EFFECT OF β-MANNANASE ENZYME ADDITION TO SOY-CONTAINING MILK REPLACERS ON GROWTH AND HEALTH OF NEONATAL DAIRY CALVES

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ABSTRACT

The increasing cost of milk proteins is forcing the milk replacer industry to evaluate alternative protein sources to make more cost effective products. Alternative proteins should contain an appropriate amount of crude protein that is digestible with an adequate amino acid profile. Among the available protein sources, soy protein appears to be good alternative because of the protein content, availability, and lower cost compared with the milk proteins. However, soybean contains anti-nutritional factors such as trypsin inhibitor, lectins, and non-starch polysaccharides and inclusion of soy proteins in milk replacer has resulted in reduced growth and health in preruminant calves. The use of exogenous enzymes such as mannanases in poultry and swine has shown improved performance in the digestibility of soy-based feeds. The aim of this study was to evaluate the effects of soy-containing milk replacer with the addition of mannanase-based enzymes on growth and health of pre-ruminant Holstein bull calves. Two milk replacers were used for this experiment, a commercially available whey based milk replacer (28% CP and 15% fat) and a specially formulated soy protein containing milk replacer with fifty percent of the protein replaced by the soy protein concentrate. Fifty-six calves (n=14 per treatment, four treatments) were assigned to treatments consisting of either the all whey protein, or the soy protein milk replacer without mannanase based enzymes or two additional treatments fed two concentrations of mannanase based enzymes. The calves were provided 0.28 Mcal gross energy/kg $BW^{0.75}$ the first seven days and 0.32 Mcal gross energy/kg $BW^{0.75}$ until day 21 when the absolute amount was held constant to maintain adequate inventory of milk replacer for the 56-day study. On day 36 a commercial calf starter was provided to the calves on an ad libitum basis, and a step down weaning process weaning occurred at day 56 with complete starter removal and

weaning by day 63. Initial (53.6 ± 6.4) and final (111.2 ± 10.7) body weights were not significantly different, thus average daily gain (0.91 ±0.14 kg/d) was not different among treatments. Among treatments, dry matter intake (1.64 ±0.25 kg/d) and overall feed efficiency (gain:feed, 0.56 ±0.07) was not different. Differences were not detected among treatments for health, fecal score and body condition score ($P > 0.10$). A cost analysis was conducted and the cost per kilogram of gain of calves fed the soy protein concentrate milk replacer with the mannanase based enzymes was approximately 30% lower than calves fed the whey based milk replacer. Therefore, in this experiment, the use of soy protein concentrate with the mannanase-based enzymes provided similar animal performance as whey proteins and decreased the cost of production.

Key words: calves, milk replacer, soy protein concentrate, average daily gain, feed conversion efficiency, haptaglobin.

BIOGRAPHICAL SKETCH

Luis Alberto Nabté-Solís was born on February 3, 1978 in Guadalajara, Jalisco, México. After living in that city for 15 years, he moved to the city of Mérida, Yucatán, México where he lived until 2005. In high school and college he attended the Autonomous University of Yucatan, where he graduated in 1995 with major in Biological Sciences, and as a biologist in 2000 respectively, he graduated from College with the thesis entitled "Factors altering growth in bovine cattle under tropical conditions", focusing in the use of models. In September of 2000 worked for a private laboratory where he was in charge of the microbiology department for three years, in the meanwhile he also worked for a couple of months giving security at work and food management courses and carrying out revisions to some national supermarket chains in Mexico. In 2003, he joined the Technological Institute in Yucatan where he worked organizing social service student's activities and some plots of plant production. There he studied a Master in Horticulture and plant breeding from the graduated with honors in 2005. In September 2005 he enrolled in the department of Animal Science in Cornell University, with major in animal nutrition and minor in international agriculture, where he based his research on growth and health aspects of calves fed with different sources of protein milk replacers and the use of exogenous enzymes.

For all your love, patience, encouragement

For being part of my past, present, and future

For being always present in so many different forms

For being the engine of my life

My inspiration

For being you

This is for you, because of you,

All my love

All my life

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

I. INTRODUCTION

Economics of animal production are becoming a main component in farm management. Finding opportunities to reduce cost without compromising animal performance is a high priority in dairy farming. Nutrition, health, reproduction, and calving management are areas where improvement can be achieved. The first months of the calves' life are the most demanding of the animal's life. Newborn calves depend completely on the feed they receive to survive, therefore they need high quality feed and intensive care.

Among the costs to raise a heifer, feed accounts for 49% of the total costs invested, followed by labor that accounts only for 17%. The most inefficient stage is from birth to 90 kg (pre-weaning), when animal's feed and management cost is high, but weight gain accumulated in that period is less than 8% of the total weight accumulated in animal's life (Karszes, 2005). During the pre weaning period, calves need a high quality liquid feed, normally provided as milk or milk replacer. Milk replacer is a very acceptable source of nutrients due to the consistency and nutrient content of the product.

The milk replacer industry is based on the use of whey protein concentrate as main source for protein, however increases in the demand for dairy proteins are increasing the price of whey protein, and consequently milk replacers. Increased costs of milk replacer is leading farmers to change some management practices, including increased feeding of waste milk, reducing weaning age, and lowered amount of feed received by the calves. The consequences of these practices can be reduced growth rates, increased problems in health, and longer time to achieve desired weight for first calving.

Alternative sources of protein for milk replacers are being tested on calves, however the evolutionary adaptation of calves to digest nutrients of dairy origin is only one constraint, and growth obtained with those alternative sources is less (Tanan, 2005). Among all the alternative sources currently used, soybean protein demonstrates some desirable characteristics such as availability, price, and chemical composition (Toullec et al., 1994). However, substitution 40% of the CP from whey protein with soy protein generally decreases growth rates in preruminant calves. The causes for this decrease are attributed to the presence of anti-nutritional compounds, lowered digestibility, and increased endogenous protein production. Processing can modify the anti-nutritional compounds and digestibility of soy protein, however increased endogenous protein production is not well understood (Drackley et al., 2006; Kanjanapruthipong, 1998; Lallès et al., 1995b; Montagne et al., 2001, 2000; Nyachoti et al., 2000).

Also, growth depression has been observed in other species. Swine and poultry have shown the same responses when soy protein is included in diets for young animals (Hahn et al., 1995; Sakomura et al., 1998; Vohra and Kratzer, 1964). Use of exogenous enzymes has improved growth responses in swine, chickens, and turkey. Experiments carried out on swine fed soy-based milk replacer and with the addition of mannanase based enzymes showed improvement in average daily gain, dry matter intake and feed conversion efficiency (Daskiran et al., 2004; Hahn et al., 1995; James et al., 1998; Sakomura et al., 1998). These observations suggest an improvement in the use of soy based protein with the addition of the enzymes, besides of a reduction of the anti-nutritional effects of some nonstarch polysaccharides in the milk replacer. Despite having well documented data on the effects of soy proteins on digestibility, intestinal morphology, and endogenous protein production in calves, there are no

studies about the use of exogenous enzymes to overcome some of these detrimental effects.

Therefore, the objective of this study was to test the hypothesis that supplementing soy protein concentrate containing milk replacers with mannanase-based enzymes would improve calf growth, nutrient digestibility, and health. At the same time, an economic analysis was conducted to compare feed costs of whey protein-based and soy protein milk replacers.

II. LITERATURE REVIEW

Growth and development

Growth is defined as the increase in mass or live weight of the animal primarily due to the accretion of compounds in the body different from water. All growth processes occur at the cellular level, predominantly through the production of new cells. However, the increase in mass includes not only increasing cell proliferation (hyperplasia) but also in the size of the cells (hypertrophy). Hypertrophy results from the increase in deposition of macromolecules or storage material (Brameld, 2005; Owens et al., 1993)

Typically, growth curves are represented by a sigmoid curve (Johnson and Sissom, 2005). With sigmoidal growth curves, an accelerated rate of growth occurs in the first stages of the animal life and decrease when the animal reaches puberty and is closer to its maturity. Two important phases in the growth curve are the prepubertal self-accelerating, and the post-pubertal, self-inhibiting phases (Fig. 1.1). Although only a small percentage of the animal's total life span, the period from point b to c in Fig. 1.1 (from birth to weaning at 6 - 8 weeks of age), is the period when the calf faces some of the greatest physical and metabolic challenges. Birth and the adaptation to an external source of feed, changes in metabolism in support of growth, and the development of

the rumen are some of those challenges (Davis and Drackley, 1998; Owens et al., 1993). Careful management of nutrition, health, and the environment of the calf all are required to minimize the effects of those challenges.

Growth is determined by the increase of four primary chemical components in the animal body: water, protein, fat, and ash. These components continually change during the various stages of growth, and are affected by factors such as rate of gain (Berg and Butterfield, 1976; Johnson and Sissom, 2005; Owens et al., 1993), nutrient intake (Diaz et al., 2001), protein intake and protein energy ratio (Bartlett et al., 2006) and source of energy (Donnelly and Hutton, 1976a, b; Tikofsky et al., 2001). All these components are important descriptors of how growth and nutrition are dynamically related in the animal (Bradley and Sissom, 2005).

During early growth, fat and protein are retained at different rates, and both rates can be modified by the protein and fat content in milk replacer, energy to protein ratio, rate of gain, and source of protein. Diaz et al. (2001) determined that calf growth was directly related to the intake of milk replacer when dietary protein was a not limiting factor. Blome et al. (2003) demonstrated that increasing the amount of CP in isocaloric milk replacers fed at 1.5% of BW daily (DM basis) linearly increased ADG, feed conversion efficiency (gain to feed, G:F), and accretion of lean tissue in viscera-free carcass. Further, Donnelly and Hutton (1976a, b) found that manipulating protein-to-energy ratios in milk replacers based on skim milk affected growth rates and body composition in preruminant dairy calves. The concept that protein intake affects composition of gain and rate of gain was further elucidated in the study by Bartlett et al. (2006). Thus, as energy intake increases, protein requirements increase accordingly to meet the demand created by greater energy allowable growth (Van Amburgh and Drackley, 2005). Thus dietary protein quality is an important variable in formulating diets for pre-ruminant calves.

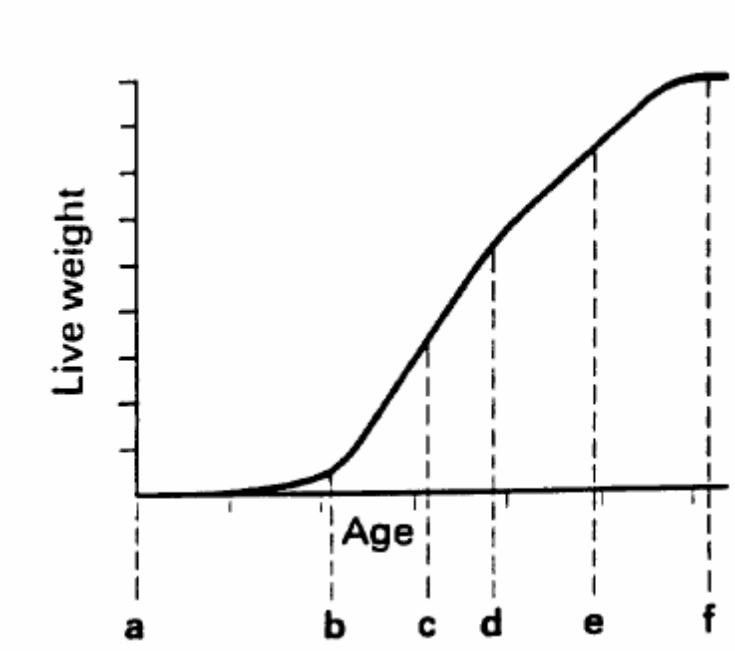

Figure 1.1. Growth curve showing the characteristic sigmoid form. a) conception, b) birth, c) self-accelerating phase, d) inflection point often associated with puberty, e) self retarding phase, and f) maturity. From Owens et al. 1993.

Calves at birth possess all the physical characteristics of an adult ruminant (omasum, abomasum, reticulum, and rumen); however, the presence of a fold of tissue that leads from the base of the esophagus to the reticulo-omasal orifice (oesophageal groove) causes young calves to behave as simple stomached animals. For this reason, newborn calves depend more on dietary ingredients that are easier to digest than their adult counterparts (Blum, 2006; Church, 1998). In this early period of pre-ruminant digestion, the abomasum is the primary gastric compartment, comprising 50 to 70% of the total volume and because of this the activity and digestive capacity of the other three compartments is minor (Davis and Drackley, 1998; Tomkins and Jaster, 1991). Besides the physical modifications in the gastrointestinal tract, the predominance of the abomasum and intestines in digestion of young animals leads to the production of certain enzymes, which forces the calf to behave as a monogastric animal. These enzymes are more adapted to the digestion of milk components because of evolutionary adaptation. Therefore, the utilization of milk-based diets improves digestion and assimilation of nutrients compared to dry diets in veal calves due to higher digestibility and nutrient profile in ingredients of milk origin in the milk replacer (Blum, 2006; Davis and Drackley, 1998; Heinrichs, 2005; Heinrichs and Leismeister, 2005; Montagne et al., 2002).

Nutrient Intake, Formulation and Cost

After birth, animal survival depends upon intake of a balanced diet consisting of carbohydrates, protein, fat, vitamins, and minerals from an external source. Availability from such sources also depends on other characteristics as digestibility, availability, and price (Davis and Drackley, 1998; Tanan, 2005).

To ensure survival and improve the performance of the animal, an adequate quantity of high-quality colostrum to the calf is the most important factor in the early

stages of life; colostrum must be the newborn calf's first meal. Colostrum is especially rich in immunoglobulins (Ig), providing the calf with early immune defense mechanisms critical for disease and fighting survival (Davis and Drackley, 1998; Tanan, 2005). Colostrum also helps enhance development of the digestive tract (Tanan, 2005) and there are other data suggesting that a lack of colostral antibodies reduces pre-pubertal growth and first lactation milk production (DeNise et al., 1989; Faber et al., 2005; Robison et al., 1988).

After colostrum, nutrients are supplied either as whole milk or as milk replacer depending on management and economic decisions (Heinrichs et al., 1995). The milk replacer industry began in the early 1950's as cheese producers consolidated after World War II and large volumes of whey became available (Mike Fowler, Land O'Lakes, personal communication). The first formulations were poor quality substitutes for cow's milk and resulted in poor calf performance and health primarily due to inadequate knowledge about protein chemistry and handling, which led to low digestibility proteins. Changes in the formulation and quality of milk replacer have increased performance; this improvement led to the fact that approximately 60% of all dairy heifer calves raised in the United States are receiving milk replacer as a primary nutrient source (Heinrichs et al., 1995). Traditionally, the main advantages of milk replacer use include increased profitability due to the availability of more saleable milk; the capacity to formulate a specific amount of protein, fat, vitamins and minerals in the diet, and improvements in the management and nutrition of the young calves (Davis and Drackley, 1998; Tanan, 2005; Tomkins and Jaster, 1991; Waterman, 2005)

According to Karszes (2005), the period from birth to 90 kg accounts for 14% of the total costs of production, but only 8% of the total weight gain in the animal life occurs during this period (Fig. 1.2). Therefore, any increase in production costs at this stage, is typically considered an expenditure more than an investment in the animal

growth. However, the low return on investment is partially due to the level of nutrient intake relative to maintenance requirements – feed efficiency is low, thus the feed cost is high relative to animal growth (Karszes, 2005). Similarly, during the first eight weeks of life of the animal (from birth to approximately 100 kg) the milk replacer cost relative to the total production cost has been estimated at approximately 36% (Manitoba Department of Agriculture, 2006). Thus, this period of growth is characterized by expensive feed ingredients. Due to the high cost of feed ingredients, the traditional feeding approach is to provide nutrients just above maintenance level intake which results in high feed costs per unit of gain.

Milk Replacer Ingredients

The manufacturing of milk replacer has evolved over the last 50 years. There has been tremendous improvement in the quality of ingredients, manufacturing technology and formulation which allows the industry to control the amount, origin and quality of nutrients in milk replacer (Davis and Drackley, 1998; Heinrichs et al., 1995; Khorasani et al., 1989; Tanan, 2005; Waterman, 2005). The modern United States milk replacer industry relies on whey powder, whey protein concentrate, delactosed whey, dried skim milk, and casein as the normal by-products sources in the manufacturing of milk replacers (Davis and Drackley, 1998; Heinrichs et al., 1995).

The use of whey in milk replacer as the main source for carbohydrates and protein is now competing with an increased demand of this product for human consumption such as, bakery products, infant formulas, energy bars (as an easily available source of protein for bodybuilders and some individuals with suppressed/abnormal immune systems and/or degenerative diseases), and even other uses as films and coatings for food products and plastics (Bell, 2000; Hutchinson et al., 2003).

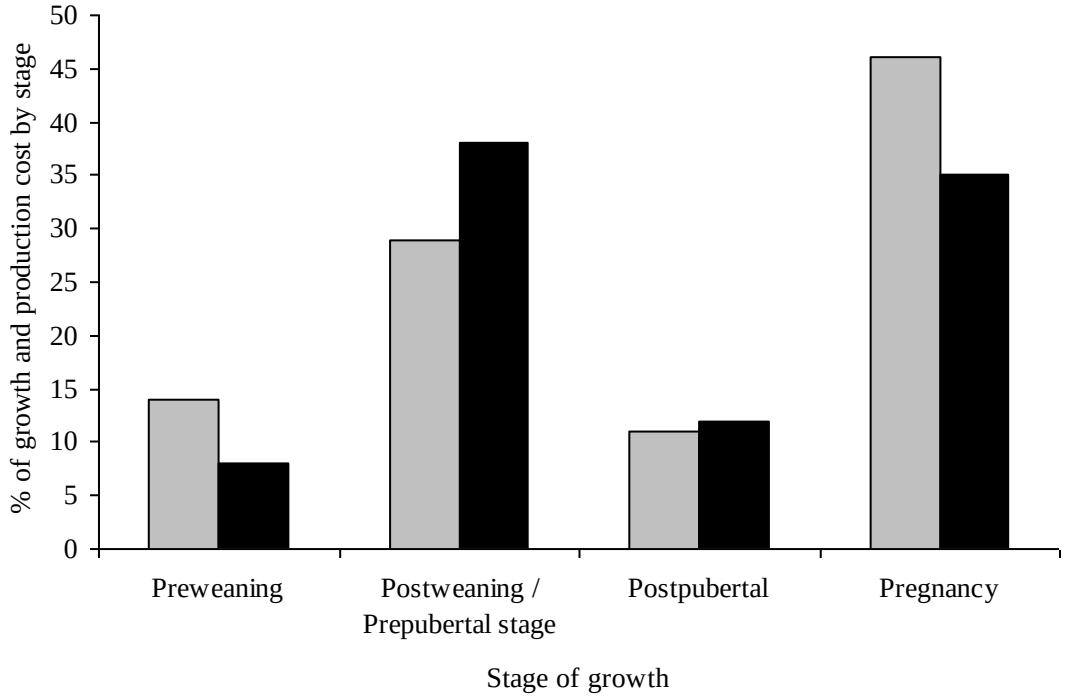

Figure 1.2. Production costs according to the stage of growth of the animal. Grey bars: percentage of the total cost invested in that stage of growth, black bars: percentage of total growth achieved in that stage (Data from Karszes, 2005).

This increased demand for whey protein has resulted in the increase in the price of those products (Fig. 1.3). An increase of 300% in the price of dry whey protein concentrate (WPC) was observed when comparing the mean price during the months of June-July of the years 2003 and 2007 (Dairy Market News, 2007). As a consequence, the increased price of WPC directly affects the formulation and final costs of milk replacer production; which can impact the feeding management decisions from birth to weaning.

Therefore, the use of alternative protein sources in milk replacers is an active area of calf nutrition research and development. Among the alternative sources originating from animals are animal plasma protein (Quigley and Bernard, 1992), egg protein (Touchette et al., 2003), fish protein (Petchey, 1982), and meat solubles (Davis and Drackley, 1998; Tanan, 2005). Alternative plant protein sources include: soy (Lallès et al., 1995b; Lallès et al., 1995c; Montagne et al., 2003), potato, wheat, pea, corn, and lupine (Branco-Pardal et al., 1995; Mbugi et al., 1989; Toullec and Formal, 1998; Toullec and Lallès, 1995).

Among alternative sources, the potential of soy protein for use in milk replacer is best understood (Davis and Drackley, 1998; Drackley et al., 2006; Gardner et al., 1990; Kanjanapruthipong, 1998; Khorasani et al., 1989; Knaus et al., 1994; Lallès, 2000; Lallès et al., 1995b; Lallès et al., 1995c; Malouf and Walker, 1981; Ouedraogo et al., 1998; Tanan, 2005; Toullec et al., 1994; Xu et al., 1997). The success of soy protein is based on availability and low cost compared with other protein sources such as skim milk and whey protein (Davis and Drackley, 1998; Lallès and Jansman, 1998).

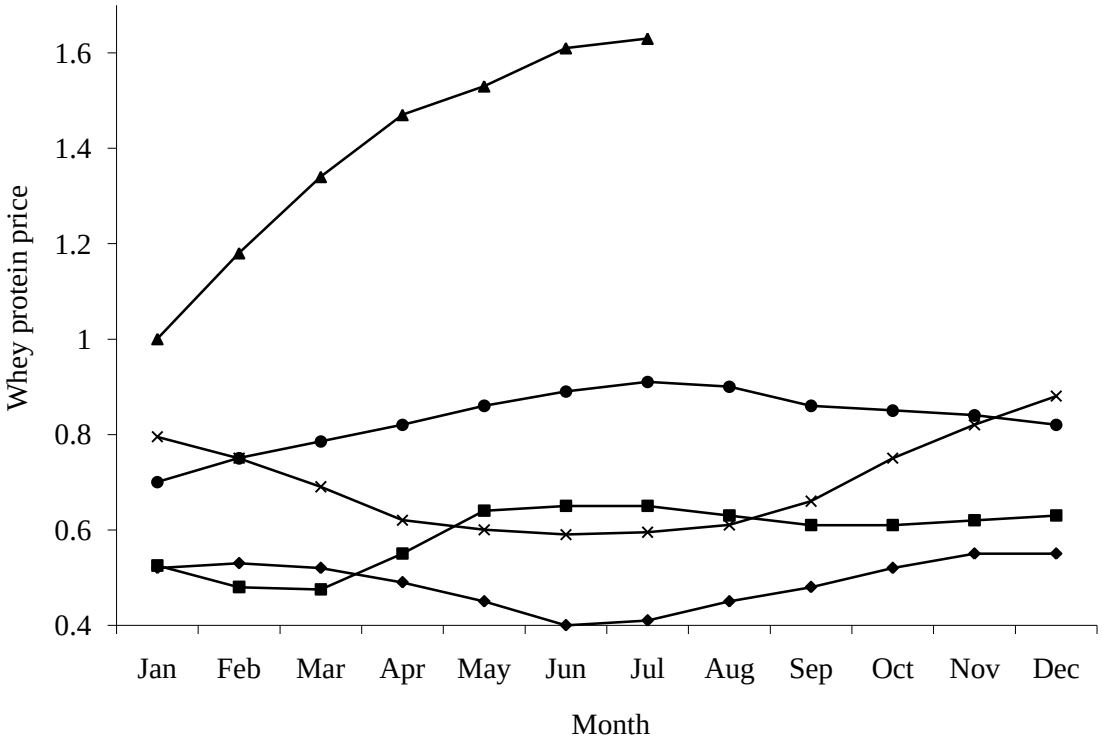

Figure 1.3. Average monthly price of Dry Whey Protein Concentrate (WPC) from January 2002 to July 2007. ♦ 2003, ■ 2004, ● 2005, × 2006, ▲ 2007 (Dairy Market News, 2007).

Processing of soybean sources removes antinutritional factors such as trypsin inhibitors, conglycinin, β-conglicinin, lectins, non starch carbohydrates and other non-desirable compounds from the feed and with each level of processing, the protein content increases (Table 2.1). In the case of soy protein isolate (SPI), the relative increase in CP can be up to 252% compared to whey protein concentrate (WPC). Despite the general increase in CP content for the soy ingredients used in milk replacer, digestibility varies depending on the overall composition of the milk replacer, origin of the ingredients and the animal's age (Table 1.2; (Davis and Drackley, 1998; Lallès et al., 1996b).

Higher CP digestibility is normally observed in skim milk, whey protein and skim milk protein concentrate; however, digestibility increases with increasing age, no matter the quality of the ingredients. When comparing whey protein sources with soy protein concentrate (SPC), hydrolyzed soy protein (HSP), and heated soy flour (SF), digestibility values decreased considerably. This decrease is more marked in younger animals (first two or three weeks of age) than in older animals (Table 1.2). Therefore, use of milk replacer with soybean protein has normally resulted in inferior calf growth when compared with milk protein use (Barr, 1981; Davis and Drackley, 1998; Drackley et al., 2006; Gardner et al., 1990; Khorasani et al., 1989). The decrease in digestibility appears to be related to the fact that soybean proteins contain different groups of anti-nutritional compounds such as trypsin inhibitors, antigenic proteins, indigestible carbohydrates, lectins, tannins, phytate, and saponins which can produce detriment in the performance of the calf (Gardner et al., 1990; Lallès, 1993; Lallès and Dreau, 1996; Lallès et al., 1995b; Pusztai et al., 1997; Xu et al., 1997).

The amount of crude fat in soy protein is relatively low. In some countries there is increased use of fat from non-animal sources in the milk replacer mainly due to

Table 1.1. Average chemical composition of the solids in milk (% dry matter) and ingredients (% on air-dry basis) on different sources of nutrients for milk replacer formulation.

Ingredient	Dry matter	Crude protein	Crude fat	Lactose
Whole milk solids	100	25.6	29.6	39.2
Dried skim milk	98	34	0.1	54
Whey protein concentrate	98	34	3.5	52
Soy protein isolate	94	86	0.5	---
Soy protein concentrate	95	67	0.3	---
Soy flour	95	53	0.2	---

(Davis and Drackley, 1998; Hansen et al., 1993; Tomkins and Jaster, 1991; Tomkins et al., 1994)

Table 1.2. Source of protein, age of calves, amount of crude protein replaced, and protein digestibility of milk replacer containing alternative protein sources.

Protein Source	Age of Calves[1]	CP replaced[2]	CP digestibility	Author
Skim milk	11–21	–	94.4	Babella et al., 1998
Whey protein concentrate	11–21	100	90.2	Grongnet et al., 1981
Skim milk	3–8	–	87	
	28–33	–	97	
Skim milk	8–14	–	92.1	Strudsholm, 1988
	22–28	–	98.7	
Whey proteins	8–14	100	89.9	
	22–28	100	91.6	
Skim milk	56–63	–	94.1	Tolman and Demeersman, 1991
Soy protein concentrate	14–21	75	58.7	Dawson et al., 1988
Whey protein concentrate	14–21	–	82.2	
Soy protein concentrate	42–48	50	87.4	Erickson et al., 1989
Skim milk	9–14	–	90.4	Lalles et al., 1995
Hydrolyzed SP isolate	9–14	56	89.1	
Heated soy flour	9–14	72	67.8	

[1]Age in days

[2]In percentage

pressure from regulations in the European Union and Japan for products with no potential for bovine spongiform encephalothapy transmission (Tanan, 2005).

Performance of calves fed milk replacers containing soy proteins.

The performance of calves fed milk replacer containing SPC differs according to the animal age (Fig. 1.4). In animals younger than 3 weeks of age and with milk replacer containing 50% protein replacement with SPC, a decrease in average daily gain (32.5%) and feed conversion (33%) was observed during the first two weeks of age. However, in older animals the decrease was only 7.1% and 5.9% compared to all milk ingredient replacers (Tomkins et al., 1994).

Similarly, Drackley et al. (2006) carried out an experiment in preruminant calves from 3 days to 48 days old. The study compared the effect of milk replacer containing all milk protein compared to milk replacer containing SPC, and glutamine was added to the SPC milk replacer to provide energy to the gut tissues in order to overcome the growth depression in these animals. Crude protein content was uniform in all treatments (~20%), fat and energy content also were constant in the different treatments (15%, 4.36 Mcal/kg respectively). In this study, the addition of SPC to milk replacer was not shown to have considerable effect on the final BW, or the height, length and heart girth of the animals, but significant differences were found in the ADG and the gain:feed (Table 1.3) (Drackley et al., 2006).

Although final BW was not significantly affected by changes in protein source of the milk replacer, average daily gain was reduced by approximately 16% in the soy protein treatment. In addition, the gain:feed ratio was reduced by 15% with the addition of the soy-based protein (Drackley et al., 2006).

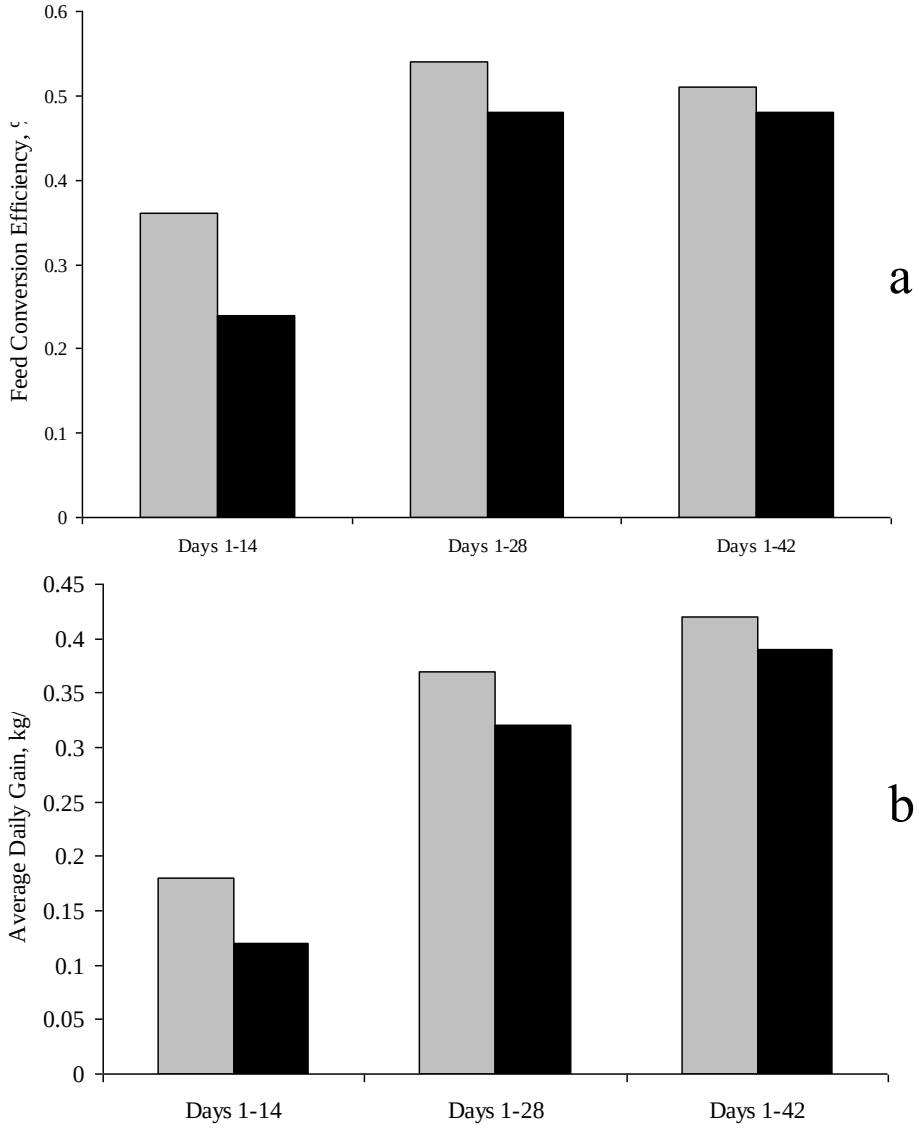

Figure 1.4. Feed conversion (a) and average daily gain (b) in different periods of growth in calves fed either whey protein-based milk replacer (grey bars) or soy protein-based milk replacer replacing 50% of milk protein (black bars) (Tomkins et al., 1994).

Table 1.3. Effect of milk replacer containing milk protein versus milk replacer containing soy protein on growth performance of pre-ruminant calves (Drackley et al., 2006.)

	Diet			
Variable	Control[1]	SPC[2]	SPC + Gln[3]	SE[4]
Initial BW, kg	42.4	41.1	41.6	1.9
Days on experiment	28.3	29.1	28.8	0.7
Final BW, kg	52.1	49.3	49.9	2.4
Average daily gain[5], kg**	0.344	0.281	0.282	0.025
DMI, kg	0.625	0.597	0.608	0.031
Gain:Feed**	0.55	0.47	0.47	0.03
Final withers height, cm*	82.4	80.8	81.4	0.9
Final body length, cm*	69.2	67.6	69.4	1.0
Final heart girth, cm*	86.8	84.6	84.5	1.3

[1]All whey protein based milk replacer.

[2]Milk replacer with 60% of whey protein replaced by soy protein concentrate.

[3]Milk replacer with 60% of whey protein replaced by soy protein concentrate and addition of glutamine.

[4]Standard error

[**]Control vs. SPC and SPC + Gln, $P < 0.05$, and * $P < 0.10$.

Similarly, Toullec et al. (1994), replaced 65% and 75% of whey protein in MR with SPC, fed to 4 to 5 week old veal calves for 101-103 days and observed a decrease in final BW of 9 to 12 %. Body weight gains were also 18, 13.5 and 17% lower in the soy based milk replacer treatments compared with the all milk control diets. All diets where formulated to contain between 20 to 21% CP and 19 – 20% fat. In this experiment (Toullec et al., 1994), animals entered the study 8 days after birth, and were fed a skim milk diet for 29 (experiment 1) and 24 (experiment 2) days. After the initial 29 or 24 days, the animals were assigned to treatment based on LW, LW gain, health status, intake and hematocrit values (Toullec et al., 1994). Results from that experiment reinforced the idea that the replacement of a large part of protein from dairy origin with soy protein depresses animal performance and diet digestibility.

Causes for growth depression in calves fed alternative proteins in milk replacer

The chemical composition of soybean indicates adequate protein levels and reasonable amino acid profiles for growth. In fact, soybean appears to possess fairly high levels of essential amino acids similar to that of cow's milk (Lallès, 1993). However, the causes of the decreased performance of calves when consuming soy protein have often been associated with decrease in apparent digestibility (Barr, 1981; Branco-Pardal et al., 1995; Bush et al., 1992; Guilloteau et al., 1986; Lallès, 1993; Malouf and Walker, 1981; Montagne et al., 2001; Nielsen et al., 1988; Sakomura et al., 1998). Decreases in digestibility are normally attributed to the presence of antinutritional factors and protein fractions associated with low digestibility (Barr, 1981; Lallès, 1993; Lallès and Dreau, 1996; Lallès and Jansman, 1998; Toullec et al., 1994). The anti-nutritional factors like ß galactomannans and lectins can disturb the physiological processes of digestion and absorption of nutrients, and cause

immunological reactions in the gut that decrease energy supply to the tissues (Huisman and Jansman, 1991).

Several studies have found that the anti-nutritional factors (trypsin inhibitor, lectins, antigenic proteins, polyphenols, oligosaccharides, phytates, saponins) found in soy proteins cause decreased growth, feed conversion efficiency and health (Barr, 1981; Gardner et al., 1990; Huisman, 1989; Huisman and Jansman, 1991; Khorasani et al., 1989; Lallès et al., 1995a; Lallès et al., 1995b; Ouedraogo et al., 1998). The factors associated with reduced performance in soy protein sources containing anti-nutritional factors can be protein digestion and absorption (trypsin inhibitors, lectins, antigens), but also the anti-nutritional factors can influence carbohydrate digestion, mineral utilization and vitamin availability (Barr, 1981; Huisman, 1989; Lallés, 1994).

Evolutionarily calves have developed an increased affinity to milk components. This is shown by curd formation when whole milk is used as main feed in preruminant calves. However there is little to no curd formation in calves fed milk replacer because most ingredients used in most milk replacers (whey and other sources as soy) do not promote the formation of a curd due to a lack of casein content. Formerly, it was thought that the decrease in clot formation was the cause of the decreased performance and increased diarrhea in young calves. The replacement of casein in milk replacer will increase the flow of digesta through the stomach and small intestine (Caugant et al., 1992); however research has indicated that this increase in flow does not generally cause diarrhea (Guilloteau et al., 1986; Longenbach and Heinrichs, 1998; Petit et al., 1987).

Clot formation results from the action of chymosin on casein and fat. Clot formation increases the residence time of casein and fat in the abomasum, causing an increase in time for the digestion and absorption of its chemical components (Longenbach and Heinrichs, 1998). However, further evaluation of whey-based milk

replacers, which do not contain casein, demonstrated similar performance as skim milk-based milk replacers indicating that clotting was unnecessary for efficient digestion.

Animals fed soy-containing milk replacers showed morphological abnormalities in the villi of the small intestine. Soy components are known to enhance mucosal abrasion and cell desquamation in the small intestine, thus increasing endogenous protein losses at the end of the small intestine (Leterme et al., 1998). These abnormalities are associated with reduced absorption and digestion of the feed components (Seegraber and Morrill, 1986). Other factors constraining animal performance from soy-based MR are the improper or inadequate processing of the soybean and reconstitution at high temperatures (Longenbach and Heinrichs, 1998).

Nonstarch Polysaccharides

Polysaccharides are the major components of plant materials. These compounds contain macromolecular polymers of monosaccharides linked by glycosidic bonds. Ninety to ninety-five percent of the polysaccharides present in plants are starch. The nonstarch polysaccharides (NSP) are complex, high molecular weight carbohydrates. The NSP include various fiber types such as lignin, celluloses, hemicelluloses, pectins, β-glucans, arabinoxylans (pentosans), uronic acid, galactose, and mannose (Aman and Graham, 1990; Annison and Choct, 1991; Bedford and Classen, 1993; Choct, 2002; Classen, 1996, 1991; Daskiran et al., 2004; Edwards et al., 1988; Meng et al., 2002). Non-starch polysaccharides can be classified in two categories: as water insoluble, which are normally indigestible in most monogastric animals, and water soluble, which are digestible. Seeds of legumes contain large amounts of NSP as part of the cell wall. Soybeans might contain up to 23% in a dry matter basis (Chesson, 1987)

The NSP are known to possess anti-nutritional properties by encapsulating nutrients, thus causing decreases in the overall nutrient digestibility through gastrointestinal modifications, decreasing the apparent metabolizable energy and the feed conversion ratio. Some models explain the antinutritional role of the NSP, one suggesting that the encapsulation of starch, fat, and protein by the NSP inhibits the access of digestive enzymes and another suggesting that the increased viscosity of the intestinal contents by the presence of NSP in the intestinal lumen. However, the effect is not fully described, but may involve both mechanisms (Bedford and Classen, 1992).

Polysaccharides become viscous solutions when dissolved in water. This is demonstrated when the intestinal viscosity is increased in an exponential manner with the increase of carbohydrates of high molecular weight in the small intestine (Annison and Choct, 1991; Choct, 2002). In the same way, depression in growth of broilers and swine have been correlated with the viscosity of digesta (Bedford and Classen, 1993).

Viscous polysaccharides are also known to cause physiological and morphological changes to the digestive system in various species (Edwards et al., 1988; Ikegami et al., 1990; Jorgensen et al., 1996; Morgan et al., 1985). The increase in mass of the gastrointestinal tract has contributed to the basal metabolic rate; therefore, any reductions in gastrointestinal size and viscosity could reduce heat production and improve net energy of the diet. Diets containing high levels of NSP significantly increase the length and weight of the gastrointestinal tract (Jorgensen et al., 1996), enlarge digestive organs, increase secretion of digestive enzymes, and depress apparent ileal protein digestibility (Ikegami et al., 1990). However, the addition of soluble NSP to broiler diets significantly elevated the fermentation in the small intestine and depolymerization of the NSP almost completely eliminated this problem (Annison and Choct, 1991; Castanon et al., 1997; Choct, 2002; Fang et al., 2007).

Among NSP, mannans are comprised of several forms: glucomannans, galactomannans, glucogalactomannans, and glucuronomannans (Aman and Graham, 1990). In β-mannan, repeating D-mannose units with β-1,4 bonds and D-galactose units are attached as sidechains by α-1-6 linkages in a 2:3 ratio (Carrè, 2002; Hsiao et al., 2006; Pettey et al., 2002; Ward and Fodge, 1996; Whistler and Saarnio, 1957). Several studies have identified the negative effects of dietary β-mannan found in palm kernel meal, copra meal, guar gum, and guar meal (Furuse and Mabayo, 1996; Morgan et al., 1985; Rainbird et al., 1984; Ray et al., 1982; Verma and McNab, 1982). These NSP have been shown to diminish growth performance and inhibit nutrient absorption in poultry (Daskiran et al., 2004; Hsiao et al., 2006; Jackson et al., 1999; James et al., 1998; McNaughton et al., 1998; Verma and McNab, 1982) and swine (Blackburn and Johnson, 1981; Daskiran et al., 2004; Hahn et al., 1995; LeMieux et al., 2003; Pettey et al., 2002). The effect of these compounds on growth and digestion of calves has not been extensively analyzed as in swine and poultry.

Dietary enzyme addition

Exogenous enzymes have been used to supplement endogenous deficiencies and enhance digestive capacity to the host animal (Baidoo et al., 1997; Classen, 1996). The feeding of exogenous enzymes cleaves polysaccharide chains to greatly reduce solution viscosity thus enhancing nutritive value (Blackburn and Johnson, 1981; Larsen et al., 1993; Xu et al., 2005).

Recent research has shown that the dietary addition of enzyme mixtures containing cellulase, xylanase, pectinase, hemicellulase, glucanase, phytase, mannanase, or protease increases the digestibility of protein, fat, starch and minerals that are incorporated in the plant cell (Baidoo et al., 1997; Bedford and Classen, 1993; Castanon et al., 1997; Classen, 1996, 1991; Wirayawan and Dingle, 1999). The

metabolizable energy (ME) content of soybean meal is very low in comparison with its gross energy, especially for monogastrics. This low ME is mainly a result of poor digestibility of the carbohydrate fractions (Pierson et al., 1980). Therefore, the digestibility of protein sources high in NSP might be improved by supplementation with exogenous enzymes. Indeed, some studies have shown that the application of commercial enzymes to a corn and soybean diet improved the ileal digestibility of energy, starch, fat and protein (Sakomura et al., 1998; Zanella et al., 1999). In the same way, addition of enzymes to a corn-soybean diet in broilers improved the ADG and size and weight uniformity of broilers (Schang et al., 1997; Wyatt et al., 1997; Zanella et al., 1999).

A commercially available β-mannanase (1,4-β-D-mannan mannanohydrolase; EC: 3.2.1.78) preparation is currently being marketed under the trade name Hemicell (ChemGen, Gaithersburg, MD). Addition of this β-mannanase to diets containing soybean meal for broilers and turkeys has increased gain and improved feed efficiency (James et al., 1998; McNaughton et al., 1998). Initial studies in growing-finishing pigs have shown improvements in G:F and gain of fat free muscle (Hahn et al., 1995).

Pettey et al. (2002), using one hundred fifty-six pigs, tested the effect of adding two levels of β-mannanase preparation (0 vs. 0.05%) and soybean oil on corn-soybean meal diets to evaluate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). They found that the addition of β-mannanase to diets improved feed efficiency and increased daily gain and feed efficiency in growing-finishing pigs. The addition of β-mannanase also improved gain of tissues other than fat in finishing pigs.

In another experiment, Omogbenigun et al. (2004), used multi-enzyme preparations to evaluate the nutrient digestibility, growth performance, and P utilization of young pigs fed diets containing ingredients high in NSP. They found that with the adequate

formulation of a multi-enzyme combination, young pigs were able to improve feed utilization and therefore growth performance with lower quality ingredients. When compared with control diet, enzyme supplementation significantly improved ileal digestibility of DM (60 vs. 66%), GE (62.8 vs. 70.4%), CP (62 vs. 72%), starch (86.7 vs. 94.2%), nonstarch polysaccharides (NSP; 10.1 vs. 17.6%), and phytate (59 vs. 70%) (Omogbenigum et al., 2004).

Thus far, the use of exogenous enzymes to degrade indigestible dietary components has yielded inconsistent results mainly because of the presence of complex substrates in feedstuffs and the use of enzyme activities often not suitable for effective hydrolysis of such components (Slominski, 2000). Previous research with poultry (Cleophas et al., 1995) and pigs (Graham et al., 1988) suggests that a combination of different enzyme activities is required for complete degradation of complex NSP and improved nutrient utilization. Recent in vitro studies showed that a combination of carbohydrase enzymes was more effective in NSP depolymerization of soybean meal, canola meal, and peas than when the individual carbohydrases were used (Meng et al., 2002). Therefore, the use of enzymes to improve performance in farm animals is now widely used, especially β-mannanase in poultry (Daskiran et al., 2004; Jackson et al., 1999; James et al., 1998; McNaughton et al., 1998), turkey (James et al., 1998), and swine (Baidoo et al., 1997; Fang et al., 2007; Hahn et al., 1995; Pettey et al., 2002). However, in spite of observed limitations in calves fed soy-based milk replacers, similar experiments for calves have not been conducted.

CHAPTER 2

I. INTRODUCTION

With continually increasing costs of whey and other milk proteins, the use of milk proteins in products such as milk replacer are becoming cost prohibitive. Alternative proteins for use in milk replacers are available, but do not necessarily provide the same level of performance as products made from whey and skim milk (Dawson et al., 1988; Davis and Drackley, 1998; Tanan, 2005). The most nutritionally effective milk replacers are still produced from all milk proteins consisting of whey proteins and various whey protein fractions (Davis and Drackley, 1998; Tanan, 2005).

Soy protein concentrate is a economical alternative for milk proteins in milk replacer and due to the processing for the production of SPC, most of the anti-nutritional factors in soybeans such as trypsin inhibitor, glycinin and ß conglycinin are rendered inactive (Lallès, 1993; Tomkins et al., 1994; Lallès et al., 1995b; Lalles and Toullec, 1998; Montagne et al., 2001).

Studies investigating whey replacement with SPC in milk replacers for calves have formulated milk replacers between 19 and 23% CP and offered them at energy levels equivalent to 1.2 to 1.4 times maintenance level intake (Drackley et al., 2006). The presence of plant proteins in the gastrointestinal tract of the calf is known to stimulate increased mucin production, endogenous protein loss and immune responses (Lallès, 2000; Montagne et al., 2000, 2001). The net result of these effects would be increased protein synthesis and energy costs associated with the protein synthesis accounted as a maintenance energy expenditure. Thus, if calves are fed at energy levels just in excess of maintenance, the decreased growth performance previously observed in calves fed alternative proteins such as SPC might be a direct result of the energy associated with the adaptive response of the gut that increases both the energy and the protein

requirement of the calf. Drackley et al. (2006) attempted to overcome the assumed additional energy requirement through the inclusion of glutamine in the diet, however calf performance was not affected by the glutamine.

There are other factors potentially affecting the performance of calves fed SPC containing milk replacers. Although negative effects of SPC are not well understood, it is known that certain non-starch carbohydrates, notably β-galactomannans, which occur in soybean meal, are extremely anti-nutritive in monogastric animals such as broiler chickens, laying hens, turkeys, and pigs (Anderson et al., 2007; Hahn et al., 1995; Sakomura et al., 1998; Vohra and Kratzer, 1964; Zou et al., 2006).

Enzymes have been developed that catalyze the breakdown of β-galactomannans (ChemGen Co., Gaithersburg, MD) and have been found to improve feed efficiency and reduce the immune system response in poultry and swine. In pigs this reduced immune response was measured by the blood levels of α-1 acid glycoprotein, an acute phase protein (Anderson and Hsiao, 2006; Anderson et al., 2007; Jackson et al., 2004).

Therefore, the objective of this study was to evaluate the performance of a high protein, SPC-containing milk replacer fed to pre-ruminant calves with or without β -mannanase-based enzymes prior to and through the weaning phase of growth. Our hypothesis was that performance of calves fed the soy protein containing milk replacer would be decreased compared to the whey protein based milk replacer and the addition of the mannanase based enzymes to the SPC containing replacer would improve the performance by reducing the anti-nutritional characteristics of the milk replacer. A simple economic analysis will be conducted to compare the cost per unit of gain of the calves feed the various treatments.

II. MATERIALS AND METHODS

Animal Management and Feed Intake

The Cornell University Animal Care and Use Committee approved all procedures and animal management methods used in this study. Fifty-six Holstein bull calves were purchased within the first two days of age from dairy farms surrounding Ithaca, NY, and transported to the Cornell Dairy Teaching and Research Center. Transportation time was up to one hour. Upon arrival, animals were weighed, and offered an oral electrolyte solution (~1.5 L) for rehydration. Calves were housed in individual pens with wire mesh and bedded on sawdust and straw in a modified greenhouse barn with sidewall curtains and a clear polycarbonate roof.

Prior to initiating the dietary treatments, all calves were fed an all milk protein milk replacer (Excelerate, MSC, Inc. Dundee, IL) until the start of the trial at an intake energy (IE) level of 0.25 Mcal of gross energy (GE)/kg metabolic body weight (MBW) (0.25 Mcal GE/kg MBW). Milk replacer was mixed at 15% solids between 43 and 48 °C and was fed at 0530 hr and 1730 hr. For the first three days in the barn the calves were bottle fed and then trained to drink from buckets. Animal health was monitored several times throughout the day and body temperatures were measured daily for the first 10 days calves were in the barn and then as deemed necessary. Electrolytes were offered to calves that appeared dehydrated or were not consuming all of their offered diet. If body temperature exceeded 39.7 °C, then calves were treated according to label indications with a non-steroidal anti-inflammatory drug (flunixin meglumine, Schering-Plough Animal Health, Union, NJ). If the fever persisted and other clinical signs of disease were observed (lethargy, off feed, or dehydration) oral or subcutaneous fluids and antibiotics were used. All treatments were recorded.

Calves were acquired starting in February 2007 at which time severe weather conditions occurred. Ambient temperatures averaged -18 °C with wind between 5 and 96 kph and low temperatures were approximately -30 °C. Due to this significant cold stress, frostbite and pre-treatment losses were greater than anticipated and the severe cold appeared to affect parturition in herds around Ithaca for approximately 1 wk which impacted the rate at which the experiment could be populated. Thus, a portion of the calves were older at the initiation of treatments. Calves were weighed, ranked by weight and then randomly assigned to one of four treatments in a complete randomized block design controlling for initial weight. Due to the effects of cold stress on calf acquisition, the animals were approximately 14 ± 12 d of age at the initiation of treatments. The mean weight at assignment of treatments was 48 ± 5.33 kg.

Warm water was offered to the calves 30 min after milk replacer was offered and they had access to the water for at least 30 min, then buckets were cleaned. As ambient temperatures approached 0 °C, the internal barn temperature increased above freezing and water was offered free choice.

After assignment to treatment, IE supplied by milk replacer was provided to each animal at 0.28 Mcal GE/kg $BW^{0.75}$ during the first seven days and then increased to 0.32 Mcal GE/kg $BW^{0.75}$. Thus, the amount of milk replacer provided was adjusted weekly based on the weekly weight to maintain the desired amount of energy delivered. However due to a limited supply of a single lot of experimental milk replacer, a decision was made to maintain the DM intake level achieved by day 21 for the remainder of the study to maintain inventory adequate to complete the study.

Table 2.1. Description of the treatments used in the experiment, and experimental design.

Treatment	Protein source	Enzyme source	Number of Animals
A (PCtrl[1])	Whey	Only salt solution added	14
B (EnzA[2])	Whey + SPC[5]	Enzyme A plus salt solution	14
C (EnzB[3])	Whey + SPC	Enzyme B plus salt solution	14
D (NCtrl[4])	Whey + SPC	Only salt solution added	14

[1]Positive control.

[2]Enzyme A application.

[3]Enzyme B treatment

[4]Negative control

[5] SPC = soy protein concentrate

Treatment A was the all whey protein positive control diet. Calves assigned to Treatments B, C, and D were transitioned onto the soy protein concentrate (SPC) containing milk replacer in a 50:50 ratio of the two milk replacers for 7 d and then 100% of the SPC containing milk replacer. During this transition, the enzymes were also added to the calves fed treatments B and C. Treatment D served as the negative control that received the SPC containing milk replacer with no added enzymes (Table 2.1).

Milk replacer formulation

The target growth rate for calves on this study was 1 kg/d, thus the nutrient content of the milk replacers chosen were consistent with the nutrient requirements suggested by Van Amburgh and Drackely (2005) for calves in this weight range. A commercially available milk replacer formulated with all milk protein ingredients (Excelerate, MSC Specialty Nutrition, Dundee, IL) was used as the positive control diet (Treatment A) (Table 2.2). The treatment diet was formulated to be isocaloric and isonitrogenous and be similar in amino acid content as the positive control milk replacer and was formulated with 50% of the milk protein replaced by a SPC (Profine VF, Solae Inc. St. Louis, MO) (Treatments B, C and D). Prior to formulation, to ensure the anti-nutritional compound of interest was present the SPC was analyzed for β-galactomannans and was found to contain approximately 0.97% on a dry matter basis (Emily Helmes, personal communication, ChemGen, Corp. Gaithersburg, MD).

To overcome the potential confounding effects of different amino acid profiles, crystalline amino acids were added to the milk replacer formulation containing the SPC (Table 2.4). The quantities of all ingredients including the amino acids added to the formulation are found in Table 2.4. Kanjanapruthipong (1998) added the amino acids Thr, Met and Lys to calves fed a soy based milk replacer and observed improved

growth performance. To maintain iso-amino acid balances in this SPC containing formulation, other amino acids, Trp, Val, Leu and Ile, were also added in addition to the Thr, Met and Lys to make ensure that the formulations contained a similar amino acid profile.

Experimental enzyme products

Two different levels of a developmental feed enzyme product, SZP (ChemGen Corp., Gaithersburg, MD) were added to the SPC containing milk replacer at the time of mixing and feeding. The enzyme product SZP contained fermentation solubles from a strain of *Bacillus lentus*; the active ingredients were endo-1,4-β-D-mannanase (EC 3.2.1.78), with side enzyme activities including: endo-1,3-β-glucanase (EC 3.2.1.39); endo-1,4-(1,3:1,4)-β-D-glucan 4-glucanohydrolase (EC 3.2.1.4); endo-1,4-β-xylanase (EC 3.2.1.8); and α-galactosidase (EC 3.2.1.22) and others. The SZP enzyme products were provided in concentrated liquid form with instructions for dilution of SZP product with clean water, salt (1:23), and subsequent mixing into the calf milk replacer. After mixing well in the SPC-containing milk replacer, the β-D-mannanase activity in the liquid milk replacer was approximately 50,000 Units/kg for Treatment B (Enzyme A) and 20,000 Units/kg for Treatment C (Enzyme B), respectively (ChemGem Corp, Gaithersburg, MD). One Unit of β-D-mannanase activity is defined as the amount of β-mannanase enzyme that generates 0.72 microgram of reducing sugars per minute from a mannose-containing substrate such as locust bean gum at pH 7.5 and 40° C. Since the dilute enzyme solutions contained approximately 4.35% salt, a solution of salt was made and mixed with the milk replacers offered to calves fed Treatment A and D to ensure that all calves received the same level of salt among treatments. The enzymes used in this study were heat sensitive, so water temperature was measured at each feeding and maintained between 43 and 48 °C to prevent

denaturation of the enzymes. Dilute enzyme and salt solutions were prepared weekly and kept refrigerated at a 4º C. Reconstituted milk replacers were sampled randomly and pooled throughout the study to measure enzyme activity. Enzyme treatments were unknown to the investigators at the time of the experiment.

After reconstitution, the milk replacer for each calf was weighed out in tared buckets and the weight recorded. Refusals were measured after 30 min of feed offered and also recorded.

During the first 35 d of the study all four treatments received their reconstituted MR as the only source of nutrients. This was done to provide a test of the enzyme addition to the SPC containing milk replacer. On day 36 a commercially available calf starter (22% CP, Cargill, Inc. Binghamton, NY) was offered on an ad libitum basis, measured daily, and the amount of milk replacer offered remained the same until d 49 of treatment (Table 2.5, 2.6). On day 49 milk replacer was reduced to 50% of the dry matter offered and fed once per day at 1730h. On day 56 of the study milk replacer was removed from the diet and only starter was available for the calves. Thus, calves were given 21 days to consume starter and develop rumen function prior to initiating the weaning process.

Calves were weighed (Way Pig 15 digital scale, Raytec, Ephrata, PA,) and data recorded every Monday at 1200 hrs for the duration of the study. Hip heights (HH) were measured and body condition scores (BCS) (one to five scale, Wildman et al., 1982) were assessed and recorded at the beginning, at week four and eight of the study. Height measurements were taken in centimeters and with the animal standing up straight on a level surface.

Table 2.2. Milk replacer chemical composition used during the experiment on a dry matter basis

	Diet	
Ingredient[1]	Whey Protein[2,3]	Soy protein[4]
Crude Protein %[4]	29.4	29.5
Crude Fat %	18.9	18.8
Crude Fiber %	NA[5]	0.66
Gross energy (Kcal/Kg)[6]	5022.73	5042.82
Lactose (%)	36.84	32.85
Calcium (%)	0.82	0.84
Phosphorus (%)	0.59	0.59
Potassium (%)	1.82	1.72
Iron (ppm[7])	92	133
Zinc (ppm)	94	106

[1]DM-basis – all values measured except for crude fiber and lactose. Crude fiber was provided by the manufacturer and lactose by difference

[2,3]Milk replacer offered to calves during prior to start of the treatments (MS Specialty Nutrition, Dundee, IL) and to calves assigned to treatment A.

[4]Milk replacer offered to calves assigned to treatments B, C, and D (MS Specialty Nutrition, Dundee, IL).

[5]Not applicable.

[6]Measured value by bomb calorimeter (Model 1281 Parr Instrument Company Moline, Ill)

[7]Parts per million

Table 2.3. Measured amino acid composition of the milk replacers used.

	Diet	
Amino acid (mg AA/ g DM)	Whey Protein MR[1,2]	Soy-based MR[3]
Alanine	13.60	13.93
Arginine	7.73	8.01
Aspartate	31.10	30.09
Cystine	10.45	9.82
Glutamate	47.33	48.21
Glycine	5.72	6.08
Histidine	5.04	5.18
Isoleucine	13.55	14.04
Leucine	27.06	28.30
Lysine	22.52	22.89
Methionine	4.02	3.89
Phenylalanine	8.75	9.09
Proline	14.80	15.93
Serine	15.62	16.79
Threonine	18.86	20.30
Tryptophan	4.45	4.96
Tyrosine	7.37	7.51
Valine	13.91	13.89

[1,2]Milk replacer offered to all calves prior to treatments and to calves assigned to Treatment A (Excelerate, MS Specialty Nutrition, Dundee, IL).

[3]Milk replacer offered to calves assigned to treatment B, C, and D (MS Specialty Nutrition, Dundee, IL).

Table 2.4. Ingredients used in the formulation of the milk replacer used in the treatments.

	Diet	
Component (kg/1000 kg)	Whey Protein MR[1,2]	Soy-based MR[3]
Dried Whey (11.84% CP)	769.48	673.60
Delactosed whey (20.5% CP)	150.00	150.00
Whey protein concentrate (70% CP)	596.93	253.11
Dry fat blend 7% CP/60% fat	432.80	443.40
Emulsifier	20.00	20.00
Limestone	12.50	12.50
Dihydrate Dicalcium Phosphorus	6.00	6.00
Vitamin E Premix 100,000	0.95	0.95
B-Vitamin blend	1.60	1.60
MR Base Mineral	7.5	7.50
MR Base Mineral PRE	1.25	1.25
Dry MS Butter Flavor	1.00	1.00
Profine VF (Low Ant) soy protein concentrate	-	394.61
L-Lysine (HCL) 98.5%	-	9.94
DL-Methionine	-	6.30
Threonine	-	7.06
98% Tryptophan	-	1.94
L-Valine	-	0.22
L-Leucine	-	7.5
Isoleucine	-	1.53

Table 2.4. (Continued)

[1,2]Milk replacer offered to all calves prior to treatments and to calves assigned to Treatment A (Excelerate, MS Specialty Nutrition, Dundee, IL).

[3]Milk replacer offered to calves assigned to treatments B, C, and D (MS Specialty Nutrition, Dundee, IL).

[4]Values are expressed in terms of percentage.

[5]Amino acids were added only to soy protein MR to match the concentration of the whey protein based milk replacer.

Table 2.5. Ingredients as a percent of the final formulation of the starter grain used in this experiment (Cargill, Inc. Binghamton, NY).

Ingredient	% of Product*
Wheat Midds By-Product	31.00
Distillers grain	20.46
Corn germ meal	15.00
Dakota gold high protein	10.07
Amino Plus	5.47
Condensed molasses	5.00
Cereal tailings	4.80
Citrus pulp	3.35
Calcium carbonate	1.87
Sodium bicarbonate	0.75
Salt	0.65
Active dry yeast	0.30
BioMos	0.25
Flash dry blood	0.24
Maxibond	0.20
Dairy ADE	0.07
Trace mineral blend	0.04
Rumensin 80	0.03
Sel-plex 2000	0.03

Table 2.6. Chemical composition of the starter used in the study.

Nutrient	Unit	Concentration, as-fed
Crude protien	%	22.50
Crude fat	%	4.31
Non fiber carbohydrate	%	27.50
Starch	%	12.00
Sugars	%	7.75
Acid detergent fiber	%	7.59
Neutral detergent fiber	%	24.96
Calcium	%	1.00
Phosphorus	%	0.73
Sulfur	%	0.32
Magnesium	%	0.29
Potassium	%	0.94
Sodium	%	0.60
Chlorine/Chloride	%	0.57
Copper	mg/kg	35.06
Manganese	mg/kg	100.00
Cobalt	mg/kg	1.43
Zinc	mg/kg	135.00
Selenium	mg/kg	0.89
Vitamin A	IU/g	38.70
Vitamin D	IU/g	6.50
Vitamin E	IU/g	188.48
Monensin	g/ton	50.00
Biotin	mg/kg	0.52

Fecal scores were assigned and recorded daily based on a scale of form of one to three: 1) solid, semi solid; 2) runny but not abnormal; 3) watery (Diaz et al., 2001). At the same time, respiration scores were also assessed and recorded based on a scale of one to four: 1) normal; 2) runny nose; 3) heavy breathing; 4) chronic coughing (Diaz et al., 2001).

Blood samples were collected once weekly via jugular venipuncture using one 10 ml tube with no anticoagulant, for serum; one 10 ml tube containing heparin for plasma; and one 7 ml tube containing Na fluoride/K oxalate for glucose measurement (Vacutainer, Becton Dickinson Vacutainer Systems USA, Rutherford, NJ). Samples were taken every Wednesday at approximately 1400 hrs for the duration of the experiment. After collection, tubes were placed on ice and for collection of plasma, were centrifuged at 3000 rpm (1620 g) for 15 min and transferred to 1.5 ml micro centrifuge tubes (Fisher Scientific Inc., Pittsburgh, PA, USA) and stored at -20ºC until analysis. For collection of serum, collection tubes were allowed to sit under refrigeration (4ºC) for up to 24 hours after collection and then serum was collected and transferred to 1.5 ml micro centrifuge tubes (Fisher Scientific Inc., Pittsburgh, PA, USA) and stored at -20ºC for analysis.

Blood was assayed weekly for plasma levels of urea nitrogen, and at weeks five and eight for plasma acute phase proteins (haptaglobin). Plasma concentrations of urea nitrogen (PUN) were determined based on Sigma kit No. 640, which combines procedures described by (Chaney and Marbach, 1962; Fawcett and Scott, 1960). Haptaglobin analysis, designed to detect the acute phase protein in serum and plasma, were carried out with the Phase Range Haptaglobin Kit (Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland, based on Eckersall et al., 1999) per kit instructions.

Gross energy content of the milk replacers was determined in a bomb calorimeter (Parr model 1281, Parr Inc., Moline, IL). Crude protein content of the milk replacers

was estimated from N content using a N combustion analyzer (Leco FP528, Leco Inc. St. Joseph, MI). Crude fat was measured by ether extract (AOAC, 1981).

The amino acid contents of the milk replacers was determined by three hydrolysis procedures (hydrochloric acid (HCl), performic acid (PA) preoxidation prior to HCl hydrolysis and alkaline hydrolysis with barium hydroxide) in duplicate on each sample. Samples were defatted with hexane after hydrolysis. For the three hydrolysis procedures, an aliquot of milk replacer containing approximately 3 mg N was weighed into Teflon-lined screw top culture tubes (20 x 125 mm; 16 x 125 mm for alkaline hydrolysis). Norleucine was added as an internal standard to each tube to yield 125 nM/ml or 250 nM/ml in the analyzed sample for the HCl and PA or $Ba(OH)^2$ samples, respectively, when diluted, which is in the approximate range of AA expected at this retention time. For acid hydrolysis, 6 M HCl was added, the mixture flushed with nitrogen, loosely capped and placed in boiling water for 10 min to remove oxygen. Upon removal from the water, the cap was tightened and the tube placed in a heating block at 110º C for 21 h. At the end of hydrolysis the tube was cooled slightly and the contents were transferred quantitatively and filtered through a Whatman 541 filter paper into a 50-ml volumetric flask. An aliquot of the filtrate was evaporated at 65º C as rapidly as possible using a stream of N gas to prevent further loss of serine, threonine and tyrosine (Gehrke et al., 1985). The residue was rinsed with distilled water and evaporated until no HCl was detectable. Sample buffer (0.05 M lithium hydroxide, 0.1415 M lithium chloride, 0.0457 M citric acid and 0.1% phenol, pH 2.8) was added to the residue and the mixture filtered through a 0.2 µm nylon filter into a sample vial, covered with a septa lined cap and frozen at -20ºC until analyzed.

Cystine and methionine, were pre-oxidized with performic acid (PA) (Moore, 1963) and analyzed as cysteic acid and methionine sulfone, respectively. The PA was prepared according to Mason et al. (1980) with the following modification: to

produce 5 ml PA, 4.5 ml 88% formic acid, 0.5 ml 30% hydrogen peroxide and 25 mg phenol were combined, incubated for 1 h at room temperature and moved to an ice bath at 4ºC for 15 min. The tubes containing the 3 mg samples of N were placed in an ultrasonic bath filled with ice and 1.5 ml of the PA solution was added to each. The contents of the tubes were sonicated for 15 min and transferred to an ice bath at 4ºC for 16 h. The oxidizing reaction was stopped and the excess PA reduced with 0.3 ml concentrated HCl. After standing for 15 min at room temperature the tubes were placed under vacuum using a water aspirator (Elkin and Griffith, 1985) to remove the residual PA and HCl. The pre-oxidized samples were then subjected to the HCl hydrolysis procedure as described previously.

Tryptophan was determined using a barium hydroxide hydrolysis. A 3 mg sample was added to a screw top culture tube (16 x 125 mm) containing 1.2 g barium hydroxide ($Ba(OH)^2 \bullet 8H_2O$. Two ml water was added to each tube (1.90 M barium hydroxide) and the tube flushed for 10 sec with N gas, loosely capped and placed in boiling water for 15 min to remove the oxygen. Upon removal from the water, the cap was tightened and the tube placed in a heating block at 110ºC for 16 h. At the end of hydrolysis the tubes were removed, cooled and the contents transferred quantitatively to a 16 x 100 tube with a small amount of concentrated HCl. The pH was adjusted to 2.8 with additional HCl. The barium was precipitated with the addition of 2 ml of 18.2% sodium sulfate and the volume adjusted to 10 ml with water. An aliquot was centrifuged in a microfuge (Eppendorf 5465C; Brinkman Instruments, Inc; Westbury, NY 11590) at 12,000 x g for 10 min at room temperature. The supernatant was filtered through a 0.2 µm nylon filter into a sample vial, covered with a septa lined cap and frozen at -20ºC until analyzed.

Amino acid analysis

Amino acids were separated on a lithium cation exchange column (4 x 100 mm, P/N 0354100, Pickering Laboratories, Mountain View, CA) using a three-buffer step gradient (Li292, Li365 and Li375, Pickering Laboratories, Mountain View, CA) and a column temperature gradient (33, 42, 60 and 70ºC). Detection was at 560 nm following ninhydrin post column derivation on an HPLC System Gold with 32 Karat software (Beckman-Coulter, Inc., Fullerton, CA). Standards (250 nM ml-1) for Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, NH3, Lys, His, Arg and Cys (125 nM ml-1) were prepared by diluting a purchased stock (Amino acid standard H, #20088; Pierce Chemical; Rockford, IL 61105) with the sample buffer. Standards (250 nM ml-1) for cysteic acid, Met sulfone, norleucine and tryptophan were prepared in sample buffer and combined with the others. The volume of samples and standards loaded on the column was 50 μL.

Statistical and Economic Analysis

Effect of diet and week on body weights, feed intake, ADG, feed efficiency (gain:feed), fecal and respiratory scores, BCS, hip height, as well as PUN and haptaglobin were analyzed as a completely randomized block design, using the general linear model (GLM) procedure in SAS (SAS version 9.1, SAS Inst. Inc., Cary, NC). Block (treatment) was designated as a random effect, whereas MR was a fixed effect. Initial measurements of BW and hip height were used as covariates. Sums of squares were partitioned to treatment and day. Model was $Y = \mu + T_i + C_{ij} + D_k + \varepsilon_{ijk}$, where Y is the dependent variable; T_i effect of the i^{th} treatment; C_{ij} effect of the j^{th} calf on the i^{th} treatment; D_k effect of the k^{th} day of study; and ε_{ijk} the residual. Interactions among calf, treatment, and day were tested and found to be non-significant and dropped from the model.

Mean separation analysis was carried out when the *F*-test suggested a potential interaction ($P < 0.10$). The variables fecal and respiratory scores and BCS are not continuous, normally distributed data and were analyzed using a non-parametric Kruskall-Wallis test.

Body weights were regressed on day to obtain the growth rates (ADG) of individual calves over the treatment period. Growth rates were calculated weekly, and from one to 21, 21 to 35, 35 to 49, one to 49, 49 to 63 days and an overall ADG was calculated.

Dry matter intake was used to calculate the total cost of feeding the calves. Only feed prices will be analyzed because all management practices were similar among the treatments. The cost per unit of weight gain was analyzed.

In all cases when means were analyzed, significance was declared at $P < 0.05$.

RESULTS

General Health

Prior to initiating treatment diets, but after allocation to treatment, 3 calves died of frostbite related causes due to extreme cold temperature conditions, one calf from Treatment C and two calves from Treatment D. Average ambient temperatures during the experiment were varied and ranged from -23 ºC (February 7), to 26 ºC (April 23, Fig. 2.1). Minimum and maximum daily temperatures during the experimental period varied by as much as 25°C, which influenced respiratory scores among all treatments during the course of the study. Two calves (one each from Treatments B and C) were diagnosed with pneumonia and died during the treatment period. One calf on Treatment A died on day 56 of treatment of spontaneous bloat and no further diagnosis was made.

Fecal and respiratory scores were analyzed and found to be similar among treatments ($P = 0.17$ and $P = 0.13$). Average fecal and respiratory scores were 1.72 ± 0.04 and 1.24 ± 0.04, respectively, indicating that the differences in protein source or enzyme treatments did not affect those measures. There was a measurable difference in fecal scores as the experiment progress indicating changes in digestive function as the calves increased in age and fecal appearance and color values decreased numerically after weaning but similarly among treatments and was normal with the changes in sources of nutrients. Overall, days treated were not significantly different among treatments (1.45 ± 0.30) and generally, once calves overcame the initial cold stress conditions, calf behavior and general well-being was excellent as indicated by the growth performance.

. **Enzyme Activity and Quality Control**

To verify that the enzymes were active and functional, β-D-Mannanase enzyme levels in two sets of pooled samples of reconstituted calf milk replacer were analyzed. At a minimum, the planned level of enzyme activity was confirmed to have been achieved (ChemGen Corp., internal data) in the reconstituted milk replacer samples with added enzymes, thus enzymes were not denatured during the mixing period. Further, the milk replacers were tested for native enzyme activity and no activity was found. Further, due to outstanding technical assistance in formulation, the diets were isocaloric, isonitrogenous and very similar in AA content (Table 2.3).

Dry Matter Intake and Body Growth

Overall, DMI was not significantly different among treatments and averaged 1.65 ± 0.08 kg over the 63-day study period (P = 0.26; Table 2.7). Also, milk replacer intakes were similar among treatments (P = 0.3; Figure 2.2). Evaluating DMI by day of treatment identified the days of milk replacer removal as the period when DMI differed significantly. Starter intake was greater (P = 0.03) for calves fed the SPC containing milk replacer for the seven days milk replacer offered was reduced (Figure 2.3, 2.4; Table 2.7). The enzyme treatments did not significantly affect DMI.

Consistent with the DMI, the ADG of calves among treatments were not different (P = 0.28; Table 2.8). Growth performance of all calves on treatment was exceptional, especially given the average ambient temperatures and wind chill experienced during the period of calf acquisition and during the early phase of the study (Figure 2.5). Overall ADG by treatment was approximately 0.90 kg per day.

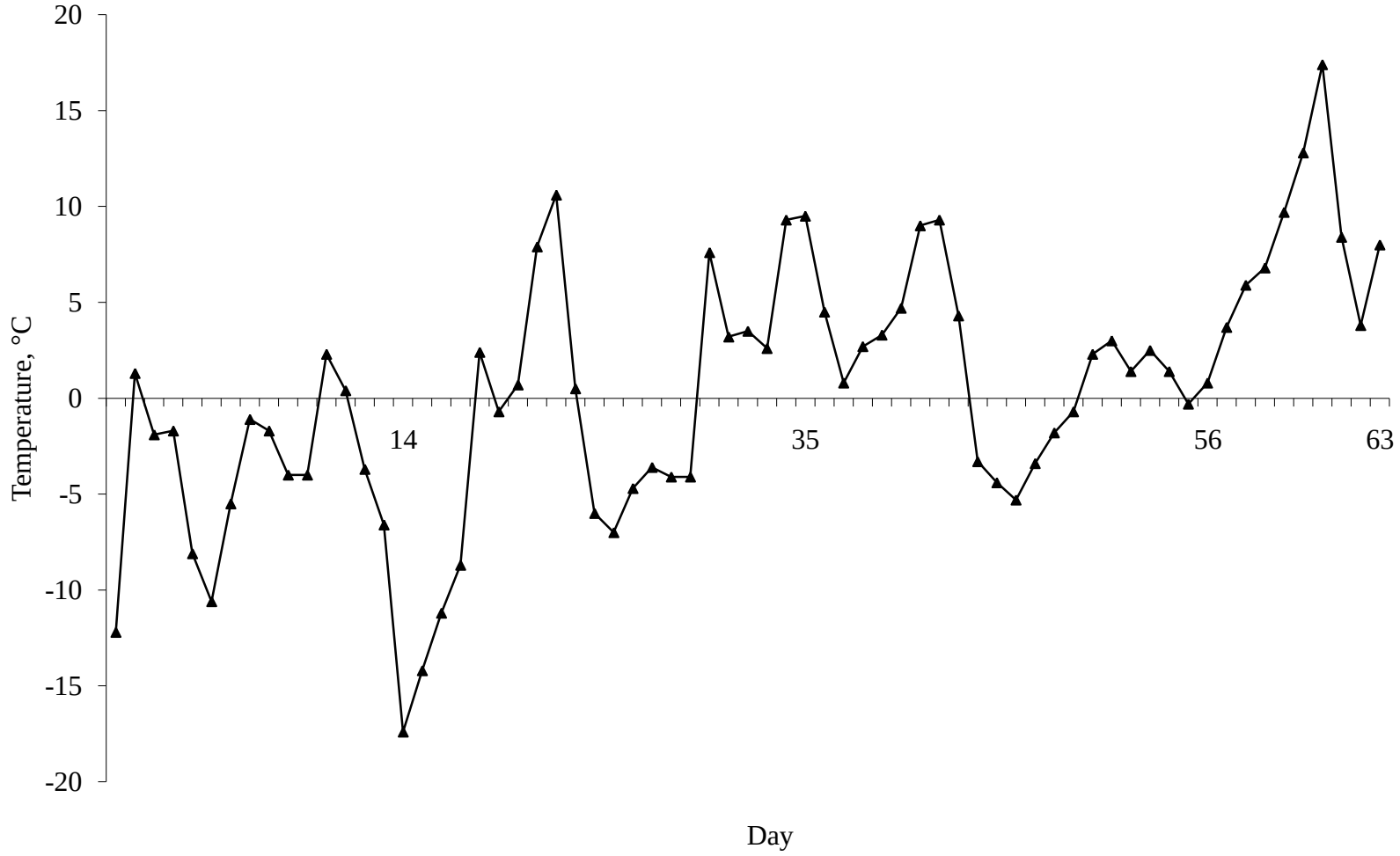

Figure 2.1. Average ambient daily temperatures during the experimental period.

Due to greater DMI of starter grain during the step down period of milk replacer intake, the calves fed the SPC containing milk replacer demonstrated greater ADG ($P < 0.05$) independent of the enzyme treatments (Table 2.8). During the 7 d step down period, ADG decreased to 0, 0.28, 0.29, and 0.24 kg/day for treatments A, B, C, and D respectively ($P < 0.05$) (Figure 2.6) suggesting that calves fed the SPC containing milk replacer had greater capacity to consume and utilize the starter grain compared to calves fed the whey based milk replacer. Finally, although not significantly different, the calves offered Treatment C were 7.4% heavier than calves offered the positive control Treatment A.

Similar to the ADG data, hip height (HH; $P = 0.52$) and BCS ($P = 0.55$) were not different among treatments (Table 2.9). Overall, calves were 95.6 ± 3.8 cm tall and the difference in HH among treatments was less than 2 cm. Among treatments, HH gain averaged 0.18 cm/d over the experimental period. Initial and final BCS were not different by treatment and the average final BCS was 2.68 ± 0.52 (Table 2.9)

Also, consistent with the ADG data the feed efficiencies were not significantly different among treatments ($P = 0.33$) although there was a trend ($P < 0.08$) for calves fed Treatment C to have a higher feed efficiency than calves fed Treatment D (Table 2.10). Overall values of feed efficiency were 0.56, 0.56, 0.59, and 0.54 for Treatments A, B, C, and D, respectively.

During the step down period for weaning week during the eight week of treatment, feed efficiency notably decreased and differences were 100, 71, 68, and 71% compared to week 7 for treatments A, B, C, and D respectively (Table 2.10).

Table 2.7. Effect of age on dry matter intake (DMI) of calves fed different sources of protein in milk replacer and with the enzymes addition.

	Treatment A[1]		Treatment B[2]		Treatment C[3]		Treatment D[4]	
Week	DMI[5]	SEM	DMI	SEM	DMI	SEM	DMI	SEM
Day 1 -35	1.56	0.04	1.49	0.04	1.43	0.06	1.40	0.07
Day 36 - 49	1.76	0.07	1.85	0.07	1.87	0.06	1.83	0.06
Day 50 - 63	2.14	0.15	2.46	0.14	2.51	0.15	2.31	0.15
MR	1.49	0.05	1.40	0.05	1.38	0.05	1.36	0.05
Starter*	0.79[b]	0.06	1.01[a]	0.07	1.02[a]	0.07	0.90[ab]	0.07
Overall	1.66	0.03	1.73	0.05	1.64	0.08	1.55	0.09

[a,b]Within a row, means without a common superscript letter differ with $^{*}P < 0.10$.

[1]Treatment A: all whey protein MR, no enzymes, only salt added.

[2]Treatment B: soy protein based MR, enzyme A added.

[3]Treatment C: soy protein based MR, enzyme B added

[4]Treatment D: soy protein based MR, no enzyme, salt added.

[5]All values are in kg/day.

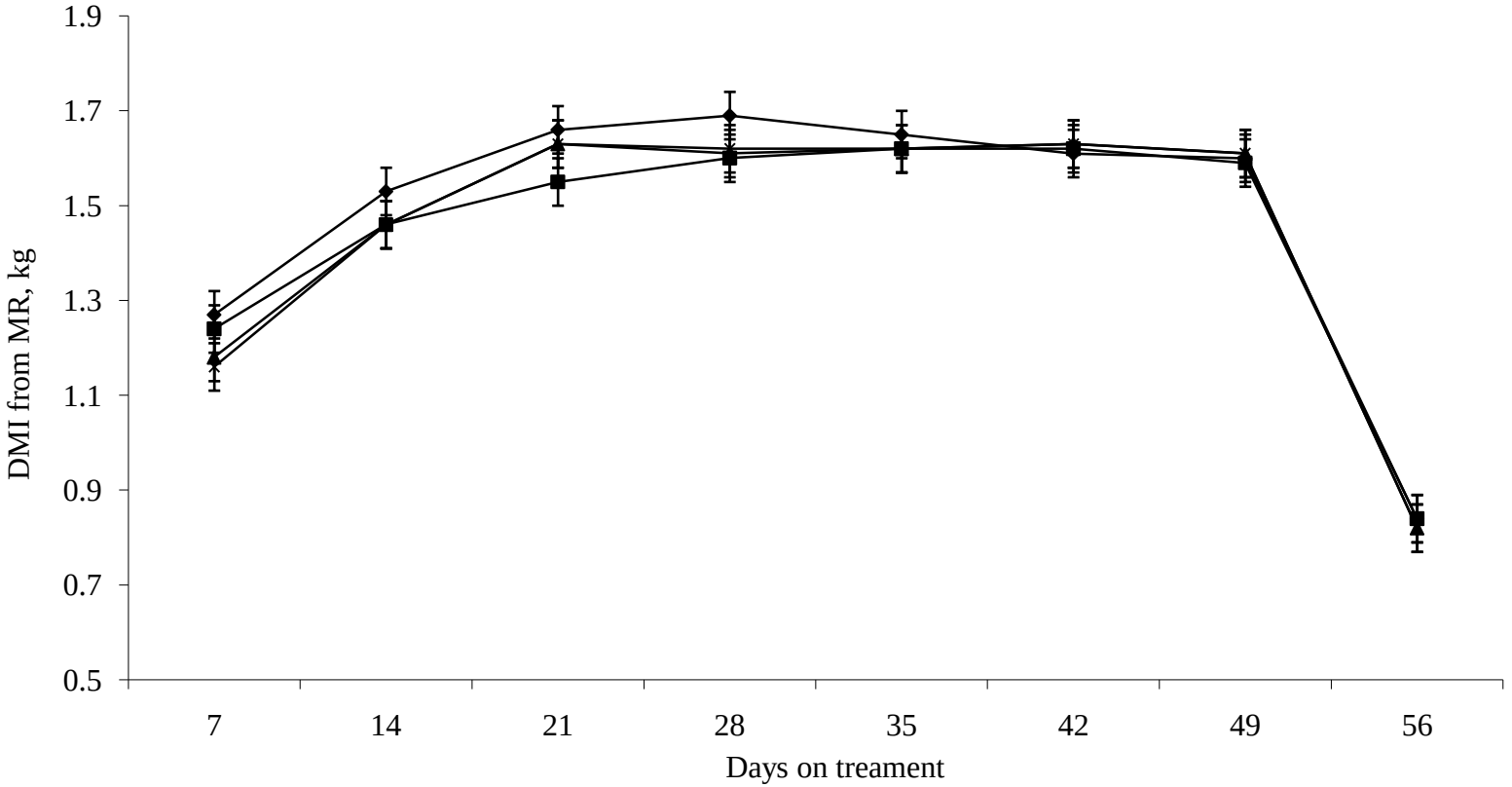

Figure 2.2. Effect of days on treatment and treatment on DMI from milk replacer (MR) for calves fed either whey protein based MR or soy protein based MR with or without added enzymes for 9 weeks. Treatment A (all whey protein MR, ♦), treatment B (soy protein based MR, enzyme A added, ■), treatment C (▲soy protein based MR, enzyme B added), treatment D (X, soy protein based MR, no enzyme, salt added).

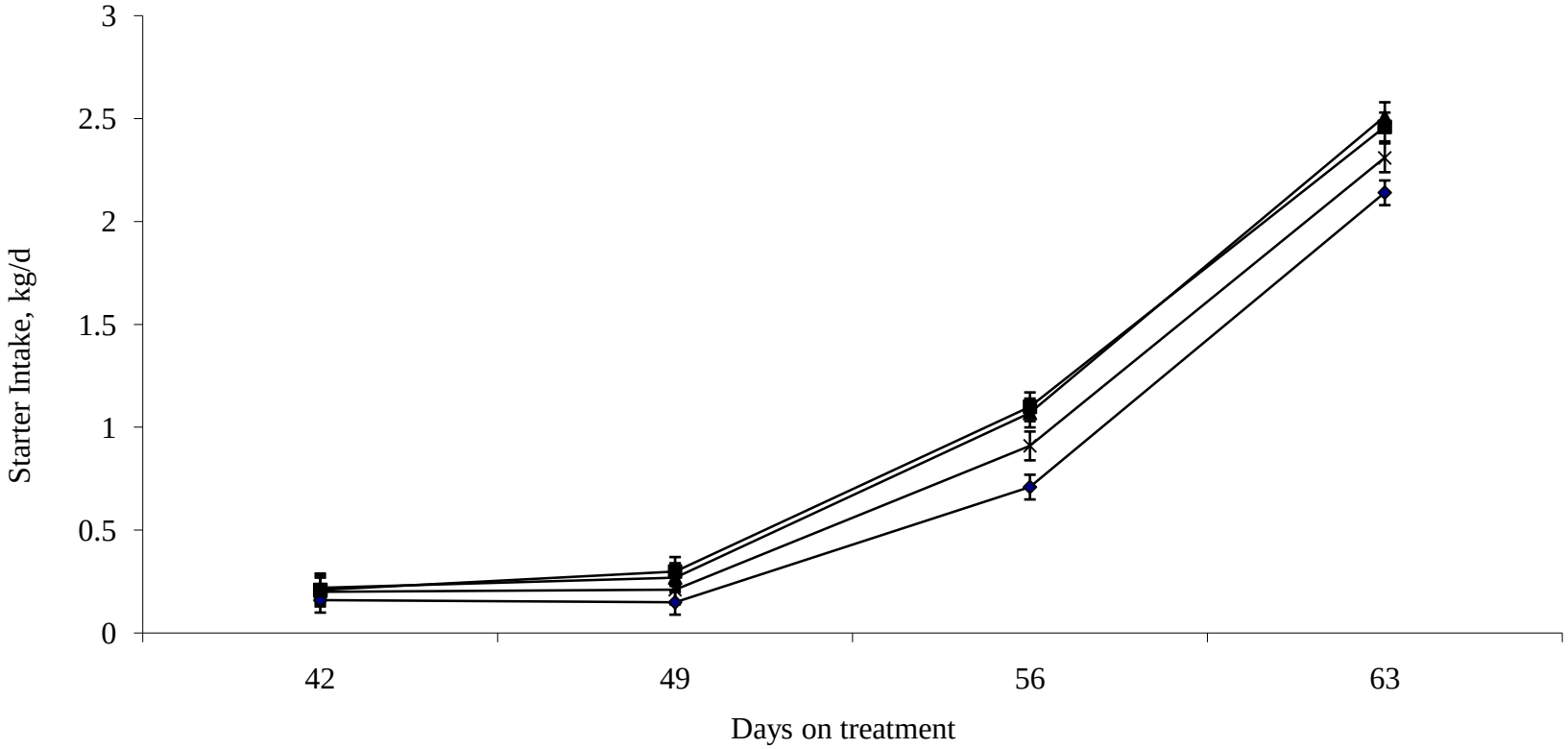

Figure 2.3. Effect of days on treatment and treatment on DMI from starter for calves fed either whey protein based MR or soy protein based MR with or without added enzymes for 9 weeks. Treatment A (all whey protein MR, ♦), treatment B (soy protein based MR, enzyme A added, ■), treatment C (▲soy protein based MR, enzyme B added), treatment D (×, soy protein based MR, no enzyme, salt added).

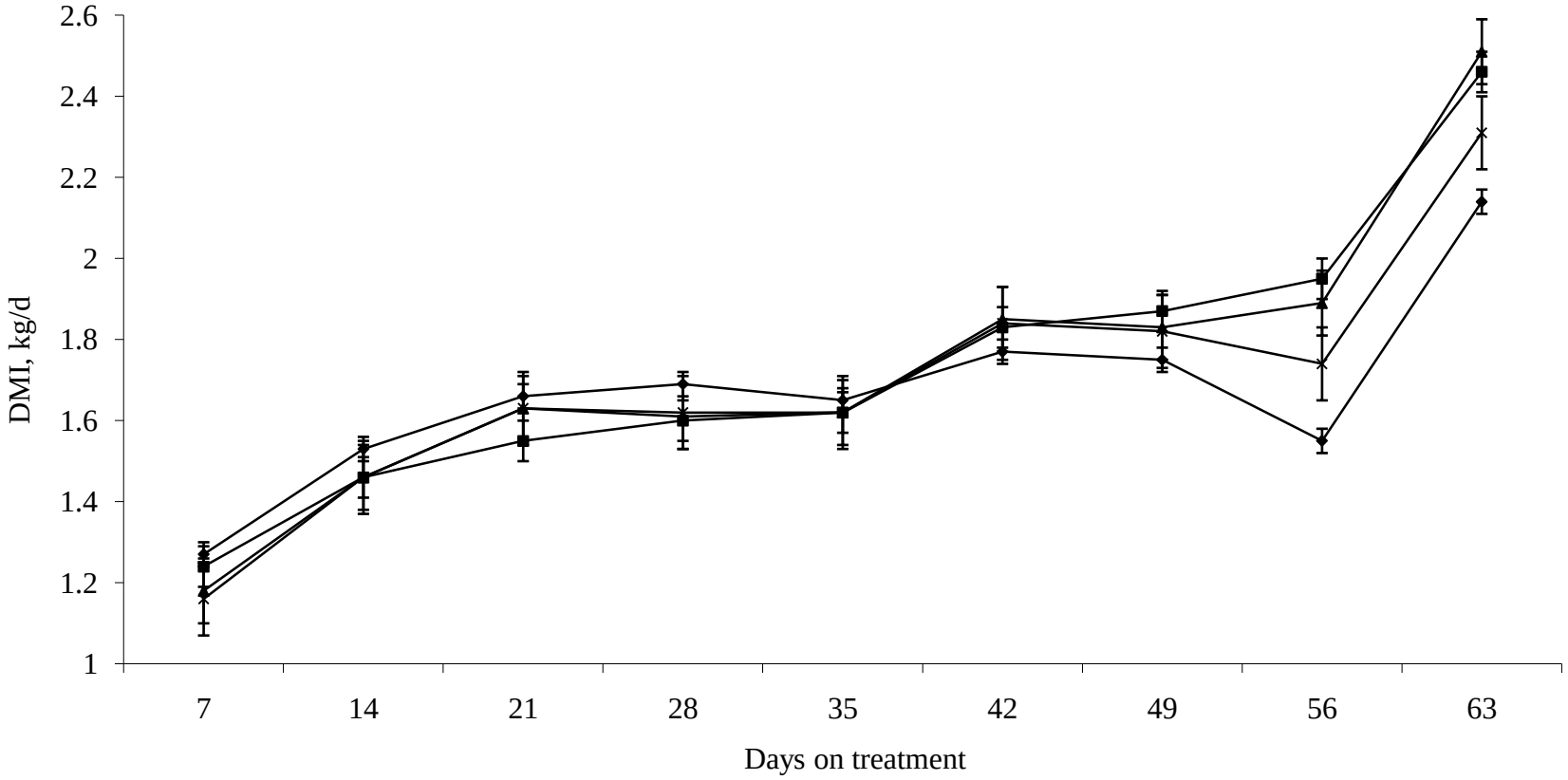

Figure. 2.4. Effect of days on treatment and treatment on DMI on calves with either whey protein MR or soy protein based MR and added or not with enzymes during 9 weeks. Treatment A (all whey protein MR, ♦), treatment B (soy protein based MR, enzyme A added, ■), treatment C (▲soy protein based MR, enzyme B added), treatment D (×, soy protein based MR, no enzyme, salt added).

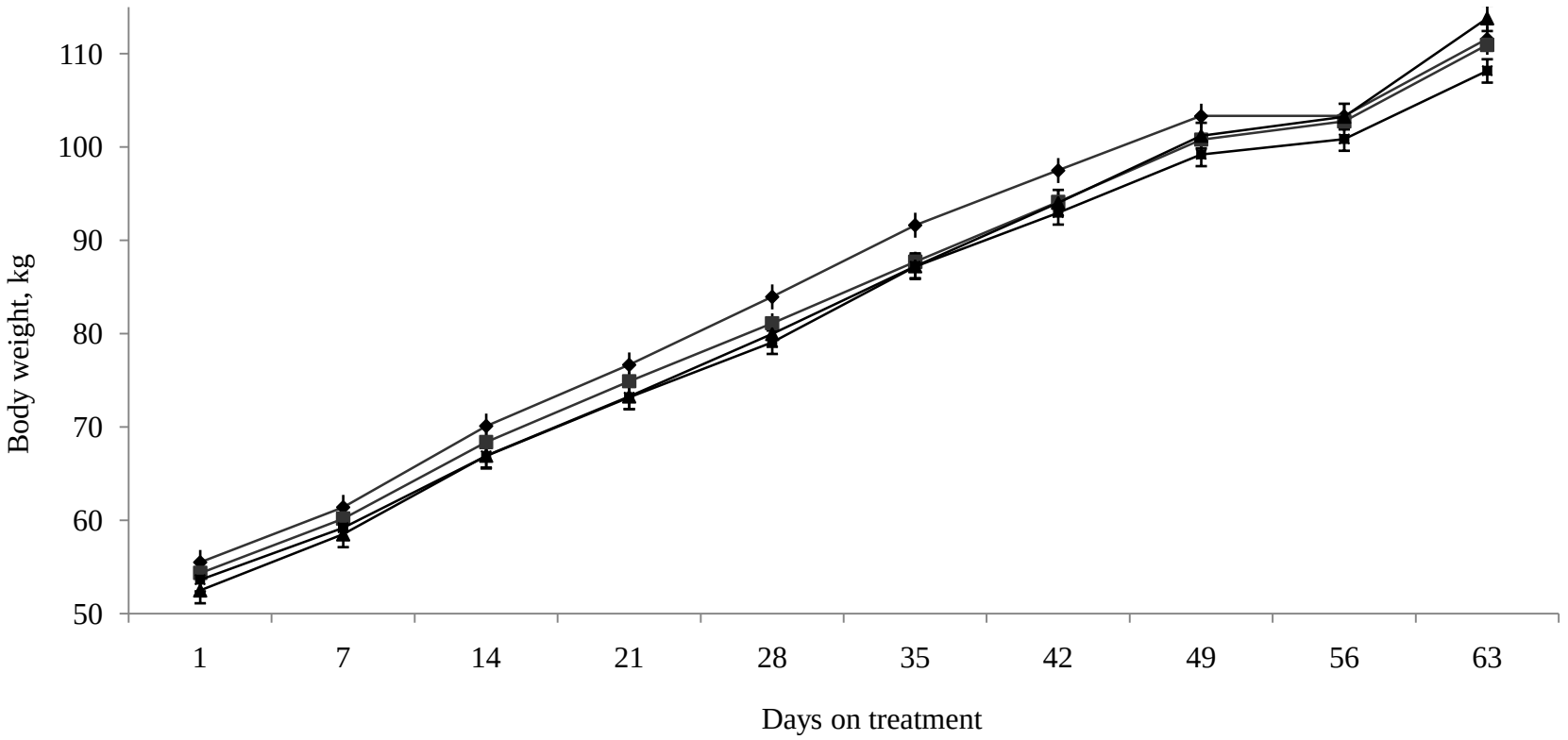

Figure 2.5. Relationship between days on treatment and body weight by treatment. Calves were assigned to all whey protein MR diet with no enzymes added (Trt A, ♦), soy protein based MR with enzyme A (Trt B, ■), soy protein based MR with enzyme B (Trt C, ▲), and soy protein based MR with no enzymes added (Trt D, **X**). No differences were observed among treatments ($P > 0.05$).

Table 2.8. Effect of age on average daily gain (kg) of calves fed two milk replacers (MR), an all whey based milk replacer (Treatment A) and a soy protein concentrate containing milk replacer (Treatments B, C and D) with two levels of β-mannanase enzymes.

	Treatment A[1]		Treatment B[2]		Treatment C[3]		Treatment D[4]	
Week	ADG[1]	SEM	ADG	SEM	ADG	SEM	ADG	SEM
Day 1 – 35	1.01	0.05	0.95	0.05	0.99	0.05	0.96	0.05
Day 36 – 56	0.61	0.08	0.72	0.08	0.76	0.09	0.65	0.09
Day 56 – 63	1.18	0.15	1.17	0.15	1.51	0.16	1.04	0.17
Day 49 - 63	0.59	0.16	0.725	0.10	0.89	0.10	0.64	0.13
Day 1 – 49	0.98	0.04	0.95	0.03	0.99	0.04	0.93	0.05
Overall	0.89	0.03	0.89	0.03	0.97	0.05	0.86	0.05

[1]Treatment A: all whey protein MR, no enzymes, only salt added.

[2]Treatment B: soy protein based MR, enzyme A added.

[3]Treatment C: soy protein based MR, enzyme B added

[4]Treatment D: soy protein based MR, no enzyme, salt added.

[5]Values are in kg

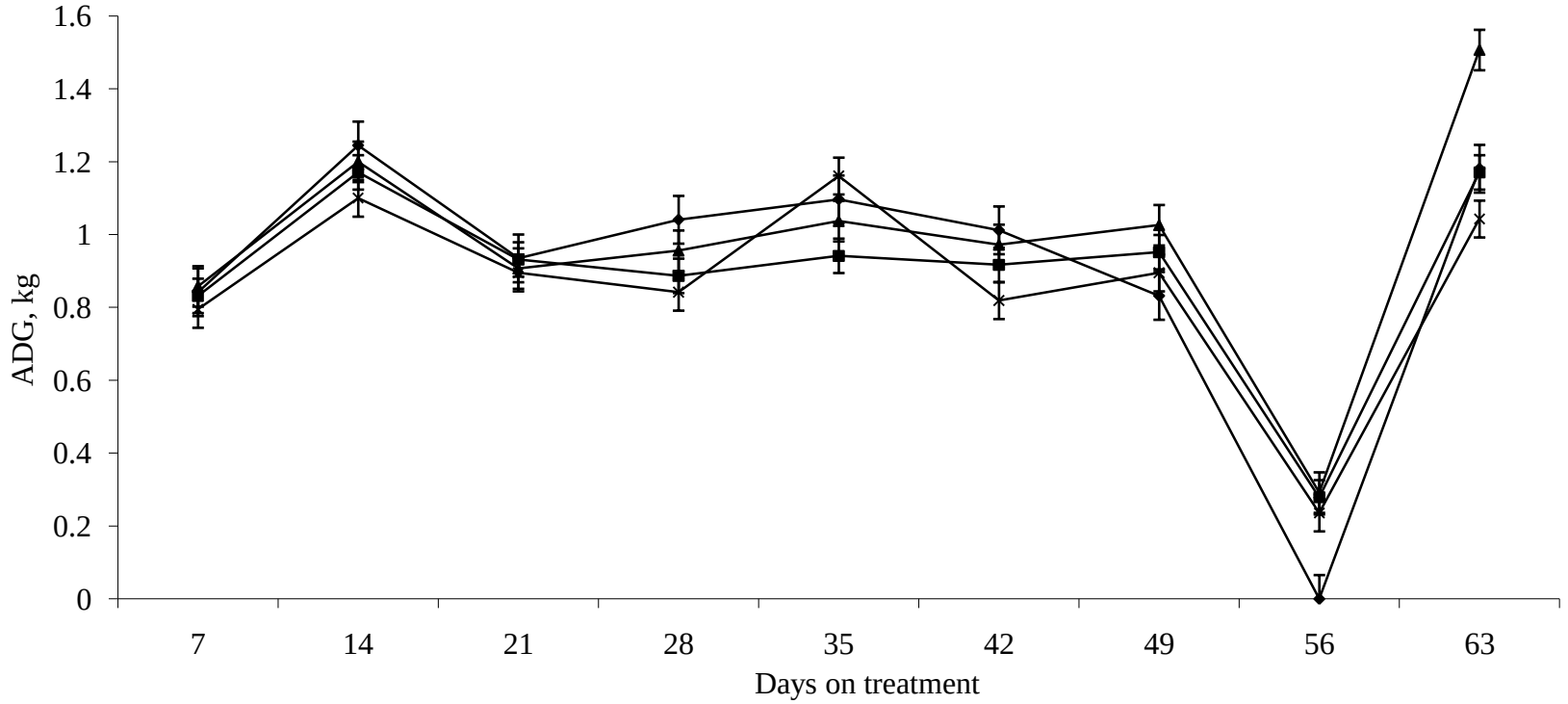

Figure 2.6. Effect of age and treatment on average daily gain (ADG) of calves fed with either all milk MR or soy protein added to MR and enzymes addition. Treatment A (all whey protein MR, ♦), treatment B (soy protein based MR, enzyme A added, ■), treatment C (▲soy protein based MR, enzyme B added), treatment D (X, soy protein based MR, no enzyme, salt added) Days 1 to 7, 0.28 Mcal gross energy/kg $BW^{0.75}$, from days 8 to 21, 0.32 Mcal gross energy/kg $BW^{0.75}$. Day 49 to 56 milk replacer restriction (50% of previous intake) and weaning.

Table 2.9. Effect of treatment on hip height and body condition score in calves fed milk replacers containing either a whey based or soy protein concentrate containing milk replacer and offered two levels of β-mannanase enzymes.

	Hip height					Body condition score				
Treatment	Initial cm[5]	SD	Final cm[5]	SD	Change cm/d	Initial[5]	SD	Final[5]	SD	Change in BCS/d
A[1]	85.75	3.16	96.96	3.71	0.20	1.74	0.26	2.62	0.55	0.033
B[2]	85.13	3.25	95.00	4.56	0.18	1.66	0.39	2.62	0.42	0.034
C[3]	84.86	2.96	95.22	3.40	0.19	1.85	0.38	2.88	0.64	0.036
D[4]	85.23	3.25	95.06	4.56	0.17	1.72	0.25	2.63	0.45	0.030

[1]Treatment A. All whey protein MR, salt added.

[2]Treatment B, soy protein based MR, Enzyme A added.

[3]Treatment C, soy protein based MR, enzyme B added.

[4]Treatment D, soy protein based MR, salt added, no enzyme added.

[5]No statistical differences were found among treatments.

Table 2.10. Feed efficiency values of calves fed two milk replacers without or with enzymes added.

	Treatment A[1]		Treatment B[2]		Treatment C[3]		Treatment D[4]	
Week	G:F	SEM	G:F	SEM	G:F	SEM	G:F	SEM
Day 1 - 35	0.68	0.02	0.66	0.02	0.68	0.02	0.65	0.02
Day 36 - 56	0.60	0.02	0.55	0.02	0.58	0.02	0.57	0.02
Day 57 - 63	0.38	0.04	0.42	0.04	0.47	0.04	0.39	0.04
Day 49 - 63	0.32	0.04	0.35	0.04	0.42	0.04	0.33	0.05
Day 1 - 49	0.63	0.02	0.62	0.02	0.64	0.02	0.60	0.02
Overall	0.56	0.02	0.56	0.02	0.59	0.02	0.54	0.02

[1]Treatment A: all whey protein MR, no enzymes, only salt added.

[2]Treatment B: soy protein based MR, enzyme A added.

[3]Treatment C: soy protein based MR, enzyme B added.

[4]Treatment D: soy protein based MR, no enzyme, salt added.

Plasma metabolites

Plasma urea nitrogen (PUN) values differed among treatments (P = 0.004) and tended to be higher in calves fed Treatment C (Table 2.11). The differences were observed during the milk replacer feeding phase (day 1 – 35) and also during the milk replacer and starter feeding phase period (day 36 – 56)

The observed PUN levels were similar than other studies (Bartlett et al., 2006; Diaz et al., 2001). The differences were not consistent among treatments and were higher in calves fed the SPC containing milk replacer, suggesting that the enzyme might have affected the availability of protein or the crystalline amino acids were being metabolized prior to carbohydrate absorption, thus causing greater oxidation.

Soy protein is known to cause some antigenic responses, which can be similar to the responses observed during infections and then inflammations and are due to the presence of antinutrients. Haptaglobin has evolved into an acute phase protein that can be used as an indicator of an inflammatory response. Haptaglobin concentrations were 0.44, 0.61, 0.54, and 0.34 mg/ml for treatment A, B, C, and D respectively and were not significantly different (P = 0.57; Table 2.12). Thus, the dietary treatment did not affect any inflammatory response.

Costs analysis

Milk replacer prices were provided by MSC and costs were calculated simply as dollars per unit of gain through each feeding period represented by either milk replacer or milk replacer and starter grain or just starter grain. Overall costs per kg of gain were also calculated. At the current market prices, the price per kilogram for the all milk protein milk replacer was $3.09 whereas the SPC containing milk replacer was $2.62, a 15% difference primarily due to the current cost of whey proteins. In

addition, the price of the starter grain was considered in this analysis and the current market price is approximately $0.34/kg.

Although not explicit, there is some economic cost to using the enzymes investigated in this study. In an economic analysis these costs should be considered however, based on our current information, the cost of the enzyme would be minimal compared to the cost of the diet ingredients and on the order of cents per day, thus it is ignored here.

Overall, the cost per unit of gain was greater for the calves fed the whey based milk replacer however ($P = 0.07$), there were no significant differences among the treatments fed the SPC containing milk replacer (Table 2.13). These values are consistent with the DMI, ADG and feed efficiency calculations where there were no significant differences among treatments in performance of the calves. There was a numerical difference in the cost per unit of gain of the calves fed the SPC milk replacer and provided the mannanase based enzymes. This suggests that with increased feed efficiency there is some economic benefit to the use of the enzymes. This might be particularly true around the time of weaning.

On average, calves gained approximately 53.6 kg during the study. Given the differences in the price of the milk replacers, feeding the SPC containing milk replacer with the addition of the enzymes, a producer would be expected to save approximately $92 over the feeding period.

Table 2.11. Effect of protein source and enzyme addition on plasma urea nitrogen concentration (PUN) in Holstein bull calves fed with a constant amount of energy and protein.

	Treatment A[1]		Treatment B[2]		Treatment C[3]		Treatment D[4]	
Day	PUN[5]	SEM	PUN[+]	SEM	PUN[+]	SEM	PUN[+]	SEM
Day 1 – 35**	13.17[c]	0.45	13.83[bc]	0.47	15.95[a]	0.49	15.32[ab]	0.51
Day 36 – 56*	11.78[b]	0.48	12.45[ab]	0.49	13.77[a]	0.50	12.66[ab]	0.53
Day 57 – 63	13.48	0.74	15.11	0.74	16.16	0.77	14.71	0.81
Overall**	12.73[c]	0.34	13.47[bc]	0.43	15.16[a]	0.37	14.25[ab]	0.37

[a,b,c]Within a row, means without a common superscript letter differ ** ($P<0.05$) and *($P<0.10$).

[1]Treatment A: all whey protein MR, no enzymes, only salt added.

[2]Treatment B: soy protein based MR, enzyme A added.

[3]Treatment C: soy protein based MR, enzyme B added.

[4]Treatment D: soy protein based MR, no enzyme, salt added.

[5]All values are in mg/dL.

Table 2.12. Haptaglobin values by treatment on week five and eight of calves fed MR with either soy protein or whey protein and with the addition of enzymes.

Treatment	Haptaglobin[1,2] Wk five	SE	Haptaglobin Wk eight	SE
A[3]	0.444	0.08	0.232	0.048
B[4]	0.610	0.08	0.398	0.12
C[5]	0.543	0.09	0.227	0.09
D[6]	0.335	0.09	0.407	0.21

[1]This weeks correspond to period just prior to offering starter, and week eight to the weaning period.

[2]Haptaglobin values are expressed in mg/ml.

[3]Treatment A: all whey protein MR, no enzymes, only salt added.

[4]Treatment B: soy protein based MR, enzyme A added.

[5]Treatment C: soy protein based MR, enzyme B added.

[6]Treatment D: soy protein based MR, no enzyme, salt added.

Table 2.13. Effect of treatment on the cost of produce a kg of calf weight gain, on calves fed with either two sources of protein and the addition of enzymes to the diet.

	Treatment A[1]		Treatment B[2]		Treatment C[3]		Treatment D[4]	
Day	Cost[5]	SE	Cost	SE	Cost	SE	Cost	SE
1 – 35**	4.85[a]	0.20	3.51	0.20	3.29	0.21	3.87	0.22
36 – 56**	4.29[a]	0.12	3.07[b]	0.08	3.07[b]	0.08	3.05[b]	0.10
57 - 63	0.76	0.05	0.88	0.05	0.89	0.05	0.82	0.06
Overall**	5.70[a]	0.47	3.94[b]	0.52	3.99[b]	0.39	4.43[ab]	0.61

[a,b]Within a row, means without a common superscript letter differ ** P<0.05.

[1]Treatment A: all whey protein MR, no enzymes, only salt added.

[2]Treatment B: soy protein based MR, enzyme A added.

[3]Treatment C: soy protein based MR, enzyme B added.

[4]Treatment D: soy protein based MR, no enzyme, salt added.

[5]Prices are in dollars per kg BW produced.

III. DISCUSSION

The growth and health performance of calves fed milk replacers containing non-milk or vegetable proteins has generally been less than calves fed diets consisting of all milk proteins (Drackley et al., 2006; Gardner et al., 1990; Khorasani et al., 1989; Knaus et al., 1994; Xu et al., 1997). Further, due to the presence of anti-nutritive non-starch carbohydrates such as β-galactomannans, decreased performance has also been observed in monogastric animals fed soy containing diets (Anderson et al., 2007; Zou et al., 2006). In this study, we reject the hypothesis that calves fed a SPC containing milk replacer have reduced performance even at high levels of consumption. This lack of effect of the SPC containing milk replacer on growth performance obviates the remainder of the hypothesis concerning the mannanase based enzyme treatments although there were trends for increased feed efficiency in the calves fed the SPC based milk replacer with the mannanase based enzymes. The growth performance of the calves on this study was exceptional, despite cold stress.

Most studies investigating the use of SPC as a partial replacement for whey or skim milk proteins in milk replacers have observed decreases in performance (Dawson et al., 1988; Drackley et al., 2006; Lallès, 2000) and the decreased performance has been attributed to the antinutritional components of the soy protein. This study utilized a low antigen form of SPC (Profine VF) that still contained a reasonable level of the β-galactomannans. Drackley et al. (2006) pointed out that under current production methodologies, most of the antinutritional factors attributed to SPC are inactivated by the manufacturing process (Lallès, 2000). However, reduced growth performance and what might be considered premature adaptations of intestinal function and immune responsiveness of calves fed SPC containing milk replacer is still an issue (Grant et al., 1989; Lallès, 2000; Leterme et al., 1998).

For example, Drackley et al. (2006) along with others have demonstrated changes in the morphology of the intestine especially in villus height when SPC is included in milk replacer (Dawson et al., 1988; Grant et al., 1989). Further, others have observed decreases in protein synthetic capacity of the gut, reduced mucosal enzyme activity and reduced transport capacity by the gut (Grant et al., 1989; Montagne et al., 1999). These changes coupled with increased mucin secretion (Montagne et al., 2000) and increased immune activation (Lallès, 2000) suggest that energy partitioned to what would be considered maintenance requirements (altered protein synthetic activity, immune function) would be greater for calves fed diets containing SPC. Calves consumed a high level of SPC on this study, and no reduction in growth was observed suggesting that some of the previously observed responses were due to energy intake levels just in excess of maintenance. In studies where SPC has been evaluated, energy intake has ranged from 2.6 to approximately 3.7 Mcal/d ME intake (Dawson et al., 1988; Drackley et al., 2006). In this study, calves averaged approximately 6.5 Mcal/d of ME intake over the treatment period, a 43 to 60% greater caloric intake. These data suggest that if calves were provided adequate energy intake, the physiological adaptations associated with the presence of the plant protein were not inhibitory to adequate growth and nutrient partitioning.

The PUN levels were high and consistent with the level of total nutrient intake and similar to previous studies (Bartlett et al., 2006; Diaz et al., 2001) suggesting that the protein status of the calves was adequate and did not limit growth in these calves. Endogenous protein secretion and loss is higher in calves fed plant based proteins (Montagne and Lallès, 2000; Montagne et al., 2001, 2000). However, true digestibility of the protein is high despite the endogenous production and Montagne et al., (2003) has suggested that specific oligopeptides might stimulate endogenous protein secretion, but that some reabsorption occurs as the protein moves through the

intestinal tract (Montagne et al., 2003). Whey based milk replacers and SPC have different amino acid profiles, thus in this study we added crystalline amino acids to minimize the differences among the two replacers. There are essentially no studies in which to compare these treatments based on amino acid composition. In Drackley et al. (2006) methionine and lysine were supplemented in the SPC containing milk replacer, but growth performance was still reduced in calves fed the SPC milk replacer. They speculated that other amino acids, specifically threonine, might have been limiting based on the data of Kanjanapruthipong (1998). In the study of Kanjanapruthipong (1998), threonine, methionine and lysine were added to a soy flour based milk replacer and growth performance of the calves was improved by over 26% suggesting that amino acid balance or profile is critical when evaluating responses between all milk and vegetable protein based milk replacers. The lack of significant growth or performance differences in this study suggests that the addition of amino acids to the SPC containing milk replacer was central to the animal response and warrants further investigation.

One additional consideration of this study design was the amount of protein fed to these calves. Although the amino acid addition to the milk replacer might be important, one other consideration is the protein content of the base diet. This is the only study we are aware of where such a high level of protein was formulated into the replacer and exchanged for SPC. In most other studies, the protein levels of the basal diets ranged from 19.6 to 23% (Dawson et al., 1988; Drackley et al., 2006; Kanjanapruthipong, 1998; Lallès et al., 1995b; Lallès et al., 1995c), so it is difficult to draw direct comparisons to previous studies and conclude if the observed performance was due to the basal diet protein content or the additional amino acids. This should be the topic of a subsequent study while remembering that the SPC used in this study was considered to be of low antigen content.

Calves fed soy containing milk replacers have exhibited sub-clinical immune responses, suggesting there were antigenic carbohydrate or protein moieties inducing these responses (Barratt et al., 1979; Lallès, 2000). Circulating levels of acute phase proteins can be used as an indicator of pathogen challenge or immune status in neonatal calves (Deignan et al., 2000; Ganheim et al., 2007; Orro et al., 2007). Haptaglobin, although primarily associated with hemolytic anemia, is considered an acute phase protein and has been used as a general indicator of health status (Ganheim et al., 2007) or acute infection (Deignan et al., 2000). Previous work evaluating SPC or SP isolate in calf milk replacers has demonstrated immune stimulation (Lallès, 2000), so we considered an immune response might be partially responsible for the redirection of nutrients towards maintenance activities and partly responsible for the decreased performance of calves fed diets containing SPC. However, there was no effect of treatment on circulating levels of haptoglobin in calves on this study. In fact, the average values suggest the calves were quite healthy. The SPC used in this study was considered low antigen by the manufacturer, thus the lack of acute phase response might be a function of the SPC.

Inclusion of soy proteins in milk replacers for calves has been identified with several negative characteristics such as partial atrophy of the villi in the intestines, decreased digestion and absorption, increased passage rate, and increase in scours (Dreau and Lalles, 1999; Lallès, 1993; Lallès and Dreau, 1996; Lallès et al., 1996a; Sissons, 1982). Scours was not observed in calves on this study and given the level of performance, it was assumed that gut health and function was similar among calves fed either milk replacer.

In monogastric species, the β-galactomannans which occur in soybean are anti-nutritional (Anderson et al., 2007; Zou et al., 2006). The presence of β-galactomannans and have been found to induce immune system stress in poultry and

swine as measured by the blood levels of α-1 acid glycoprotein, an acute phase protein, (Anderson and Hsiao, 2006; Anderson et al., 2007; Jackson et al., 2004). Within the experimental conditions of this study, there was no effect of the mannanase based enzymes on growth performance or immune responsiveness in the calves fed the SPC based milk replacer despite the measured levels of ß-galactomannans in the milk replacer. It is possible that calves are not as sensitive to the mannans as monogastric species and that the adaptive gastrointestinal response to the presence of vegetable proteins and carbohydrates normally associated with weaning (Lallès et al., 2007) is not negative provided adequate calories are provided to the calf to allow for the response.

Weaning strategies have been developed to optimize growth and minimize post-weaning growth decreases (Khan et al., 2007). The weaning approach used in this study was designed to evaluate the effects of the enzyme addition on both milk replacer and the starter, thus the starter was withheld for the first 35 days of the study. Although there were no significant effects of the enzyme on the milk replacer or starter intake, there was a significant effect of the SPC based milk replacer and starter intake for the week of milk replacer restriction. Starter intake in the calves fed the SPC containing milk replacer was high enough to maintain weight gain compared to the calves fed the whey based milk replacer and their weight gain fell to zero the week of restriction. The week after all milk replacer was removed, dry matter intake was not different among treatments and the calves achieved growth rates similar to the pre-weaning period.

Feed efficiency was moderately increased by the addition of the mannanase enzymes and this was most apparent during the period starter grain was available to the calves. Increases in efficiency could be a function of several factors. The main effect of the addition of mannanase to soy containing diets is not well understood, however, recent

work showed the potential of viscous polysaccharides associated with the presence of B galactomannans to decrease glucose absorption and production of insulin and IGF-I (Hahn et al., 1995; McNaughton et al., 1998; Zou et al., 2006).

The effect of mannanase on β-galactomannan is to break the molecule and make mannan oligosaccharides and galactose. This results in greater galactose availability and the creation of some mannan oligosaccharides, which have been proved to be useful to reduce infections (LeMieux et al., 2003). Further, this dissociation reduces the viscosity of the feed which reduces adhesion to the wall of the intestinal tract and decreases endogenous protein production, thus reducing energy output by the gut (Larsen et al., 1993; Xu et al., 2005). This would allow for greater energy partitioning towards tissue growth and thus improved feed efficiency.

Finally, given the cost of whey proteins, finding functional alternatives would be beneficial to the industry. Under these study conditions, the SPC based milk replacer with the added enzymes provided similar growth performance at a significant economic benefit. Further evaluation of this formulation approach would be a useful alternative for the dairy industry.

Conclusions

Replacing 50% of the whey proteins in a 28% CP all-milk milk replacer with a low antigen SPC had no significant effect on calf growth or health. The addition of mannanase based enzymes to the SPC containing milk replacer did not enhance the performance of the milk replacer but increased the feed efficiency of the calves after the introduction of the starter grain to the diet. Supplementing a low antigen SPC with amino acids appeared to enhance the performance of the SPC milk replacer, but given the differences in level of intake in this experiment and the basal protein content of the milk replacer, another study should be conducted to determine if the

performance was simply an energy response or a combination of energy and amino acid balance.

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CHAPTER III.

I. IMPLICATIONS FOR RESEARCH AND TECHNOLOGY APPLICATION IN PRODUCTION SYSTEMS IN THE GULF REGION OF MEXICO

The beef and dairy industries in Mexico show a deficit, where production is being surpassed by population growth and increased consumption per capita. Therefore, improvement in production systems through a yield increase is needed. As mentioned by Baba (2007) management limitations are affecting animal production units in the Gulf of Mexico. Issues in energy management, calf weaning weights, and calving intervals are present and affecting productivity in the system. However, priority research considerations on animal production should be carried out taking into account holistic, integrative strategies to generate improvement in profitability (Baba, 2007; Reynoso-Campos et al., 2004).

The region of the Gulf of Mexico presents a diverse group of productive systems and management methods, however the main types used currently in the area are the dual purpose system and beef production, both based mainly in grazing and seasonal availability of resources.

The dual purpose cattle in the gulf region of Mexico yields 30% of the national milk production, possesses approximately 67% of the dairy national herd and most farmers in the tropic use this technique due to the seasonality of water and forage growing conditions. The primary characteristic of a dual purpose system is the simultaneous production of milk and beef, demanding the use of animals resistant to climate and management. Due to dependence on grazing productivity and calves extraction rate is relatively low. In economical terms calf production in the from birth to weaning is the least profitable activity, fattening calves after weaning is

intermediate in profitability, and milk production with dual purpose systems artificial rearing is the most profitable (Odermatt and Santiago, 1997).

There are different systems of calf rearing in the Gulf of Mexico region. The traditional approach is manual milking and leaving the calf on one teat, which results in an ADG of approximately 0.3 kg/d. There is also restricted suckling, offering limited amounts of milk to the calf, along with a protein supplement, and ADG of approximately 0.5 kg; and the artificial rearing approach where calves are fed from three days of age with milk replacer or whole milk, weaned at 90 days with an observed ADG around 0.6 and 0.7 kg. However, most of farmers are still using the traditional method (Livas, 2002; Teyer-Bobadilla et al., 2002).

Nevertheless, beef production is also present in the region with extensive zones used for fattening animals. Calves for beef are maintained with the dam and weaned at approximately 8 months. Calf growth depends upon quality and quantity of feed available in a given time and not on management and desired growth rates. In those systems, can be observed restricted feed intake, poor quality forage overall in the dry season, and a big reliance on the body tissue reserves (Baba, 2007).

Under tropical conditions, quality and quantity of feed available during the different seasons of the year is one of the main constraints. Seasonal variations affect reproduction, growth, end efficiency of the productive system; therefore is important to identify ways to improve feed resources and nutrition, to allow production systems to become sustainable and profitable according to the ecosystem characteristics (Magaña-Monforte et al., 2006; Teyer-Bobadilla et al., 2002).

The rearing method can affect production and reproduction. In an experiment comparing restricted suckling (RS) and artificial rearing (AR) on production and reproduction in south-east of Mexico, RS calves had higher weights at 90 days and that difference was observed until 120 days, but posterior growth showed no

difference among groups. Total milk yield was not affected, but in groups RS age at first calving and calving interval were higher. At the same time, RS cows had bigger weight losses up to 120 days post partum, affecting reproductive behavior, which is a disadvantage in tropical conditions for dual purpose, dairy, and beef cattle (Magaña-Monforte et al., 1996). At the same time, decreases in growth and delayed puberty has been found in calves under traditional systems (Coppock and Sovani, 1999).

Changes in calf nutrition and management therefore can be translated into improved cow performance and calf growth. Results obtained in our experiment, showed weight gains up to 1.2 kg/d in calves fed only MR, which indicates that methodologies used in the present experiment are useful to improve growth in animals artificially reared and with out affecting milk yield in the farm. It is known that calves can drink from 25 to 40% of the milk production of the dam in the traditional management method used in dual-purpose systems, affecting milk production and profitability of the system (Teyer-Bobadilla et al., 2002).

Calves fed milk replacer including soy protein and with the addition of mannanase-based exogenous enzymes, have similar performance as the whey-based milk replacer. However, as shown in this study, the difference in price can be up to 33% and according to the trends in whey protein price, that difference can be greater in a near future. In Mexico, the price paid to the producer per liter of milk is around $0.30 USD (SAGARPA, 2007) whereas the cost to feed 1 L of reconstituted milk replacer in present study, was $0.46 and $0.39 USD for all milk protein and soy protein milk replacer respectively. In US, milk price paid to producer per liter during the month of July was $0.47 USD (based on a price of $21.35 per cwt, Dairy Market News, 2007). In US conditions, the use of milk replacer allows the producer to sell more milk, however, utilization of the of soy-protein milk replacer would translate to a 33% cost reduction when feeding a calf one liter of milk. Under Mexican conditions, the milk

replacer price is less than in the US, a 10 kg bag of MR can cost approximately $20.00 USD (GGAVATT Tepetzintla, personal communication), which means a price of $0.30 USD per liter of reconstituted MR (dilution rate of 15%, as used in present experiment) therefore, MR price per liter would be the same than a liter of milk sold. If soy-based milk replacer could be used, and the difference in price was similar to the observed difference in our study, this could be translated to a 25% reduction in price of feeding a calf a liter of milk replacer.

And aside from the potential increase in saleable milk and the associated cost reduction of feeding a calf with milk, the nutritional aspects could be managed in a better way. As mentioned by Baba (2007) some of the opportunity areas for improvement of the system in tropical areas of the Gulf of Mexico include increase in feed intake that could reduce the heavy reliance on body reserves, improvement in diet formulation, knowledge of the chemical composition of the feed provided to the animals, reduce the restriction of feed intake, and use of CNCPS to evaluate and predict responses in animals.

Despite of the lack of formulation methods and institutions to establish nutrient requirements and diet formulations on animal systems, like in the U.S. (NRC, 2001; Van Amburgh and Drackley, 2005), UK (AFRC), France (INRA), and Australia (CSIRO), some models like the CNCPS have proved to be useful to predict animal performance under tropical conditions, using different management practices and feed composition. However, the right chemical composition of feed should be know, thing that is possible with the use of milk replacers.

In the same way, the use and correct formulation of milk replacers in Mexico can allow establishing nutrient requirements for growing calves, due to the possibility to design milk replacer with pre-established amounts of protein, fat, and amino acids.

Therefore, trials on the effect of increased crude protein and the interaction of protein and fat content could be carried out under tropical conditions.

In the same way, milk replacer use would help establishing pre-determined growth rates, and target weights for calves, achieving higher rates of gain, decrease age to first calving, and reducing negative effects on reproductive aspects on the dam are other advantages that could be achieved with the use of milk replacer.

In addition, seasonal variations in feed quality and quantity could be avoided with a feeding regimen like the one used in the present experiment. In the same way, difficult weaning responses could be alleviated with the use of soy protein milk replacer, as observed in present study.

In spite of the results observed in the present study, the inclusion of changes in management techniques and feeding will depend upon milk replacer quality, climatic factors, and availability of the resources. First step to the application of artificial rearing with the use of soy-based milk replacer should be to carry out trials under tropical conditions to obtain a more precise appraisal of the usefulness of using artificial rearing under those conditions.

Research agencies and industry in the Gulf region of Mexico could benefit from the knowledge generated in this study, the use of artificial rearing, diet formulation, feeding rates, and energy management given to the calves could be used for further studies. Studies in protein content in feed, energy use and efficiency could be conducted.

Increased profitability in dual purpose systems could be achieved under management practices used in our study, due to a 20% milk production increase per animal due to decrease in calf consumption, and to cost reduction in feed. In the same way, reduction of adverse reproductive and growth effects in cow and calf, and a better control in nutrition and management could be achieved. However, comparisons

and extrapolations of the results obtained in this study with the expected outcomes in the Gulf region of Mexico should conducted carefully.

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