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The effects of feeding fermented soybean meal in calf starter on growth and performance of dairy calves

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

GENERAL INTRODUCTION

Thesis Organization

The following thesis is organized into 4 chapters. Chapter 1 is a review of the alternative proteins available for use in dairy calf diets, problems associated with feeding these alternative proteins, incorporation rates of these alternative proteins, processing methods used for soybeans, and a brief review of the acquired immune system development in the calf. Chapter 2 is a summary of research conducted to evaluate the effects of the use of a fermented soybean product in dairy calf starter diets. Chapter 3 presents general conclusions about the research conducted. Chapter 4 contains acknowledgements.

Review of Literature

Introduction

Soybeans have been a significant source of plant origin proteins for both the livestock feed and human industries for many years. Soybean meal is the most popular protein source in the animal feed industry because of its high protein content and wide availability (Easter and Kim, 1999; Baker, 2000). Unfortunately, the use of soybean meal in animal diets is primarily limited to adult animals due to the inefficient digestibility of soy proteins by young animals and the susceptibility of young animals to antinutritional compounds in soybeans that are either not properly processed or undercooked (Jiang et al., 2000; Baker, 2000). These antinutritional compounds include trypsin inhibitors, lectins, flatulence producing compounds, and many other allergenic proteins (Kim and Baker, 2003; Baker, 2000; Dunsoford et al., 1989). These antinutritional compounds can be denatured by fermentation thereby enabling the use of

soybean meal in piglet diets (Feng et al., 2007*b*). Fermented soybean meal can successfully replace animal-derived protein sources such as plasma protein and dried skim milk in piglet nursery diets without adversely affecting the growth performance of the piglets (Kim et al., 2009). In addition, piglets fed the fermented soy responded with increased feed intake, higher nutrient digestibility and absorption, improved growth performance, and reduced diarrhea compared to piglets fed other animal-derived protein sources (Kim et al., 2009). The researchers speculated that these fermented soy products could be incorporated into diets of pre-ruminant calves, ruminants, pets, as well as aquaculture diets (Kim et al., 2009).

In calf rearing programs, the high cost of milk replacers and the relatively low cost of calf starter diets economically favor an early weaning program (Quigley, 1997). Furthermore, the protein ingredients utilized in milk replacers and starter diets constitute a significant portion of the cost associated with these feeds (Quigley et al., 1999). The single most important factor impacting age at weaning is voluntary starter consumption. Therefore, the factors that influence early intake of starter are of great importance to the dairy industry. There are numerous factors that work cooperatively to influence starter consumption by calves, including palatability of the diet. Finding a cost effective and palatable alternative protein for dairy calf starter diets is therefore of great importance. If soy-fermented proteins could be incorporated into calf starter diets and yield similar positive results as occurs in nursery piglets (Kim et al., 2009), the effect on the dairy industry could be profound.

Alternative Proteins in Milk Replacers and Starter Diets for Dairy Calves

The adequacy of a processed soy protein in meeting the nutrient requirements of the calf is dependent on the quality of the protein after processing of the whole soybean (Akinyele and Harshbarger, 1983). The use of alternative sources of vegetable and animal protein in milk

replacers and starters for calves, instead of using milk or milk protein based products, has increased as a result of increased milk prices and costs of raising calves, along with the increasing importance of milk protein for humans (Barr, 1981). Since casein is not essential in the diet for the young calf, alternative proteins that may be more affordable can be used in milk replacers and starters for dairy calves (Leibholz, 1967), however, typically the substitution with cheaper vegetable sources results in reductions in growth rates and digestibility with an increased incidence of digestive disturbances (Huber, 1968). Therefore to ensure proper growth and health of the calf, processing and subsequent solubility of the protein source are crucially important for determining the suitability of alternative proteins in milk replacers and starters (Whitelaw and Preston, 1963).

Digestive disturbances in calves fed alternative proteins are often due to the inability of the calf to secrete the appropriate enzymes for the digestion of non-milk nutrients (Noller, et al., 1956*b*; Hinks et al., 1975; Jenkins, 1981; Caugant et al., 1992). Disturbances that occur as a result include inhibition or acceleration of abomasal emptying, impaired curd formation in the abomasum, altered rate of digesta flow through the small intestine, altered gastrointestinal tract morphology, abnormal salt and water exchange, decreased nitrogen absorption, and the creation of antinutritional factors (Shoptaw et al., 1937; Smith and Sissons, 1975; Colvin et al., 1969; Williams et al., 1976). Other factors that influence calf performance on alternative protein based milk replacers and starters are the proportion of the milk protein replaced with the alternative protein and the age of the calf (Akinyele and Harshbarger, 1983; Caugant et al., 1993; Ramsey and Willard, 1975*a*; Noller et al., 1956*b*; Huber and Campos, 1982; Campos and Huber, 1982*b*); younger calves and those fed a higher proportion of alternative protein sources are more likely to respond poorly.

Alternative Proteins Used In Calf Feeds. Alternative proteins for use in calf milk replacers and starters are typically selected based on how close their amino acid profile matches that of milk proteins. Calves require a high quality protein to grow properly (Huber and Slade, 1967) although calves are able to use non-milk proteins more effectively with age (Akinyele and Harshbarger, 1983; Caugant et al., 1993; Ramsey and Willard, 1975*a*; Noller et al., 1956*b*, Campos and Huber, 1982*b*; Hinks et al., 1975; Seegraber and Morrill, 1979). Essential amino acids are often 5 to 10% lower in diets containing alternative protein sources than in all milk diets (Huber and Campos, 1982). However, supplementing diets with essential amino acids won't counteract completely the antinutritional effects of some alternative proteins because the protein in these alternative sources becomes completely digestible only after heating (Rackis, 1974). This deficiency of essential amino acids and the limited proteolytic digestion because of trypsin inhibitor activity leads to a greatly enhanced need for amino acids, especially sulfur amino acids, for re-synthesis of protein in the pancreas due to enhanced pancreatic juice secretion and pancreas enlargement (Rackis, 1974).

Akinyele and Harshbarger (1983) reported that as calves grew older, protein digestibilities improved for milk protein replacers and soy protein concentrate replacers, but not as much for calves fed full fat soy flour replacers. They attributed this to lack of the correct enzyme combinations or low secretion rates or particular enzymes required to digest the soy protein. Seegraber and Morrill (1979) reported that soy protein may cause digestive disturbances during early life because intestinal absorption capacity does not improve even as the animal gets older. Akinyele and Harshbarger (1983) reported that this was true when full-fat soy flour was used in milk replacers, but not when soy protein concentrate was fed.

They hypothesized that this was due to the diet impairing the functions of the digestive tract which led to inadequate quantities of essential amino acids being absorbed for tissue synthesis. Calves cannot properly utilize many alternative proteins until they are at least 4 weeks of age (Noller et al., 1956*b*; Campos and Huber, 1982*b*; Harshbarger and Gelwicks, 1965; Nitsan et al., 1971). The early work of Shaw et al. (1918) reported similar results and they concluded that 4 to 7 day old calves were able to digest only one-fifth of the starch consumed, but by 3 to 4 weeks of age the calves were able to digest over 90% of the starch. This has led some early researchers to term this as the "critical period" in the calf's life (Noller et al., 1956*a*). During this period, little to no weight gain occurs in calves fed conventionally (Noller et al., 1956*a*). The end of the critical period is marked by the initiation of rumen function and by an increase in feed consumption, increased body weight, improved appearance of the calf, and a change in odor and appearance of the feces (Noller et al., 1956*a*). The end of the critical period is also characterized by an improvement of protein utilization (Noller et al., 1956*a*; Bell and Adams, 1974; Huber, 1968), which is to be expected since the rumen microbes are capable of breaking down complex proteins and thus improving digestibility of alternative protein sources. It is also possible that increased digestibility of feed by the older calves may be due to physiological changes, such as increased enzyme activity in the alimentary tract (Noller et al., 1956*b*; Huber, 1968). Nonetheless, crude vegetable protein is not completely digestible by calves until they are 9 weeks old (Archibald, 1928; Noller et al., 1956*b*).

Retention of digested nitrogen is often lower for calves receiving non-milk proteins not properly processed when compared with calves fed milk protein (Campos and Huber, 1982*a*; Campos and Huber, 1982*b*). This suggests poor availability of essential amino acids

in these non-milk proteins. In legumes such as soybean protein and pea protein, cystine and threonine are often the least digestible amino acids. Gorrill and Nicholson (1969) reported that methionine was not a limiting amino acid in the calves fed soybean protein diets as it was in rats, chicks, and pigs. Calves fed replacers or starters containing fish protein may need vitamin E supplemented in their ration since vitamin E deficiency is associated with feeding of fish proteins (Huber, 1974; Michel et al., 1972). Fish protein is also associated with deficiencies in tryptophan, histidine, isoleucine, and valine (Patureau-Mirand et al., 1974; Genskow et al., 1969).

Another way to overcome the poor availability of amino acids in non-milk proteins besides processing is by combining multiple sources of proteins in one milk replacer or starter diet. Stein et al. (1954) reported acceptable rates of growth in calves fed a mixture of plant and milk protein. Cruywagen and Horn (1984) found that dairy calves can be reared successfully until weaning on mixtures of soy flour, whey powder, and colostrum instead of whole milk. Morrill et al. (1969) reported that a milk replacer containing 68% dried sweet whey and 22% soy protein concentrate resulted in calf performances equivalent to calves fed an all milk replacer. The complementary effect of protein sources was also evident in data from Huber and Campos (1982) that showed combining enzymatic hydrolysate of fish and soy protein concentrate to make 33% of the total protein in the replacer diet improved gains and feed efficiency compared to soy protein concentrate or enzymatic hydrolysate of fish alone. However, not all combinations of alternative proteins or incorporation rates of these combinations are effective. When fish protein concentrate and soy protein concentrate are combined to replace all protein in milk replacers, calves have reduced nitrogen retention (Gorrill et al., 1972). They concluded that the reduced digestibility indicated that the amino

acid balance of the fish and soybean proteins combined was inferior to that of milk proteins. Additionally, Morrill et al. (1969) reported that gains of calves were significantly lower when they received a replacer containing 77% dried sweet whey and 44% of the total protein was provided by soy.

Concurrent feeding of hay and/or grain along with non-milk protein replacers also improved growth rates of calves consuming alternative protein replacers of lower protein quality or with higher proportions of the milk proteins being replaced. Concurrent feeding accomplishes this by lowering the calf's dependency on one type of protein (Huber, 1974; Harshbarger and Gelwicks, 1965; Nitsan et al., 1971). It may be possible that offering hay along with the non-milk protein replacer helps to initiate rumen function earlier by acting as a scratch factor, thus improving digestibility of non-milk protein replacers sooner than if fed alone. This is supported by McMeekan's (1954) study in which calves on pasture began ruminating as early as 7 days of age and rarely later than 3 weeks.

Soybean protein is often used in calf milk replacers and starters instead of milk proteins because of its low cost and its essential amino acid content is similar to cow's milk (Caugant et al., 1993). Although soybean protein is most widely used and thoroughly researched as the main alternative protein used for calf diets, there are many other alternative proteins available. Alternative protein ingredients, including soy, wheat, fish, corn, pea, whey, rapeseed, blood, meat, bone, egg, sunflower, barley, oats, beet pulp, and potato, have also been used and researched as protein sources in milk replacers (Quigley et al., 1999; Akinyele and Harshbarger, 1983; Stein et al., 1954; Bhatty and Christison, 1980; Cruywagen and Horn, 1984; Stobo et al., unpublished data; Gorrill et al.,1976; Touchette et al., 2003; Mandibaya et al., 1999; Cafrey and McAlesse, 1964).

Digestive Enzymes. When calves are born, and for several weeks thereafter, the only areas of the digestive tract in which digestion take place are the abomasum and intestine. This is due to two things: a non-functioning rumen and because many liquids stimulate the closure of the esophageal groove which shunts the feed directly into the abomasum (Hoffman, 1956). Due to this, when only liquid diets are fed, the vegetable-based protein sources in the milk replacer reach the abomasum without any prior digestion (Kwiatkowska, 1972). The abomasum is very limited in its capacity to digest complex food materials such as non-milk proteins (Larsen et al., 1956). When the rumen is functioning normally, the protein in the feed reaches the abomasum after being partially digested by rumen microbia (Kwiatkowska, 1972). Due to different amino acid compositions of alternative proteins, calves often do not have the proper digestive enzymes and/or quantities of these enzymes to breakdown non-milk proteins before their rumen is functioning (Zieliniski et al., 1978). Processing can help to breakdown non-milk protein sources prior to consumption to make them more digestible and utilizable for the young calf.

In studies with raw soybean meal as the main source of protein in young animals, proteins and large peptides that were not digested are found in the intestinal contents of the animals (Bielorai et al., 1972; Caugant et al., 1993). This undigested protein often contains essential amino acids that the animal requires for proper tissue protein synthesis and growth (Campos and Huber, 1982*b*). This results in a negative nitrogen balance, which results in the animals' body needing to break down its own stores of proteins in order to maintain nitrogen equilibrium (Akinyele and Harshbarger, 1983). This has been reported in chicks (Bielorai et al., 1972), calves (Akinyele and Harshbarger, 1983; Sleiman and Huber, 1971; Campos and Huber, 1982*b*), rats (Stein et al., 1954), and piglets (Barratt et al., 1978). Decreased nutrient

digestibility and/or absorbability and abnormal digestion flow leads to poor calf growth and diarrhea (Barr, 1981; Gorrill and Thomas, 1967; Seegraber and Morrill, 1985; Stein et al., 1954; Colvin and Ramsey, 1968; Shoptaw et al., 1937; Sissons, 1982; Smith and Sissons, 1975) and increased rates of mortality (Campos and Huber, 1982*a*; Huber and Slade, 1967).

Pre-ruminant calves have very limited ability to digest proteins, starch, and lipids due to limited digestive enzyme activities (Porter, 1969; Williams et al., 1976; Roy et al., 1977; Noller et al., 1956*b*; Caugant et al., 1992). The small intestines of newborn calves contain low activities of maltase, sucrase, and amylolytic enzymes (Bhatty and Christison, 1980), as well as proteolytic enzymes (Williams et al., 1976; Roy et al., 1977). Additionally, the pepsin-gastric intestinal protease complex that is necessary to digest non-milk proteins may not even exist in calves (Henschel et al., 1961). Non-milk sources of starch, protein, and lipids need to be broken down into more digestible components prior to consumption by young animals (Bhatty and Christinson, 1980; Gorrill and Nicholson, 1969).

The replacement of a portion of milk proteins with alternative proteins that are improperly processed, such as soy protein and fish protein, results in faster gastric emptying, impaired curd formation in the abomasum, and reduced secretion of hydrochloric acid, renin, and pepsin (Shoptaw et al., 1937; Williams et al., 1976; Campos and Huber, 1982*a*; Caugant et al., 1993). This demonstrates a possible interaction between increased rate of digesta flow and other digestive disturbances such as reduced digestive enzyme secretions. Caugant et al. (1993) also concluded that since soybean protein entered the intestine in larger amounts and sooner after the meal than milk protein, the rate of proteolysis by pancreatic and intestinal enzymes and the subsequent absorption of the amino acids are decreased. Calves fed whole milk have greater total trypsin and chymotrypsin activities in intestinal contents compared to

calves fed other non-milk based diets (Magee, 1961). This may be explained in part by the lower pH of abomasal and upper intestine contents of calves fed non-milk based diets (Gorrill and Thomas, 1967), which also impairs curd formation in the abomasum (Shoptaw et al., 1937). A noticeable reduction in secretion of enzymes in pancreatic juice collected from calves fed alternative protein based diets was also attributed to the greater total trypsin and chymotrypsin activities (Magee, 1961). Furthermore, poor growth associated with alternative protein based diets is attributed to the hyposecretion of pancreatic enzymes and deficiencies of essential amino acides for protein and enzyme synthesis (Gorrill and Thomas, 1967).

Antinutritional and Allergenic Factors. Other factors in addition to protein digestibility also reduce calf performance when fed alternative proteins. Some alternative protein sources have high protein digestibility, but still result in poor growth in calves when they are providing a high percentage of the protein in the diet due to antinutritional factors, allergic reactions, or amino acid deficiencies associated with feeding of that particular protein.

Antinutritional factors contribute to reduced growth rates in calves. Trypsin inhibitors with high methionine content have been found in soybeans (Hwang et al., 1977). The growth depression caused by trypsin inhibitor is from loss of endogenous nitrogen rather than poor protein digestibility (Alumot and Nitsan, 1961). Birk (1961) demonstrated that trypsin inhibitor from soybeans may pass through the abomasum undamaged and reach the site of tryptic and α-chymotryptic activity. The pancreas reacts to the presence of the trypsin inhibitor in the intestine by secreting more enzymes to compensate for the effect of the inhibitor (Alumot and Nitsan, 1961). Because these pancreatic enzymes are rich in sulfur-

containing amino acids, pancreatic hypertrophy causes a drain on the body tissue of these particular amino acids in order to meet an increased need for the synthesis of the trypsin and chymotrypsin (Liener, 1981), which leads to further growth depression. Pancreatic hypertrophy additionally leads to an excessive fecal loss of the endogenous protein secreted by the pancreas (Rackis, 1974). Trypsin inhibitors also reduce availability of amino acids, vitamins, and minerals, leading to even greater reductions in performance (Liener, 1979). Trypsin inhibitors not only limit the extent of digestion, but they also suppress further digestion of intermediate products by proteolysis (Bielorai and Bondi, 1963). Heating of soy products destroys both antinutritional factors and the negative effects that they have on the pancreas (Liener, 1981; Struthers et al., 1983).

Factors which contribute to limited soy protein utilization in milk replacers and starters are the complexity of these proteins, their susceptibility to denaturation, and their variability due to differences in processing conditions (Morr, 1979). Soy proteins are heterogeneous and have complex quaternary structures that undergo dissociation-association reactions which depend upon ionic conditions in solution (Schmidt and Morris, 1984). Increased feeding efficiency with alternative proteins, such as soybeans, has been attributed to increased accessibility of protein to enzyme attack as a result of changes in the complex protein's original conformation (Fukushima, 1968) and inactivation of proteolytic inhibitors, primarily trypsin inhibitors (Liener, 1969).

The nutritional value of soy protein increases when antigrowth factors, such as trypsin inhibitor, are inactivated (Baker and Mustakas, 1973; Ramsey and Willard, 1975*b*). Delobez et al. (1971) found that trypsin inhibitors are bound prior to digestion and are set free by gastric enzymes. It is nutritionally important for these inhibitors to be released prior

to their release during digestion (Wang et al., 1972). Trypsin inhibitor leads to reductions in the levels and concentrations of pancreatic trypsin and chymotrypsin secretion in the calf (Gorrill and Thomas, 1967). This stimulates more trypsin production by the pancreas. Trypsin inhibitors in soybeans have been found to inhibit trypsin in the intestinal tract by 90-100% (Struthers and MacDonald, 1983). Soybean trypsin inhibitors also significantly decrease fat absorption, decrease carbohydrate and amino acid metabolism, decrease protein digestibility, and increase pancreatic hypertrophy (Rackis, 1974). There are two major types of trypsin inhibitors: Kunitz inhibitors have activity against trypsin and Bowman-Birk inhibitors inhibit both trypsin and chymotrypsin (Sessa and Bietz, 1986; Steiner and Frattali, 1969). Alkaline conditions in small intestine may possibly increase trypsin inhibitory activity through the release of bound inhibitor (Ramsey and Willard, 1975*a*). Trypsin inhibitor evokes hypersecretion of pancreatic enzymes by forming trypsin-trypsin inhibitor complexes that suppress the activity of trypsin already secreted by the pancreas and reduces protein digestibility (Rackis, 1981; Lepkovsky et al., 1971). Poorly digested protein and endogenous protein forms trypsin-protein complexes that also accelerate pancreatic secretion (Rackis, 1981). Trypsin inhibitors induce a nonspecific increase in pancreatic enzyme synthesis by hormonal negative feedback (Liener, 1981). Liener (1977) found that trypsin inhibitors induce enlargement of the pancreas only in species in which the pancreas normally comprises more than 0.3% of the body weight such as rats, but not calves.

Lipoxygenase is an enzyme in soy proteins that oxidizes lipids and results in rancidity and off-flavors (Mustakas et al., 1969). Inactivation of lipoxygenase in soybeans enhances both palatability and storage stability (Baker and Mustakas, 1973). Since palatability is an important factor in early starter consumption, inactivation of lipoxygenase is crucial. Urease

is another enzyme found in soybeans that catalyzes the conversion of urea into ammonia and carbon dioxide, which can be a problem in rations containing urea (Baker and Mustakas, 1973). Since inactivation rates of antinutritional factors vary so much during processing, urease could be completely inactivated while considerable trypsin inhibitor is still present (Baker and Mustakas, 1973). Other antinutritional factors such as hemagglutins, saponins, phytohemagglutins (lectins), goitrogens, anti-vitamins, phytases, estrogens, allergens, and unidentified antigrowth factors are also present in alternative proteins, such as soybeans (Schingoethe, 1970; Liener, 1981). Hemagglutins results in poor protein and fat digestibility (Arnold et al., 1971). Lectins that are found in legumes are able to bind carbohydrates (Liener, 1976). When lectins interact with red blood cells, the glycoproteins located on the surface of the cells cause agglutination of the red blood cells (Liener, 1981). Phytic acid readily chelates with di- and tri-valent metal ions such as calcium, magnesium, zinc, copper, and iron (Liener, 1981; Rackis, 1974). Such complexes are poorly absorbed across the epithelium of the small intestine and result in reduced availability of these minerals (Liener, 1981). Despite the negative effects exerted by their presence, Holm et al. (1973) concluded that the nutritional impact of these antinutritional factors' in animal feeding programs is small. Kakade et al. (1976) likewise concluded that trypsin inhibitors in soybeans play a minor role in calf nutrition as well when used in place of casein in milk replacers for calves under 3 weeks old.

Insufficiently processed alternative proteins can contain antinutritional factors and allergenic proteins (Huisman and Jansman, 1991). Sissons (1982) found that calves are particularly prone to mount immune responses to alternative proteins. Antigen-induced responses may be associated with a lack of protective emigration of neutrophils from the

lamina propria into the lumen, where antigens are either inactivated or destroyed (Seegraber and Morrill, 1985). Intestinal dysfunction occurs when the antibody to the soy protein antigen has been synthesized and antigen enters the lumen of the gut (Barratt et al., 1979). Kilshaw and Slade (1980) reported that it is possible that the absorption of abnormally large quantities of alternative protein antigens and bacterial products during hypersensitivity reactions in the gut might perpetuate the hypersensitivity condition and possibly initiate secondary pathogenic reactions either locally or in peripheral tissues. When alternative proteins are associated with allergenic responses, there is a strong allergenicity for the alternative protein hypersensitivity mediated by the immunoglobulin E antibody (Koshiyama et al., 1981). Serum IgG response to ingested soy antigens tends to increase with the time of exposure to the diet, leading Barratt et al. (1978) to conclude that local immune mechanisms may have little effect in blocking access to the antigen. Allergenic factors in alternative proteins in diets cause villous atrophy, increased crypt cell mitosis, crypt hyperplasia and, thereby, malabsorption syndrome (Kenworthy and Allen, 1996). One of the hypersensitivity responses is to glycinin proteins and β-conglycinin in alternative proteins (Miller et al., 1994). Glycinin and β-conglycinin are the major storage globulins in proteins bodies of soybeans (Barratt et al., 1978). Glycinin and β-conglycinin initiate humoral immune responses in calves sensitized to dietary soy proteins (Barratt et al., 1978). Sissons et al., (1984) found that β-conglycinin, but not glycinin, was unaffected by pepsin. They also found that both antigens were fairly resistant to rennin and trypsin and that the solubility of glycinin and β-conglycinin remained high over pH ranges likely to be encountered in the calf digestive tract.

Soy antigens are resistant to proteolysis and somewhat resistant to the microbial action of rumen fluid (Barratt et al., 1978). Additionally, delaying the introduction of the soy antigen to the calf from 1 week to 4 weeks of age significantly decreases the allergic response in the calf (Barratt and Porter, 1979). This may be because the young calf exhibits a less complete mucosal barrier than the older animal and an exceptionally poor intestinal antibody response (Barratt and Porter, 1979). Gorrill and Thomas (1967) reported that trypsin inhibitor activity in soy flour remains high, which results in poor growth rates. They also reported that soy protein concentrate contained only trace amounts of trypsin inhibitor, and feeding soy protein concentrate led to higher growth rates than feeding soy flour. In their studies, they determined that contents from the lower intestine contained less free trypsin inhibitor than contents from the upper and middle sections which may be due to trypsin inhibitor being destroyed or inactivated as the digesta transverse the small intestine of the calf. Lalles et al. (1995) reported significant negative simple linear correlations between apparent digestibility of nitrogen in soybean and concentrations of native protein, antitryptic activity, glycinin, α-conglycinin, and β-conglycinin, and that low levels of β-conglycinin was the best predictor of improved digestibility of nitrogen in soy protein. Additionally, Visser and Tolman (1993) found that antitryptic activity, lectin, and aggregated protein, but not antigenic proteins, appeared to be the most important factors in explaining variation between soy protein products and their apparent digestibilities of dietary nitrogen. However, Lalles et al. (1995) concluded that the involvement of local immune reactions cannot be excluded in the explanation of increased fluxes of endogenous protein. Smith and Sissons (1975) found that soy protein isolate induced lower amounts of antibodies to soy protein than soy flour. If soy flour is heated suitably, destruction of trypsin inhibitor and other deleterious factors

would occur (Vest et al., 1966). Rachitogenic and perotic factors are concentrated in soy protein isolate, while growth-promoting, antiperotic, antirachitogenic, and antithyrotoxic factors are present in soybean meal extracts (Rackis, 1974).

Soybeans processed by fermentation have been found to have a different type of trypsin inhibitor present in them in comparison to soybeans processed by other methods (Wang et al., 1975). This trypsin inhibitor contains three unsaturated fatty acids that other soybean products do not have: oleic, linoleic, and linolenic (Wang et al., 1975). Wang and Hesseltine (1966) concluded that fermentation by *R. oligosporus* produces extracellular hydrolysis of soybean oil that in turn yields free fatty acids during fermentation. Free fatty acids have been reported to inhibit various enzymes, such as glycolytic, gluconeogenic, lipogenic, and proteolytic enzymes, some hydrolases including trypsin, and fatty acid synthesis (Wang et al., 1975; Weber et al., 1966; Korchak and Masoro, 1964; Bargoni, 1960). Their effect on enzymes appeared to be nonspecific (Wang et al., 1975). However, Feng et al. (2007*a*), found that fermentation by *Bacillus subtilis* completely inactivated all trypsin inhibitors in soybean meal because there was a complete breakdown of the 3 subunits from β-conglycinin and both polypeptides from glycinin (Kiers et al., 2000). Glycinin and β-conglycinin are the major storage molecules in soy protein and are trypsin inhibitors (Barratt et al., 1978). β-conglycinin also causes a delayed-type, cell-mediated immune response in the small intestine when ingested (Lalles et al., 1995).

Deleterious factors have also been researched with fish products as an alternative protein source (Genskow, 1968). Protein digestibilities determined for fish flour were higher than those observed for replacers containing relatively large amounts of fish meal and cereal grains (Raven and Robinson, 1959), soybean meal (Noller et al., 1956), or distiller's dried

soluble (Jacobson et al., 1965). Most of the detrimental effects of isolated fish protein on weight gains and protein digestibility in young calves are from irreversible heat denaturation of the protein during its preparation, which results in a reduction of its water-holding and emulsification capacity (Opstvedt et al., 1978).

Altered Gastrointestinal Tract Morphology. In calves fed milk proteins, intestinal villi are long, tapering, and uniform (Seegraber and Morrill, 1985). Gradual deterioration and abnormal confirmation of villi occurs in calves fed soy or fish proteins in place of milk proteins (Seegraber and Morrill, 1985; Campos and Huber, 1982a; Silva and Huber, 1985). The process of deterioration of villi begins with epithelial damage, which is followed by broadening and shortening of villi with fusion initiating at the bases and progresses to complete villous atrophy and a flat intestinal mucosa (Loehry and Creamer, 1969; Seegraber and Morrill, 1982). More severe degeneration leads to decreased surface area for absorption of nutrients (Seegraber and Morrill, 1985). More rapid degeneration has been observed in calves fed soy protein concentrate than in those fed soy flour (Seegraber and Morrill, 1985). Thickened intestinal walls also occur in calves fed whey, fish, and soy proteins (Roy et al., 1977; Stobo, unpublished data). Changes to intestinal morphology after feeding of alternative proteins can occur in as little as 7 days (Barratt et al., 1978). Antinutritional factors and allergic reactions in the digestive tract of the calf can cause the intestinal morphology to become distorted. Numerous alternative proteins can cause these changes in intestinal morphology. The morphological changes caused by feeding alternative proteins resemble those seen with enteric viral infections in calves (Mebus et al., 1975). Often, these changes are reversible if the calves are switched back to all-milk milk replacers (Seegraber and Morrill, 1985).

Decreased or inhibited digesta flow through the small intestine and more basic pH values in the abomasum, which impairs curd formation, occur more frequently in calves fed milk replacers with alternative proteins, such as fish and especially soybean diets (Shoptaw, 1937; Colvin et al., 1969; Guilloteau et al., 1986; Smith and Sissons, 1975; Kwiatkowska, 1972), than in calves fed milk replacers with all-milk proteins. Proteins that produce a firm coagulum in the abomasum of the calf enhance nutrient digestibility by allowing proper flow of digesta from the stomach to the small intestine (Hill et al., 1970). The difference in flow rates between proteins is due to differences in digesta composition upon entering the duodenum (Smith and Sissons, 1975). In order for the diet to form a curd in the abomasum, the pH must drop significantly to around a pH of 1.5 (Shoptaw et al., 1937) and glycopeptides must be released from K-casein (Roy, 1970). This does not happen when feeding milk replacers with significant amounts of most alternative proteins (Colvin et al., 1969; Gorrill and Nicholson, 1972). Since alternative proteins do not form a curd, they can easily be expelled from the abomasum before the pH drops sufficiently to allow effective pepsin activity (Jenkins et al., 1980). Dietary proteins that escape clotting and digestion in the abomasum also tend to promote the proliferation of pathogenic organisms in the upper small intestine (Tagari and Roy, 1969). Curd formation in the abomasum is also important to stimulate the secretion of digestive enzymes in the small intestine (Williams et al., 1976; Caugant et al., 1992). The lack of drop in pH of the digesta indicates that the gastric mucosa was not capable of secreting sufficient hydrochloric acid to acidify the digesta both because of the quantity of digesta that is released and because of the higher initial pH of the digesta (Gorrill and Nicholson, 1972). Low digestibility and absorption of protein, low nitrogen retention, and increased diarrhea are also associated with lack of curd formation, increased

abomasal emptying rates, and higher pH of digesta (Smith and Sissons, 1975). In some cases of extreme digestive disturbances with young calves fed milk replacers containing soy flour and soybean protein isolate, there was a complete inhibition of digesta flow for a period of time in the small intestine (Smith and Sissons, 1975).

For the first 3-4 weeks of life, calves have limited digestive capabilities in their gastrointestinal tract (Berridge et al., 1943). Intestinal contents from calves suffering diarrhea contain very low levels of proteolytic enzymes (Campos and Huber, 1982a). Release of secretin and cholecystokinin, previously called pancreozymin, triggers the secretion of exocrine fluid and digestive enzymes from the pancreas (Harper et al., 1961). Pancreozymin activity in calves fed alternative protein based diets is limited due to the limited proteolysis associated with the alternative proteins. This leads to diarrhea in calves (Campos and Huber, 1982a). Additionally, cholecystokinin-pancreozymin (CCK-PZ) can form complexes with dietary proteins which are resistant to proteolysis, even in the presence of high concentrations of intestinal proteolytic enzymes and further reduce digestibility of alternative proteins (Laporte and Fontaine, 1971). Inhibition of intestinal proteolysis would limit the absorption of amino acids needed for synthesis of digestive enzymes as well as all other proteins (Campos and Huber, 1982a). This impaired absorption was confirmed in projects with soy-fed calves using the xylose absorption test (Seegraber and Morrill, 1985). Jenkins et al. (1980) found that proteolytic enzymes present in the abomasum work more effectively on milk proteins than non-milk proteins and that the protein portion of milk replacers play an important role in the digestibility of other nutrients and the overall health of the calf. Additionally, increased permeability of the gut to macromolecules has been associated with feeding soy proteins (Kilshaw and Slade, 1980). Also, trypsin and chymotrypsin activities of

the pancrease and intestinal contents from calves fed the milk replacer diet containing 50% of the protein from soy protein were less than those from calves fed all milk (Gorrill and Thomas, 1967). Proteins are broken down in the stomach by pepsin (Bergmann and Fruton, 1941) and hydrochloric acid and in the intestinal lumen by chymotrypsin (Bergmann and Fruton, 1941), trypsin (Sanger and Tuppy, 1951), and carboxypeptidases (Putnam and Neurath, 1946). Peptide bonds of some alternative proteins are less accessible to cleavage by these enzymes, which further decreases their digestion (Hansen and Johnston, 1976). Digestion rates of heat-treated soy flour proteins by trypsin and carboxypeptidase-B are decreased in comparison to soy flour not heat-treated (Hansen and Johnston, 1976). Nesheim and Carpenter (1967) found that a significant proportion of the proteins and peptides which escape digestion and absorption in the small intestine, enter the ceca, and are fermented in such a way that nitrogen is absorbed as ammonia or in some other form with no nutritional value. If absorbed peptides are large, they may go directly into the circulatory system and be taken to the kidneys to be excreted in the urine (Hansen and Johnston, 1976).

These digestive disturbances and morphological changes are only seen in sensitized calves, or calves that have been previously fed alternative proteins. When calves are first fed the alternative proteins associated with digestive disturbances, calves digest them just as they would milk proteins (Smith et al., 1970). It was only after several feedings that abomasal emptying was inhibited and digesta flow rates changed (Smith and Wynn, 1971; Smith et al., 1970), which suggests a possible allergic reaction (Colvin et al., 1969). This allergic reaction causes a decreased transit time through small intestine, abnormal water and salt exchange in the small intestine, and decreased nitrogen absorption throughout the small intestine to the ileum (Smith et al., 1970). Additionally, some soybean products contain a factor which

survives digestion in the abomasum and duodenum that causes an allergic response to varying extents in different calves (Smith and Sissons, 1975).

The digestive disturbances associated with alternative protein sources in diets are only apparent until the animal matures enough to better handle those protein sources. Xylose absorption tests have shown that calves at 6 weeks of age on lower quality diets have no impaired ability to absorb nutrients from the digestive tract (Campos and Huber, 1982a). In fact, when low quality proteins are used in milk replacers, there is often compensatory weight gain between weaning and week 15, while the calf is consuming starter (Gorrill et al., 1976).

Incorporation Rates of Alternative Proteins. Since two of the main factors that influence calf performance on alternative protein based milk replacers and starters are the proportion of the total protein replaced with the alternative protein and the age of the calf, incorporation rates of alternative proteins in calf diets profoundly impacts how the calves perform on the alternative protein. Many alternative proteins are suitable for young calves as long as they do not exceed a certain incorporation percentage. This maximum incorporation rate is different for each alternative protein source, the manner in which it is processed, and whether it is being incorporated into the calf's milk replacer or the starter diet.

Even though milk proteins are better digested by the calf than soy proteins (Gorrill and Nicholson, 1972; Gorrill et al., 1971; Lalles, 1993; Nitsan et al., 1971; Silva and Huber, 1985), many studies have shown that properly processed soy protein can supply a large portion of the protein in milk replacers for young calves. Gorrill and Nicholson (1969) confirmed this conclusion in their research with soy protein concentrate. Additionally, Akinyele and Harshbarger (1983) found that gains for calves fed soy protein concentrate milk replacers were better when only 30% of the milk protein was replaced by soy protein

concentrate compared to calves fed milk replacers containing over 84% soy protein. Campos and Huber (1983), however, found that replacement of 50% of the milk protein with soy protein concentrate did not result in significantly lower average daily gains in comparison to calves fed the all milk replacer. Colvin and Ramsey (1969) also found that satisfactory growth could be achieved in calves fed with a milk replacer containing 86% soy protein concentrate and Gorrill and Nicholson (1969) found that 70% could be replaced with soy protein concentrate. If grain and hay is provided in addition to the milk replacer, up to 90% of the milk protein can be replaced with soy protein concentrate. In summary, upwards of about 85% incorporation of soy protein concentrate is acceptable when just milk replacer is supplied to the calf and up to 90% of the protein can be replaced if concentrates and hay are offered.

Soy protein isolates and concentrates are better digested by the young calf than soy flour (Akinyele and Harshbarger, 1983). The use of high levels of soy flour in milk replacer decreased growth rates in calves (Stein, et al., 1954). Additionally, relatively poor growth, digestibility, and nitrogen retention resulted when cooked soybeans, soybean meal, or lightly cooked soybean flour were used (Porter and Hill, 1963) and several studies have shown that diets containing more than about 30-40% of their protein in the form of heated, fat-extracted but otherwise untreated, soy flour nearly always proved unsuccessful when fed to calves and have led to diarrhea, weight loss or very poor growth, and sometimes even death (Stein et al., 1954; Gorrill and Thomas, 1967; Colvin and Ramsey, 1968). This poor performance could be due to the high levels of starch in soybean meal and soy flour, which require further processing other than cooking to become digestible to the calf (Gorrill and Nicholson, 1969).

Caugant et al. (1993) found the differences in amino acid digestibilities of soy flour and soy protein concentrate diets were minimal, suggesting that the processing used to prepare soy flour can vastly improve its nutritional value by breaking down starches that are indigestible to the young calf. Colvin and Ramsey (1967) presented data from calves fed milk replacers containing soy flour that would support this observation as well. When they treated soy flour with acid, they doubled the growth of the calves fed the milk replacer containing acid-treated soy flour in comparison with the calves on the untreated soy flour. Another way to overcome the poor digestibility of the soy flour is to feed it in combination with another higher quality protein. Rindsig and Bodoh (1977) used soy flour in combination with whey powder and colostrum to create a milk replacer that was suitable for young calves.

Ground, raw soybeans are not an acceptable source of protein for young calves in milk replacers when incorporated at a level of 40% (Williams and Knodt, 1950). Kwiatkowska (1972) found that up to 30% of protein from the milk replacer could be supplied in the form of solvent-extracted soybean meal with no significant differences in nitrogen retention. When soybean meal supplied 73% of the total protein in a milk replacer, calves grew poorly due to low protein digestibility and decreased fat and ash absorption (Nitsan et al., 1971).

Since 75% of a calf's growth during the first 4 to 6 weeks of life is due to starter intake, high quality protein in the starter ration is very important (Barr, 1981). Soy protein is often used in starter diets to replace dried milk and/or corn or other grains. Pardue et al. (1962) found that the addition of dried skim milk provided little benefit over a vegetable source of protein in the starter of early weaned calves. One form of soy protein that has been

studied in starter ration incorporation is condensed soy solubles; they can be substituted for corn in starter rations without any adverse effects (Cline et al., 1976). The growth of calves between 5 and 11 weeks of age has been found to be similar with meat meal, dried skim milk, soybean meal or fish meal as the sole protein supplement to grain diets (Whiting and Clark, 1955; Pardue et al., 1962; Whitelaw and Preston, 1963; Leibholz, 1967).

Fish protein is also used in calf diets. Fish protein concentrate incorporated into the milk replacer at 35% resulted in acceptable growth and performance of calves (Huber and Slade, 1967; Campos and Huber, 1982a). Previously, Sleiman and Huber (1971) found that an incorporation rate of 40% fish protein concentrate did not adversely affect calf performance, and Opstevedt et al. (1978) concluded even higher levels could be fed if grain and hay were also available to the calf. Huber (1974) found that calves performed satisfactorily when fed milk replacers containing 70% fish protein concentrate if grain and hay were available as well. Gorrill and Nicholson (1969) found that calf performance was similar for calves fed whole milk, milk replacer containing all milk protein, or milk replacer with 50% of the protein coming from isopropanol-extracted fish protein concentrate and 50% coming from milk proteins. Although calves on this study performed similarly no matter the protein source, Huber and Campos (1982) found that calves fed milk replacers containing 33% fish hydrolysate or soy protein concentrate had a lower feed to gain ratio than calves on all milk replacers (Morrill et al., 1971; Roy et al., 1977). Huber and Slade (1967) also found that feed conversion rates were lower for calves on fish flour than calves on all milk replacers and Makdani et al. (1971b, 1974) found similar results with calves fed fish protein concentrate prepared by various processing methods. Campos and Huber (1982b) concluded that incorporating spray-dried fish solubles at even 10% was excessive and resulted in 30%

mortality rates, but in a different study they found that spray-dried fish solubles could replace 8-16% of protein satisfactorily (Huber and Campos, 1982). Once again, this shows how important proper processing is to ensure maximum calf performance. Processing fish to create partially hydrolyzed fish protein (PHFP) results in fish protein that is a highly digestible and an available source of protein in milk replacers for young calves (Jenkins et al., 1981). The PHFP could supply about one-half of the dietary protein in calf diets without lowering their performance or feed efficiency in comparison to calves fed all milk replacers. The water soluble fraction of fish protein concentrate is not an acceptable source of protein for incorporation into young calf diets due to its poor amino acid profile (Huber, 1974). However, satisfactory growth of calves has been reported when 40% of the milk replacer protein was supplied by fish flour that was prepared by dichloroethane extraction of fish meal, but 60 and 67% incorporation rates resulted in death of the calf (Huber and Slade, 1967). Also, poor performance occurred when the milk replacer contained protein only from fish flour (Huber and Slade, 1967) or only from fish protein concentrate (Makdani et al., 1971a).

Several other sources of alternative proteins have been studied less extensively as well. Bhatty and Christison (1980) found that pea protein isolate could replace up to 50% of milk proteins in a milk replacer without any major adverse effects in calf growth or performance. They additionally concluded that pea protein concentrate contained higher levels of indigestible starch and oligosaccharides that prevented it from being utilized at the same rates of incorporation as pea protein isolate.

Whey powder, which is made from the liquid material created as a by-product of cheese production, can be substituted for whole milk proteins in all-milk replacers

successfully in calf diets at a rate of up to 60% of the total protein (Cruywagen and Horn, 1984; Stobo et al., unpublished data). Acid whey powder can also be added to milk replacers at a level of 23% to help adjust the pH (Gorrill and Nicholson, 1972). Adjusting the pH improves calf performance by slowing the rate of abomasal emptying and lowering chymptrypsin and trypsin activities in the intestinal digesta (Gorrill and Nicholson, 1972).

Gorrill et al., (1976) found that rapeseed flour and oil could be incorporated at a level of 30% into young calf diets, as well as rapeseed protein concentrate prepared by dehulling, heating, and water- and solvent-extracting low-glucosinolate rapeseed. They also found that dehulled commercial high-glucosinolate rapeseed meal or rapeseed flour didn't appear to be a suitable source of nutrients for calf milk replacers due to poor digestibility and high contents of antinutritional and allergenic factors.

Potato (Hinks et al., 1975) and evaporated milk (Noller et al., 1956b) can also be used in place of cow's milk proteins in calf rations. These alternative proteins have been used successfully in place of whole milk proteins in calf milk replacers.

Quigley et al. (1999) has done several studies with blood protein and found that calves on diets including hydrolyzed spray-dried red blood cells performed equally to calves on all milk replacers, even with higher levels of incorporation.

Liquid egg is another alternative protein that can be used in milk replacers and can effectively replace up to 10% of the milk proteins in the calf diet (Touchette et al., 2003). Quigley (2001) reported that spray-dried whole eggs did not provide enough nutrients to support adequate calf growth when incorporated into a milk replacer as the sole source of protein.

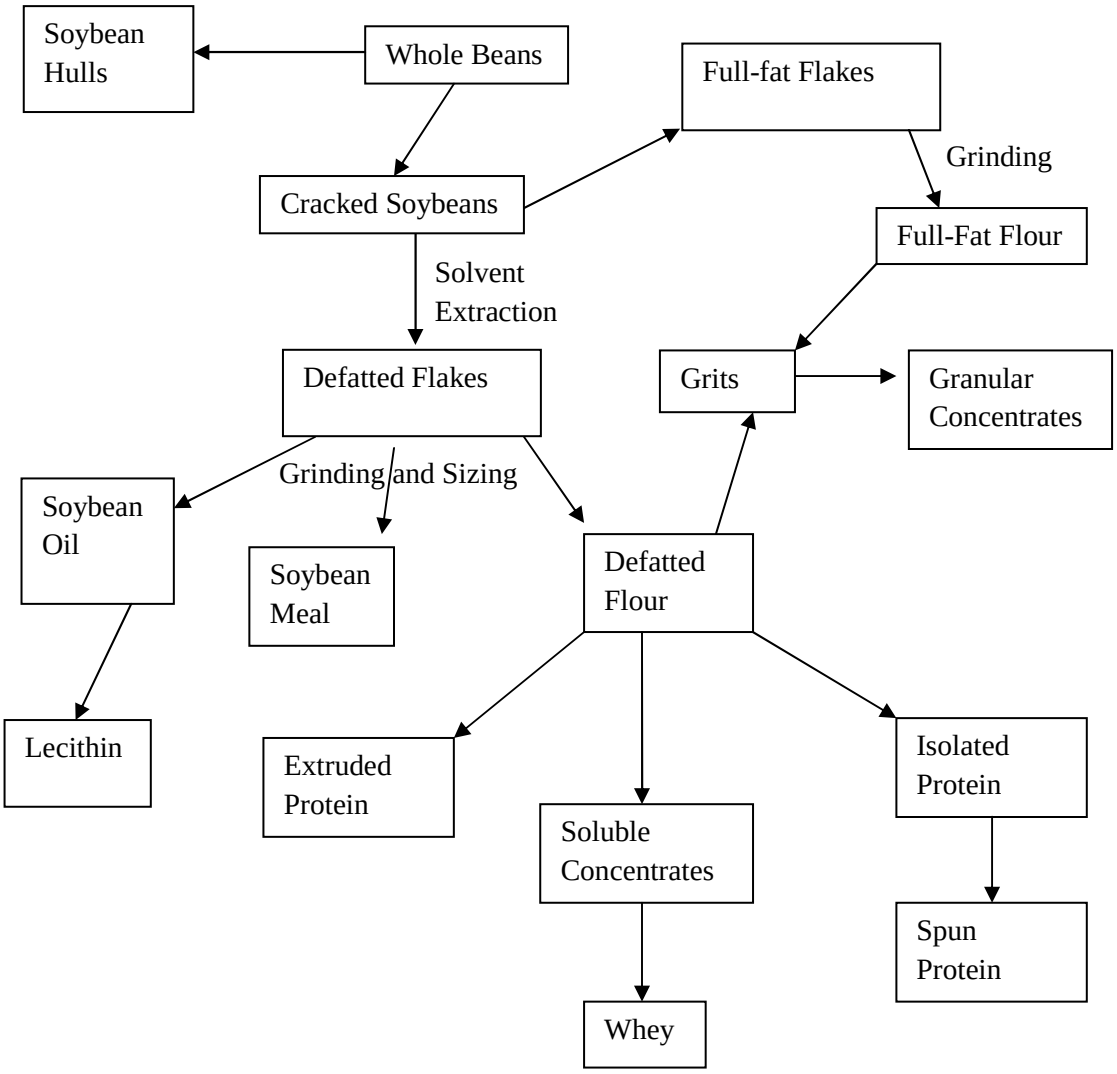
Finally, Cafrey and McAlesse (1964) found that starters can be based on barley, oats, or molassed beet pulp without affecting calf performance. They also found that of the protein supplements they examined, soybean meal and linseed flakes were the most palatable with skim milk powder, and fish meal and meat and bone meal were least palatable, which is valuable information since palatability is the main factor in getting calves to consume starter rations at an early age. In addition, sunflower-based meals are also suitable for feeding young calves in starter rations in place of milk proteins (Mandibaya et al., 1999).

Processing Methods of Soybeans and Their Products

Soybeans must be processed prior to consumption by the calf to effectively be utilized and produce desirable growth and performance. Unprocessed soybeans contain nutrients that the calf is unable to break down due to not having the appropriate digestive enzymes or antinutritional and allergenic factors that inhibit digestion of the nutrients (Birk and Gertler, 1961; Barr, 1981). In calves with completely functioning rumens, antinutritional factors and complex proteins are not as detrimental, and thus processing is not as crucial (Abdelgadir et al., 1984).

Soybean Products. Many products are the result of different processing methods of soybeans. How these products are created is illustrated in the flow diagram in Figure 1. Often, these products go onto further processing to make them more digestible to animals or to denature antinutritional and allergenic factors still present in them.

Figure 1. Processing methods of soybeans and soybean products flow diagram. Modified from the National Soybean Research Laboratory flow diagram for soy processing products and how they are used (National Soybean Research Laboratory, University of Illinois, Urbana, IL).



Sometimes, reduced performance occurs because calves are unable to utilize essential nutrients in alternative proteins, rather than performance impairment occurring because of toxic substances inhibiting digestion. Rackis (1974) observed this response in trials feeding raw full-fat and defatted soy flour; these diets inhibited growth, depressed fat absorption, reduced protein digestibility, caused pancreatic hypertrophy, stimulated hyper- and hyposecretion of pancreatic enzymes, and reduced amino acid, vitamin, and mineral availability. As a result, metabolizable energy in these diets was reduced.

Soy flour contains the second highest trypsin inhibitor activity on a dry weight and protein basis (Charpentier and Lemmel, 1984). Acid or alkali treatment of soy flour improved growth of calves fed a milk replacer containing only soy protein (Gorrill and Nicholson, 1969). Trypsin inhibitor inactivation was accelerated by base and retarded by acid addition (Baker and Mustakas, 1986). Unfortunately, treatment of soy flour with acid or alkali does not affect its rate of passage through the abomasum or the pH changes occurring in the abomasum (Colvin et al., 1969). However, with either acid or base additives, the initial inactivation of urease and lipoxygenase was accelerated significantly (Baker and Mustakas, 1973). Since soybean globulins are fairly resistant to denaturation by ethanol at high and low concentrations, a mixture of equal proportions of ethanol and water is often necessary to dissociate the globulins and improve the suitability of soy as an alternative protein (Sissons et al., 1982).

Age differences contribute to which processing method is most effective. This difference in utilization of soy proteins was observed when Kwiathowska and Zielinski (1975). They observed significantly increased digestibility in one month old calves, but not

in two month old calves, when the soy oilmeal was processed by toasting or other physiochemical processes. Zieliniski et al. (1959) previously found that the processing of flour from solvent extracted, toasted soybean oilmeal considerably increased the digestibility of soy components in the milk replacer. Additionally, a very fine partitioning of the plant material, making the protein substances more accessible to the enzymes of the alimentary tract is another way to improve the digestibility of proteins from soy proteins (Kwiathowska and Zielinski, 1975). The high degree of partitioning of the plant material may slow the passage rate of the digesta through the intestines (Kwiathowska and Zielinski, 1975). This prolongs both the time of action of digestive enzymes on the substrate and period of intestinal absorption, thus increasing digestibility (Kwiathowska and Zielinski, 1975). The difference in digestibility with age has also been observed in studies involving full-fat soybeans. When calves' rumens were not functioning, they benefitted more from processed full-fat soybeans in comparison to soybean meal (Abdelgadir et al., 1984).

Further Processing Methods of Soybeans and Their Products. Many other processing methods are used in addition to those that create byproducts of soybeans to improve soybean utilization. Expansion, extrusion, popping, heating, boiling, acid/alkali treatment, and toasting are a few processing methods that have been studied. Micronization, ultrafiltration, thermoalkali treatment, supplementation, microwaving, fermentation, soaking, and cooking are more processes that have been studied. Regardless of the processing method utilized, the process should minimize activity of antinutritional factors and achieve maximum availability of nutrients for digestive enzyme access to maximum protein utilization (Abdelgadir et al., 1984).

Boiling of soybeans inactivates of up to 98% of the trypsin inhibitor, thus improving soy protein digestibility (Collins and Sanders, 1976). Extruded soybeans were equal to soybean meal as a source of protein for young calves (Daniels et al., 1973; Stutts, 1982), and expanded, extruded soybeans were digested more efficiently in calves than soybean meal plus fat (Daniels and Flynn, 1976). Micronization processing offers a rapid alternative method for processing whole soybeans that effectively destroys urease activity and trypsin inhibitors while increasing protein digestibility (Hutton and Foxcroft, 1974). A micronizing temperature of between 200˚ and 225˚ C is required for optimal processing of whole soybeans (Hutton and Foxcroft, 1974). With soy proteins, increasing drying temperature to double the rate of destruction of trypsin inhibitor would increase the destruction of many nutrients by four- to fivefold (Rackis, 1974). Ultrafiltration is processing method that is a separation technique and effectively removes phytate with little or no loss of protein (Okubo et al., 1975).

Heated Soybeans. Heating is one method of processing, and even though trypsin inhibitors are readily inactivated by steam heat (Rackis, 1966), heating of soybeans is not the most effective processing method to achieve this goal. This is because many of the antinutritional factors present in soybeans require such a high temperature to become denatured that the proteins and amino acids present in the soybeans also become denatured (Logenecker et al., 1964; Rios Iriarte and Barnes, 1966; Arnold et al., 1971).

Since extreme heat denatures nutrients, soy protein that is heat treated to improve digestibility and remove deleterious factors often needs further processing to further improve the digestibility (Vest et al., 1966). With soybean protein, unless the heat treatment is followed by very fine grinding or flaking, the maximum feeding potential of the whole

soybeans cannot be achieved (Arnold et al., 1971). Miller and Ramsey (1978) found that for calves fed a milk replacer containing soy flour as the only source of protein, maximum calf growth was obtained when the flour was heated for 90 minutes. This treatment gave better results than the commercially available "fully-cooked" soy flour product. Hansen and Johnson (1976) concluded that the highest pepsin and trypsin digestion rates for soy flour proteins were for flour processed with 13% moisture content at 108˚ C f or 2 min. They also found that more severe processing resulted in a progressive reduction in digestion rates, probably due to denaturation of the protein.

Rackis (1974) found that processing by the form of short cooking time in an extruder minimizes damage to nutritional properties, but adequately destroys the growth inhibitors. The processing method of moist heat has a beneficial effect upon the nutritive value of soy protein isolates (Rackis, 1974). When treating defatted soybean meal with steam to inactivate antinutritional substances, the nutritive value of the product still remained markedly inferior to milk protein (Gorrill and Thomas, 1967).

The effectiveness of heat treatment on the nutritional characteristics of soy protein largely depends on water activity, pH, heating time, and processing temperature (Johnson et al., 1980). Certain combinations of these factors create products that, when included in calf diets, promote weight gains and feed efficiencies superior to those of the raw protein (Arnold et al., 1971). Nearly all vegetable proteins and products derived from them are consumed after some degree of heat treatment (Rackis, 1966). Processing of soybeans alters flavor, color, texture, and other functional properties of the proteins, along with altering the digestibility of the protein.

Alkali Treatment. Soy protein sources treated with alkali improves calf performance (Barr, 1981). This additional processing removes antigenic properties and enhances utilization (Barr, 1981). Alkaline processing conditions were found to render trypsin inhibitors more heat-labile and therefore easier to destroy during heat-processing (Badenhop and Hackler, 1970). The conditions of the ethanol treatment, such as time, temperature, and relative amounts of ethanol and water, may affect the extent of removal of the deleterious factors (Smith and Sissons, 1975). Borowska and Kozlowska (1986) found that a pH of 8.2 was optimal for soybean flour extraction. Treatment with hot aqueous ethanol has also improved soybean protein utilization (Sissons et al., 1979). Additionally, moist heating under mild alkaline conditions improves soy protein quality, but this processing method can form a toxic amino acid, lysinoalanine, during the process (Woodard and Short, 1973). Sissons et al. (1982) concluded that glycin and β-conglycinin levels were best denatured when soy protein was treated in 65% ethanol at a temperature of 78˚ Celcius. In studies done by Kilshaw and Sissons (1979), it was furthermore shown that the antigenic activity of soy protein can be completely eliminated by treatment with hot aqueous alcohol. Several studies have proven that calves given a milk replacer containing protein from soy protein concentrate prepared by ethanol extraction had significantly better performances than calves receiving a milk replacer containing protein from heated soy flour (Gorrill and Thomas, 1967; Gorrill and Nicholson, 1969; Nitsan et al., 1971). Wolf (1970) concluded that this improvement was due to the removal of oligosaccharides, which are soluble in aqueous alcohol, from soybean meal. Additionally, phytase activity is decreased by 50-70% with alkaline environments compared to non-modified soybean isolate (Borowska and Kozlowska, 1986).

Acid Treatment. Colvin and Ramsey (1968) have found that calves fed acid-treated soy flour grew at nearly twice the rate of those receiving untreated soy flour. They observed improved calf performance when fully cooked soy flour was treated at pH of 4 versus 6.4. Sudweeks and Ramsey (1972) found similar results in that acid treatment did improve calf growth rates on soy flour based milk replacers, but they did not see any differences between acid-treated diets at different pHs. They also found that the carbohydrate fraction of the soy flour was not any more available to calf digestion, and therefore was not involved in the improved growth rates observed. Trypsin inhibitor, but not other detrimental factors, was degraded by proper treating with acid (Barr, 1981). Wilson and Ramsey (1972) also found that soy flour treated with anhydrous hydrochloric acid had improved nutrient value; however, they could not establish an optimum level of acid treatment. Nutritional quality of soy protein concentrate is improved by thermoalkali processing for 5 min, but increasing the length of time of processing to 25, 30, or 60 min was of little benefit (Coblentz et al., 1976).

Supplementation of Soy Protein. The protein digestability of soy protein has not been improved by addition of proteolytic enzymes or by amino acid supplementation (Fries et al., 1958; Otterby and Linn, 1981). This has sometimes even depressed growth (Colvin and Ramsey, 1968). Also, the addition of DL-methionine to soy protein based milk replacers did not increase calf growth or nitrogen retention (Gorrill and Nicholson, 1969). Limestone incorporation was hypothesized to be able to neutralize the abomasal pH and thus increase digestibility of soy protein, but it was ineffective in studies testing this hypothesis (Campos and Huber, 1983).

Toasting Treatment of Soy Protein. Processing using dry roasting, or toasting, produces a very palatable, nutritious food from soybeans (Badenhop and Hackler, 1971).

Poor results from feeding trials utilizing raw soybean meal are related to trypsin and chymotrypsin inhibitors which are inactivated by toasting (Nitsan et al., 1971). The process of toasting additionally improves digestibility and availability of both protein and carbohydrates (Nitsan et al., 1971). During the roasting process, the temperature attained is considerably higher than needed to destroy trypsin inhibition and may indeed be detrimental to the nutritional quality of the protein (Badenhop and Hackler, 1971). Since palatability is the number one factor in early weaning consumption, this protein loss is balanced by the benefit of increased consumption. The toasting process includes a rapid dehydration followed by a partial pyrolysis (Badenhop and Hackler, 1971). Soybeans toasted at 146˚ C are better digested than untoasted soybean meal (SBM) (Abdelgadir, 1996). Trypsin inhibitor activity is very low in toasted soybean meal and in isolated protein commercially processed (Rackis, 1966). Kunitz-type trypsin inhibitors, which have activity against trypsin, and Bowman-Birk type trypsin inhibitor, which inhibit both trypsin and chymotrypsin, accounted for most residual trypsin inhibitor activity of toasted soybean flour (Sessa and Bietz, 1986; Steiner and Frattali, 1969). Soybeans processed at 138˚ C with or without tempering or at 171˚ C are all similar in digestibility (Abdelgadir et al., 1984). Additionally, calves consuming the starters containing soy protein processed at 171˚ C consumed more feed, gained weight faster, had lower fecal scores, and less mortality than calves consuming soybeans processed at 138˚ C with or without tempering (Abdelgadir et al., 1984). Digestibility has been shown to be higher in toasted soybeans than microwave-cooked soybeans (Prasad and Morrill, 1976).

Fermentation of Soy Protein. Fermentation is another processing method that produces a highly palatable product. Fermentation also often increases the availability of

nutrients and amino acids such as lysine, methionine, and tryptophan in the blends (Chompreeda and Fields, 1984). In addition to reducing antinutritional factors and eliminating trypsin inhibitors (Feng et al., 2007*a*), fermentation also reduced raffinose and stachyose in soybean meal (Chompreeda and Fields, 1984), which increases nutrient digestibility.

The most important factor in having a product ideal for fermentation is the heat treatment given to soy proteins at any stage during its preparation before inoculation with the organisms for fermentation (Patel et al., 1980). The best heat treatment found by Patel et al. (1980) was treatment at 100˚ C for 20 minutes. Certain minimal heat treatment is necessary to eliminate the lipoxydase activity and to destroy trypsin inhibitor found in raw soybean meal whether the product is being further processed by fermentation or not (Patel et al., 1980).

Soybean processing treatments like soaking and cooking, which are often employed in soymilk preparation, substantially reduce the content of fermentable, soluble carbohydrates needed for the fermentation microorganisms (Patel et al., 1980). Fortification of soy proteins with certain sugars like lactose and glucose is imperative to increase the sugar substrates present for the microorganisms to utilize (Patel et al., 1980). Sucrose supplementation seems to be particularly suitable for certain lactobacilli like *L. acidophilus*, alone or in combination with *S. thermophilus* (Patel et al., 1980).

Fermentation of soybean meal using several *Bacillus* spp. has increased digestibility of soy proteins as well (Kiers et al., 2003). Kiers et al. (2003) also found that complete breakdown of 3 subunits from β-conglycinin and both polypeptides from glycinin occurred after fermentation with *B. subtilis*. Feng et al. (2007*a*) also found that fermentation improved

the nutritional value of soybean meal and reduced or eliminated some important antinutritional factors, such as glycinin and β-conglycinin. Active trypsin inhibitors have been shown to be liberated from a heat-resistant, inactive, bound form during fermentation by *R. oligosporus* proteases (Wang et al., 1972); however, this trypsin inhibitor was readily inactivated by heat.

Fermented soybeans are not only highly digestible and nutritious by contributing important nutrients including calcium, vitamin A and B vitamins, but fermented soybeans also have functional properties, such as immunomodulatory and anti-cancer effects (Lee, 1998). Since fermentation can vastly improve the palatability of soy proteins along with increasing its digestibility, it is a very promising processing method for the industry.

Acquired Immunity Development.

The most important factor dictating whether a calf becomes sick when exposed to a compromised environment is the strength of its immune system (Osburn et al., 1974). Since there is no significant placental transfer of immunoglobulins (Osburn et al., 1974), calves are born immunosupressed and must consume colostrum and absorb antibodies that are present in the colostrum to acquire passive immunity. Passive immunity lasts a few weeks until the calf's own immune system begins to function. Often, maternal colostrum contains antibodies to soy protein that cause a rapid response to soy proteins once they are consumed (Barratt and Porter, 1979; Barratt et al., 1979).

The development of cell-mediated immunity in young calves is under the influence of the thymus (Outteridge, 1985). T lymphocytes are important components of the calf's acquired immune system. One important function of T lymphocytes is to mediate cellular immunity (Osburn et al., 1974). When the antigen that a specific T lymphocyte reacts with

comes into contact with the complimentary receptor site on the T lymphocyte, the lymphocyte is stimulated to release lymphokines (Dumonde and Mairi, 1971). Lymphokines amplify the immune system's response to the antigen by stimulating corresponding B and T lymphocytes (Claman and Chaperon, 1969). However, some physiologic events that occur during the birthing process may suppress the cellular immune response (Zeman et al., 1972). At parturition there is production of large quantities of glucocorticoids which are immunosuppressive towards T lymphocytes (Zeman et al., 1972).

Activation, differentiation, trafficking, and migration of T lymphocytes to sites of inflammation or infection are essential for an effective immune response (Foote et al., 2005). Neonatal calves often do not have lymphocytes that function correctly (Foote et al., 2005). Often, the activation and homing mechanisms in the lymphocytes are defective, thus compromising the immune system of the calf even further (Foote et al., 2005). As the calf ages, its acquired immune system develops and begins to function more effectively. This is reflected by increases in the percentages of mononuclear and polymorphonuclear leukocyte in peripheral blood (Foote et al., 2007).

Several studies have demonstrated that nutrition impacts the development and responsiveness of a calf's immune system. Of current interest are feeds containing biologically active components that produce a biological effect or health benefit that is above and beyond the nutritive value of that feedstuff. Antibodies in colostrum fed after the first 48 hours of life, instead of being absorbed into the blood circulation like they are during the first 48 hours of life, have been found to increase intestinal immunity (Drew, 1994; Fowler et al., 1995). This is an example of a biologically active feedstuff. These biologically active feed components are often present in the polypeptide chains of proteins. These fragments remain

inactive in the sequences of their own precursors, but when they are released by proteolytic enzymes that they may interact with, they are able to regulate certain physiological functions (Dziuba and Darewiez, 2007). One common proteolytic enzyme that releases these bioactive peptides is trypsin (Yamamoto et al., 1994). They may also be released by processing methods, such as fermentation (Möller et al., 2008). Some biologically active proteins and peptides are able to regulate the immune system (immunomodulatory effects). Other effects that bioactive components may have are antihypertensive, osteoprotective, opiate, antioxidative, and antimicrobial (Möller et al., 2008). Additonally, many proteins and peptides interact directly in the intestinal tract and via receptors and cell signaling (Möller et al., 2008).

Fementation of soybean meal not only improves digestion and destroys antinutritional factors like trypsin inhibitors, glycinin, and β-conglycinin, but also releases many bioactive components that are immunomodulatory, antipathogenic, and enhance phagocytosis (Lee, 1998; Kim et al., 2009, Magalhães et al., 2008; Hong et al., 2004). Fermented soybean meal also contains live microorganisms that are beneficial to intestinal tract health (Kim et al., 2009). The functions of the bioactive components released via microbial fermentation should increase overall calf health, mostly because of its immunomodulatory and intestinal tract benefits.

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CHAPTER TWO

THE EFFECTS OF FEEDING FERMENTED SOYBEAN MEAL AS PART OF A STARTER RATION ON GROWTH AND PERFORMANCE OF DAIRY CALVES

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INTRODUCTION

Soybeans have been a significant source of plant origin proteins for both the livestock feed and humans industries for many years. Soybean meal is the most popular protein source in the animal feed industry because of its high protein content and wide availability (Easter and Kim, 1999; Baker, 2000). Unfortunately, the use of soybean meal in animal diets is primarily limited to adult animals due to the inefficient digestibility of soy proteins by young animals and the susceptibility of young animals to antinutritional compounds that are present in soybeans that are either not properly processed or undercooked (Jiang et al., 2000; Baker, 2000). These antinutritional compounds include trypsin inhibitors, lectins, flatulence producing compounds, and many other allergenic proteins (Kim and Baker, 2003; Baker, 2000; Dunsoford et al., 1989). These antinutritional compounds can be denatured by fermentation thereby enabling the use of soybean meal in young animal diets (Feng et al., 2007*b*).

Fermentation of soybean meal not only improves digestion and destroys antinutritional factors like trypsin inhibitors, glycinin, and β-conglycinin, but also releases many bioactive components that are immunomodulatory, antipathogenic, and enhance phagocytosis (Lee, 1998; Kim et al., 2009, Magalhães et al., 2008; Hong et al., 2004). Fermented soybean meal also contains live microorganisms that are beneficial to the intestinal tract health (Kim et al., 2009). The bioactive components released by microbial

fermentation potentialy could increase overall calf health, mostly because of their immunomodulatory and intestinal tract benefits.

Fermented soybean meal can successfully replace animal-derived protein sources such as plasma protein and dried skim milk in piglet nursery diets without adversely affecting the growth performance of the piglets (Kim et al., 2009). In addition, piglets fed the fermented soy responded with increased feed intake, higher nutrient digestibility and absorption, improved growth performance, and reduced diarrhea compared to piglets fed other animal-derived protein sources (Kim et al., 2009). The researchers speculated that these fermented soy products could be incorporated into diets of pre-ruminant calves, ruminants, pets, as well as aquaculture diets (Kim et al., 2009).

In calf rearing programs, the high cost of milk replacers and the relatively low cost of calf starter diets economically favor an early weaning program (Quigley, 1997). Furthermore, the protein ingredients utilized in milk replacers and starter diets constitute a significant portion of the cost associated with these feeds (Quigley et al., 1999). The single most important factor impacting age at weaning is voluntary starter consumption. Therefore, the factors that influence early intake of starter are of great importance to the dairy industry. There are numerous factors that work cooperatively to influence starter consumption by calves, including palatability of the diet. Finding a cost effective and palatable alternative protein for dairy calf starter diets is therefore of great importance. If fermented soybean meal could be incorporated into calf starter diets and yield similar positive results as occurs in nursery piglets (Kim et al., 2009), the effect on the dairy industry could be profound.

The objective of this study was to evaluate the suitability of fermented soybean meal for use in dairy calf starter diets in place of soybean meal. Growth rates, weaning age, and

health and immunological parameters were obtained to evaluate the effects of feeding fermented soybean meal in place of soybean meal in dairy calf starter diets.

METHODS AND MATERIALS

Animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University in Ames, Iowa.

Animals

Eighty dairy bull calves were obtained prior to 5 days of age from dairies in Northeast Iowa and Southwest Wisconsin. Fourteen calves died prior to ingesting significant amounts of starter, and therefore before ingesting significant amounts of either treatment diet (less than 1.0 lb per day). Death losses, therefore, were assumed to be unrelated to treatment. Calves were transported to the research site in Ames, Iowa, where they were housed individually in hutches. Upon arrival, each calf received an ear tag and was administered a single dose of colostrum replacer and electrolytes (Acquire; American Protein Company, Inc., Ankeny, IA; Merrick's Blue Ribbon Calf Electrolytes; Merrick's Animal Nutrition, Inc., Middleton, WI). The calves were allowed to acclimate for 6 days, and on the 7^{th} day they were weighed, a blood sample was collected, and assigned to treatment groups. Calf attitude, appetite, and fecal scores were recorded daily, along with amount of starter consumed and all treatments administered. Fecal scores, attitude scores, and appetite scores of calves on milk replacer were based on a scale of 1 to 3 as described in Table 2. Any amount of milk replacer not consumed by the calf was fed via an esophageal feeder. Weights and blood samples were obtained weekly. Pens were bedded daily and fresh water was given twice daily.

Dietary Treatments

Before and during the trial, calves were fed a 23% crude protein, 15% fat all milk replacer twice daily via a bottle at a total 10% of their birth weight daily (Table 3) (Vigortone VigorMilk 20NT; Vigortone Ag Products, Hiawatha, IA) reconstituted to 2 L with warm tap water at 0630 and 1830 h. Calves were randomly assigned to one of two treatments upon arrival and starter treatments were started on the Sunday following their arrival (40 calves per treatment). All calves were weighed and a blood sample was collected prior to beginning the treatments. The treatments were both complete texturized calf starters with soy protein providing about 45% of the total protein (Table 4). The control starter contained soybean meal as the source of protein and the treated product contained fermented soybean meal in place of soybean meal as the source of protein (Vigortone Calf Starter; Vigortone Ag Products, Hiawatha, IA; PepSoyGen; Nutra-Flo Protein and Biotech Products, Sioux City, IA). Starters were provided daily at a level to ensure *ad libitum* access up to 6 lbs. of starter per day. Dry feed refusal was recorded daily. Once calves consumed 1.5 lbs or greater of starter for three consecutive days they were immediately weaned from milk replacer.

Fermented Soybean Meal

Fermented soybean meal was prepared by a commercial company (Nutra-Flo Protein and Biotech Products, Sioux City, IA) as described by Kim et al. (2009) and Hong et al. (2004). Dried soybean meal was soaked with distilled water for 60 minutes in order to achieve a 35% moisture concentration. Hydrated soybean meal was then cooked in a steam tank at 60 to 70°C for 1 h. Cooked soybean meal was cooled to room temperature for 1 h, and then inoculated with *Aspergillus oryzae* GB-107 and *Bacillus subtilis* GR-101. The soybean meal was next mixed and fermented in a bed-packed incubator for 48 h. After

fermentation, fermented soybean meal was dried at 50˚ to 60°C to achieve a moisture concentration of approximately 10%. The fermented soybean meal was then ground by a hammer mill. Nutrient composition of soybean meal and fermented soybean meal used in this study is shown in Table 4. The manufacturing process is summarized in Figure 2. This manufacturing process enables the fermentation microbes used in fermentation to remain viable and are fed to the calf as direct-fed microbials.

Passive Immunity

Passive immunity was measured in all calves upon arrival at the research site. Blood was collected via jugular venipuncture and tested using the Midland BioProducts Corporation® MBS QTII (Boone, Iowa). The QTII tester used a turbidometric assay to determine IgG concentrations.

Clinical Measurements

Peripheral blood samples were obtained via jugular venipucture weekly from wk 0 through wk 6 of the study from calves 1-48 using 10 ml evacuated test tubes (Vacutainer brand tubes, Becton-Dickinson, Franklin Lakes, NJ) containing 143 USP units sodium heparin. Due to death loss during the study, by the end of the study the number of samples we obtained weekly was n=19 in the control group and n=20 in the treatment group. Samples were collected in the morning prior to the calf receiving milk replacer. Blood samples were used for cell population counts determined by a Mascot™ Hemavet® 850 machine (CDC Technologies Inc., Oxford, CT) and for mitogen proliferation assays, and separate aliquots were assayed by flow cytometry for T- and B-cell subsets.

Mitogen Proliferation

A proliferation assay is used to non-specifically measure lymphocyte activation and to determine the immunocompetence of an animal (Mond and Brunswick, 1994). To prepare the blood for use in the proliferation assay for T-cell function and B-cell function, along with a cytokine secretion assay, whole blood was diluted 1:10 using RPMI 1640 (Life Technologies, Grand Island, NY) that was supplemented with 50µg/ml gentamicin, 25 mM HEPES, and 2 mM of L-glutamine (supplemented media). Diluted whole blood was plated in 96-well cell culture plates (Corning® Costar®, Cambridge, MA) with stock mitogens that were diluted in supplemented media. Equal volumes of blood and mitogens were plated in triplicate to attain final concentrations of mitogens as follows: Concanavalin (Con A) (Sigma-Aldrich® Cell Culture Reagents, St. Louis, MO) at concentrations of 0, 1.0, and 2.5 µg/ml; Lipopolysaccharide (LPS) (Sigma-Aldrich® Cell Culture Reagents, St. Louis, MO) at concentrations of 0, 0.1, and 1.0 µg/ml; and for LPS 0.1 plus corticosterone 1.0 µg/ml (Sigma-Aldrich® Cell Culture Reagents, St. Louis, MO) to represent functionality under stress conditions. The plates containing concavalin A were then incubated for 62 hrs at 37˚ C, 100% humidity, and 7% CO_2. The LPS plates were incubated for 86 hrs at 37˚ C, 100% humidity, and 7% CO_2. The plates were then pulsed with 1 µCi/well of [^{3}H] thymidine (PerkinElmer Life Sciences, Inc., Boston, MA) and incubated again for 9-10 hrs. The cellular DNA was harvested onto glass-fiber paper (Skatron Instruments Inc., Sterling, VA) using a Skatron Combi Cell Havester (Skatron Instruments Inc., Sterling, VA). The dried samples on the glass-fiber paper were suspended in 1.5 ml of CytoScint ES Scintillation Cocktail (ICN Biomedicals, Costa Mesa, CA) and analyzed by a Packard TriCarb 2100TR Liquid Scintillation Analyzer (Packard BioScience Company, Downers Grove, IL).

Flow Cytometry

Flow cytometric analysis was used to examine the proportion of CD4 and CD8 cells in comparison to a memory marker, CD45RO and B-cells (Noguchi, 1994). Whole blood (100 µl) was diluted in an equal volume of phosphate buffered saline (PBS; pH 7.2-7.4; 1.9 mM NaH_2PO_4; 8.1 mM $Na_2H\ PO_4$; 154 mM NaCl) + 0.1% sodium azide in a 5 ml polystyrene round bottom tube (BD Falcon™, Franklin Lakes, NJ). The primary antibodies were then added to the tubes to create one tube for co-analysis of CD4+ (total T-helper) and CD45RO+ (memory T-helpers), one tube for co-analysis of CD8+ (total T-cytotoxic) and B cell+ population, and one tube for an isotype control. After incubation with primary antibodies for 40 minutes at 4˚ C, red blood cells were removed using an ammonium chloride lysing solution (150 mM NH_4Cl; 10 mM $NaHCO_3$; 1 mM EDTA). The cells were next washed with PBS + 0.1% sodium azide. Appropriate secondary antibodies were incubated for 40 minutes at 4˚ C. At the end of this incubation, the cells were washed with PBS + 0.1% sodium azide, vortexed, and Strepavidin-cychrome was then added to the CD4/CD45RO and the isotype tubes with a final incubation period of 40 minutes at 4˚ C. After the final wash with PBS + 0.1% sodium azide, all sets of tubes were fixed with PBS + 1% formaldehyde (methanol free; Polysciences, Inc., Warrington, PA) until flow cytometric analysis was performed at the Iowa State University Cell Hybridoma Facility.

Statistical Analysis

Analysis of variance was performed using the general linear model and mixed procedures of SAS (SAS Institute, 2003). Dependent variables included weight gain, attitude scores, appetite scores, fecal scores, health, and amount of feed being consumed. The data was sorted using PROC SORT by treatment and by amount of feed being consumed.

Significance was declared at $P < 0.05$ unless otherwise noted and probability values between 0.05 and 0.15 were defined as tendency towards significance. Standard errors presented were for the differences among least squares means.

RESULTS

Results were analyzed using four sets of parameters. The first parameter analyzed the data according to treatment. There were 34 calves in the control group and 32 calves in the FSBM (fermented soybean meal) group. The second parameter analyzed the data according to treatment and whether or not the calf received any medical treatments (electrolytes, antibiotics, or antivirals). If the calf had received one or more medical treatments over the course of the study, it was classified in the "sick" category. If the calf had not received any medical treatments over the course of the study, it was classified in the "healthy" category. In the control group, there were 15 calves in the healthy group and 19 calves in the sick group. In the FSBM group, there were 12 calves in the healthy group and 20 calves in the sick group. Even though the difference in medical treatments between the two groups was not significantly different (Table 6), the analysis was designed to determine whether interactions between health status and treatment occurred. The third set of parameters included treatment and feed group by week. There were 4 possible feed groups. The first feed group included calves that consumed less than 1 pound of starter per day during that particular week. The second feed group included calves that consumed between 1 and 2 pounds of starter daily. The third feed group included calves that consumed more than 2 pounds of starter per day, but were not weaned. Finally, the fourth feed group included calves that were weaned during that week. Data was analyzed according to these feed groups because treatment was in the starter diet, so the amount of starter that the calf was consuming

might impact the response to the treatment. Finally, immunological data was analyzed according to a treatment and health interaction by feed groups.

During week 3 of the trial, there was a severe drop in temperatures. This caused data from all calves to show a decrease in performance and immunological measures. This decrease was seen equally across treatments, and therefore did not affect the results.

Passive Immunity

Blood IgG concentrations were measured on all calves, and the calves that died tended to have significantly lower blood IgG concentrations when compared to the calves that survived (p=.1240) (Table 1). Additionally, calves that received one or more medical treatments during the course of the study had significantly lower blood IgG concentrations than calves that received no medical treatments during the course of the study (Table 1). Calves on the control diet did not have significantly lower blood IgG concentrations than calves on the treatment diet (Table 1). Blood IgG concentration upon arrival profoundly impacted calf performance.

Weight Gain

In this study, weekly weight gains and total weight gain were not significantly different by treatment, nor was the total weight gained over the course of the study (Table 5 and Figure 3). Additionally, health status within treatment did not affect weight gain of the calves in this study (Table 6 and Figures 4 and 5). This is not surprising since the feed eaten weekly was not significantly different between the control and FSBM groups (Tables 5 and 6 and Figures 6 and 7). Feeding FSBM in place of SBM did not increase voluntary starter consumption or feed efficiency.

Weaning Age

The most important factor affecting the cost of raising dairy calves is weaning age. In this study, weaning age was adversely affected by feeding the FSBM diet. The mean age at weaning, in days, was significantly older for calves on the FSBM diet in comparison to the SBM diet (P=0.0422; Table 5). When data was analyzed according to calf health within treatment, there was no significant difference between weaning ages (Table 6).

Attitude, Appetite, and Fecal Scores

Attitude, appetite, and fecal scores were not significantly different between groups of calves whether analyzed by treatment, health status or feed intake (Tables 5-7 and Figures 8-13). This suggests that calves fed FSBM did not have increased digestive disturbances in comparison to calves fed SBM based starters.

Mitogen Proliferation

For the mitogen proliferation assays, concavalin A, lipopolysaccharide (LPS), and lipopolysaccharide with corticosterone was used to stimulate the cells in the samples. Concavalin A stimulates T-lymphocytes, while LPS stimulates B-lymphocytes. Corticosterone was added to simulate a stress response. We stimulated the cells in the samples at two different concentrations of mitogens: sub-optimal and optimal. Concavalin A at 1.0 µg/ml and LPS at 0.1 µg/ml are considered suboptimal conditions, and demonstrate the ability of lymphocytes to respond to low levels of antigen. Concavalin A at 2.5 µg/ml and LPS at 1.0 µg/ml concentrations, however, are considered optimal conditions and should stimulate a majority of lymphocytes capable of proliferation to respond to the antigen. Before analyzing the data obtained from the mitogen proliferation assays, we corrected the data to account for the number of lymphocytes present in the sample, as counted by the

Mascot™ Hemavet® 850 machine (CDC Technologies Inc., Oxford, CT). For the statistical analysis, we compared week to week differences in data according to feed groups and between treatments. Although there were some feed groups that had significant differences between treatments, there were no consistent patterns suggesting that the FSBM diet was able to improve the efficiency of the calf's immune system or accelerate the development of adaptive immunity (Table 8) or that the health status of the animal interacted with the treatment (Table10). Because feed groups were based on calf starter intake, the number of calves in each group changed on a weekly basis. Therefore, each week, some groups did not have adequate numbers of animals to permit comparison of immunological parameters with other groups by statistical analysis.

Flow Cytometry

For the statistical analysis of the data obtained from the flow cytometer, week to week differences in data were analyzed between feed intake groups and between treatments. Within the data collected from the flow cytometer, no trends suggesting improved immunologic responses were evident (Table 9) or that the health status of the animal interacted with the treatment to produce different results (Table 11). Because feed groups were based on calf starter intake, the number of calves in each group changed on a weekly basis. Therefore, each week, some groups did not have adequate numbers of animals to permit comparison of immunological parameters with other groups by statistical analysis.

Table 1. IgG blood concentrations.

	[1]Calves that lived	[2]Calves that Died	P-value
IgG mg/dl	1102.46562	845.33125	0.124

	[3]Health Calves	[4]Sick Calves	P-value
IgG mg/dl	1309.58083	1024.14206	0.0397

	[5]SBM	[6]FSBM	P-value
IgG mg/dl	1182.33778	1151.38512	0.8205

[1]Calves that lived: Data from all calves that survived during the course of the trial.
[2]Calves that died: Data from all calves that died during the course of the trial.
[3]Healthy calves: Data from all calves that received no medical treatments during the course of the study.
[4]Sick calves: Data from all calves that received one or more medical treatments during the course of the study.
[5]SBM: Data from calves in the control group fed a starter diet with soybean meal as the main protein ingredient.
[6]FSBM: Data from calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient.

Table 2. Response criteria for subjective clinical measures.[1]

	Clinical Score		
Clinical Measure	1	2	3
Fecal	normal, firm	soft, spreads easily	Very runny or watery, liquid consistency
Attitude	Alert and responsive	Nonactive and moderately lethargic	Severely lethargic; recumbent and will not rise
MR Appetite	Calf suckled aggressively	Calf suckled, but not aggressively and may not have finished the bottle	Calf did not suckle at all

[1]Scoring was conducted by a common person throughout the study

Table 3. Milk replacer composition.

Nutrient	
Crude Protein, min	23%
Crude Fat, min	15%
Crude Fiber, max	0.15%
Calcium, min	0.75%
Calcium, max	1.25%
Phosphorus, min	0.70%
Vitamin A, min (IU/lb)	40,000
Vitamin D_3, min (IU/lb)	10,000
Vitamin E, min (IU/lb)	125.00%

Ingredients: Dried Whey, Dried Whey Protein Concentrate, Dried Whey Product, Animal Fat (preserved with BHA, BHT, Citric Acid & Ethoxyquin), Propylene Glycol, Dried Skimmed Milk, L-Lysine, Calcium Carbonate, DL-Methionine, Dicalcium Phosphate, Sodium Silico Aluminate, Artificial Flavor, Vitamin E Supplement, Ferrous Sulfate, Magnesium Sulfate, Choline Chloride, Maltodextrin, Selenium Yeast, Zinc Sulfate, Vitamin A Supplement, Manganese Sulfate, Copper Sulfate, Vitamin D3 Supplement, Ascorbic Acid, Niacin Supplement, Calcium Pantothenate, Menadione Sodium Bisulfite Complex (source of Vitamin K activity), Biotin, Riboflavin Supplement, Thiamine Mononitrate, Pyridoxine Hydrochloride, Vitamin B12 Supplement, Ethylenediamine Dihydriodide, Folic Acid, Cobalt Sulfate.

Table 4. Diet compositions[1]: soybean meal based starter diet and fermented soybean meal based starter diet (as-fed basis).

Item	SBM	FSBM
Ingredient %		
SBM	71.5	-
PepSoyGen	-	61.5
Corn, ground	13.15	23.15
Vit/min Premix	7.85	7.85
Wheat Middlands	7.5	7.5
Composition		
Crude Protein (%)	35.61	35.8
Dry Matter (%)	90.52	91.49
DE (Kcal/lb)	1503	1482.14
ME (Kcal/lb)	1400.89	1402.18
NFC (%)	29.4	30.24
NE Maint (Mcal/lb)	0.16	0.25
NE Gain (Mcal/lb)	0.11	0.17
ADF (%)	6.17	5.81
Calcium (%)	2.26	2.24
Phosphorus (%)	0.7	0.71
Cal:Phos Ratio	3.24:1	3.16:1
Potassium (%)	1.63	0.98
Salt (%)	1.42	1.42
Vit A, Added (IU/lb)	21.41	21.41
Vit D_3, Added (IU/lb)	4.28	4.28
Vit E, Added (IU/lb)	70.65	70.65
Se Added (ppm)	0.86	0.86
Copper (ppm)	47.89	52.19
Zinc (ppm)	177.41	201.4

[1] Diet compositions were calculated by Vigortone prior to manufacturing the diet (Vigortone Calf Starter; Vigortone Ag Products, Hiawatha, IA).

Table 5. Mean growth performance of dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration.

Item	SMB	FSMB	P-value
Weaning Age (d)	22.007	26.05	0.0422
Total Medicine Treatments	3.47	2.78	NS
Weight (kg)			
Initial	44.42524	43.83938	NS
Week 1	45.06618	44.77575	NS
Week 2	48.59135	47.74094	NS
Week 3	49.96671	49.68463	NS
Week 4	56.22924	56.55138	NS
Week 5	61.10306	60.382	NS
Week 6	68.60741	67.70275	NS
Total Weight Gain	24.40976	23.90673	NS
Feed Eaten (kg)			
Week 1	0.076277	0.085976	NS
Week 2	0.300014	0.292319	NS
Week 3	0.613186	0.661822	NS
Week 4	1.246897	1.276354	NS
Week 5	1.797535	1.785882	NS
Week 6	2.209066	2.15021	NS
Attitude Score			
Week 1	1.223629	1.169643	NS
Week 2	1	1.008929	NS
Week 3	1	1	NS
Week 4	1	1	NS
Week 5	1.016807	1.004464	NS
Week 6	1.005882	1	NS
Appetite Score			
Week 1	1.050633	1.013393	NS
Week 2	1	1.008929	NS
Week 3	1	1	NS
Week 4	1	1	NS
Week 5	1	1	NS
Week 6	1	1	NS
Fecal Score			
Week 1	1.818565	1.767857	NS
Week 2	1.634454	1.558036	NS
Week 3	1.647059	1.589286	NS
Week 4	1.47479	1.4375	NS
Week 5	1.243697	1.267857	NS
Week 6	1.229412	1.225	NS

[1]SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient.

[2]FSMB: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient.

Table 6. Mean growth performance data for dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on a treatment by health status interaction.

Items	[1]SMBH	[2]FSBMH	P-value	[3]SBMS	[4]FSBMS	P-value
Total Weight Gain (kg)	26.47683	24.01388	NS	22.3427	23.79959	NS
Mean Medical Treatments	0	0	NS	6.947	5.55	NS
Mean Weaning Age (d)	21.0114	26.267	NS	23.011	25.825	NS
Mean Daily Feed Consumed (kg)						
Week 1	0.090618	0.043735	0.0457	0.065073	0.111321	0.0457
Week 2	0.290318	.227908	NS	0.2724	0.330966	NS
Week 3	0.661889	0.628682	NS	0.574737	.681849	NS
Week 4	1.393824	1.279415	NS	1.132248	1.27449	NS
Week 5	1.964566	1.768762	NS	1.665668	1.796154	NS
Week 6	2.25527	2.065574	NS	2.172589	2.200992	NS
Attitude Score						
Week 1	1.047619	1.047619	NS	1.363636	1.185714	NS
Week 2	1	1	NS	1	1.014286	NS
Week 3	1	1	NS	1	1	NS
Week 4	1	1	NS	1	1	NS
Week 5	1	1	NS	1.030075	1.007143	NS
Week 6	1.013158	1.013158	NS	1	1	NS
MR Appetite Score						
Week 1	1.028571	1.011905	NS	1.068182	1.014286	NS
Week 2	1	1.02381	NS	1	1	NS
Week 3	1	1	NS	1	1	NS
Week 4	1	1	NS	1	1	NS
Week 5	1	1	NS	1	1	NS
Week 6	1	1	NS	1	.	NS
Fecal Score						
Week 1	1.619048	1.690476	NS	1.977273	1.814286	NS
Week 2	1.6	1.440476	NS	1.661654	1.628571	NS
Week 3	1.428571	1.380952	NS	1.819549	1.714286	NS
Week 4	1.228571	1.190476	NS	1.669173	1.585714	NS
Week 5	1.161905	1.154762	NS	1.308271	1.335714	NS
Week 6	1.131579	1.116667	NS	1.308511	1.29	NS

[1]SMB H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that received no medical treatments during the course of the project.

[2]FSBM H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that received no medical treatments during the course of the project.

[3]SBM S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that received one or more medical treatments during the course of the project.

[4]FSBM S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that received one or more medical treatments during the course of the project.

Table 7. Mean performance data of dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on starter diet consumption.

Item	[1]SBM0	[2]FSMB0	P-value	[3]SBM1	[4]FSBM1	P-value	[5]SBM2	[6]FSBM2	P-value	[7]SBM3	[8]FSBM3	P-value
Attitude Score												
Week 1	1.238	1.167	NS	1	1.214	NS	-	-	-	-	-	-
Week 2	1	1	NS	1	1.022	NS	-	1	-	-	-	-
Week 3	1	1	NS	1	1	NS	1	1	NS	1	1	NS
Week 4	1	-	-	1	1	NS	1	1	NS	1	1	NS
Week 5	1.429	-	-	1	1	NS	-	-	-	1.004	1.005	NS
Week 6	1	-	-	-	-	-	1	-	-	1	1	NS
MR Appetite Score												
Week 1	1.054	1.014	NS	1	1	NS	-	-	-	-	-	-
Week 2	1	1.016	NS	1	1	NS	-	1	-	-	-	-
Week 3	1	1	NS	1	1	NS	1	1	NS	1	1	NS
Week 4	1	-	-	1	1	NS	1	1	NS	1	1	NS
Week 5	1	-	-	1	1	NS	-	-	-	1	1	NS
Week 6	1	-	-	-	-	-	1	-	-	-	-	-
Fecal Score												
Week 1	1.825	1.8	NS	1.714	1.286	NS	-	-	-	-	-	-
Week 2	1.65	1.571	NS	1.612	1.56	NS	-	1.286	-	-	-	-
Week 3	1.619	1.929	NS	1.724	1.626	NS	1.143	1.321	NS	1.648	1.582	NS
Week 4	1.286	-	-	1.714	1.524	NS	1.643	1.429	NS	1.444	1.417	NS
Week 5	1.143	-	-	1	1.571	NS	-	-	-	1.254	1.258	NS
Week 6	1.5	-	-	-	-	-	1.4	-	-	1.213	1.444	NS

[1]SBM 0: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.
[2]FSMB 0: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.
[3]SBM 1: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.
[4]FSBM 1: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.
[5]SBM 2: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.
[6]FSBM 2: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.
[7]SBM 3: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week.
[8]FSBM 3: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week.

Table 8. Mean mitogen proliferation data for dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on starter diet consumption.

Item	[1]SBM0	[2]FSMB0	P-value	[3]SBM1	[4]FSBM1	P-value	[5]SBM2	[6]FSBM2	P-value	[7]SBM3	[8]FSBM3	P-value
Δ Con A 0												
Week 1	862.7	1406.48	0.014	-	-	-	-	-	-	-	-	-
Week 2	759.81	634.86	NS	-	-	-	-	-	-	-	-	-
Week 3	311.96	724.22	<0.001	431.54	695.91	0.1259	-	-	-	-	-	-
Week 4	-	-	-	233.56	1105.56	0.0003	-	-	-	540.95	1043.82	NS
Week 5	-	-	-	336.68	1441.87	NS	-	-	-	782.6	1363.99	NS
Week 6	-	-	-	-	-	-	-	-	-	1003.78	676.92	NS
Δ Con A 1.0												
Week 1	289225	535243	0.021	-	-	-	-	-	-	-	-	-
Week 2	70038	74423	NS	-	-	-	-	-	-	-	-	-
Week 3	24923	24960	NS	10296	66115	NS	-	-	-	-	-	-
Week 4	-	-	-	21598	33851	NS	-	-	-	17566	37842	NS
Week 5	-	-	-	61256	221660	0.024	-	-	-	336204	500869	NS
Week 6	-	-	-	-	-	-	-	-	-	544599	303134	0.076
Δ Con A 2.5												
Week 1	744884	1729058	NS	-	-	-	-	-	-	-	-	-
Week 2	226117	222720	NS	-	-	-	-	-	-	-	-	-
Week 3	63505	80070	NS	61396	203366	NS	-	-	-	-	-	-
Week 4	-	-	-	57287	140347	0.036	-	-	-	66553	106379	NS
Week 5	-	-	-	473294	349441	NS	-	-	-	514438	840247	0.1088
Week 6	-	-	-	-	-	-	-	-	-	880838	994393	NS
Δ LPS 0												
Week 1	1915.22	2383.84	NS	-	-	-	-	-	-	-	-	-
Week 2	1028.17	2189.24	.005	-	-	-	-	-	-	-	-	-
Week 3	791.95	1907.39	0.004	2426.32	2221.54	NS	-	-	-	-	-	-
Week 4	-	-	-	810.69	1175.25	0.047	-	-	-	475	2207.52	0.1208
Week 5	-	-	-	1348.18	1308.21	NS	-	-	-	1184.3	1684.07	NS
Week 6	-	-	-	-	-	-	-	-	-	946.72	1149.08	NS

[1]SBM 0: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.
[2]FSMB 0: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.
[3]SBM 1: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.
[4]FSBM 1: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.
[5]SBM 2: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.
[6]FSBM 2: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.
[7]SBM 3: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week.
[8]FSBM3: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week.
Week 1: SBM 0 n=20; FSMB 0 n=19
Week 2: SBM 0 n=19; FSMB 0 n=17; SBM 1 n=1; FSMB 1 n=3;
Week 3: SBM 0 n=14; FSMB 0 n=9; SBM 1 n=6; FSMB 1 n=9; FSMB 2 n=1;
Week 4: SBM 0 n=2; FSMB 0 n=1; SBM 1 n=14; FSMB 1 n=7; SMB 2 n=1; FSMB 2 n=2; SMB 3 n=3; FSMB 3 n=9;
Week 5: SBM 0 n=1; FSMB 0 n=0; SBM 1 n=2; FSMB 1 n=2; SMB 2 n=1; FSMB 2 n=1; SMB 3 n=16; FSMB 3 n=15;
Week 6: SBM 0 n=1; FSMB 0 n=0; SBM 1 n=1; FSMB 1 n=1; SMB 2 n=0; FSMB 2 n=0; SMB 3 n=18; FSMB 3 n=18;

Table 8. (continued)

Item	[1]SBM0	[2]FSMB0	P-value	[3]SBM1	[4]FSBM1	P-value	[5]SBM2	[6]FSBM2	P-value	[7]SBM3	[8]FSBM3	P-value
Δ LPS 0.1												
Week 1	3729.47	6043.4	NS	-	-	-	-	-	-	-	-	-
Week 2	3271.96	7278.49	NS	-	-	-	-	-	-	-	-	-
Week 3	1957.28	4695.88	0.135	9516.07	10525	NS	-	-	-	-	-	-
Week 4	-	-	-	1584.33	4245.43	0.0876	-	-	-	2304.78	2304.38	NS
Week 5	-	-	-	14275	6920.21	NS	-	-	-	11947	8890.29	NS
Week 6	-	-	-	-	-	-	-	-	-	6757.19	7731.63	NS
Δ LPS 0.1+Cort 1.0												
Week 1	4785.4	6833.47	NS	-	-	-	-	-	-	-	-	-
Week 2	972.52	1561.79	NS	-	-	-	-	-	-	-	-	-
Week 3	3537.26	5556.01	NS	1891.72	3321.52	NS	-	-	-	-	-	-
Week 4	-	-	-	539.12	941.24	0.044	-	-	-	956.9	978.12	NS
Week 5	-	-	-	663.28	6466.05	NS	-	-	-	3411.42	3438.46	NS
Week 6	-	-	-	-	-	-	-	-	-	1707.74	2345.45	NS
Δ LPS 1.0												
Week 1	3729.47	6043.4	NS	-	-	-	-	-	-	-	-	-
Week 2	3271.96	7278.49	NS	-	-	-	-	-	-	-	-	-
Week 3	1957.28	4695.88	0.135	9516.07	10525	NS	-	-	-	-	-	-
Week 4	-	-	-	1584.33	4245.43	0.088	-	-	-	2304.38	1635.78	NS
Week 5	-	-	-	14275	6920.21	NS	-	-	-	11947	8890.29	NS
Week 6	-	-	-	-	-	-	-	-	-	6757.19	7731.63	NS

Table 9. Mean percent of cells with specific markers as analyzed by flow cytometry for dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on starter diet consumption.

Item	[1]SBM0	[2]FSMB0	P-value	[3]SBM1	[4]FSBM1	P-value	[5]SBM2	[6]FSBM2	P-value	[7]SBM3	[8]FSBM3	P-value
Δ CD 4												
Week 1	40.883	39.17	NS	-	-	-	-	-	-	-	-	-
Week 2	34.598	34.596	NS	-	-	-	-	-	-	-	-	-
Week 3	22.429	24.222	NS	23.072	25.53	NS	-	-	-	-	-	-
Week 4	-	-	-	39.329	36.566	NS	-	-	-	37.802	37.733	NS
Week 5	-	-	-	36.443	42.057	NS	-	-	-	39.019	39.186	NS
Week 6	-	-	-	-	-	-	-	-	-	34.268	33.337	NS
Δ CD4/45												
Week 1	13.403	13.037	NS	-	-	-	-	-	-	-	-	-
Week 2	12.405	13.286	NS	-	-	-	-	-	-	-	-	-
Week 3	10.321	10.071	NS	12.977	11.709	NS	-	-	-	-	-	-
Week 4	-	-	-	11.765	15.147	0.009	-	-	-	14.619	14.407	NS
Week 5	-	-	-	12.25	16.7	NS	-	-	-	12.64	12.438	NS
Week 6	-	-	-	-	-	-	-	-	-	12.396	14.543	NS
Δ CD8												
Week 1	14.046	13.283	NS	-	-	-	-	-	-	-	-	-
Week 2	14.875	14.209	NS	-	-	-	-	-	-	-	-	-
Week 3	20.755	22.714	NS	25.983	23.087	NS	-	-	-	-	-	-
Week 4	-	-	-	14.158	16.992	NS	-	-	-	14.057	13.904	NS
Week 5	-	-	-	15.956	21.03	NS	-	-	-	16.506	15.779	NS
Week 6	-	-	-	-	-	-	-	-	-	17.93	16.992	NS
Δ B cell												
Week 1	6.997	6.466	NS	-	-	-	-	-	-	-	-	-
Week 2	7.109	8.285	0.015	-	-	-	-	-	-	-	-	-
Week 3	6.899	7.001	NS	8.312	10.335	NS	-	-	-	-	-	-
Week 4	-	-	-	7.745	8.674	NS	-	-	-	10.153	8.607	NS
Week 5	-	-	-	11.261	6.993	NS	-	-	-	10.176	9.063	NS
Week 6	-	-	-	-	-	-	-	-	-	11.132	11.996	NS

Δ: Signifies the marker subtracted from the isotype

[1]SBM 0: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.

[2]FSMB 0: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week.

[3]SBM 1: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.

[4]FSBM 1: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week.

[5]SBM 2: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.

[6]FSBM 2: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week.

[7]SBM 3: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week.

[8]FSBM3: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week.

Week 1: SBM 0 n=20; FSMB 0 n=19

Week 2: SBM 0 n=19; FSMB 0 n=17; SBM 1 n=1; FSMB 1 n=3;

Week 3: SBM 0 n=14; FSMB 0 n=9; SBM 1 n=6; FSMB 1 n=9; FSMB 2 n=1;

Week 4: SBM 0 n=2; FSMB 0 n=1; SBM 1 n=14; FSMB 1 n=7; SMB 2 n=1; FSMB 2 n=2; SMB 3 n=3; FSMB 3 n=9;

Week 5: SBM 0 n=1; FSMB 0 n=0; SBM 1 n=2; FSMB 1 n=2; SMB 2 n=1; FSMB 2 n=1; SMB 3 n=16; FSMB 3 n=15;

Week 6: SBM 0 n=1; FSMB 0 n=0; SBM 1 n=1; FSMB 1 n=1; SMB 2 n=0; FSMB 2 n=0; SMB 3 n=18; FSMB 3 n=18;

Table 10. Mean mitogen proliferation data for dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on starter diet consumption with a treatment by health interaction.

Item	[1]SBM0 H	[2]FSMB0 H	P-value	[3]SBM0 S	[4]FSMB0 S	P-value	[5]SBM1 H	[6]FSBM1 H	P-value	[7]SBM1 S	[8]FSBM1 S	P-value	[9]SBM2 H	[10]FSBM2 H	P-value	[11]SBM2 S	[12]FSBM2 S	P-value	[13]SBM3 H	[14]FSBM3 H	P-value	[15]SBM3 S	[16]FSBM3 S	P-value
Δ Con A 0																								
Week 1	565.88	1181.76	0.1244	1068.7	1459.9	0.1373	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
Week 2	1142.66	548.87	NS	534.73	662.93	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3	312.68	835.57	0.0011	311.14	635.57	0.0109	248.94	-	-	508.16	702.44	NS	-	-	-	-	-	-	-	-	-	-	-	-

Δ: Signifies the marker subtracted from the isotype

[1]SBM 0 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[2]FSMB 0 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[3]SBM 0 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[4]FSMB 0 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[5]SBM 1 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[6]FSBM 1 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[7]SBM 1 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[8]FSBM 1 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[9]SBM 2 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving no medical treatments during the course of the study.

[10]FSBM 2 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving no medical treatments during the course of the study.

[11]SBM 2 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving one or more medical treatments during the course of the study.

[12]FSBM 2 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving one or more medical treatments during the course of the study.

[13]SBM 3 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week and receiving no medical treatments during the course of the study.

[14]FSBM3 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week and receiving no medical treatments during the course of the study.

[15]SBM 3 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week and receiving one or more medical treatments during the course of the study.

[16]FSBM3 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week and receiving one or more medical treatments during the course of the study.

Week 1: SBM 0 H n=8; FSMB 0 H n=4; SBM 0 S n=12; FSMB 0 S n=13; FSBM 1 S n=2.

Week 2: SBM0 H n=4; FSMB 0 H n=4; SBM 0 S n=8; FSMB 0 S n=5; SBM 1 H n=3; SBM 1 S n=5; FSMB 1 S n=9.

Week 3: SBM 0 S n=2; FSMB 0 S n=1; SBM 1 H n=2; FSMB 1 H n=3; SBM 1 S n=6; FSMB 1 S n=4; SBM 2 H n=1; SBM 3 H n=5; FSBM 3 H n=1; SBM 3 S n=4; FSBM 3 S n=8.

Week 4: SBM 0 S n=1; FSBM 1 H n=1; SBM 1 S n=2; FSMB 1 S n=2; SBM 2 S n=1; FSBM 2 S n=1; SBM 3 H n=8; FSBM 3 H n=3; SBM 3 S n=8; FSBM 3 n=12.

Week 5: SBM 0 S n=1; FSMB 1 H n=1; SBM 1 S n=1; SBM 3 H n=8; FSBM 3 H n=3; SBM 3 S n=10; FSBM 3 n=15.

Week 6: SBM 0 S n=1; SBM 2 S n=1; SBM 3 H n=8; FSBM 3 H n=4; SBM 3 S n=10; FSBM 3 n=15.

Table 10. (continued)

Item		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Δ Con A 1.0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Δ Con A 2.5
[1]SBM0 H	-	723805	302865	82648	-	-	-	-	291784	75195	38817	-	-	-	-
[2]FSMB0 H	-	902614	64560	73153	-	-	-	-	584920	61782	28978	-	-	-	-
P-value	-	NS	0.1285	NS	-	-	-	-	0.1398	NS	NS	-	-	-	-
[3]SBM0 S	-	777117	186834	44861	-	-	-	-	288687	66949	13950	-	-	-	-
[4]FSMB0 S	-	1934899	266320	92463	-	-	-	-	521062	78387	22631	-	-	-	-
P-value	-	NS	NS	NS	-	-	-	-	0.0787	NS	NS	-	-	-	-
[5]SBM1 H	53853	8317.82	-	-	-	-	-	-	-	-	1460.85	18620	-	-	-
[6]FSBM1 H	108794	-	-	-	-	224083	-	-	-	-	-	48035	-	-	-
P-value	NS	-	-	-	-	-	-	-	-	-	-	NS	-	-	-
[7]SBM1 S	58504	87754	-	-	-	61173	-	-	-	-	14673	22526	-	-	-
[8]FSBM1 S	164403	203446	-	-	-	220532	48035	-	-	-	66133	48035	-	-	-
P-value	0.0471	NS	-	-	-	NS	NS	-	-	-	NS	NS	-	-	-
[9]SBM2 H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[10]FSBM2 H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P-value	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[11]SBM2 S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[12]FSBM2 S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P-value	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[13]SBM3 H	62837	-	-	-	-	273894	488049	-	-	-	-	16546	856.12	856.12	620.23
[14]FSBM3 H	18862	-	-	-	-	475361	2183.08	-	-	-	-	282.03	604.31	604.31	215.25
P-value	NS	-	-	-	-	NS	0.1291	-	-	-	-	NS	NS	NS	NS
[15]SBM3 S	-	-	-	-	-	400477	594815	-	-	-	-	-	1127.29	1127.29	693.94
[16]FSBM3 S	118712	-	-	-	-	505937	339944	-	-	-	-	42990	683.02	683.02	1434.69
P-value	-	-	-	-	-	NS	0.1125	-	-	-	-	-	NS	NS	NS

Table 10. (continued)

Item	[1]SBM0 H	[2]FSMB0 H	P-value	[3]SBM0 S	[4]FSMB0 S	P-value	[5]SBM1 H	[6]FSBM1 H	P-value	[7]SBM1 S	[8]FSBM1 S	P-value	[9]SBM2 H	[10]FSBM2 H	P-value	[11]SBM2 S	[12]FSBM2 S	P-value	[13]SBM3 H	[14]FSBM3 H	P-value	[15]SBM3 S	[16]FSBM3 S	P-value
Week 5	-	-	-	-	-	-	-	152614	-	512503	561259	NS	-	-	-	-	-	-	520767	778952	NS	508200	855511	NS
Week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	963799	611911	NS	822902	983102	NS
Δ LPS 0																								
Week 1																								
Week 2		979.21	2395.95	0.0565	1058.53	2123.98	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3		1020.46	1862.24	0.142	624.04	1937.95	0.0089	796.21	-	3034.62	2324.91	NS	-	-	-	-	-	-	-	-	-	-	-	-
Week 4	-	-	-	-	-	-	-	815.18	1226.73	809.95	1134	NS	-	-	-	-	-	-	443.48	2665.95	NS	-	2155.53	-
Week 5	-	-	-	-	-	-	-	-	1369.46	1354.58	1271.2	NS	-	-	-	-	-	-	1140.42	1136.94	NS	1227.59	1821.25	NS
Week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	969.97	1180.4	NS	928.41	1144.71	NS
Δ LPS 0.1																								
Week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 2	1267.02	2916.11	NS	2772.08	9798.08	0.0757	-	1861.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3	2404.59	8568.08	NS	4226.3	7217.66	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 4	-	-	-	-	-	-	1542.59	7540.1	-	0.0207	2012.64	NS	-	-	-	-	-	-	264.63	3157.48	0.1074	-	2900.43	-
Week 5	-	-	-	-	-	-	-	75974	-	-	5657.63	5513.52	NS	-	-	-	-	-	8917.37	2445.07	NS	11931	16651	NS

Table 10. (continued)

Item	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Δ LPS 0.1+Cort 1.0						
[1]SBM0 H	-	-	4084.54	-	-	-
[2]FSMB0 H	5383.32	1139.56	7029.23	-	-	-
P-value	NS	NS	NS	-	-	-
[3]SBM0 S	4394.93	872.59	3088.57	-	-	-
[4]FSMB0 S	7119.99	1508.47	4481.8	-	-	-
P-value	0.1218	NS	NS	-	-	-
[5]SBM1 H	-	-	1293.61	458.78	-	-
[6]FSBM1 H	-	-	-	824.66	9437.39	-
P-value	-	-	-	NS	-	-
[7]SBM1 S	-	-	2158.98	571.75	2149.19	-
[8]FSBM1 S	-	-	3337.42	1027.45	3494.48	-
P-value	-	-	-	0.0941	NS	-
[9]SBM2 H	-	-	-	-	-	-
[10]FSBM2 H	-	-	-	-	-	-
P-value	-	-	-	-	-	-
[11]SBM2 S	-	-	-	-	-	-
[12]FSBM2 S	-	-	-	-	-	-
P-value	-	-	-	-	-	-
[13]SBM3 H	-	-				23781
[14]FSBM3 H	-	-				31410
P-value	-	-				NS
[15]SBM3 S	-	-				8515.66
[16]FSBM3 S	-	-				23940
P-value	-	-				NS
Δ LPS 1.0						
[1]SBM0 H	4488.87	1812.84	1384.15	-	-	-
[2]FSMB0 H	4690.47	5741.17	7719.7	-	-	-
P-value	NS	NS	0.0225	-	-	-
[3]SBM0 S	3240.98	4132.3	2295	-	-	-
[4]FSMB0 S	6414.72	7781.74	2424.23	-	-	-
P-value	NS	NS	NS	-	-	-
[5]SBM1 H	-	-	1311.66	1868.77	-	-
[6]FSBM1 H	-	-	-	8308.65	72186	-
P-value	-	-	-	0.0039	-	-
[7]SBM1 S	-	-	13326	1464.31	34462	-
[8]FSBM1 S	-	-	10671	1213.63	26286	-
P-value	-	-	NS	NS	NS	-
[9]SBM2 H	-	-	-	-	-	-
[10]FSBM2 H	-	-	-	-	-	-
P-value	-	-	-	-	-	-
[11]SBM2 S	-	-	-	-	-	-
[12]FSBM2 S	-	-	-	-	-	-
P-value	-	-	-	-	-	-
[13]SBM3 H	-	-	-	2455.51	10869	10157
[14]FSBM3 H	-	-	-	458.87	56.0409	10221
P-value	-	-	-	NS	NS	NS
[15]SBM3 S	-	-	-	-	13245	4028.13
[16]FSBM3 S	-	-	-	1739.14	10981	7405.66
P-value	-	-	-	-	NS	NS

Table 11. Mean percent of cells with specific markers as analyzed by flow cytometry for dairy calves fed diets with or without fermented soybean meal replacing soybean meal in the starter ration separated into groups based on starter diet consumption with a treatment by health interaction.

Item	[1]SBM0 H	[2]FSMB0 H	P-value	[3]SBM0 S	[4]FSMB0 S	P-value	[5]SBM1 H	[6]FSBM1 H	P-value	[7]SBM1 S	[8]FSBM1 S	P-value	[9]SBM2 H	[10]FSBM2 H	P-value	[11]SBM2 S	[12]FSBM2 S	P-value	[13]SBM3 H	[14]FSBM3 H	P-value	[15]SBM3 S	[16]FSBM3 S	P-value
Δ CD 4																								
Week 1	40.1563	37.6742	NS	41.3024	39.6216	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 2	32.1347	36.0901	NS	36.0392	34.1328	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3	23.2252	21.1125	NS	21.8248	26.72	NS	27.009	-	-	21.2529	25.4634	NS	-	-	-	-	-	-	-	-	-	-	-	-

Δ: Signifies the marker subtracted from the isotype

[1]SBM 0 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[2]FSMB 0 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[3]SBM 0 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[4]FSMB 0 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed less than 1.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[5]SBM 1 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[6]FSBM 1 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving no medical treatments during the course of the study.

[7]SBM 1 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[8]FSBM 1 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed between 1.0 and 2.0 lbs daily of starter for that week and receiving one or more medical treatments during the course of the study.

[9]SBM 2 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving no medical treatments during the course of the study.

[10]FSBM 2 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving no medical treatments during the course of the study.

[11]SBM 2 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving one or more medical treatments during the course of the study.

[12]FSBM 2 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that consumed 2lbs or more daily of starter for that week and receiving one or more medical treatments during the course of the study.

[13]SBM 3 H: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week and receiving no medical treatments during the course of the study.

[14]FSBM3 H: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week and receiving no medical treatments during the course of the study.

[15]SBM 3 S: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that were weaned completely off of milk replacer and only consuming starter for that week and receiving one or more medical treatments during the course of the study.

[16]FSBM3 S: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that were weaned completely off milk replacer and only consuming starter for that week and receiving one or more medical treatments during the course of the study.

Week 1: SBM 0 H n=8; FSMB 0 H n=4; SBM 0 S n=12; FSMB 0 S n=13; FSBM 1 S n=2.

Week 2: SBM0 H n=4; FSMB 0 H n=4; SBM 0 S n=8; FSMB 0 S n=5; SBM 1 H n=3; SBM 1 S n=5; FSMB 1 S n=9.

Week 3: SBM 0 S n=2; FSMB 0 S n=1; SBM 1 H n=2; FSMB 1 H n=3; SBM 1 S n=6; FSMB 1 S n=4; SBM 2 H n=1; SBM 3 H n=5; FSBM 3 H n=1; SBM 3 S n=4; FSBM 3 S n=8.

Week 4: SBM 0 S n=1; FSBM 1 H n=1; SBM 1 S n=2; FSMB 1 S n=2; SBM 2 S n=1; FSBM 2 S n=1; SBM 3 H n=8; FSBM 3 H n=3; SBM 3 S n=8; FSBM 3 n=12.

Week 5: SBM 0 S n=1; FSMB 1 H n=1; SBM 1 S n=1; SBM 3 H n=8; FSBM 3 H n=3; SBM 3 S n=10; FSBM 3 n=15.

Week 6: SBM 0 S n=1; SBM 2 S n=1; SBM 3 H n=8; FSBM 3 H n=4; SBM 3 S n=10; FSBM 3 n=15.

Table 11. (continued)

Item	[1]SBM0 H	[2]FSMB0 H	P-value	[3]SBM0 S	[4]FSMB0 S	P-value	[5]SBM1 H	[6]FSBM1 H	P-value	[7]SBM1 S	[8]FSBM1 S	P-value	[9]SBM2 H	[10]FSBM2 H	P-value	[11]SBM2 S	[12]FSBM2 S	P-value	[13]SBM3 H	[14]FSBM3 H	P-value	[15]SBM3 S	[16]FSBM3 S	P-value
Week 4	-	-	-	-	-	-	-	35.4975	NS	40.9374	37.6678	NS	-	-	-	-	-	-	38.0846	34.8277	NS	-	37.9898	-
Week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38.2534	42.3963	NS	39.7817	38.3859	NS
Week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33.9648	31.1885	NS	34.5112	33.7669	NS
Δ CD4/45																								
Week 1	-	13.9882	14.0784	NS	12.8951	14.0784	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 2	-	11.9849	10.9419	NS	12.7696	13.715	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3	-	10.3332	9.6554	NS	10.311	10.3486	NS	11.8103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 4	-	-	-	-	-	-	-	-	14.6764	-	11.7639	13.9362	-	-	-	-	-	-	-	-	-	-	-	-
Week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.7948	12.7948	NS	12.0527	14.9463	NS
Δ CD8																								
Week 1	-	14.4524	12.75	NS	13.7677	13.4312	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 2	-	16.6701	15.5785	NS	13.9835	13.7818	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3	-	18.5002	21.3762	NS	22.4863	23.7207	NS	7.3247	-	-	30.2821	0.1293	-	-	-	-	-	-	-	-	-	-	-	-
Week 4	-	-	-	-	-	-	-	11.9093	17.7003	NS	14.8339	16.458	NS	-	-	-	-	-	14.1037	8.0573	NS	-	14.7188	-

Table 11. (continued)

Item	[1]SBM0 H	[2]FSMB0 H	P-value	[3]SBM0 S	[4]FSMB0 S	P-value	[5]SBM1 H	[6]FSBM1 H	P-value	[7]SBM1 S	[8]FSBM1 S	P-value	[9]SBM2 H	[10]FSBM2 H	P-value	[11]SBM2 S	[12]FSBM2 S	P-value	[13]SBM3 H	[14]FSBM3 H	P-value	[15]SBM3 S	[16]FSBM3 S	P-value
Week 5	-	-	-	-	-	-	-	17.3199	-	15.2659	23.5741	0.0618	-	-	-	-	-	-	16.9258	14.3638	NS	16.0881	16.1634	NS
Week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.9154	17.6598	NS	17.148	16.8545	NS
Δ B cell																								
Week 1																								
		8.0217			6.2968	6.2892																		
			NS				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 2																								
	6.5071	8.1731	0.0659	7.4171	8.3123	0.1117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 3																								
	5.786	9.2753	NS	7.5959	5.6692	NS	10.0998	-	-	6.645	10.311	0.1168	-	-	-	-	-	-	-	-	-	-	-	-
Week 4																								
	-	-	-	-	-	-	-	10.2561	-	7.9761	4.5834	NS	-	-	-	-	-	-	10.1526	-	-	-	-	-
Week 5																								
	-	-	-	-	-	-	-	9.8054	-	11.6926	5.1547	NS	-	-	-	-	-	-	11.4025	7.7089	NS	8.9539	9.4293	NS
Week 6																								
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.1905	11.9157	NS	11.8693	12.0224	NS

Figure 2. Manufacturing process flow diagram depicting how soybean meal is made into the fermented soybean meal product (PepSoyGen, Nutra-flo Protein and Biotech Products, Sioux City, Iowa). Modified from Nutraferma Flier (Nutra-flo Protein and Biotech Products, Sioux City, Iowa).

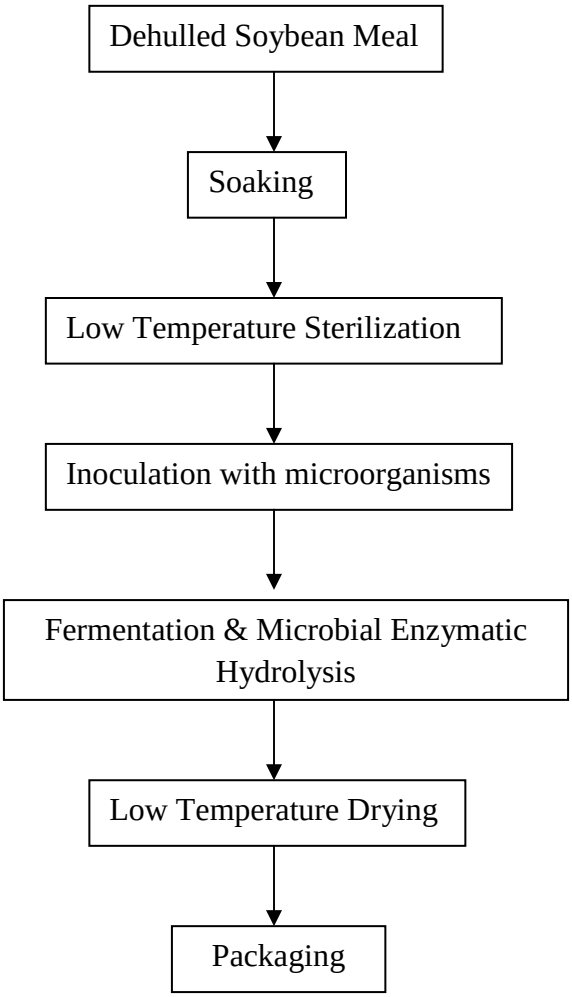
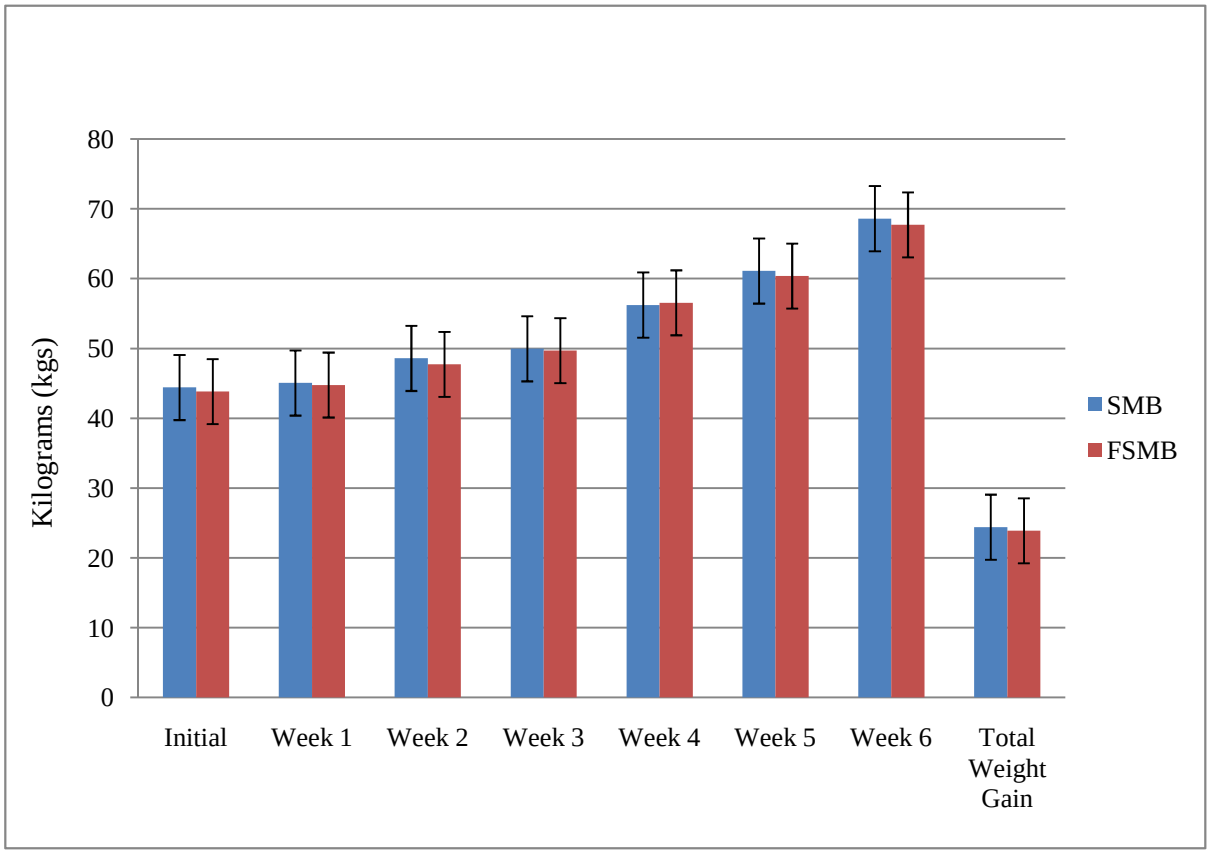


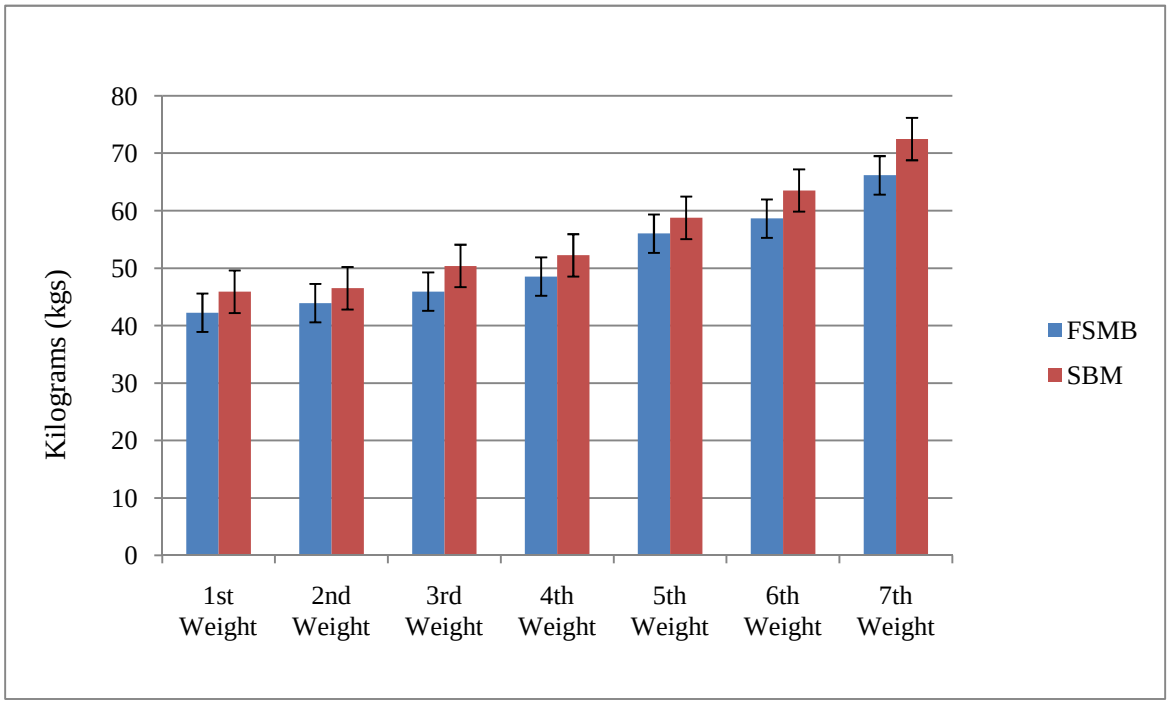
Figure 3. Mean weekly weights in kilograms of calves on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (n=32).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (n=34).

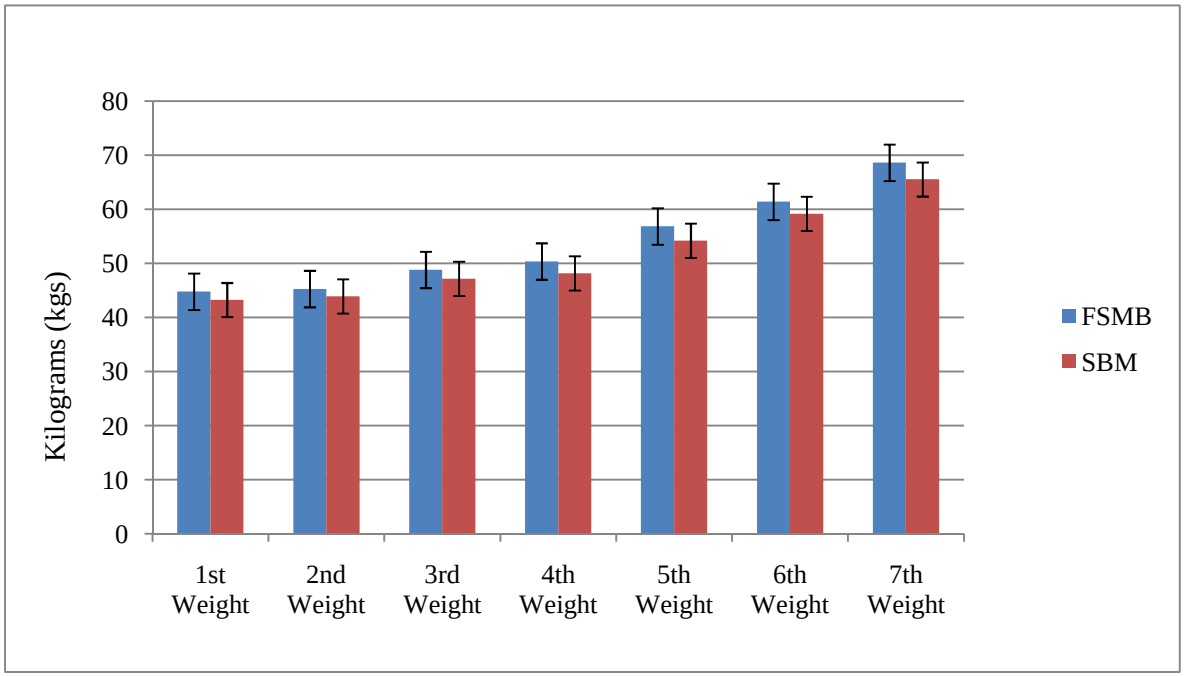
Figure 4. Mean weekly weights in kilograms of calves that received no medicine treatments during the course of the project that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that received no medicine treatments during the course of the project (n=12).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that received no medicine treatments during the course of the project (n=15).

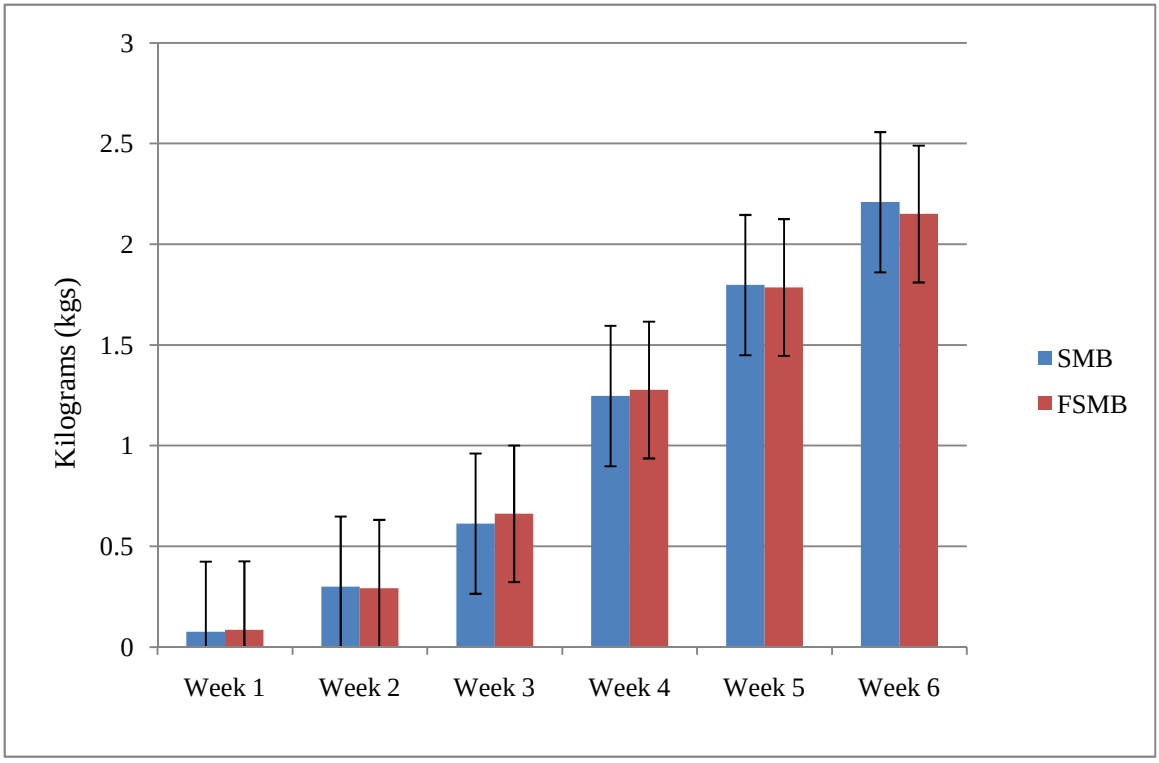
Figure 5. Mean weekly weights in kilograms of calves that received one or more medicine treatments during the course of the project that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient that received one or more medicine treatments during the course of the project (n=20).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient that received one or more medicine treatments during the course of the project (n=19).

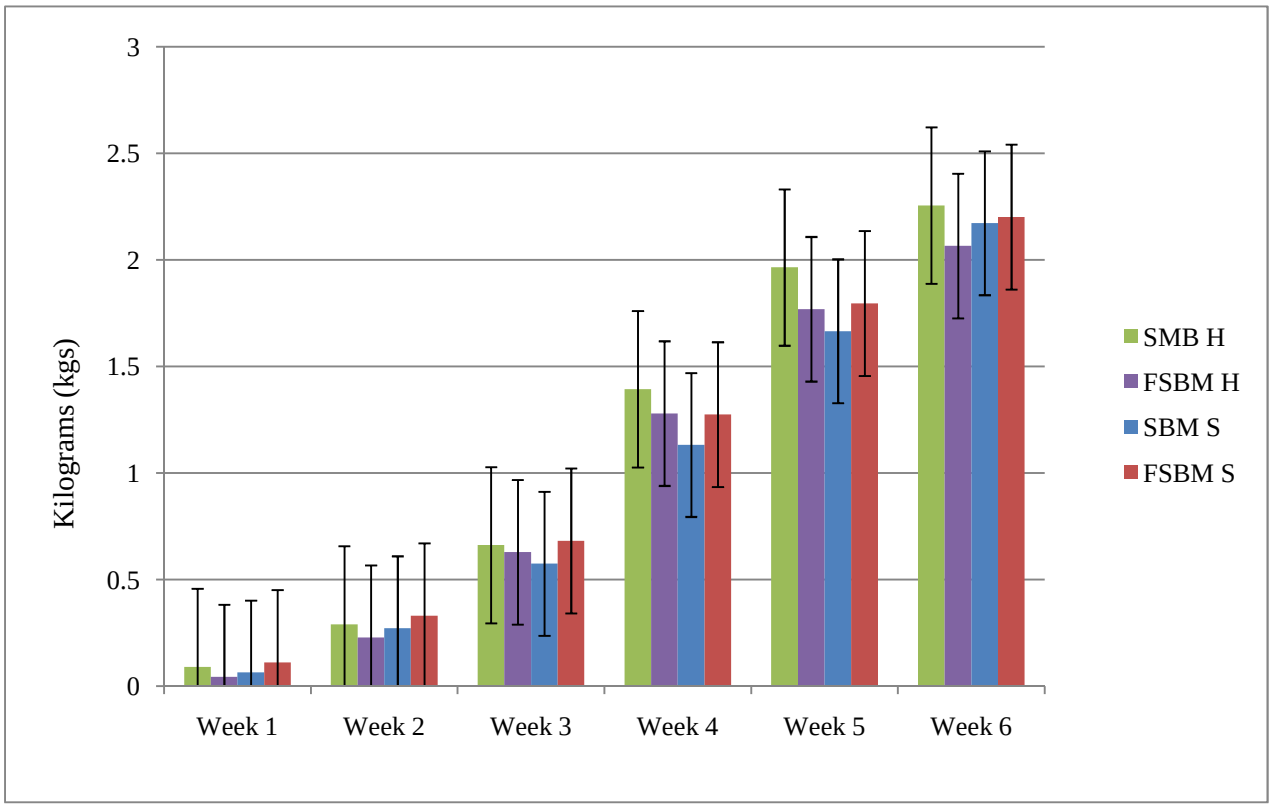
Figure 6. Mean weekly starter diet consumption in kilograms for calves that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (n=32).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (n=34).

Figure 7. Mean weekly starter diet consumption in kilograms for calves that received either no medicine treatments during the course of the project or one or more medicine treatments that were on either the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



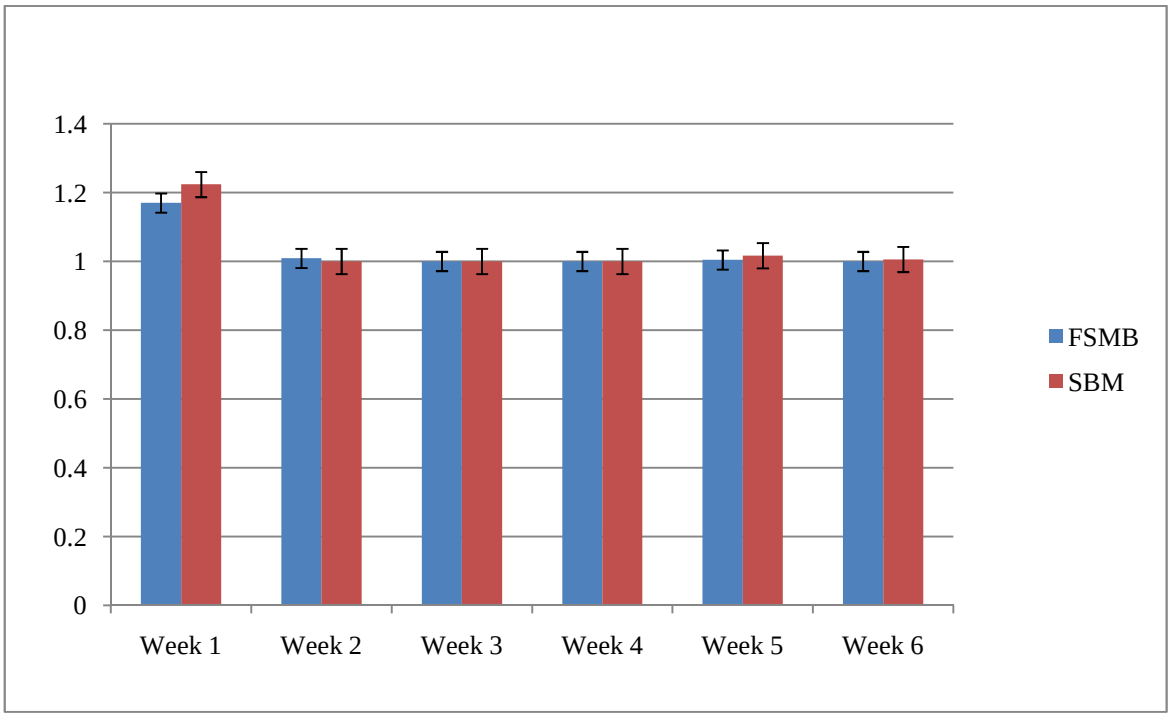
"H" denotes healthy calves, which are calves that had no medical treatments during the course of the project.

"S" denotes sick calves, which are calves that had one or more medical treatments during the course of the project.

FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (H: n=12; S: n=20).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (H: n=15; S: n=19).

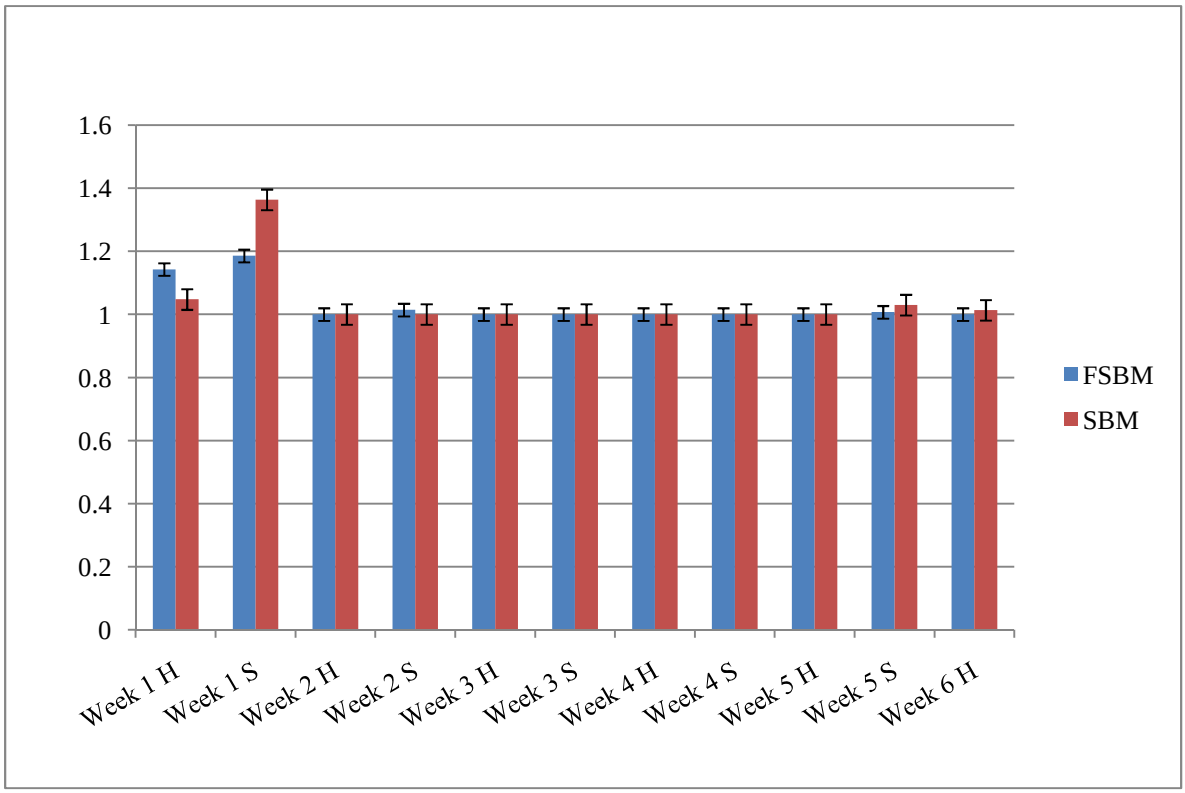
Figure 8. Mean weekly attitude scores for calves that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (n=32).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (n=34).

Figure 9. Mean weekly attitude scores for calves that received either no medical treatments during the course of the project or one or more medical treatments that were on either the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



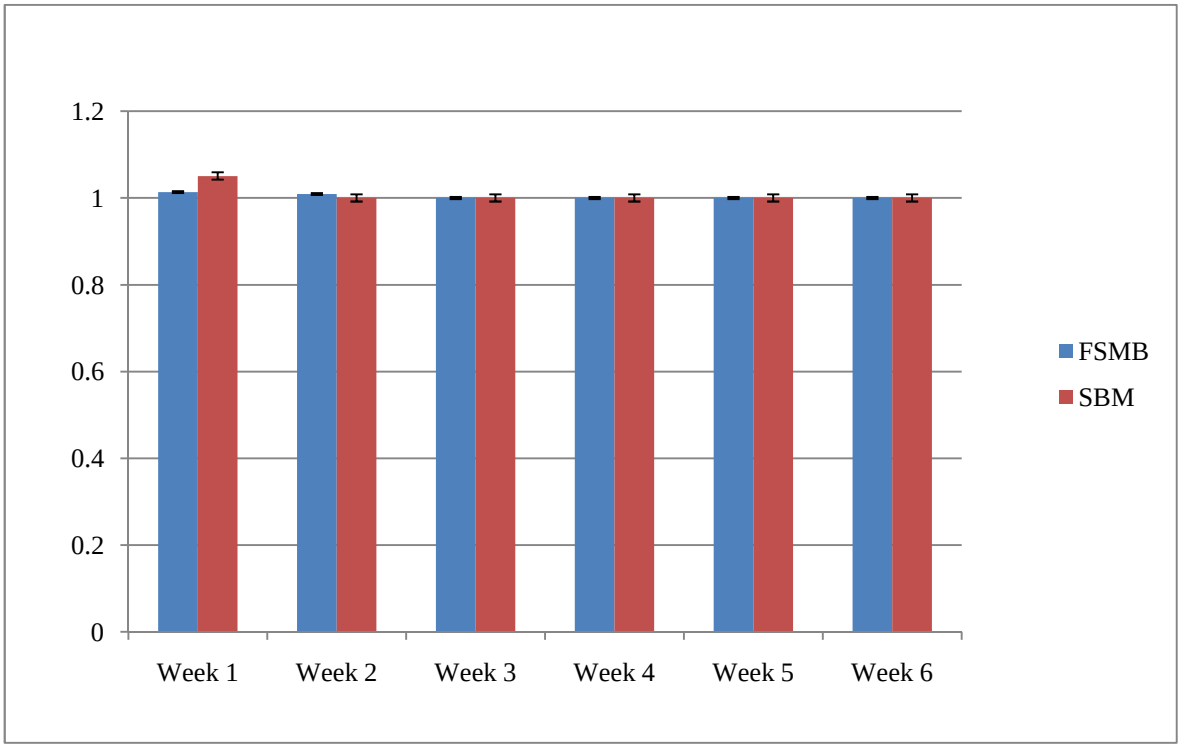
"H" denotes healthy calves, which are calves that had no medical treatments during the course of the project.

"S" denotes sick calves, which are calves that had one or more medical treatments during the course of the project.

FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (H: n=12; S: n=20).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (H: n=15; S: n=19).

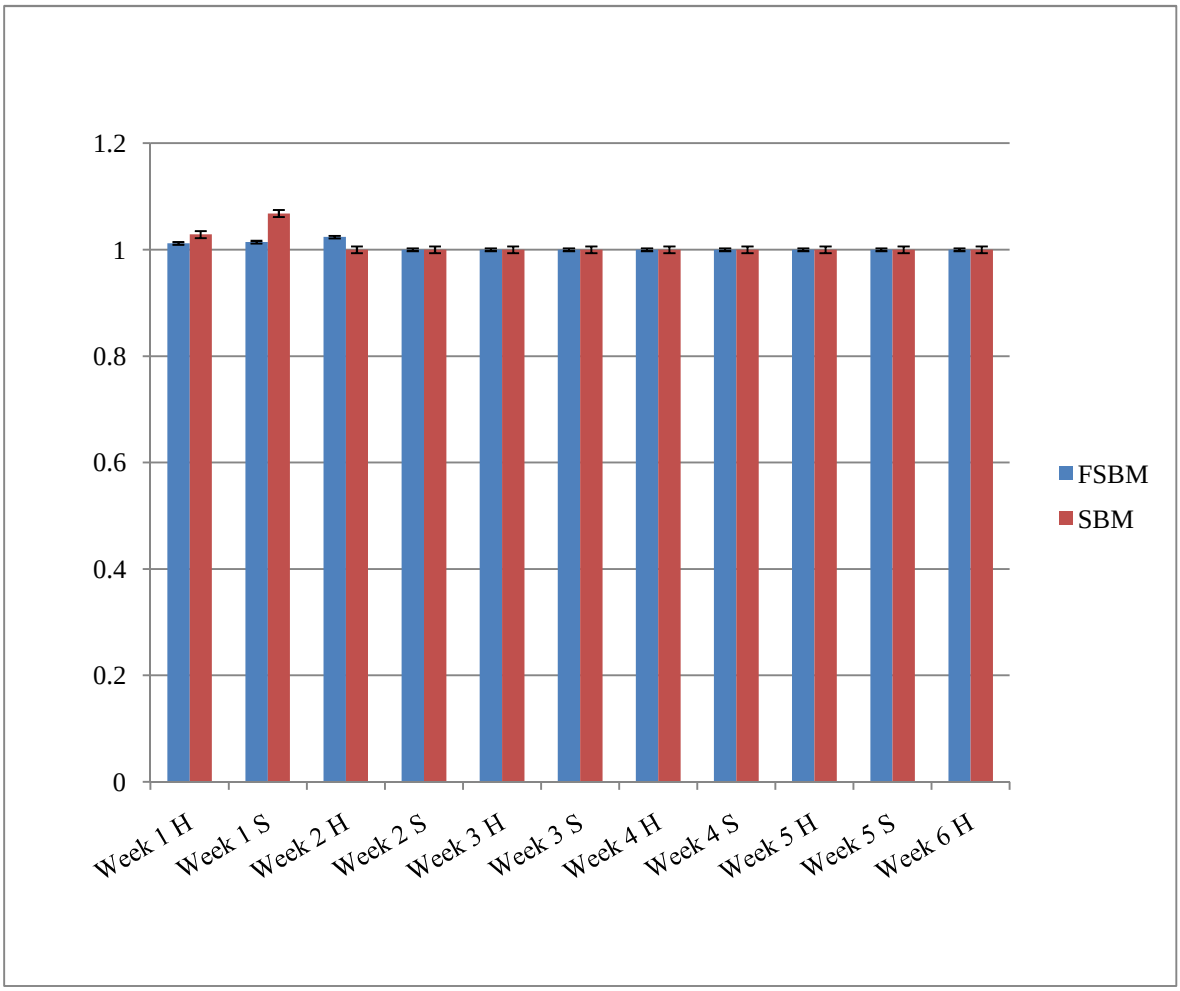
Figure 10. Mean weekly milk replacer appetite scores for calves that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (n=32).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (n=34).

Figure 11. Mean weekly milk replacer appetite scores for calves that received either no medical treatments during the course of the project or one or more medical treatments that were on either the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



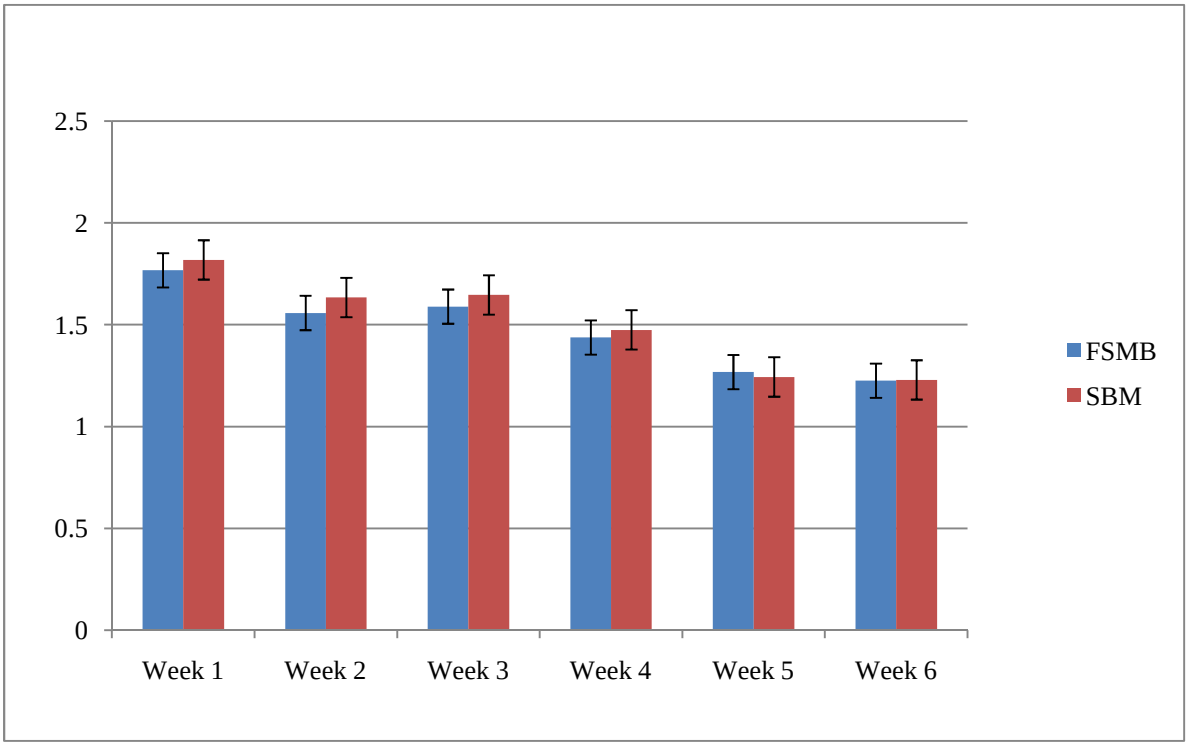
"H" denotes healthy calves, which are calves that had no medical treatments during the course of the project.

"S" denotes sick calves, which are calves that had one or more medical treatments during the course of the project.

FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (H: n=12; S: n=20).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (H: n=15; S: n=19).

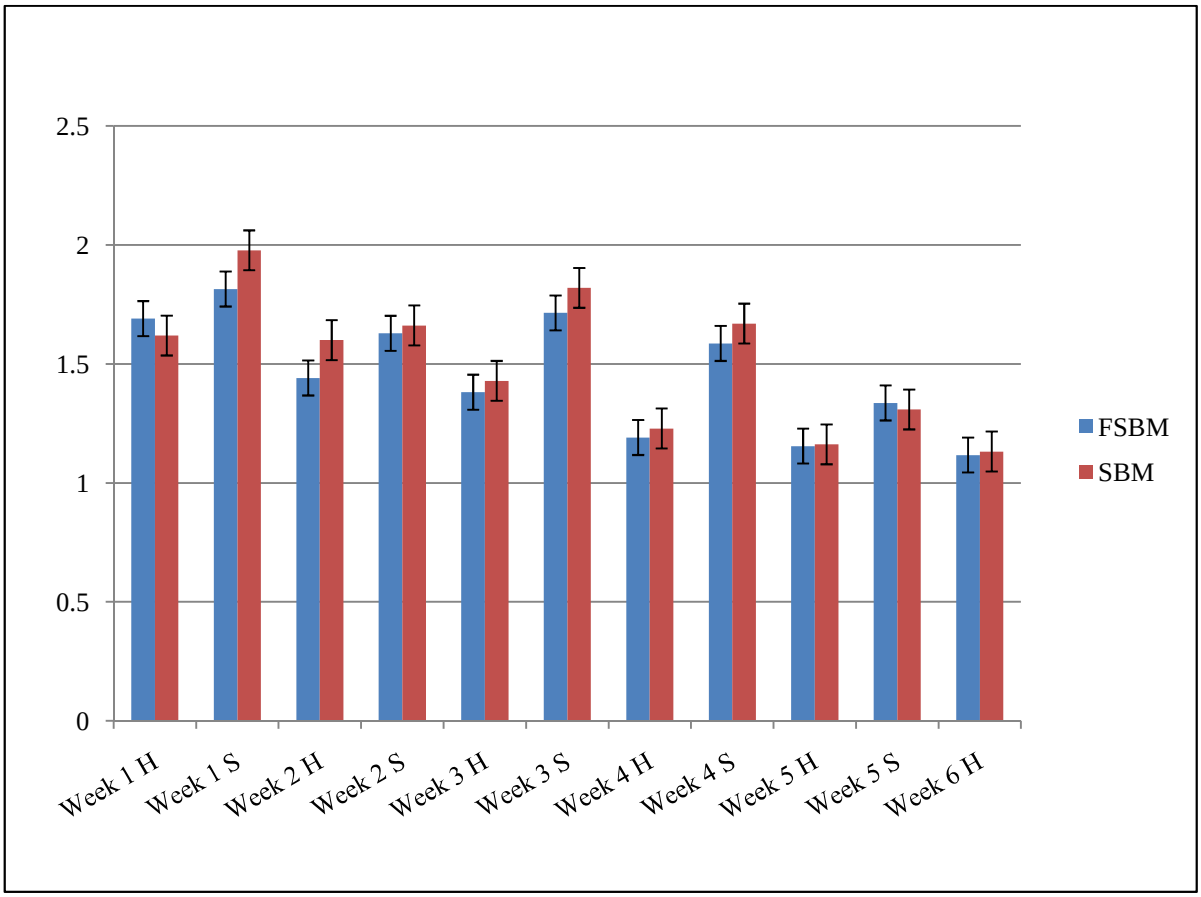
Figure 12. Mean weekly fecal scores for calves that were on the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (n=32).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (n=34).

Figure 13. Mean weekly fecal scores for calves that received either no medical treatments during the course of the project or one or more medical treatments that were on either the standard soybean meal based starter diet and of calves on the fermented soybean meal based starter diet (least squares means ± standard error of the mean).



"H" denotes healthy calves, which are calves that had no medical treatments during the course of the project.

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FSBM: Calves in the group fed a starter diet with fermented soybean meal as the main protein ingredient (H: n=12; S: n=20).

SMB: Calves in the control group fed a starter diet with soybean meal as the main protein ingredient (H: n=15; S: n=19).

DISCUSSION

Data from this study, such as no significant difference in weight gain, feed intake, fecal, attitude, and appetite scores, and immunological parameters, suggests that fermented soybean meal (FSBM) based starter diets are similar to soybean meal (SBM) based diets, contrary to observations in nursery piglet studies (Kim et al., 2009). The data in this study associated with weight gain, fecal scores, and attitude scores did show that FSBM could successfully replace SMB without any adverse affects on growth or digestive processes. This is similar to findings in piglets from other studies as well (Kim et al., 2009; Feng et al., 2007*b*).

Fermentation by *Aspergillus oryzae* and *Bacillus subtilis* decreases peptide size by carbohydrases released by *Aspergillus oryzae* and by proteases and peptidases released by *Bacillus subtilis* produced during fermentation that aid in the breakdown of complex nutrients in soy protein (Hong et al., 2004). Hong et al. (2004) concluded that most of the peptides in FSBM were smaller than 10 kDa, whereas most peptides in SBM were between 20 and 250 kDa. Fermentation by these microorganisms was able to effectively denature antinutritional and allergenic factors in the soy protein, such as glycin and β-conglycinin, since most of the subunits in these antinutritional factors are greater than 20 kDa (Helm et al., 2000; Richert et al., 2004; Deak et al., 2006). Kim et al. (2009) found that fermentation of soybean meal by *Aspergillus oryzae* destroyed about 40% of glycin and β-conglycinin. In the present study, however, this decrease in peptide size and antinutritional and allergenic factors did not appear to increase digestibility or feed efficiency in the calf. This may be due to the fact that by the time they start consuming starter, their rumens are beginning to function and may have the ability to produce similar fermentative products in their rumen.

Immunological data showed fairly equal development and responsiveness of the immune system between groups of calves receiving different treatments. This suggests that bioactive peptides that were released from the soybean meal by fermentation did not have more of an immunomodulatory effect on the calf than did the soybean meal. Although bioactive peptides may have been present in the FSBM, once again, it may be possible that by the time the calves start consuming enough of the starter for the bioactive peptides in the FSBM to have an effect on the calf's immune function, their rumens are beginning to function and may have the ability to produce similar fermentative products in their rumen from the SBM based starter. This would explain why the beneficial results in piglet studies were not observed in this study (Kim et al., 2009; Hong et al., 2004). Magalhães et al. (2008) found similar responses in dairy calves fed yeast culture supplemented grain. Different results may be obtained if the FSBM was incorporated into milk replacers that would allow the bioactive peptides created from fermentation to bypass rumen fermentation and be absorbed by the calf. In addition, the concentration of bioactive peptides fed could be standardized for consistent intake on a daily basis at an earlier age; milk replacer would be consumed at the same amounts on a daily basis starting at one day of age compared to highly variable intakes of calf starter starting at much older ages.

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CHAPTER 3

GENERAL CONCLUSIONS

This research study represents part of a continuing endeavor to find non-milk proteins that can effectively be used in calf diets without resulting in adverse effects on growth or health. It also represents a continuing effort to better understand bioactive feedstuffs and their effects on animals at different ages. The protein ingredient in calf diets represent the majority of the cost associated with feeds. The objective of this study was to observe the effects of fermented soybean meal in place of soybean meal in calf starter diets on calf growth, performance, and immune function.

This study provides additional support for the suitability of fermented soybean meal as an alternative protein in young calf diets, as well as its impacts on immune system development and responsiveness. Although weaning age was older as a result of replacing soybean meal in calf starter diets with fermented soybean meal, growth and performance was shown to be equal to that of soybean meal. Additionally, immune system development and responsiveness was not improved with feeding fermented soybean meal in calf starter diets, perhaps because the calf was essentially ingesting an already fermented product into a rumen that was beginning to function as a fermentation vat.

Further studies with fermented soybean meal in calf diets is needed before firm conclusions can be made about the availability of bioactive peptides to the calf. It is possible that the fermentation of the soybean meal did in fact release bioactive peptides, but these bioactive peptides are also released by the calves consuming the soybean meal based diets by microbes inhabiting the calf's rumen. It is also possible that the fermented soybean meal may have lower concentrations of allergenic and antinutritional factors than the soybean meal

initially, but the rumen ferments the soybean meal based diet to make it equal to the fermented soybean meal before the diets reach the site of absorption in the small intestines. This hypothesis of the rumen fermenting the soybean meal after consumption would confound the results from any similar feeding trial because rumen function was not measured in the calves. Ideally, this fermented soybean meal would be incorporated into a milk replacer and then tested against an all milk replacer and an unfermented soy protein milk replacer in a future study. This would eliminate the possibility of the diet being fermented in the rumen of the calf because milk replacers bypass the rumen via the esophageal groove (Hoffman, 1956). Such a study would clearly demonstrate if fermented soybean meal could be efficiently used by the calf and if it could improve immune function.

Our study can provide further support for fermented soybean meal use in calf diets and can possibly lead to further studies that could clarify the effects of fermenting soybean meal prior to consumption by the calf on growth and performance. The industry could profoundly benefit from the finding of an alternative protein that could serve also as a bioactive feedstuff that could be used to improve health in the young dairy calf. This could possibly reduce calf morbidity and mortality while lowering the cost to raise the calves.

CHAPTER 4

ACKNOWLEDGEMENTS

My graduate education experience wouldn't have gone as well as it did without the support and guidance I received from my major professor and fellow graduate students. First of all, I cannot thank Dr. Howard Tyler enough for not only his assistance and involvement in my graduate program, but also for his guidance in issues involving everyday life. He has helped me to remember that every decision I make should be made in hopes of bettering myself and those that I care about. I also want to thank Dr. Joan Cunnick for her patience in her assistance and guidance in the laboratory. She was a fundamental part of my research and taught me a lot about measuring and understanding immunological parameters. I cannot thank my committee members enough for supporting me in my research, in my graduate program, and most of all for their guidance and advice in life. They were very flexible and supportive in my decision to get married and have a baby while completing my Master's degree.

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