observations
[2] Standard deviation

Table 3. Statistical significance of effects for all variables in the study

	Treatment effect		Day effect		treatment x day effect	
Variable	F value	Pr>F	F value	Pr>F	F value	Pr>F
Body weight[1]	0.95	0.394	118.53	<0.0001	0.31	0.963
Initial body weight	0.81	0.5	N/A[4]	N/A	N/A	N/A
Final body weight	0.68	0.51	N/A	N/A	N/A	N/A
Total weight gain	0.42	0.661	N/A	N/A	N/A	N/A
Overall average daily gain	0.42	0.661	N/A	N/A	N/A	N/A
Average daily gain[1]	0.09	0.9123	5.97	0.0008	0.27	0.9502
Overall total milk replacer intake	4.48	0.0168	N/A	N/A	N/A	N/A
Total milk replacer intake[2]	5.22	0.0075	1321.22	<0.0001	1.57	0.0845
Daily milk replacer intake	4.46	0.017	N/A	N/A	N/A	N/A
Overall total calf starter intake	1.27	0.2908	N/A	N/A	N/A	N/A
Total calf starter intake[2]	0.69	0.5094	93.3	<0.0001	1.11	0.345
Daily calf starter intake	1.27	0.291	N/A	N/A	N/A	N/A
Overall total feed consumption	0.87	0.424	N/A	N/A	N/A	N/A
Total feed consumption[2]	0.41	0.6648	45.03	<0.0001	1.08	0.3748
Overall feed efficiency	0.83	0.442	N/A	N/A	N/A	N/A
Feed efficiency[1]	0.05	0.9549	0.36	0.7839	0.26	0.9555
Days to consume 1 kg of calf starter	1.5	0.233	N/A	N/A	N/A	N/A
Cost of Baytril	0.11	0.898	N/A	N/A	N/A	N/A
Cost of Fluazine	0.52	0.596	N/A	N/A	N/A	N/A
Total medication cost	0.11	0.9	N/A	N/A	N/A	N/A
Overall average fecal score	0.06	0.937	N/A	N/A	N/A	N/A
Average fecal score[2]	0.13	0.8794	29.69	<0.0001	0.9	0.552
E. coli	0.24	0.7878	3.62	0.0052	1.5	0.1363
Salmonella[5]	25.95	0.0128	32.5	0.0114	.	.
Campylobacter	0.02	0.9788	6.77	<0.0001	0.94	0.4883
Clostridia	3.13	0.0669	15.01	<0.0001	0.68	0.6867
Lactic acid bacteria	0.16	0.8498	19.51	<0.0001	3.3	0.0011
Overall average fecal pH	1.03	0.374	N/A	N/A	N/A	N/A
Average fecal pH [3]	0.99	0.3872	10.57	<0.0001	0.39	0.948
Average fecal DM content[3]	0.02	0.9838	4	0.0026	0.87	0.5627

[1]Measurements on each variable made bi-weekly.
[2] Measurements on each variable made weekly.
[3] Measurements on each variable made on day 0, 7, 13, 28, 41, and 57 of the study.
[4] N/A = not applicable.
[5]Invalid analysis

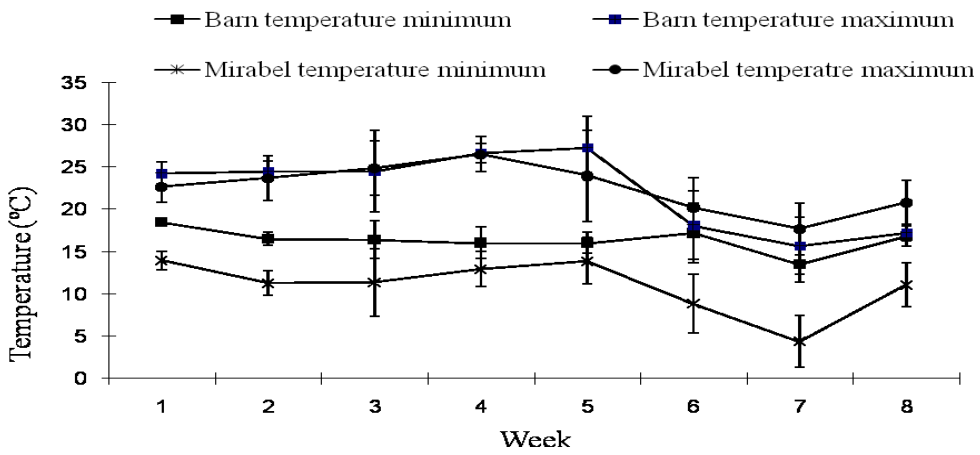

Figure 2. Ambient temperature inside the barn and in Mirabel[1] over the 8 week study period
[1]Data obtained from Environment Canada 2008.

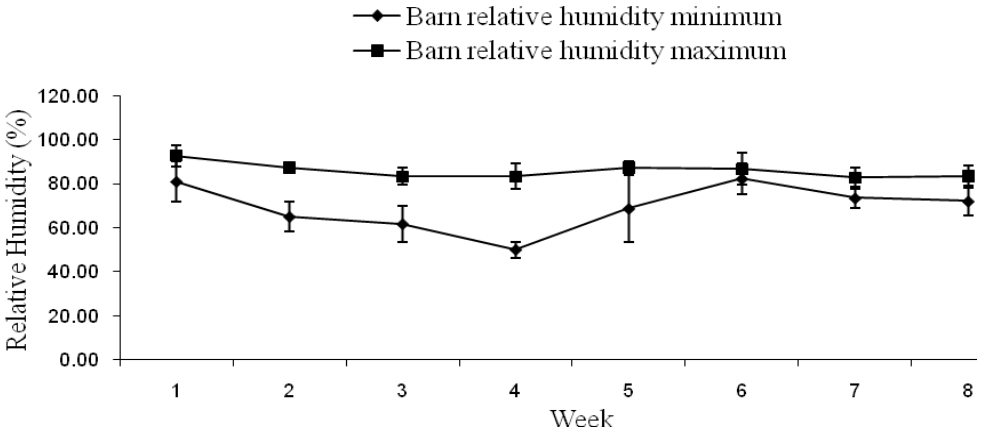

Figure 3. Relative humidity inside the barn

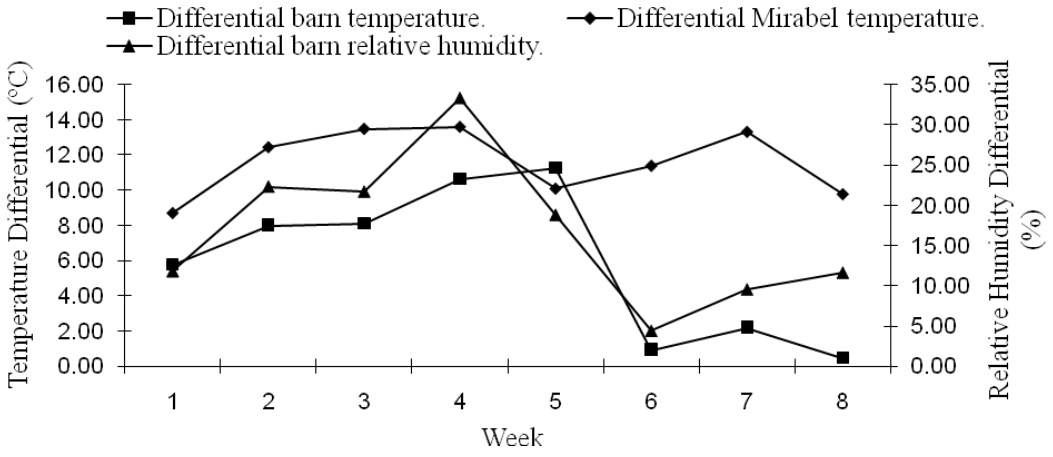

Figure 4. Differential temperature and relative humidity

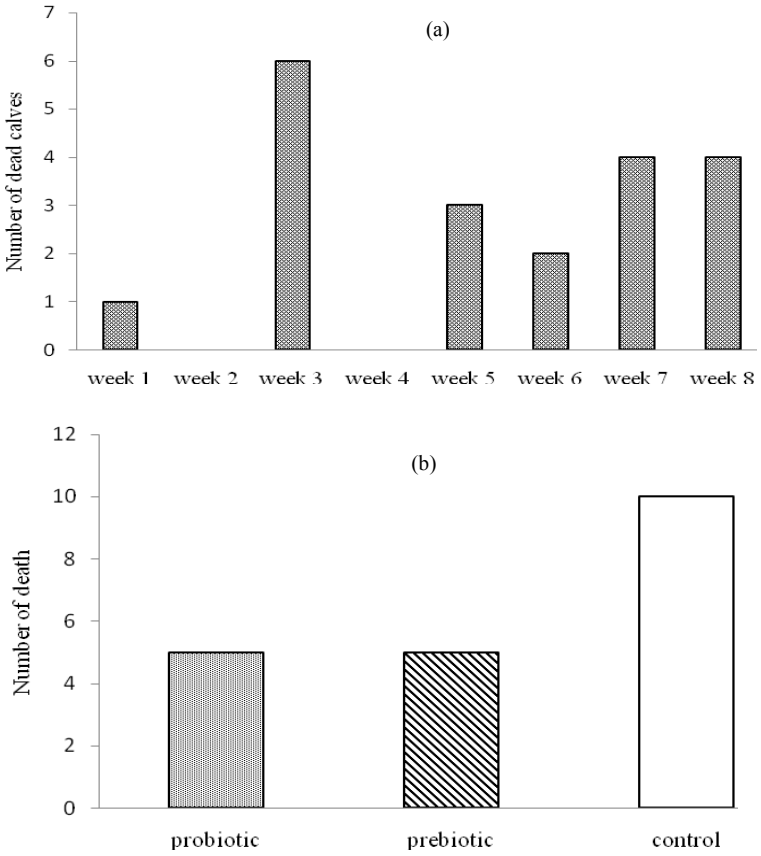

Figure 5. Calf mortality throughout the study
a) Total number of calves that died from day 0 to day 57.
b) Total number of calves that died in each treatment group from day 0 to day 57; probiotic (17 calves at the start of the study), prebiotic (n=17) and control (n=34).

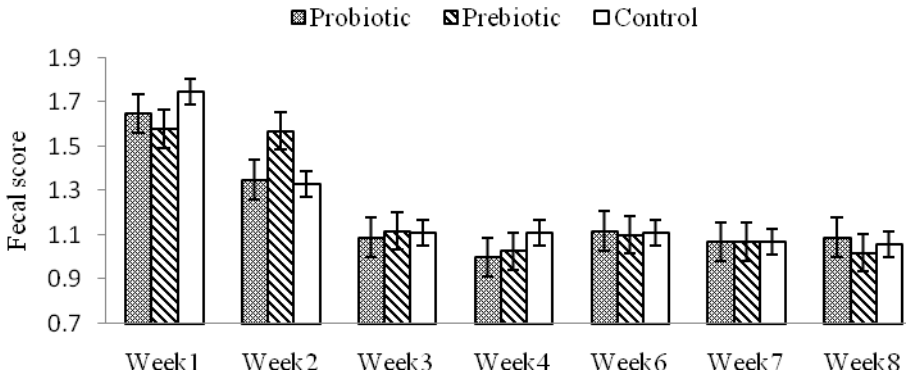

Figure 6. Least square means (LSM) (±standard error) of treatment x day for fecal score of veal calves fed milk replacer containing either probiotics or prebiotics.

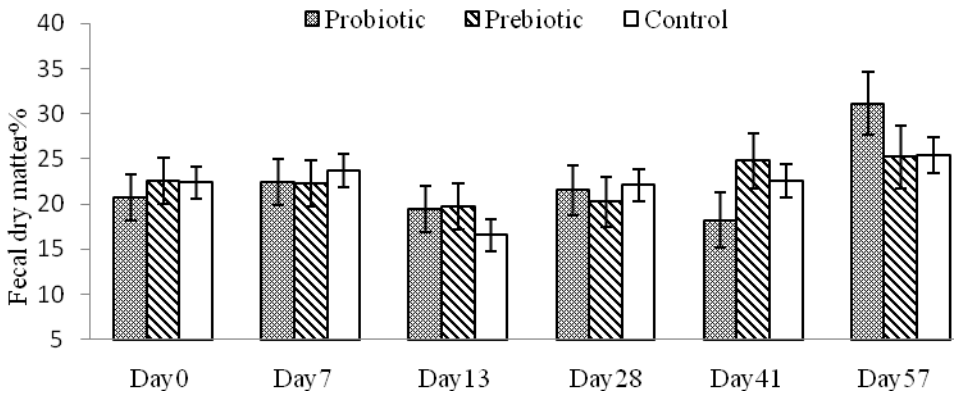

Figure 7. Least square means (LSM) (±standard error) of treatment x day for fecal dry matter (%) of veal calves fed milk replacer containing either probiotics or prebiotics.

Table 4. Least square means (LSM) (±standard error) of medication cost (dollars) for veal calves fed milk replacer containing either probiotic (PRO) or prebiotic (PRE)

	Treatment			Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Baytril cost	15.7±3.08	13.1±2.99	17.1±2.08	0.553	0.366
Fluazine cost	0.5±0.24	0.7±0.24	0.6±0.17	0.52	0.934
Total medication cost	29.5±3.12	26.7±3.02	30.7±2.11	0.522	0.394

[1] Control diet, without any additive.

Table 5. Least square means (±standard error) of overall1 performance traits for veal calves fed milk replacer containing either probiotic (PRO) or prebiotic (PRE)

	Treatment			Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Initial body weight, kg	45.4±0.78	46.4±0.75	45.3±0.52	0.342	0.423
Final body weight, kg	80.5±3.67	83.9±3.51	78.9±2.43	0.503	0.356
Total weight gain, kg	35.9±3.63	37.5±3.48	33.6±2.41	0.638	0.447
Overall average daily gain, kg	0.62±0.06	0.66±0.06	0.59±0.04	0.638	0.447
Overall total milk replacer intake, kg	41.5±0.6	39.6±0.57	41.6±0..40	0.0233	0.0818
Daily milk replacer intake, kg	0.78±0.01	0.7±0.01	0.78±0.0007	0.0234	0.0825
Overall total calf starter intake, kg	41.4±6.15	47.1±5.89	35.9±4.08	0.503	0.162
Daily calf starter intake, kg	0.73±0.11	0.84±0.11	0.64±0.073	0.503	0.162
Overall total feed intake, kg	83.0±6.17	86.7±5.91	77.4±4.09	0.658	0.22
Daily total feed intake, kg	1.52±0.11	1.59±0.10	1.42±0.07	0.667	0.223
Overall feed efficiency	2.7±0.17	2.4±0.16	2.5±0.11	0.206	0.861
Days to consume 1kf of calf starter	43.7±2.81	41.1±2.69	46.6±1.86	0.5	0.123

[1] Values for each variable, except initial body weight and final body weight, are based on measurements taken over the entire 8 week (57d) trial period.
[2] Control diet, without any additive

Table 6. Least square means (LSM) (±standard error) of treatment x day for total milk replacer intake (kg) of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Week1	3.91±0.107	3.93±0.103	3.96±0.0712	-0.016±0.1495	-0.05511±0.103	0.915	0.593
Week2	5.20±0.107	5.11±0.103	5.26±0.0712	0.083±0.1485	-0.11±0.103	0.576	0.307
Week3	6.07±0.107	6.08±0.103	6.05±0.0712	-0.015±0.1485	0.024±0.103	0.922	0.816
Week4	6.09±0.107	5.70±0.103	6.04±0.0712	0.40±0.149	-0.14±0.103	0.0083	0.173
Week5	6.23±0.107	5.75±0.103	6.11±0.0712	0.48±0.149	-0.12±0.103	0.0014	0.2552
Week6	6.08±0.107	5.73±0.103	6.07±0.0712	0.35±0.149	-0.17±0.103	0.0192	0.107
Week7	6.05±0.107	5.69±0.103	6.08±0.0712	0.35±0.149	-0.21±0.103	0.0187	0.047
Week8	1.89±0.107	1.58±0.103	1.99±0.0712	0.31±0.149	-0.25±0.103	0.0373	0.0145

[1] Control diet, without any additive.

Table 7. Least square means (LSM) (±standard error) of treatment x day for total calf starter intake (kg) of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Week1	0.78±1.203	1.12±1.151	0.81±0.798	-0.34±1.665	0.15±1.153	0.839	0.9
Week2	1.70±1.203	2.003±1.151	1.47±0.798	-0.31±1.665	0.38±1.153	0.854	0.745
Week3	2.42±1.203	2.32±1.151	2.14±0.798	0.095±1.665	0.23±1.153	0.954	0.841
Week4	2.77±1.203	3.36±1.151	2.74±0.798	-0.59±1.665	0.32±1.153	0.724	0.781
Week5	4.14±1.203	4.34±1.151	3.68±0.798	-0.19±1.665	0.56±1.153	0.908	0.631
Week6	6.53±1.203	7.77±1.151	5.35±0.798	-1.24±1.665	1.81±1.153	0.46	0.122
Week7	9.06±1.203	9.91±1.51	7.13±0.798	-0.85±1.665	2.36±1.153	0.611	0.0449
Week8	13.99±1.203	16.32±1.151	12.56±0.798	-2.33±1.665	2.59±1.153	0.166	0.0278

[1] Control diet, without any additive.

Table 8. Least square means (LSM) (±standard error) of treatment x day for total feed intake (kg) of veal calves fed milk replacer containing either probiotic (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Week1	4.63±1.219	5.05±1.219	4.78±0.845	-0.42±1.724	0.060±1.2069	0.811	0.961
Week2	6.76±1.219	7.12±1.219	6.73±0.845	-0.35±1.724	0.206±1.2069	0.838	0.865
Week3	8.29±1.219	8.40±1.219	8.19±0.845	-0.12±1.724	0.16±1.2069	0.947	0.897
Week4	8.54±1.219	9.06±1.219	8.78±0.845	-0.52±1.724	0.019±1.2069	0.764	0.987
Week5	9.81±1.219	10.08±1.219	9.79±0.845	-0.27±1.724	0.16±1.207	0.875	0.896
Week6	11.77±1.219	13.50±1.219	11.42±0.845	-1.73±1.724	1.22±1.207	0.319	0.316
Week7	13.98±1.219	15.61±1.219	13.21±0.845	-1.63±1.724	1.59±1.207	0.348	0.194
Week8	14.56±1.219	17.90±1.219	14.55±0.845	-3.34±1.724	1.68±1.207	0.0568	0.169

[1] Control diet, without any additive.

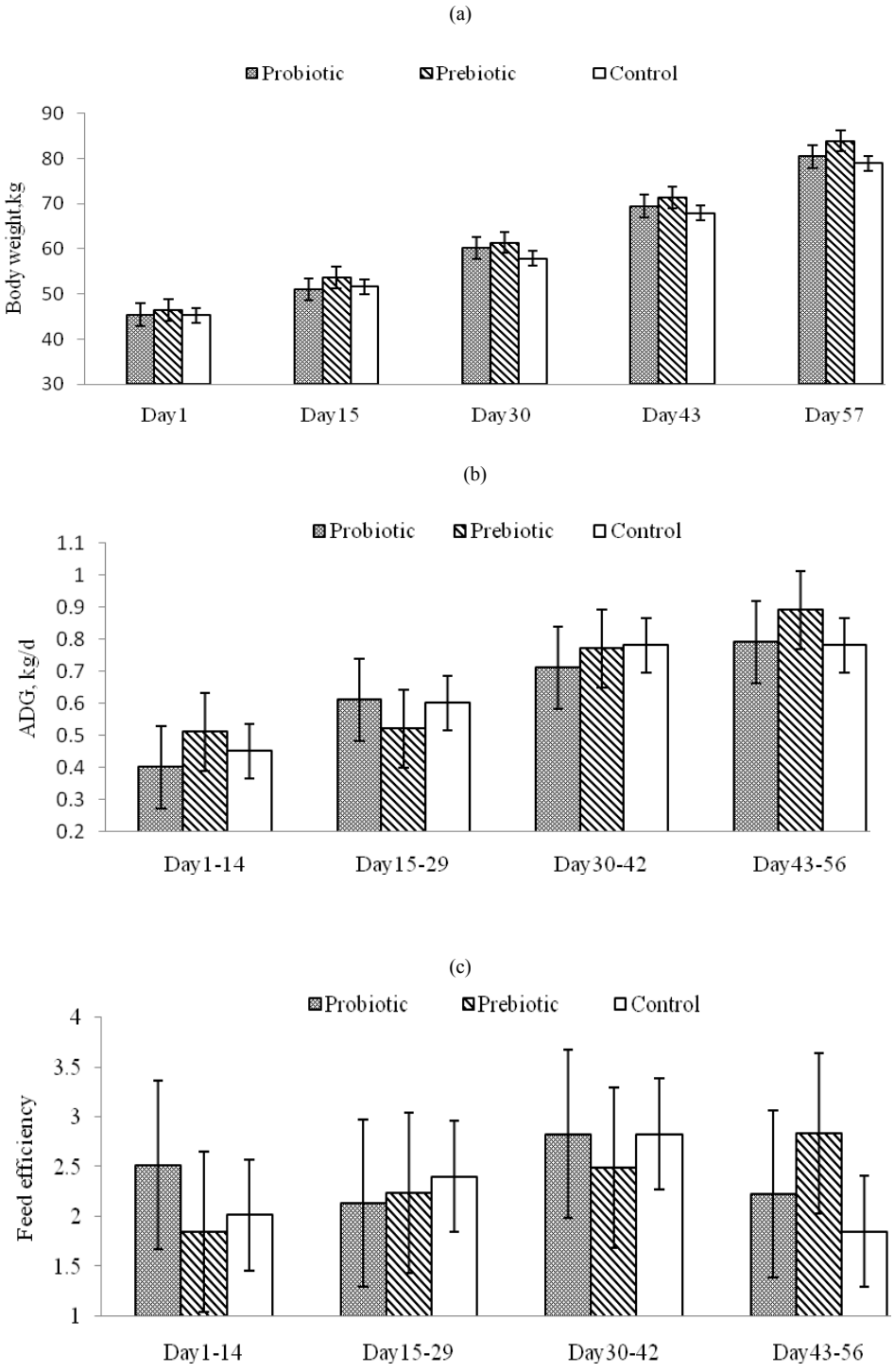

Figure 8. Least square means (LSM) (±standard error) of treatment x day for body weight (kg), ADG (kg/d) and feed efficiency of veal calves fed milk replacer containing either probiotic or prebiotic

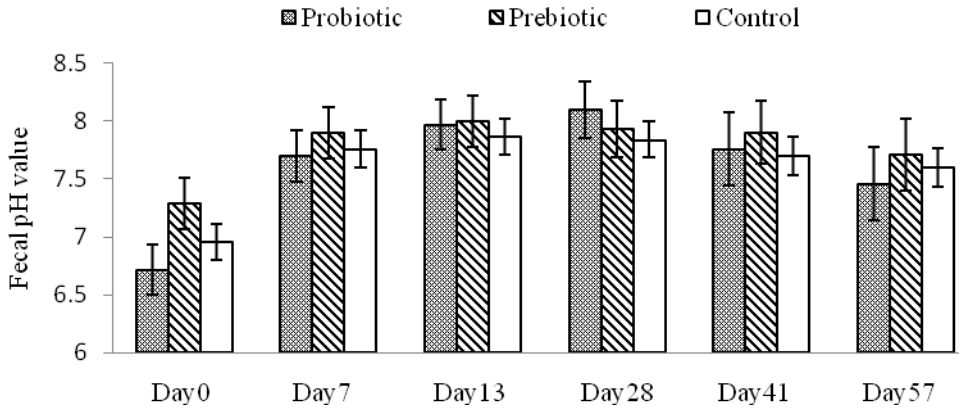

Figure 9. Least square means (LSM) (±standard error) of treatment x day for fecal pH of veal calves fed milk replacer containing either probiotics or prebiotics.

Table 9. Least square means (LSM) (±standard error) of treatment x day for fecal LAB populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotics.

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
day0	8.69±0.343(6)[2]	9.78±0.343(6)	9.57±0.242(12)	-1.09±0.502	-0.3319±0.349	0.0332	0.345
day7	8.65±0.343(6)	7.66±0.343(6)	8.64±0.242(12)	0.99±0.484	-0.48±0.343	0.0459	0.1663
day13	7.34±0.343(6)	8.27±0.343(6)	7.90±0.242(12)	-0.93±0.343	-0.99±0.343	0.0594	0.772
day28	9.01±0.368(5)	7.67±0.402(5)	8.53±0.250(12)	-0.19±0.370	7.53±0.368	0.0164	0.616
day41	7.53±0.368(5)	7.63±0.402(4)	7.67±0.250(11)	-0.097±0.545	-0.087±0.370	0.86	0.814
day57	7.36±0.450(3)	7.49±0.452(3)	7.08±0.0.270(10)	-0.13±0.638	0.35±0.418	0.841	0.41

[1] Control diet, without any additive.
[2]values in parenthesis represent the number of observation for each mean

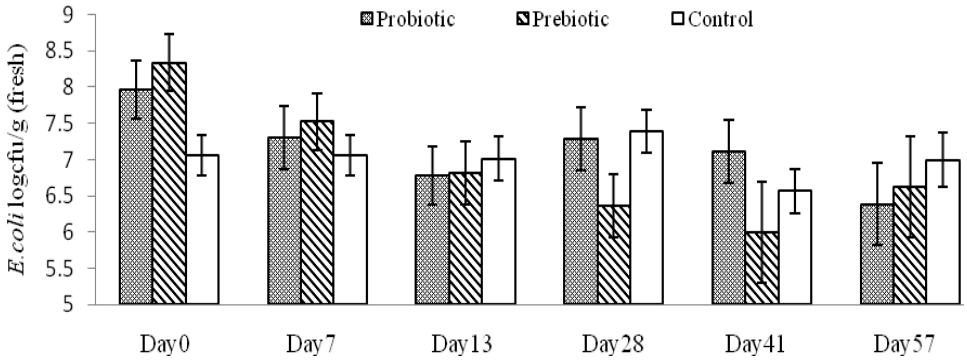

Figure 10. Least square means (LSM) (±standard error) of treatment x day for fecal *E. coli* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh)

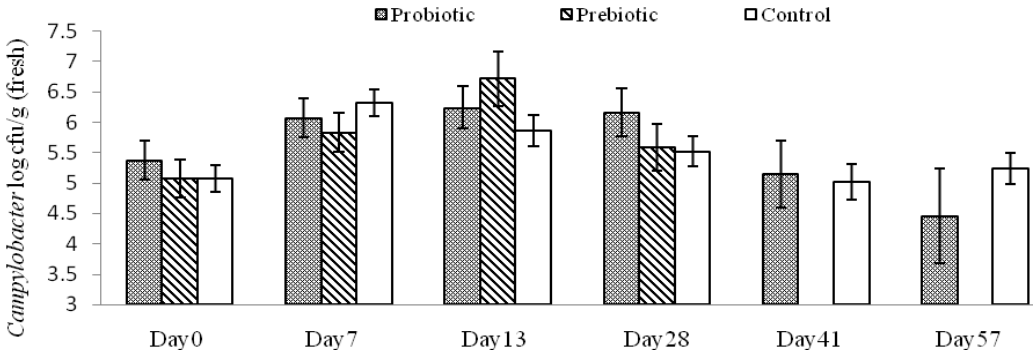

Figure 11. Least square means (LSM) (±standard error) of treatment x day for *Campylobacter* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh)

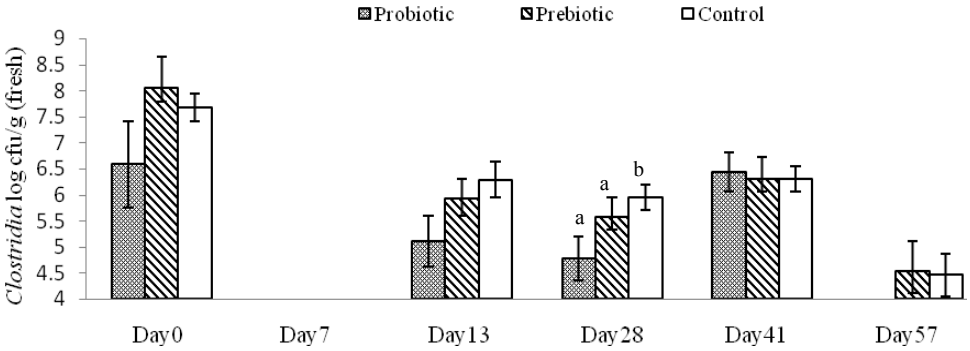

Figure 12. Least square means (LSM) (±standard error) of treatment x day for *Clostridia* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh)
[a,b] Values with different script within a group are different ($p<0.05$)

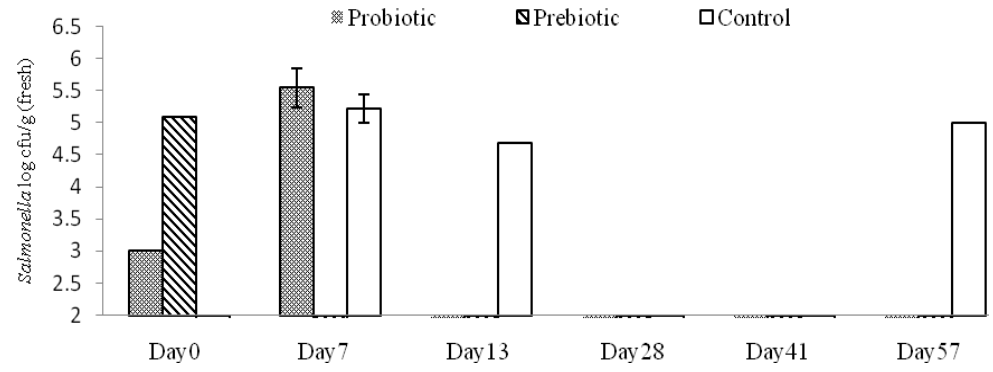

Figure 13. Least square means (LSM) (±standard error) of treatment x day for *Salmonella* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh)

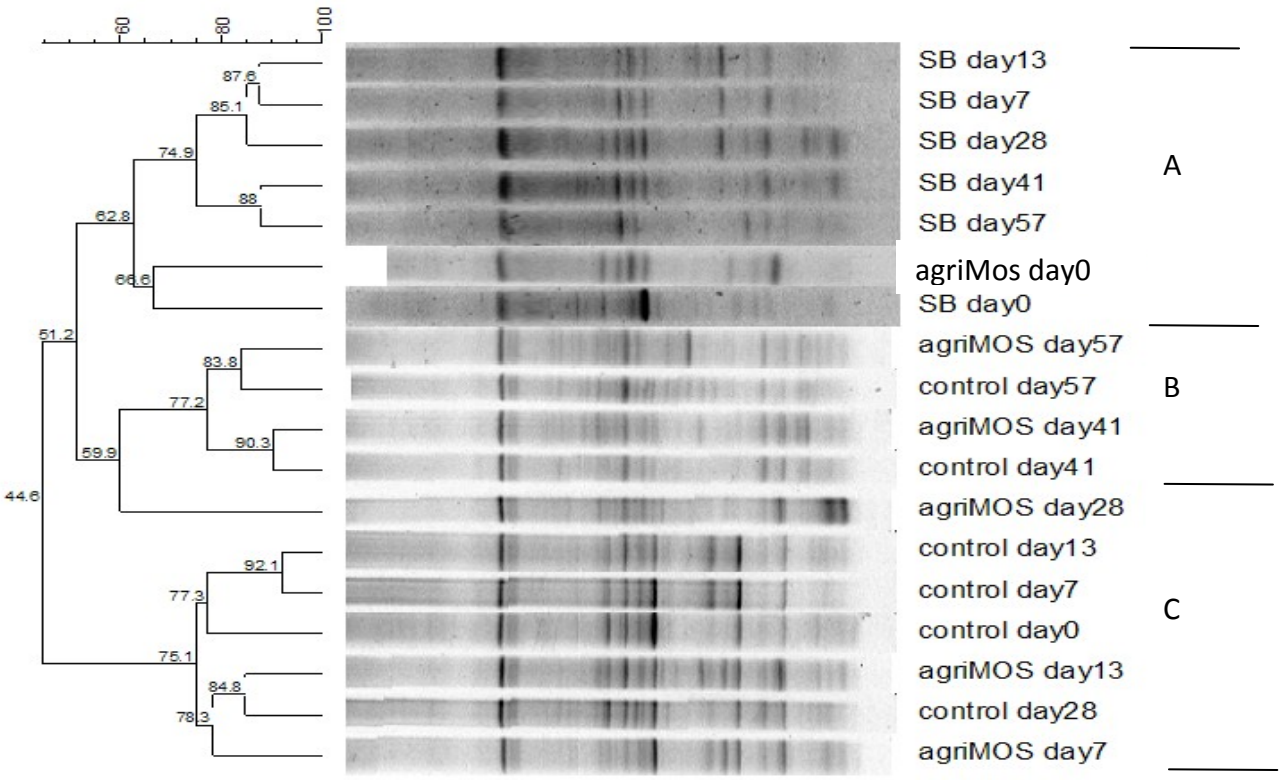

Figure 14. TTGE result by using pearson correlation (0-100%)

5. DISCUSSION

5.1 Calf Health, Mortality and Fecal Scores

In the life cycle of the calf, one of the most critical periods is the first 2-3 weeks of life and meeting the high nutrient of this young animal is of paramount importance (NRC 2001). A crude protein content of 20% and crude fat content of 20% of are among the nutrient recommendations for milk replacer (MR) fed to grain-fed veal calves (NRC 2001). Based on the consumption of MR and calf starter throughout the study, the calves met or exceeded their nutrient requirements (NRC, 2001). Therefore, inadequate nutrition is unlikely to have been a factor contributing to calf mortality. The health of the calf can be affected by numerous environmental factors and several reports have identified temperature, ventilation, relative humidity and season as important determinants of the health status of the calf (Roy et al., 1971; Waltner-Toews et al. 1986; Hillman et al., 1992; Broucek et al., 2008). Optimal housing temperature for calves ranges from 18˚C to 21˚C and the recommended relative humidity for young calves range from 50% to 60% (FASS, 1999). In the present study, which was conducted during the late summer and fall of 2008, the average barn temperature and relative humidity deviated from these standards and may have had an impact on calf health and the high incidence of respiratory illness and calf mortality. Not only was the mean barn maximum temperature high (23˚C) but there was considerable differences between the daytime and the nighttime temperatures. Large variations in temperature do predispose calves to respiratory disease (Roy et al., 1971). The study was conducted under commercial conditions of veal production in order to maximize the transferability of the research findings to production conditions. The high calf mortality rate was clearly a reflection of the prevailing management and environmental

conditions within the barn. According to Hellickson and Walker (1983) the optimum indoor ventilation for raising calves is about 50 CFM /calf. Calculations reveal that the fan capacity in the barn was approximately 213 CFM/calf; however, due to unforeseen limitations in building design and pattern of air flow, there is uncertainty about the effective ventilation rate at the level of the calves. According to the Midwest Plan Service (1987), a ventilation system is considered effective if it successfully distributes and mixes the air within the building to control the temperature, humidity and air quality.

Necropsy examinations revealed that pneumonia was the principal cause of calf mortality (Appendix Table 3). According to Bowland and Shewen (2000), pneumonia is one of the categories of bovine respiratory disease (BRD) and it is known that BRD is the main cause of health problems and economic losses in veal production (Snowder et al., 2005). The incidence of pneumonia was unrelated to dietary treatment and this finding agrees with observations by Pinos-Rodriguez et al. (2008) who found that yeast prebiotic had no effect on the incidence of pneumonia. The first peak in mortality occurred at week 3, when the calves were approximately 27day old. Snowder et al. (2005) reported that the number of calves diagnosed each day with BRD is associated with the age of calf, and their own research showed that the first peak in the incidence of BRD occurred when calves were approximately 20 days old. The second peak in pneumonia and mortality occurred when the calves were 32 days old (week 7 and 8) much earlier than the age at which a second surge in the incidence of BRD was reported to occur (Snowder et al. 2005).

Bovine respiratory disease is a prevalent and complex disease in cattle, and it is caused by viruses, bacteria and mycoplasma (Snowder et al., 2005); the latter microorganisms was among the pathogens isolated from the lungs of dead calves. Mycoplasma species belong to the

class Mollicutes, a group of bacteria that lack cell walls and are instead enveloped by a complex plasma membrane; the microorganisms typically inhabits mucosal surfaces, including those of respiratory tract, and usually form an intimate association with host cells for their survival (Maunsell and Donovan, 2009). The antibiotic, Draxxin®, is effective against most pathogens that cause pneumonia, and this medication was administered to the calves on the day of arrival. Draxxin® is effective for only 14 days (Pfizer Animal Health, 2006) so such antibiotic therapy may not have been sufficient. However, given that the study was aimed at investigating the impact of a probiotic and a prebiotic, it was important that antibiotic therapy be kept at a minimum.

Inadequate intake of colostrum at birth was clearly a factor contributing to the high calf mortality rate. Within the veal industry, calves frequently arrive at the farm with inadequate intake of colostrum and are subject to mismanagement; this leads to a high rate of calf morbidity and mortality (Pare et al., 1993; Tyler et al., 1998; Donovan et al., 1998). Evidence for inadequate intake of colostrum by calves in this study can be adduced from the fact that only 15 calves of the 68 calves received showed serum concentrations of gamma-glutamyl transferase (GGT) greater than 324U/L, the threshold value for declaring a sufficient level of passive transfer of immunity from colostrum (Jezek et al., 2008). The enzyme, GGT, is produced by the ductile cells of mammary gland; it is present in colostrum in higher concentrations than normally found in serum and it is absorbed by the calf during the period of immune globulin (Ig) absorption (Thompson et al., 1981; Braun et al., 1982). Studies have shown that for calves less than three weeks old, measurement of serum GGT in combination with total serum is an indirect and useful diagnostic test for assessing colostral Ig absorption and passive immunity in neonatal calves (Parish et al., 1997; Jezek et al., 2008). It is worthwhile to note that, based on serum GGT

concentration, the incidence of inadequate Ig intake was greater among the calves that died than those that survived; this finding reinforces the importance of colostrum intake in ensuring calf survival.

Diarrhea was not an important problem in the present study. A fecal score greater than 3.0 was used as an indicator of diarrhea, and based on this criterion, the calves generally appeared to be free of diarrhea. This may account for the failure to observe an effect of either the probiotic or the probiotic on fecal score. Previous studies have also shown no effect either a probiotic or prebiotic on diarrhea in calves (Galvao et al., 2005; Terre et al., 2007; Pinos-Rodriguez et al., 2008).

The severity of respiratory problems observed in this study highlight the hazards of acquiring animals from auction yards and the risks associated with inadequate colostrum intake by new born calves. Studies have shown that despite inadequate passive immunity, calves can be raised successfully (Wilson et al., 2000; Moore et al., 2002). There is no doubt, however, that under conditions of inadequate intake of colostrum, there would be an increase in the use of antibiotics and the cost of medication. Th total cost of medication for each calf ranged from $27 to $31, which was 52% higher than the average value ($19) for the industry as reported by FPBQ (2006). The high cost observed in the study was due to the high incidence of illness among calves. There was a large variation in medication cost within treatment and this probably reflects animal to animal variation in immuno competence and susceptibility to disease.

During the study, Baytril® was administered as curative therapy for BRD, and this antibiotic accounted for more than half of the total cost of medication. Prolonged administration of antibiotics may not only have increased the cost of medication but may have minimized any potential differences between control calves and those fed probiotic or prebiotic. Furthermore,

excessive use of antibiotics in animal production has also been linked to the development of antibiotic resistant bacteria, with implications for human health (Fuller, 1989; Phillips, 1999).

The fact that neither the probiotic nor the prebiotic influenced calf mortality or diarrhea may explain the similarity in medication cost among dietary treatments. Gorgulu et al. (2003) reported that when *Lactobacillus spp.* was used as probiotic in the morning milk for 3 day old calves, those fed the probiotic were healthier than the control group, and the mortality rate and veterinary cost were less in calves fed the probiotic. Yeast was used as the probiotic in the present study and this may have could contribute to differences observed between this study and that of Gorgulu et al. (2003).

5.2 Calf Performance

All calves were weaned at the same time in order to ensure that they all received the respective feed additive for a fixed period of time; day 56 of the study was selected as the time for weaning which would have allowed most of the calves to have consumed sufficient amount of calf starter to justify being weaned. The weaning strategy used in the present study meant that the final body weights represented the weaning weights. Terre et al. (2007) also used a fixed time for batch weaning but the calves were weaned on day 35 of the study. The age at weaning or the number of days to weaning is useful a measurement for assessing diet effects calf development on. However since there was a fixed time for weaning in the present study, the number of days to consume 1 kg of calf starter was used as the variable for assessing treatment effects on the calf development and readiness for weaning. Though not statistically significant (P=0.12) the shorter

time by taken calves fed additives to consume 1 kg of calf starter is an encouraging indication of the potential impact of probiotic and prebiotic on calf development. .

Values for overall ADG observed in this study are consistent with growth rate standards reported by NRC (2001), and fall within the range of estimates reported for calves weaned at similar body weights (Abe et al. 1995; Timmerman et al. 2005). Estimates of overall feed efficiency also fall within the range of estimates reported for pre-weaned young calves (Abe et al., 1995; Cruywagen et al., 1995; Timmerman et al., 2005; Frizzo et al., 2008).

The finding that overall ADG was not affected by probiotic is in agreement with other studies (Galvao et al., 2005; Pinos-Rodriguez et al., 2008), where different strains of *S. cerevesiae* were investigated. Abe et al. (1995) found a significant effect on body weight gain of probiotics but they were based on strains of *Lactobacillus* and *Bifidobacteria*. In studies with calves fed probiotics containing either multiple (Timmerman et al., 2005) or single (Cruywagen et al., 1995) strains *Lactobacillus*, ADG was significantly improved in the probiotic treated groups but only during the first 2 weeks of the experiments which lasted for either 6 weeks (Cruywagen et al., 1995) or 8 weeks (Timmerman et al., 2005); feed efficiency was also improved in the study by Timmerman et al. (2005) but not in the study by (Cruywagen et al., 1995). The effects of probiotics on animal performance and health have been inconsistent and variability in responses has been partially attributed to inadequate quality control and inadequacies in labeling of commercial products (Weese, 2003).

The lack of an effect of the prebiotic on ADG is consistent with the findings of Heinrichs et al. (2003) and Terre et al. (2007) who investigated the use of a mannose oligosaccharide (MOS) prebiotic in calves. This is the first report of the impact of a prebiotic on veal calves. The

scarcity of published research on the effects of prebiotics in calves highlights the need for more research with this type of feed additive.

The only positive impact of the additives on feed intake related to MR intake; intake of calf starter was unresponsive to the presenec of the additives. The higher MR intake with SB compared to AgriMOS® occurred mainly during the last four weeks of the study but the effects were not reflected in differences in ADG or feed efficiency. One possible reason that the MR intake was less in the prebiotic group than other groups is that the calves tended to show more interest in consuming the CS. The present finding agrees with results of Terre et al. (2007) indicating that during the pre-weaning period, calves fed with MOS tended to consume more calf starter than the control but there was no significant effect on MR intake. In their study with prewean calves, Heinrichs et al. (2003) reported no difference in grain intake fed between MOS and control; no measurements were reported for MR intake. Due to the limited research in calves fed prebiotics, more studies should be conducted on the impact of prebiotics on the intake of both calf starter and milk replacer.

Galvao et al. (2005) investigated the same yeast probiotic using calves, and reported that SB had a tendency to improve dry matter intake before weaning; however, the authors found no beneficial effects on feed efficiency or body weight gain of calves.

There is very little published work on the effects of yeast probiotics or prebiotics in calves so the lack of a beneficial effect must be considered in this context. There are, however, numerous studies calves fed probiotics containing on lactic acid bacteria, and the results are quite inconsistent. In a study with Holstein calves fed milk replacer containing either *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus*, Abe et al. (1995) found no difference in feed intake between the two probiotic groups or between the probiotic groups. Timmerman et al. (2005) and

Cruywagen et al. (1995) reported that young calves benefited from *Lactobacillus* probiotics during the first two weeks of milk replacer feeding but thereafter the beneficial effects on performance did not persist. In their review of the impact of probiotics in pre-ruminant calves and ruminant animals, Krehbiel et al. (2003) concluded that the effects of these additives have been mixed and their mode of action unclear; animals that are unhealthy are more likely to benefit from probiotics. In the preset study, there were clear indications of serious respiratory illness but diarrhea was not a concern. Although differences in the nature of the probiotic and the conditions of the research would complicate interpretation of studies with these feed addtives, the conditions under which probiotics and prebiotics would be beneficial for calves are not obvious. It appears that the major benefit of probiotics relates to the establishment of normal intestinal flora rather than to animal, performance. Given the scarcity of published research with yeast probiotics and prebiotic in calves, there needs to be caution before making definitive conclusions about the utility of SB in veal production.

5.3 Fecal pH and Fecal Microbiology

In the present study, fecal pH was lowest during the first week; it increased in the first two weeks and stabilized thereafter. In their study with neonatal calves, Sato and Koiwa (2008) reported higher fecal concentrations of lactate and lower fecal pH during the first two weeks of age; fecal pH increased to values between 7.20 and 7.5 when the calves were 4 to 6 weeks of age. In the present study, fecal pH in the control group was as high as 7.84 at day 28 of the study. The lower fecal pH observed during the first two weeks in this study may have been due to elevated levels of lactate produced during hindgut fermentation of carbohydrate. According to Ireland-Perry and Stallings (1993) feces exhibiting low pH had high starch content.

Previous research has explored the relationships between fecal pH and diet in feedlot cattle (Buchko et al. 2000 and Berg et al. 2004) and dairy cows (Ireland-Perry and Stallings 1993), and between fecal pH and age of the calf (Sato and Koiwa, 2008). However, this study represents the first published report of the effect of a prebiotic or probiotic on fecal pH in calves, or the combination of fecal pH and fecal microflora. The findings in this study that the additives had no effect on fecal populations of LAB are consistent with results of Schwab et al. (1980) with calves and the report by Whitley et al. (2009) who fed probiotitcs to meat goats. The results conflict however with findings of Jenny et al. (1991) who observed that mixed species of lactic acid bacteria probiotic resulted in increased fecal counts of LAB when calves were 6 weeks old. There is also a scarcity of published studies dealing with the effects of probiotics and prebiotics on fecal microflora in calves (Krehbiel et al., 2003) so the impact of SB, other probiotics as well as prebiotics on fecal LAB population deserves further study.

In the present study, there seemed to be no relationship between fecal pH and fecal counts of *E. coli*. Based on studies with feedlot cattle, Buchko et al. (2000) and Berg et al. (2004) associated a low fecal pH with inhibition of proliferation of *E. coli O157*. However, in a study with steers, manipulated through diet to experience altered hindgut fermentation, Depenbusch et al. (2008) demonstrated that fecal shedding of *E. coli O157* was not related to fecal pH.

The fact that the additives had no significant effect on fecal *E. coli* could be explained by the fact that fecal score remained normal. According to Krehbiel et al. (2003) probiotics are unlikely to have an effect on the fecal coliforms when calves are experiencing normal stool; that condition seemed to apply in this study.

There are no published reports of the effects of probiotics or prebiotics on fecal populations of *Campylobacter* but our study shows no beneficial effects of the additives on the fecal population of this pathogen. This microorganism is known to cause diarrhea in calves (Garcia et al., 1985) but the calves in this study did not experience problems of diarrhea. In a study with broilers, Line et al. (1998) reported that the SB probiotic had no effect on colonization of the cecae by *Campylobacter*.

Only on day 28 was there an effect of the additives on *Clostridia*; compared to control their effect was a marked reduction in the fecal population of this pathogen. In an extensive meta-analysis study with humans involving of different types of single strain probiotics and 7 types of probiotic mixtures, SB was found to be the only probiotic effective against *Clostridium difficule* disease in humans (McFarland, 2006). In their study the MOS prebiotic, Terre et al. (2007) observed no difference in fecal population of *Clostridia perfringens* between control calves and those fed MOS. A difference in the nature of the additives may explain the discrepancy in results between the present study and that of Terre et al. (2007).

The overall microbial diversity as well as microbial populations in feces were studied using temporal temperature gradient gel electrophoresis (TTGE) instead of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE); TTGE is a recently modified method from DGGE and TGGE and was used to generate a DNA fingerprint analysis of the microbial population at a specific time point of fecal sampling. The technique separates amplicons which encompass the V6 to V8 region of the 16S rDNA gene from different bacterial species in the sample and may be used to monitor the time course changes and treatment effects on the microbial community of the gut (Riesner et al., 1992; Zoetendal et al., 1998). Different banding patterns are obtained which acts as means of identifying key

differences in the composition of the fecal flora between different treatments. A similar DNA fingerprinting approach as used in this project was applied in a study to compare the biodiversity of the microflora in the colon of patients who had been diagnosed as sufferring from Crohn's disease (CD) with those of normal patients (Seksik et al., 2005). The study showed that biodiversity remains high in patients with CD; however, the TTGE profile was very stable over time under healthy condition but unstable in CD patients. Furthermore, *enterobacteria* were observed significantly more frequently in CD patients than in health patients. TTGE can also used in other aspects; for example, the efficacy of TTGE was proven to be useful in identifying *Clostrium* in cheese (Le Bourhis et al., 2005) and in observing the impact of yogurt on composition of human intestinal microbiota (Alvaro et al., 2006). Furthermore, TTGE is used to show the changes in the diversity of dominant bacterial communities in human in response to dietary supplementation with hormone-related compounds combined with functional foods (Clavel et al., 2005). The wide use of TTGE indicates its ability to be implemented as a detective method in different fields in determining if specific bacteria or specific pattern of microbiota involved in a particular study.

In the present study, probiotic treatment appears to have a significant effect on the composition of the microbial community with a different banding pattern associated with the supplementation of the probiotic being generated. Such a treatment effect was not observed for either the control or prebiotic groups. This would imply that the probiotic has more of an effect on altering the composition of the flora than the prebiotic since very little difference was observed in the banding pattern between the prebiotic treatment and control group. This alteration of the gut microbial community in response to probiotics has been shown in many different studies and species (Alvaro et al., 2006; Marzotto et al., 2006; Barc et al. 2008).

It is unclear from the results of this study which treatment generated a higher degree of microbial diversity since the number of bands increased over time for all of the groups. This increase in diversity and change in the flora would reflect a change in the diet as the animal gradually consumed more calf starter and reduced the intake of milk replacer, and as the gut matured. It is well known that the composition of the intestinal microflora is relatively stable and diverse overtime compared to those in the first days of life; this applies to both humans and animals (Smith, 1965; Karney et al., 1986; Palmer et al., 2007). In the present study, for each treatment group, the microbial composition changed over time, such that there were distinct microbial clusters in the early phase of the study (d7, d13, d28) and distinct microbial clusters in the latter phase of study (d47, d57) . However, some factors can influence the pattern and composition of gut microbial population such as diet (Humblot et al., 2005). This reflected in the present study where the probiotic group had a distinct treatment effect on microbial population in feces. Some specific bands were observed in the probiotic group but not in other groups or vice versa; this implies that specific bacteria might be involved in explaining the response to the different treatment groups. The reason underlying the difference in microbial population on day 0 between control and additive groups remains unclear.

In summary, there was a high incidence of respiratory disease among the calves which arrived at the site of study with biochemical evidence of inadequate transfer of passive immunity via colostrum; as a consequence the incidence of calf mortality close to 30%. Compared to control diet, the SB and AgriMOS® had no effect on health and overall performance of the calves; the only difference between SB and AgriMOS® was higher MR intake with the former additive, but this was not reflected in differences in calf health or performance. A fecal population of LAB was greater with SB than with AgriMOS® but the average effect of the

additives was no different from control. Over time, there was a marked decline in *E. coli* populations in the groups fed additives but there was no such change in the control group. The lowest population fecal *E. coli* was observed with AgriMOS® in the prebiotic group on day 41 of the study. The study also revealed that the fecal microbial population associated with SB was significant different from that associated with AgriMOS® or control groups implying an effect of the probiotic on the composition of the gut microflora.

6. CONCLUSION

Under the conditions of the present study, where most of the week old calves received an inadequate supply colostrum and there were unforeseen limitations in ventilation and barn temperature control, there was a high incidence of respiratory illness and mortality due to pneumonia. Neither a probiotic nor a prebiotic was beneficial for health and performance when compared to control. However, when compared to each other each other, SB resulted in higher fecal population of LAB than AgriMOS® and had a distinct effect on microbial composition of the feces had positive effects on the fecal populations of bacteria. Although SB seemed to have some positive effects on gut microflora, there was no consistent benefit for veal production. The study demonstrates the importance of effective calf management and high quality housing conditions to ensure the success in veal production. Yeast probiotic seem to have the potential to alter the microbial diversity of the gut and this is an area for future research in order to determine the role of probioitcs and prebiotic in gut microbiology and gut health. Much more research is required before probiotics and prebiotics can be considered useful alternatives to antibiotics in animal production and health.

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8. APPENDEIX FIGURES AND TABLES

Appendix Table 1. Diet values from La Coop Federee, Quebec

	Milk Replacer (%)	Calf Starter (%)
Crude Protein	20	19
Crude fat	20	2
Crude fiber	0.2	6
Calcium	0.75	0.9
Phosphorus	0.7	0.45
Magnesium	N/A	0.25

Appendix Table 2. Statistical data of all variables.

Variables	SAS analysis Performed		Variance components		
Performance	Point measures	Repeated Measures	σ^2_e	covariance structure	animal variation
Body weight					
Bi-weekly body weight	N/A	done	68.3117	AR=0.6482	0
Initial body weight	done	N/A	6.6820442	N/A	N/A
Final body weight	done	N/A	147.76556	N/A	N/A
Total weight gain	done	N/A	145.36236	N/A	N/A
Overall average daily gain	done	N/A	0.0455	N/A	N/A
Bi-weekly average daily gain	N/A	done (bi-weekly)	0.178	AR=0.2578	0
Feed consumption					
Overall total milk replacer intake	done	N/A	3.931		
Weekly total milk replacer intake	N/A	done (weekly)	0.1266	AR=0.6412	0
Average daily milk replacer intake	done	N/A	0.001397		
Overall total calf starter intake	done	N/A	416.554		
Weekly total calf starter intake	N/A	done (weekly)	15.9088	AR=0.8957	0
Average daily calf starter intake	done	N/A	0.1328		
Overall total feed intake	done	N/A	0.1336		
Weekly total feed intake	N/A	done (weekly)	17.8358	AR=0.9086	0
Overall feed efficiency	done	N/A	0.322		
Bi-weekly feed efficiency	N/A	done (bi-weekly)	7.5148	AR=0.04262	0.2284
Days to consume 1 kg of calf starter	done	N/A	86.73		
Medication cost					
Baytril	done	N/A	144.36968	N/A	N/A
Fluazine	done	N/A	0.488592	N/A	N/A
Total	done	N/A	164.73612	N/A	N/A

Fecal microbiology						
	Overall average fecal score	done	N/A	0.01974		
	Weekly average fecal score	N/A	done (interval)	0.08015	CS=0	0.006392
	Microbiology populations (fresh basis)					
	E. coli	data for each week done separately	done (interval)	0.9299	CS=0.01552	0.01552
	Salmonella[1]	data for each week done separately	done (interval)	.	.	.
	Campylobacter	data for each week done separately	done (interval)	0.5359	CS=0.06712	0.06712
	Clostridia	data for each week done separately	done (interval)	0.5393	CS=0.1561	0.1561
	Lactic acid bacteria	data for each week done separately	done (interval)	0.4357	CS=0.2680	0.268
	Overall average fecal pH	done	N/A	0.0593		
	Interval average fecal pH	N/A	done (interval)	0.2718	AR= -0.2000	0.01931
	Interval average fecal DM content	N/A	done (interval)	29.0572	CS=9.6179	9.6179

[1] Invalid analysis due to too many missing observation for statistical analysis

Appendix Table 3. Measurement of gamma glutamyl transferase (GGT), total serum protine, interpretation of passive transfer, hematocrits, record of date of dead and aetiolgy in the study.

Pen #	ATQ Tag #	Trt #	Date Arrival	GGT U/L	total proteines (g/L)	Interpretation	Hematocrits %	Date	Aetiolgy
1	105096263	4	29-Jul	202	59.6	Inadequate passive transfer	23		
2	105072665	3	31-Jul	106	55.3	Inadequate passive transfer			
3	101171382	4	29-Jul	120	52.1	Inadequate passive transfer	17	19-Sep	Pnemonia (Mycoplasma Bovis)
4	105484212	1	29-Jul	328	56.2	successful passive transfer	26		
5	105543510	4	29-Jul	65	54.3	Inadequate passive transfer	30		
6	105604913	3	29-Jul	119	54.2	Inadequate passive transfer	17		
7	104939086	4	31-Jul	448	57.7	successful passive transfer			
8	104962850	1	29-Jul	163	63.1	Inadequate passive transfer	27	24-Sep	Pnemonia (Mycoplasma Bovis)
9	105404314	1	31-Jul	120	53.7	Inadequate passive transfer			
10	105503438	2	29-Jul	50	59	Inadequate passive transfer	23		
11	105513920	2	29-Jul	27	55.7	Inadequate passive transfer	24		
12	104775672	4	29-Jul			N/A[1]	25% or 29%	18-Sep	Pnemonia (Mycoplasma Bovis)
13	252494650	2	29-Jul	80	52.6	Inadequate passive transfer	23	4-Sep	Pnemonia (Mycoplasma Bovis)
14	104990187	1	29-Jul	160	63.8	Inadequate passive transfer	28		
15	253764255	4	29-Jul	151	50.2	Inadequate passive transfer	26		
16	105403833	2	29-Jul	365	53.5	successful passive transfer	27		

17	253782863	1	29-Jul	705	60.6	successful passive transfer	33		
18	105368045	4	29-Jul	34	46.4	Inadequate passive transfer	30		Pnemonia (Mycoplasma Bovis)
19	103741161	3	29-Jul	36	48.2	Inadequate passive transfer	25		
20	105487906	4	29-Jul	635	61	successful passive transfer	35		
21	105314745	3	31-Jul	105	55.3	Inadequate passive transfer			
22	105173042	3	29-Jul	561	65.7	successful passive transfer	25		
23	105516980	4	29-Jul	384	64.9	successful passive transfer	36		
24	105527472	1	29-Jul	533	69.9	successful passive transfer	28		
25	252099983	4	29-Jul	31	47.5	Inadequate passive transfer	23	7-Sep	Pnemonia (Mycoplasma Bovis)
26	105172213	2	29-Jul	403	55.5	successful passive transfer	29	4-Aug	Ulcer
27	105503877	1	29-Jul	582	64.1	successful passive transfer	32		
28	105388958	2	29-Jul	60	48.7	Inadequate passive transfer	33		
29	105260638	1	29-Jul	36	55.9	Inadequate passive transfer	29		
30	105370771	2	29-Jul	144	54.3	Inadequate passive transfer	36		
31	252574751	2	31-Jul	72	57.2	Inadequate passive transfer			
32	105339034	2	29-Jul	18	50.8	Inadequate passive transfer	34		
33	105068920	3	29-Jul	56	59.8	Inadequate passive transfer	23	26-Aug	Pnemonia (Mannheimia haemolytica, Enteriobacteriacae, Proteus sp.)

34	251058123	3	29-Jul	27	47.8	Inadequate passive transfer	36		
35	7.6614E+10	4	29-Jul	53	50.3	Inadequate passive transfer	19		
36	105132584	3	29-Jul	91	56.9	Inadequate passive transfer	29		
37	105091110	2	29-Jul	91	48.6	Inadequate passive transfer	29	20-Aug	Pnemonia (Mycoplasma Bovis)
38	105134502	2	29-Jul	286	80	Inadequate passive transfer	25		
39	105334219	3	31-Jul	272	65.4	Inadequate passive transfer		25-Sep	Pnemonia (Mycoplasma Bovis)
40	104936089	4	31-Jul	36	52.7	Inadequate passive transfer			
41	105233993	3	29-Jul	65	63.2	Inadequate passive transfer	23	10-Sep	Pnemonia (Mannheimia haemolytica, Pasteurella multocida)
42	105378549	3	29-Jul	82	49.9	Inadequate passive transfer	27		
43	252140020	1	29-Jul	62	55.5	Inadequate passive transfer	22		
44	105370064	1	29-Jul	107	47.4	Inadequate passive transfer	19		
45	105232056	2	29-Jul	34	56.8	Inadequate passive transfer	27		
46	105509678	3	29-Jul	61	49.9	Inadequate passive transfer	25		
47	104258539	1	29-Jul	32	47.8	Inadequate passive transfer	24		
48	105293563	1	29-Jul	106	51.4	Inadequate passive transfer	coagulated		
49	105118204	3	31-Jul	309	56.3	Inadequate passive transfer			
50	105310890	4	29-Jul	410	61.9	successful passive transfer	36	8-Sep	Pnemonia (Histophilus Somni or Pasteurella multocida)
51	105078130	3	29-Jul	99	51.5	Inadequate passive	30		

						transfer			
52	250662938	4	29-Jul	274	63.8	Inadequate passive transfer	24		
53	105472552	1	29-Jul	432	56.5	successful passive transfer	21	15-Sep	Pnemonia (Mycoplasma Bovis)
54	105136092	4	29-Jul	34	52.3	Inadequate passive transfer	28		
55	249845331	2	31-Jul	1175	66.9	successful passive transfer			
56	105274692	3	29-Jul	85	55.3	Inadequate passive transfer	33	24-Aug	Pnemonia (Pneumonia (Mannheimia heamolytica, proteus sp., streptococcus sp., E.coli, Bacillus sp.)
57	104946539	3	31-Jul	32	48.7	Inadequate passive transfer		21-Aug	Pneumonia (Mannheimia haemolytica, Serratia sp., Pseudomonas sp., Mycoplasma arfinini)
58	104292553	1	29-Jul			N/A	25% or 29%	26-Aug	Pnemonia (Mycoplasma Bovis)
59	105171486	1	29-Jul	233	55.8	Inadequate passive transfer	27	19-Sep	Pnemonia (Mycoplasma Bovis)
60	105510331	1	29-Jul	47	49.1	Inadequate passive transfer	25	26-Aug	Pnemonia (Mycoplasma sp.)
61	105526894	4	29-Jul	286	68	Inadequate passive transfer	25		
62	105437882	2	31-Jul	110	71.1	Inadequate passive transfer			
63	105437976	2	31-Jul	28	50.2	Inadequate passive transfer		20-Sep	Pnemonia (Mycoplasma Bovis)
64	104697765	3	31-Jul	51	51.4	Inadequate passive transfer			
65	105058280	2	29-Jul	601	59.1	successful passive transfer	31	24-Sep	Pnemonia (Mycoplasma Bovis)
66	253453031	2	29-Jul	332	64	successful passive transfer	29		
67	105359529	1	29-Jul	178	51.8	Inadequate passive transfer	22	24-Sep	Pnemonia (Mycoplasma Bovis)

68	105366631	4	29-Jul	125	55.7	Inadequate passive transfer	23

[1]N/A = data not applicable

Appendix Table 4. Medication cost over the entire 8 week (57d) trial period.

Pen #	Tag #	Trt #	Baytril Frequency	Cost ($)	Flunazine Frequency	Cost ($)	Total Cost($)
37	105091110	2	1	13.92	0	0.00	21.17
51	105078130	3	2	27.85	0	0.00	41.84
57	104946539	3	2	27.85	0	0.00	35.10
63	105437976	2	2	27.85	5	3.06	43.16
21	105314745	3	1	13.92	0	0.00	27.92
55	249845331	2	0	0.00	0	0.00	13.99
49	105118204	3	2	27.85	1	0.61	42.45
50	105310890	4	2	27.85	3	1.84	36.93
56	105274692	3	2	27.85	0	0.00	35.10
60	105510331	1	2	27.85	0	0.00	35.10
25	252099983	4	2	27.85	0	0.00	35.10
8	104962850	1	1	13.92	1	0.61	26.78
17	253782863	1	1	13.92	0	0.00	27.92
18	105368045	4	2	27.85	2	1.23	46.53
16	105403833	2	2	27.85	1	0.61	42.45
12	104775672	4	2	27.85	4	2.45	42.55
9	105404314	1	2	27.85	4	2.45	51.23
29	105260638	1	2	27.85	0	0.00	41.84
3	101171382	4	3[1]	41.77	7	4.29	58.31
23	105516980	4	1	13.92	0	0.00	27.92
13	252494650	2	2	27.85	1	0.61	35.71
6	105604913	3	1	13.92	1	0.61	28.53
10	105503438	2	1	13.92	1	0.61	28.53
20	105487906	4	1	13.92	1	0.61	28.53
22	105173042	3	1	13.92	1	0.61	28.53
45	105232056	2	1	13.92	1	0.61	28.53
35	76613572057	4	2	27.85	2	1.23	43.07
44	105370064	1	1	13.92	1	0.61	28.53
42	105378549	3	2	27.85	3	1.84	43.68
11	105513920	2	2	27.85	3	1.84	47.15
32	105339034	2	1	13.92	1	0.61	28.53
7	104939086	4	2	27.85	1	0.61	42.45
14	104990187	1	1	13.92	1	0.61	28.53
62	105437882	2	1	13.92	5	3.06	30.98
43	252140020	1	2	27.85	1	0.61	42.45
59	105171486	4	2	27.85	6	3.68	43.77
34	251058123	3	3[1]	41.77	2	1.23	56.99
24	105527472	1	1	13.92	1	0.61	28.53
61	105526894	4	2	27.85	2	1.23	43.07

65	105058280	2	1	13.92	2	1.23	27.40
41	105233993	3	2	27.85	0	0.00	35.10
28	105388958	2	1	13.92	0	0.00	27.92
31	252574751	2	0	0.00	0	0.00	13.99
67	105359529	1	2	27.85	2	1.23	41.32
36	105132584	3	0	0.00	0	0.00	13.99
53	105472552	1	0	0.00	3	1.84	14.08
26	105172213	2	0	0.00	0	0.00	7.25
39	105334219	3	1	13.92	1	0.61	28.53
15	253765255	4	0	0.00	0	0.00	13.99
66	253453031	2	1	13.92	1	0.61	28.53
19	103741161	3	0	0.00	1	0.61	18.07
33	105068920	3	2	27.85	0	0.00	35.10
58	104292553	1	2	27.85	0	0.00	35.10
40	104936089	4	1	13.92	0	0.00	27.92
27	105503877	1	1	13.92	0	0.00	27.92
1	105096263	4	0	0.00	0	0.00	13.99
2	105072665	3	0	0.00	0	0.00	13.99
4	105484212	1	0	0.00	0	0.00	13.99
5	105543510	4	0	0.00	0	0.00	13.99
30	105370771	2	0	0.00	0	0.00	13.99
38	105134502	2	0	0.00	0	0.00	13.99
46	105509678	3	0	0.00	0	0.00	13.99
47	104258539	1	0	0.00	0	0.00	13.99
48	105293563	1	0	0.00	0	0.00	13.99
52	250662938	4	0	0.00	0	0.00	13.99
54	105136092	4	0	0.00	0	0.00	13.99
64	104697765	3	0	0.00	0	0.00	13.99
68	105366631	4	0	0.00	0	0.00	13.99
		Total	77	1072.21	73	44.72	1997.48

[1] Calves received too much Baytril.

Appendix Table 5. Chemical composition of feed samples[1]

Chemical composition of feed samples obtained from experiment 2 of veal project

(%)

Sample Description	DM	ash		Fat		NDF		ADF		Protein		Ca		Mg		P	
		as fed	DM basis	as fed	DM basis	as fed	DM basis	as fed	DM basis	as fed	DM basis	as fed	DM basis	as fed	DM basis	as fed	DM basis
MR[2] Week1	85.33	6.61	7.75	20.97	24.5773	N/A	N/A	N/A	N/A	19.80	23.20	0.63	0.74	0.13	0.16	0.68	0.80
MR Week2	86.02	6.32	7.34	20.75	24.1245	N/A	N/A	N/A	N/A	18.75	21.80	0.60	0.69	0.14	0.17	0.62	0.72
MR Week3	86.57	5.99	6.91	19.85	22.931	N/A	N/A	N/A	N/A	19.69	22.74	0.64	0.74	0.10	0.12	0.70	0.81
MR Week4	86.03	6.16	7.17	19.84	23.0623	N/A	N/A	N/A	N/A	20.75	24.12	0.61	0.71	0.11	0.13	0.71	0.83
MR Week5	85.62	6.60	7.71	20.26	23.6658	N/A	N/A	N/A	N/A	20.33	23.74	0.67	0.78	0.10	0.12	0.72	0.84
MR Week6	86.93	6.00	6.90	20.95	24.1022	N/A	N/A	N/A	N/A	21.88	25.17	0.70	0.81	0.11	0.13	0.75	0.87
MR Week7	86.20	6.13	7.11	21.79	25.2752	N/A	N/A	N/A	N/A	21.10	24.48	0.68	0.79	0.11	0.12	0.76	0.88
MR Week8	84.55	6.27	7.42	20.47	24.2097	N/A	N/A	N/A	N/A	21.56	25.50	0.71	0.84	0.11	0.13	0.77	0.91
Average(week1-week8)	85.91	6.26	7.29	20.61	23.99	N/A	N/A	N/A	N/A	20.48	23.84	0.66	0.76	0.11	0.13	0.71	0.83
Number of sample	8	8	8	8	8	N/A	N/A	N/A	N/A	8	8	8	8	8	8	8	8
Standard deviation	0.74	0.24	0.3266	0.65	0.77116	N/A	N/A	N/A	N/A	1.047	1.24137	0.043	0.05092	0.015	0.01777	0.0474	0.0564
CS[3] Week1	84.08	5.79	6.89	2.66	3.16051	16.48	19.60	7.65	9.09	19.84	23.60	0.91	1.09	0.21	0.25	0.55	0.66
CS Week2	88.45	7.35	8.31	2.92	3.30087	19.21	21.72	8.63	9.75	12.95	14.64	0.59	0.67	0.15	0.17	0.36	0.40
CS Week3	87.01	6.29	7.23	2.72	3.12626	18.03	20.72	7.79	8.95	11.99	13.78	0.66	0.76	0.22	0.25	0.40	0.46
CS Week4	87.54	5.48	6.26	2.89	3.30476	17.42	19.90	7.53	8.61	11.53	13.17	0.49	0.56	0.13	0.15	0.35	0.40
CS Week5	85.97	7.00	8.14	2.54	2.95881	19.25	22.39	7.85	9.13	12.84	14.94	0.78	0.91	0.16	0.19	0.44	0.51
CS Week6	85.35	6.74	7.90	2.63	3.08546	16.30	19.10	8.80	10.30	16.30	19.09	1.03	1.21	0.22	0.26	0.50	0.59
CS Week7	84.64	6.62	7.82	2.55	3.00733	16.72	19.76	8.21	9.70	11.08	13.09	0.44	0.52	0.14	0.17	0.36	0.43
CS Week8	85.21	6.39	7.50	2.83	3.32684	18.87	22.15	6.77	7.94	15.57	18.28	0.67	0.79	0.24	0.29	0.47	0.55
CS Average(week1-week8)	86.03	6.46	7.5059	2.718	3.15885	17.8	20.6666	7.902	9.1854	14.01	16.3223	0.697	0.81194	0.185	0.21578	0.4292	0.4999
Number of sample	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Standard deviation	1.51	0.61	0.6873	0.149	0.14097	1.23	1.26989	0.646	0.7359	2.999	3.70304	0.205	0.2454	0.042	0.05107	0.0731	0.09175

[1] Values in each cell is the average of duplicate analysis

[2] MR = Milk replacer

[3] CS = Calf starter

Appendix Table 6. Ambient temperature inside the barn and in Mirabel1 over 8 weeks.

	Temperature inside the barn				Temperature outside (Mirabel)			
Week	Minimum (ºC)	Standard deviation	Maximum (ºC)	Standard deviation	Minimum (ºC)	Standard deviation	Maximum (ºC)	Standard deviation
1	18.48	0.26	24.25	1.39	13.96	1.09	22.64	1.83
2	16.53	0.77	24.53	1.22	11.29	1.47	23.71	2.63
3	16.41	2.26	24.53	4.80	11.39	4.00	24.86	3.19
4	16.03	1.85	26.67	1.14	12.93	2.08	26.51	2.08
5	16.03	1.25	27.29	3.67	13.90	2.74	23.97	5.39
6	17.20	3.55	18.13	4.06	8.84	3.48	20.21	3.48
7	13.50	2.13	15.70	3.39	4.39	3.04	17.69	3.04
8	16.80	1.20	17.27	1.00	11.07	2.59	20.83	2.59

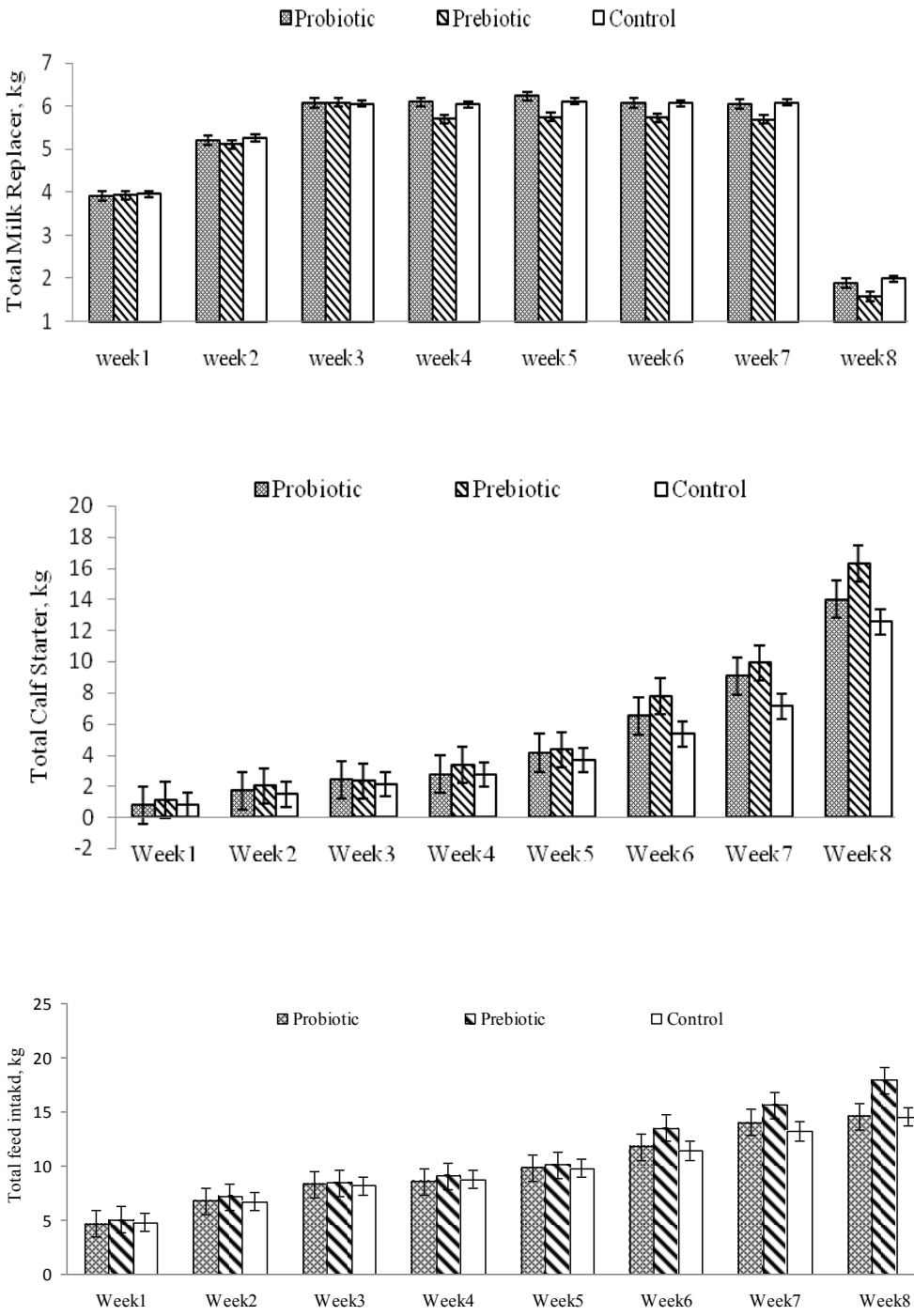
[1]Data obtained from Environment Canada 2008.

Appendix Table 7. Relative humidity inside the barn

Week	Minimum (%)	Standard deviation	Maximum (%)	Standard deviation
1	81.00	9.14	92.83	4.83
2	65.00	6.75	87.33	2.34
3	61.71	7.99	83.43	3.69
4	50.00	3.61	83.33	5.86
5	68.67	15.38	87.50	2.51
6	82.29	6.99	86.71	7.30
7	73.43	4.24	83.00	4.32
8	72.00	6.24	83.67	4.73

Appendix Table 8. Differential temperature and relative humidity

Week	Barn differential temperature (ºC)	Mirabel differential temperature (ºC)	Barn differential relative humidity (ºC)
1	5.77	8.69	11.83
2	8.00	12.43	22.33
3	8.11	13.47	21.71
4	10.63	13.59	33.33
5	11.25	10.07	18.83
6	0.93	11.37	4.43
7	2.20	13.30	9.57
8	0.47	9.76	11.67



Appendix Figure 1. Least square means (LSM) (±standard error) of treatment x day for total milk replacer (kg), total calf starter (kg) and total feed intake (kg) for veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

Appendix Table 9. Least square means (LSM) (±standard error) of treatment x day for body weight (kg) of veal calves body weight of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Day1	45.40±2.492	46.44±2.386	45.31±1.653	-1.04±3.45	0.61±2.39	0.765	0.801
Day15	51.01±2.492	53.60±2.386	51.65±1.653	-2.59±3.450	0.649±2.389	0.454	0.786
Day30	60.20±2.492	61.39±2.386	57.90±1.653	-1.18±3.450	2.90±2.389	0.733	0.228
Day43	69.48±2.492	71.40±2.386	67.87±1.653	-1.92±3.450	2.46±2.389	0.579	0.306
Day57	80.49±2.492	83.91±2.386	78.93±1.653	-3.42±3.450	3.28±2.389	0.323	0.173

[1] Control diet, without any additive.

Appendix Table 10. Least square means (LSM) (±standard error) of treatment x day for ADG (kg/d) of veal calves fed milk replacer containing either probiotic (PRO) or prebiotic (PRE)

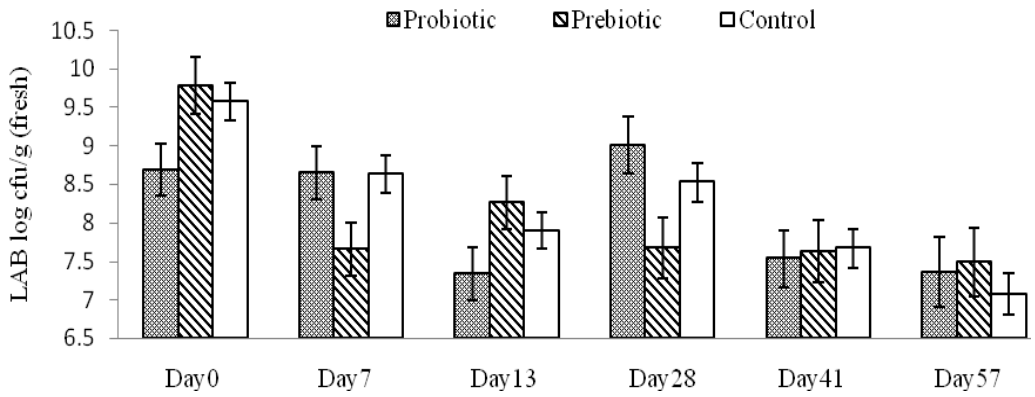
	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Day1-Day14	0.40±0.127	0.51±0.122	0.45±0.0844	-0.11±0.176	0.00302±0.122	0.595	0.98
Day15-Day29	0.61±0.127	0.52±0.122	0.60±0.0859	0.093±0.176	-0.030±0.123	0.595	0.811
Day30-Day42	0.71±0.127	0.77±0.122	0.78±0.0844	-0.057±0.176	-0.034±0.122	0.747	0.782
Day43-Day56	0.79±0.127	0.89±0.122	0.78±0.0844	-0.11±0.176	0.058±0.122	0.543	0.633

[1] Control diet, without any additive.

Appendix Table 11. Least square means (LSM) (±standard error) of treatment x day for feed efficiency fed milk replacer containing either probiotic (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Day1-Day14	2.52±0.839	1.85±0.803	2.02±0.557	0.67±1.162	0.17±0.804	0.564	0.831
Day15-Day29	2.14±0.839	2.24±0.803	2.40±0.557	-0.095±1.1616	-0.21±0.804	0.935	0.798
Day30-Day42	2.83±0.839	2.49±0.803	2.83±0.557	0.34±1.162	-0.16±0.804	0.772	0.838
Day43-Day56	2.23±0.839	2.84±0.803	1.85±0.557	-0.61±1.162	0.68±0.804	0.6	0.399

[1] Control diet without any additive.



Appendix Figure 2. Least square means (LSM) (±standard error) of treatment x day for Lactic acid bacteria (LAB) populations (cfu/g) of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE) (fresh)

Appendix Table 12. Least square means (LSM) (±standard error) of treatment x day for *E. coli* populations (cfu/g) of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE) (fresh)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
E. coli population (in day)							
Day 0	7.97± 0.397(6)[2]	8.34± 0.397(6)	7.06±0.281(12)	-0.37±0.5614	1.09±0.397	0.51	0.072
Day7	7.30±0.437(6)	7.52± 0.397(6)	7.06± 0.281(12)	-0.22±0.590	0.35±0.407	0.704	0.389
Day13	6.78± 0.397(6)	6.81±0.437(6)	7.01±0.309(12)	-0.035±0.590	-0.22±0.427	0.953	0.612
Day28	7.28± 0.436(5)	6.37± 0.436(5)	7.39± 0.294(12)	0.91±0.617	-0.57±0.426	0.143	0.186
Day41	7.12± 0.436(5)	6.00±0.699(4)	6.57± 0.309(11)	1.12±0.824	-0.0071±0.515	0.178	0.989
Day57	6.39±0.569(3)	6.63±0.700(3)	7.00±0.372(10)	-0.24±0.902	-0.49±0.585	0.793	0.406

[1] Control diet, without any additive.
[2] Values in parenthesis represent the number of observation for each mean

Appendix Table 13. Least square means (LSM) (±Standard error) of treatment x day for *Campylobacter* populations (cfu/g) of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE) (fresh)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Campylobacter population (in day)							
Day 0	5.38± 0.317(6)[2]	5.08± 0.317(6)	5.08±0.224(12)	0.30±0.448	0.15±0.317	0.506	0.644
Day7	6.07±0.317(6)	5.83± 0.317(6)	6.33± 0.224(12)	0.24±0.448	-0.37±0.317	0.592	0.245
Day13	6.24± 0.348(6)	6.72±0.449(6)	5.87±0.259(12)	-0.48±0.568	0.61±0.384	0.397	0.114
Day28	6.07± 0.389(5)	5.59± 0.369(5)	5.52± 0.246(12)	0.48±0.550	0.31±0.369	0.389	0.406
Day41	5.15± 0.549(5)	N/A	5.02± 0.293(11)	N/A	N/A	N/A	N/A
Day57	4.46±0.775(3)	N/A	5.24±0.259(10)	N/A	N/A	N/A	N/A

[1] Control diet, without any additive.
[2] Values in parenthesis represent the number of observation for each mean.

Appendix Table 14. Least square means (LSM) (±standard error) of treatment x day for *Clostridia* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Clostridia population (in day)							
Day 0	6.58± 0.823(6)[2]	8.06± 0.586(6)	7.68±0.263(12)	-1.48±1.010	-0.36±0.570	0.15	0.532
Day7	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Day13	5.12± 0.481(6)	5.93±0.373(6)	6.29±0.337(12)	-0.81±0.609	-0.77±0.454	0.188	0.0943
Day28	4.77± 0.417(5)	5.57± 0.373(5)	5.95± 0.241(12)	-0.80±0.559	-0.80±0.369	0.161	0.0386
Day41	6.12± 0.373(5)	6.31±0.417(4)	6.31±0.251 (11)	-0.19±0.559	-0.094±0.376	0.734	0.804
Day57	N/A	4.54±0.583(3)	4.47±0.412(10)	N/A	N/A	N/A	N/A

Appendix Table 15. Least square means (LSM) (±standard error) of treatment x day for *Salmonella* populations (cfu/g) for veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE) (fresh)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Salmonella population (in day)							
Day0	3.01±0(1)[2]	5.096±0(1)	N/A	N/A	N/A	N/A	N/A
Day7	5.544±0.3104(1)	N/A	5.2195±0.2195(2)	N/A	N/A	N/A	N/A
Day13	N/A	N/A	4.699±0(2)	N/A	N/A	N/A	N/A
Day28	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Day41	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Day57	N/A	N/A	5±0(2)	N/A	N/A	N/A	N/A

[1] Control diet, without any additive.
[2] Values in parenthesis represent the number of observation for each mean

Appendix Table 16. Least square means (LSM) (±standard error) of treatment x week for fecal score of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Week1	1.65±0.089	1.58±0.085	1.75±0.058	0.066±0.1228	-0.14±0.085	0.592	0.106
Week2	1.35±0.089	1.57±0.085	1.33±0.058	-0.22±0.123	-0.13±0.085	0.0734	0.128
Week3	1.09±0.089	1.12±0.085	1.11±0.058	-0.028±0.1228	-0.0036±0.08504	0.819	0.966
Week4	1.00±0.089	1.028±0.0850	1.11±0.058	-0.028±0.1228	-0.093±0.08504	0.821	0.276
Week6	1.12±0.089	1.10±0.085	1.11±0.058	0.022±0.1228	-0.0025±0.08504	0.86	0.976
Week7	1.07±0.089	1.07±0.085	1.07±0.058	-0.0065±0.1228	-0.000035±0.08504	0.958	0.997
Week8	1.09±0.089	1.02±0.085	1.06±0.058	0.067±0.1228	0.000264±0.08504	0.585	0.998

[1] Control diet, without any additive.

Appendix Table 17. Least square mean (LSM) (±standard error) of treatment x day for fecal pH of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Day0	6.72±0.220	7.29±0.220	6.96±0.156	-0.57±0.312	0.048±0.220	0.071	0.83
Day7	7.70±0.220	7.90±0.220	7.76±0.156	-0.21±0.312	0.036±0.2203	0.512	0.871
Day13	7.97±0.220	8.00±0.220	7.87±0.156	-0.038±0.3115	0.12±0.220	0.902	0.59
Day28	8.10±0.242	7.93±0.242	7.84±0.156	0.18±0.342	0.17±0.231	0.602	0.461
Day41	7.76±0.314	7.90±0.271	7.70±0.163	-0.13±0.415	0.12±0.264	0.749	0.646
Day57	7.46±0.314	7.71±0.314	7.60±0.171	-0.25±0.444	-0.014±0.2802	0.574	0.959

[1] Control diet, without any additive.

Appendix Table 18. Least square means (LSM) (±standard error) of treatment x day for day matter (%) content of veal calves fed milk replacer containing either probiotics (PRO) or prebiotic (PRE)

	Treatment			LSM difference		Contrasts (p-value)	
	PRO	PRE	CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]	PRO-PRE	½(PRO+PRE) - CON[1]
Day0	20.67±2.539	22.53±2.539	22.37±1.795	-1.86±3.591	-0.77±2.539	0.605	0.763
Day7	22.40±2.539	22.31±2.539	23.68±1.795	0.091±3.591	-1.33±2.539	0.98	0.601
Day13	19.39±2.539	19.76±2.539	16.52±1.795	-0.37±3.591	3.06±2.539	0.918	0.232
Day28	21.54±2.757	20.21±2.757	22.09±1.795	1.33±3.899	-1.21±2.650	0.735	0.648
Day41	18.20±3.045	24.76±3.045	22.58±1.865	-6.56±4.309	-1.10±2.850	0.131	0.7
Day57	31.11±3.471	25.23±3.47	25.41±1.945	5.88±4.911	2.77±3.132	0.234	0.379

[1] Control diet, without any additive.

Appendix Table 19. Least square means (±standard error) of treatment for total milk replacer (kg), total calf starter (kg) and total feed intake (kg) for veal calves fed milk replacer containing either probiotics or prebiotic

	Treatment			Significance
	Probiotic	Prebiotic	Control	p-value
Total milk replacer	5.19±0.069	4.95±0.066	5.2±0.046	0.0075
Total calf starter	5.17±1.047	5.89±1.0025	4.49±0.695	0.5094
Total feed intake	9.79±1.079	10.84±1.079	9.68±0.7478	0.665

Appendix Table 20. Least square means (±standard error) of treatment for body weight (kg), ADG (kg/d) and feed efficiency for veal calves fed milk replacer containing either probiotics or prebiotic.

	Treatment			Significance
	Probiotic	Prebiotic	Control	p-value
Body weight	61.32±1.864	63.35±1.785	60.36±1.236	0.394
ADG	0.63±0.0764	0.67±0.0732	0.65±0.0509	0.912
Feed efficiency	2.43±0.444	2.35±0.425	2.27±0.296	0.955

Appendix Table 21. Least square mean (±Standard error) of treatment of *E. coli* and LAB populations (cfu/g) for veal calves fed milk replacer containing either probiotics or prebiotic (fresh)

	Treatment			Significance
	Probiotic	Prebiotic	Control	p-value
E. coli population	7.14± 0.190	6.95± 0.222	7.02± 0.131	0.788
LAB population	8.10±0.246	8.31±0.250	8.22±0.170	0.85

Appendix Table 22. Least square means (±Standard error) of treatment of *Campylobacter* and *Clostridia* populations (cfu/g) for veal calves fed milk replacer containing either probiotic or prebiotic (fresh)

	Treatment			Significance
	Probiotic	Prebiotic	Control	p-value
Campylobacter population	5.56±0.222	N/A	5.51±0.124	0.979
Clostridia population	N/A	6.08±0.260	6.14±0.170	0.0669

Appendix Table 23. Least square means (±standard error) of treatment for total fecal score, fecal pH and fecal dry matter(%) for veal calves fed milk replacer containing either probtioc or prebiotic.

	Treatment			Significance
	Probiotic	Prebiotic	Control	p-value
Fecal score	1.19±0.04	1.21±0.039	1.22±0.027	0.879
Fecal pH	7.62±0.107	7.79±0.103	7.62±0.0665	0.387
Dry matter (%)	22.2±1.64	22.5±1.65	22.1±1.11	0.984

Appendix Table 24. Least square means (±standard error) of week of total milk replacer (kg), total calf starter (kg) and total feed intake (kg) of veal calves fed milk replacer containing either probiotics or prebiotic.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Significant (p-value)
Total milk replacer	3.94±0.0549	5.19±0.0549	6.07±0.0549	5.94±0.0549	6.03±0.0549	5.96±0.0549	5.94±0.0549	1.82±0.0549	<0.0001
Total calf starter	0.9±0.615	1.72±0.615	2.29±0.615	2.96±0.615	4.05±0.615	6.55±0.615	8. 7±0.615	14.29±0.615	<0.0001
Total feed intake	4.82±0.64	6.87±0.64	8.29±0.64	8.79±0.64	9.89±0.64	12.22±0.64	14.26±0.64	15.67±0.64	<0.0001

Appendix Table 25. Least square means (±standard error) of day for body weight (kg), ADG (kg/d) and feed efficiency for veal calves fed milk replacer containing either probiotics or prebiotic.

	Day 1	Day 15	Day 30	Day 43	Day 57	Significance p-value
Body weight	45.72±1.275	52.09±1.275	59.83±1.275	69.62±1.275	81.11±1.275	<.0001
	Day 1-14	Day 15-29	Day30-42	Day43-56		
ADG	0.46±0.065	0.58±0.065	0.75±0.065	0.82±0.065		0.0008
Feed efficiency	2.13±0.429	2.26±0.431	2.72±0.429	2.31±0.429		0.784

Appendix Table 26. Least square means (±standard error) of day of *E. coli* and Lactic acid bacteria (LAB) populations (cfu/g) for veal calves fed milk replacer containing either probiotics or prebiotic (fresh).

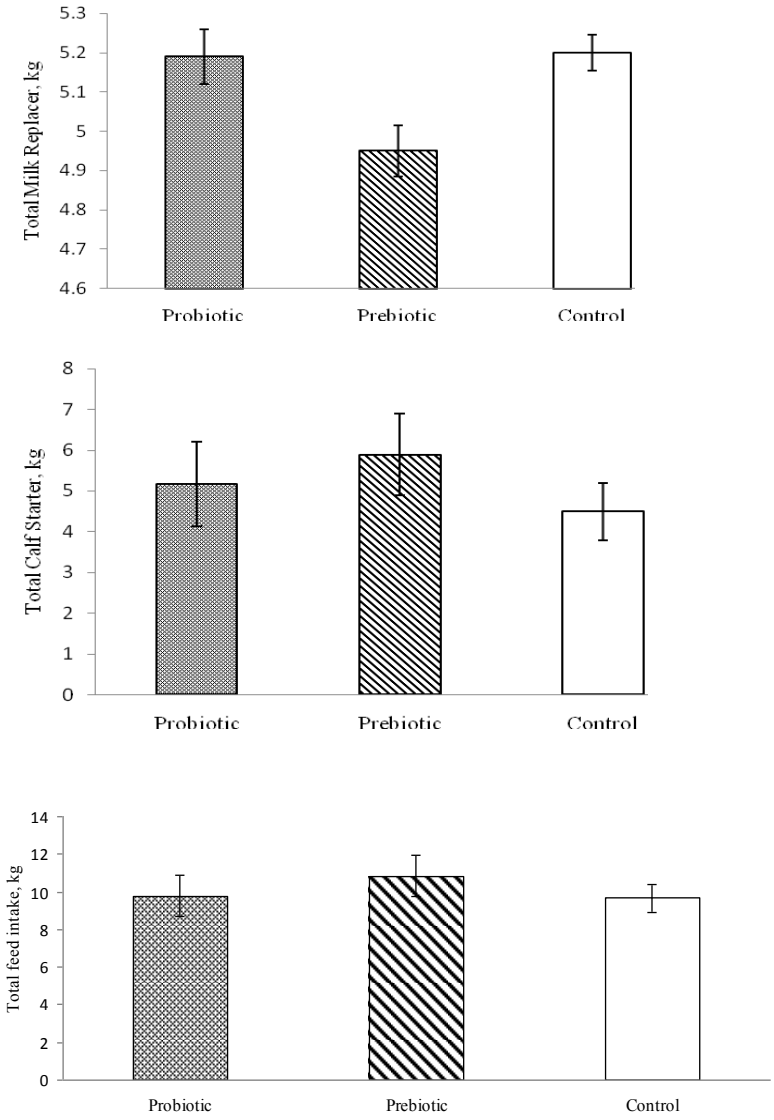
	Day 0	Day 7	Day 13	Day 28	Day 41	Day 57	Significant (p-value)
E. coli population	7.79±0.209	7.29±0.218	6.87±0.222	7.02±0.228	6.56±0.293	6.67± 0.325	0.0052
LAB population	9.345±0.180	8.32±0.180	7.83±0.181	8.40±0.200	7.61±0.200	7.31±0.231	<0.0001

Appendix Table 27. Least square means (±standard error) of day of *Campylobacter* and *Clostridia* populations (cfu/g) for veal calves fed milk replacer containing either probiotic or prebiotic

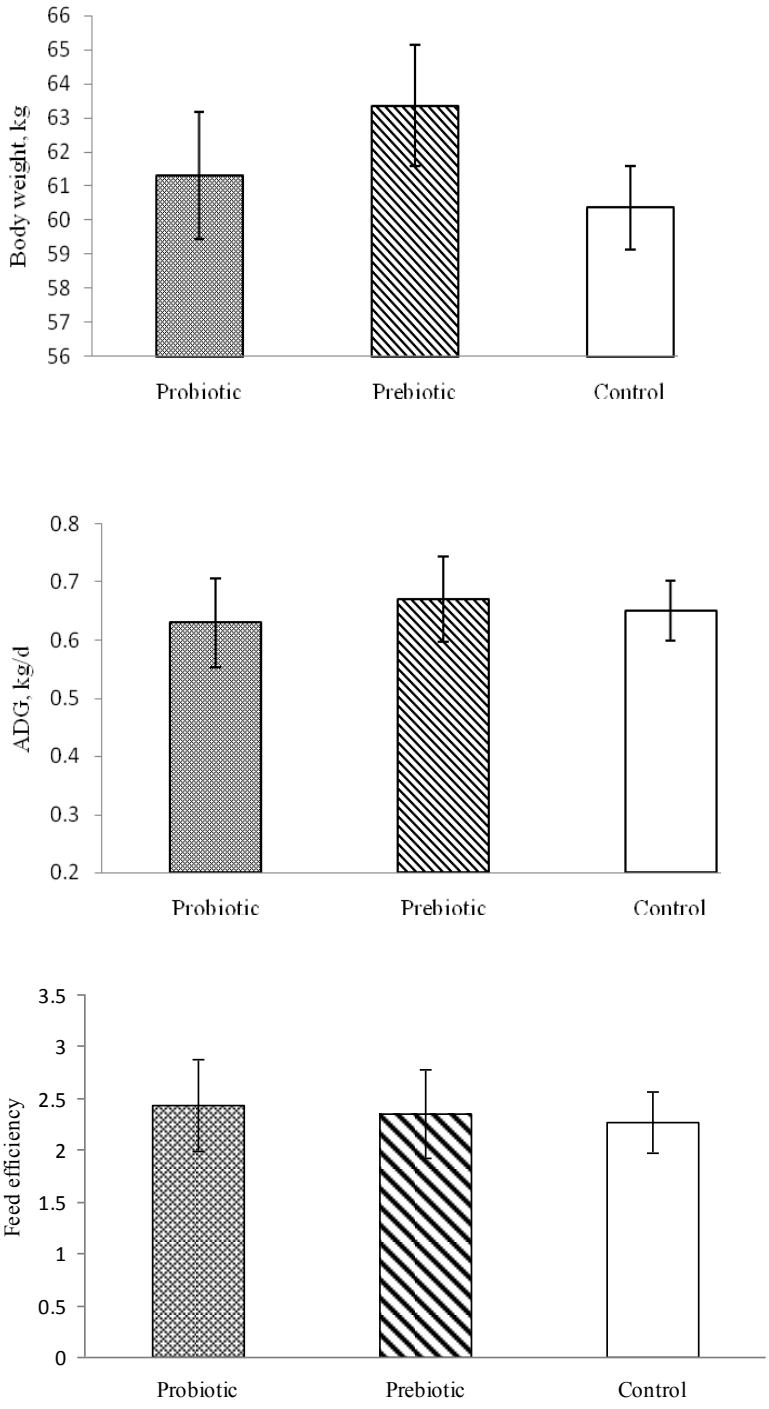
	Day 0	Day 7	Day 13	Day 28	Day 41	Day 57	Significant (p-value)
Campylobacater population	5.18±0.167	6.08±0.167	6.28±0.208	5.73±0.201	N/A	N/A	<0.0001
Clostridia population	7.44±0.348	N/A	5.78±0.232	5.43±0.203	6.24±0.204	N/A	<0.0001

Appendix Table 28. Least square means (±standard error) of week of fecal score, fecal pH and dry matter (%) of veal calves fed milk replacer containing either probiotic or prebiotic.

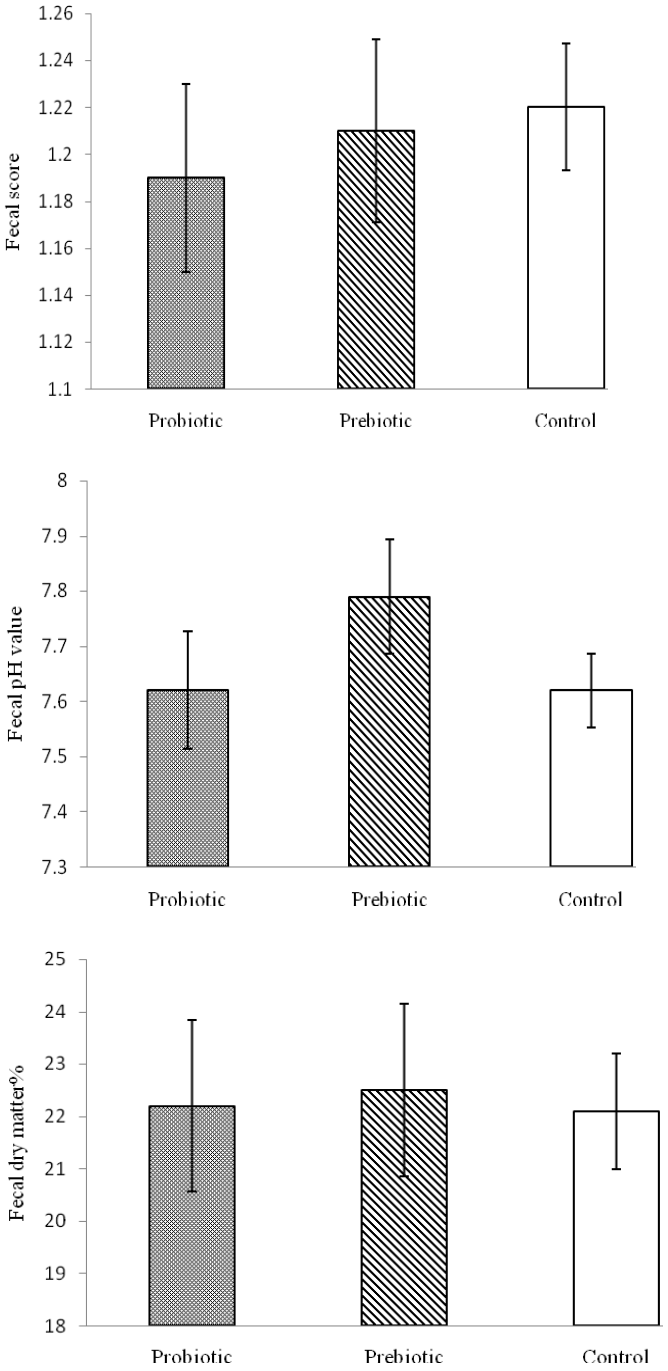
	Week 1	Week 2	Week 3	Week 4	Week 6	Week 7	Week 8	Significance p-value
Fecal score	1.66±0.045	1.42±0.045	1.11±0.045	1.04±0.045	1.11±0.045	1.07±0.045	1.06±0.045	<.0001
	Day 1	Day 7	Day 13	Day 28	Day 41	Day57		
Fecal pH	6.99±0.116	7.79±0.116	7.94±0.116	7.96±0.125	7.79±0.149	7.59±0.159		<.0001
Dry matter	21.9±1.34	22.8±1.34	18.6±1.34	21.3±1.43	21.8±1.57	27.3±1.77		0.0026



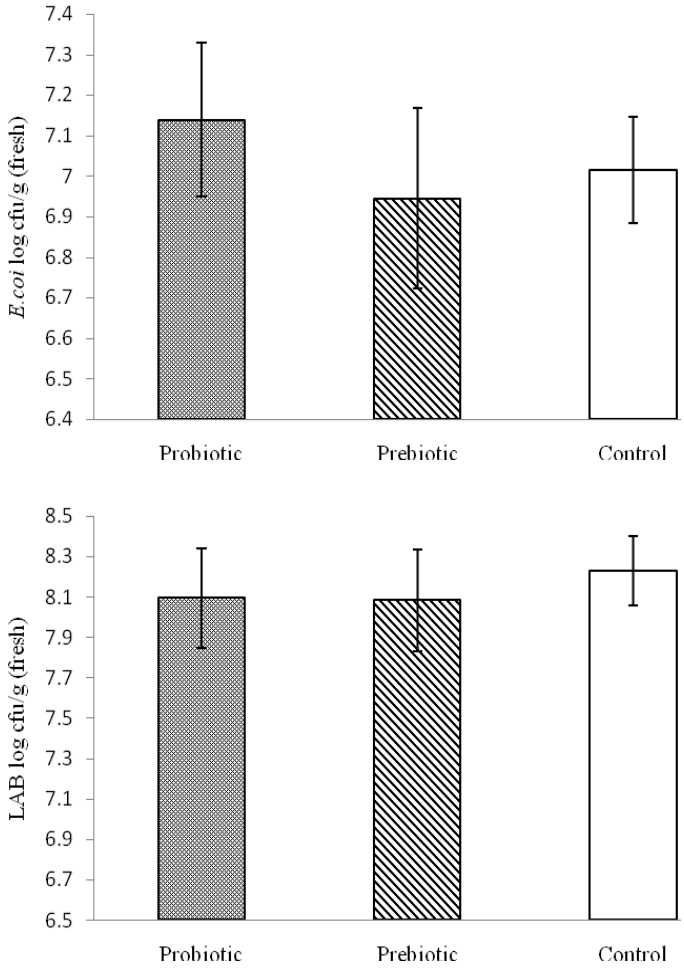
Appendix Figure 3. Least square means (±standard error)of treatment for total milk replacer (kg), calf starter (kg) and feed intake (kg) of veal calves fed milk replacer containing either probiotics or prebiotic.



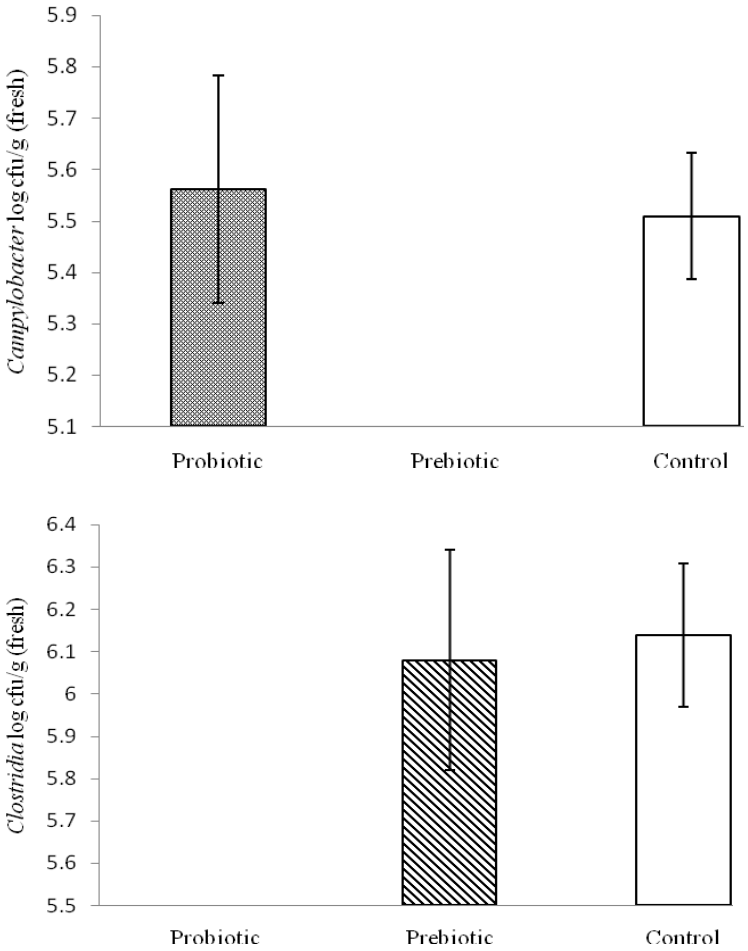
Appendix Figure 4. Least square means (±standard error)of treatment for body weight (kg), ADG (kg/d) and feed efficiency of veal calves fed milk replacer containing either probiotics or prebiotic.



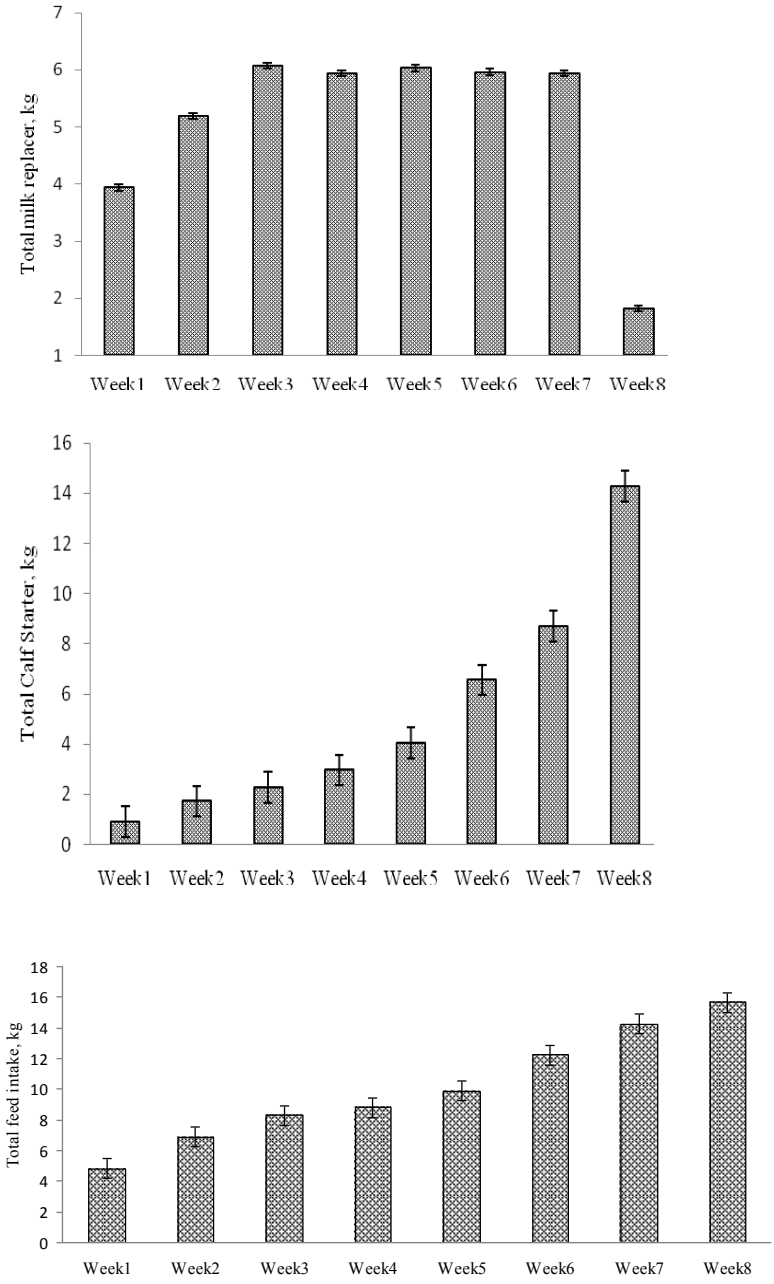
Appendix Figure 5. Least square means (±standard deviation)of treatment for total fecal score, fecal pH and fecal dry matter(%) for veal calves fed milk replacer containing either probiotics or prebiotic.



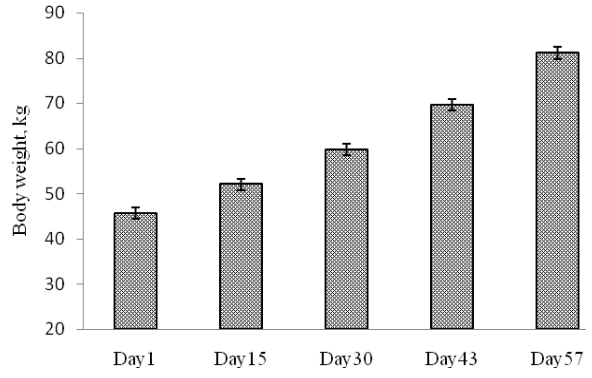
Appendix Figure 6. Least square means (±standard error) of treatment for *E. coil* and Lactic acid bacteria (LAB) populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh).

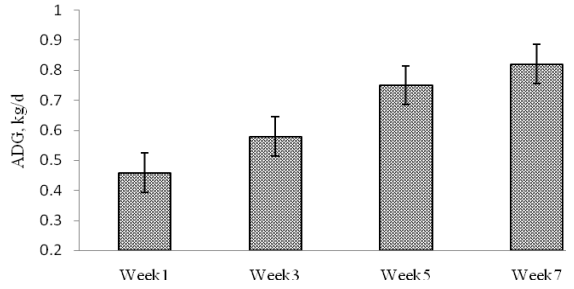


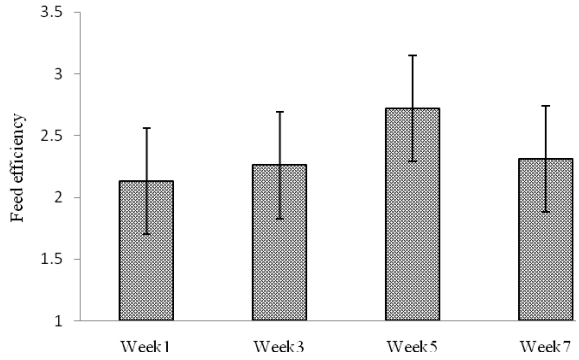
Appendix Figure 7. Least square means (±standard error) of treatment for *Campylobacter* and *Clostridia* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh).



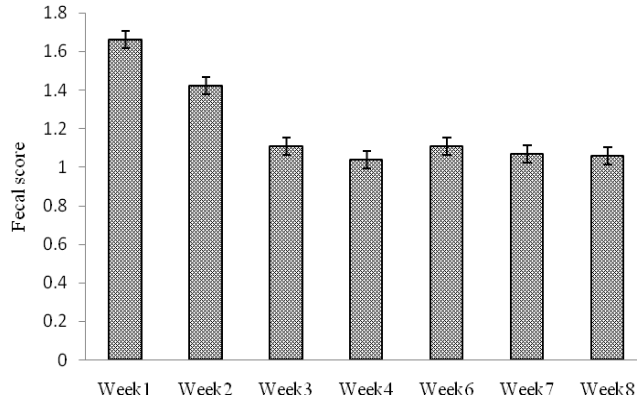
Appendix Figure 8. Least square means (±standard error) of day for total milk replacer (kg), total calf starter (kg) and total feed intake (kg) of veal calves fed milk replacer containing either probiotics or prebiotic.

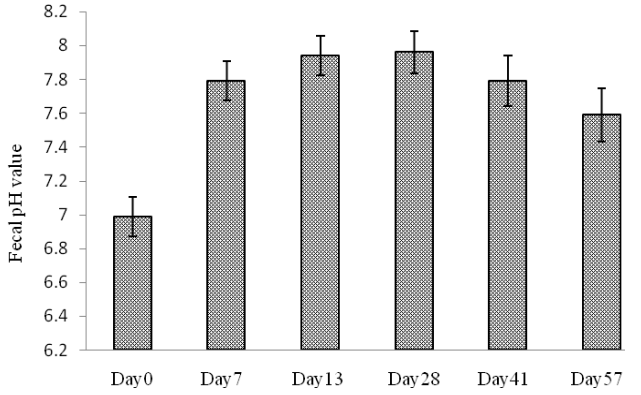


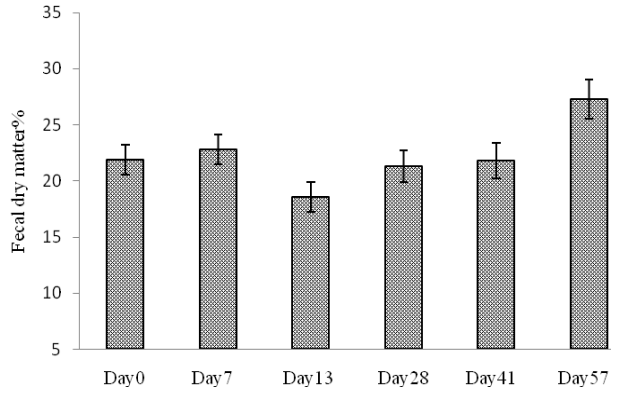




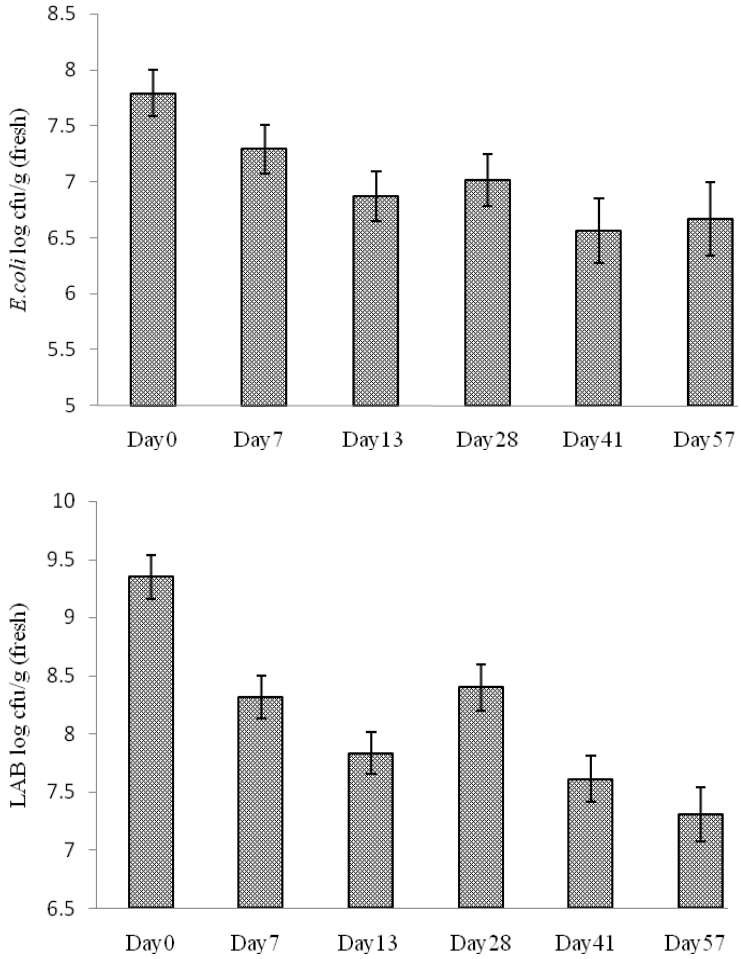
Appendix Figure 9. Least square means (±standard error) of day for total body weight (kg), ADG (kg/d) and feed efficiency for veal calves fed milk replacer containing either probiotics or prebiotic.



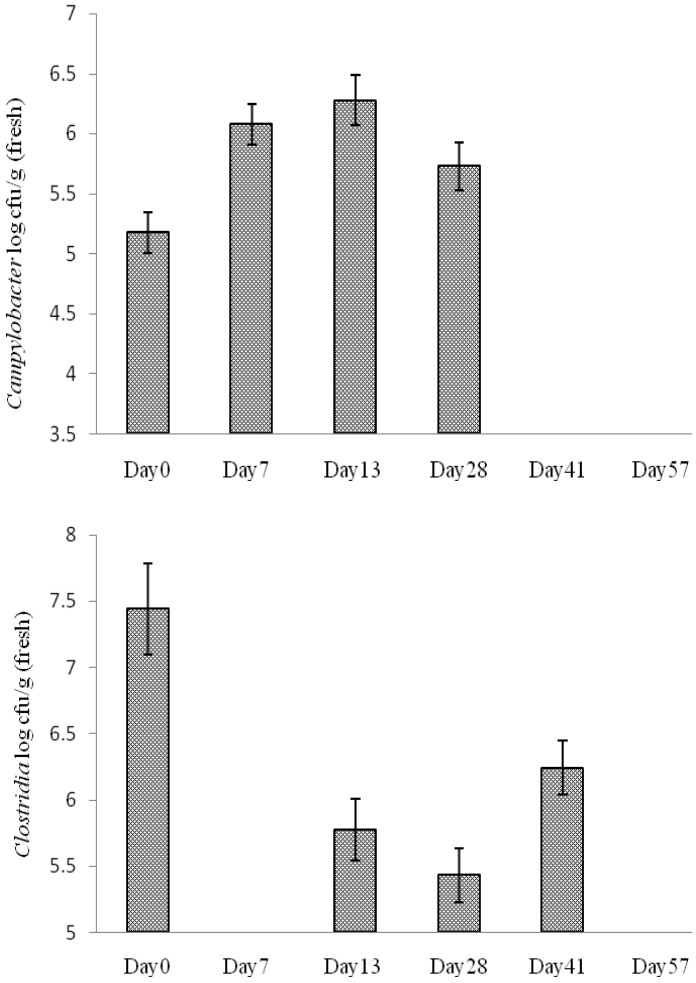




Appendix Figure 10. Least square means (±standard error) of day for fecal score, fecal pH value and fecal dry matter(%) of veal calves fed milk replacer containing either probiotics or prebiotic.



Appendix Figure 11. Least square means (±standard error) of day for *E. coli* and Lactic Acid Bacteria (LAB) populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh).



Appendix Figure 12. Least square means (±standard error) of day for *Campylobacter* and *Clostridia* populations (cfu/g) of veal calves fed milk replacer containing either probiotics or prebiotic (fresh).