DEFINING THE RANGE FOR PLASMA PROTEIN INCORPORATION IN MILK REPLACER FOR DAIRY CALVES

BY

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THESIS

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ABSTRACT

The objective of this study was to determine the effects of calf milk replacer containing from 0% to 100% of the total protein from porcine plasma protein, with or without isoleucine balanced, on calf growth and health. Four groups of 31 male Holstein calves were blocked by initial BW and plasma protein concentration and assigned to one of seven treatments. Treatments were as follows: A: control, all-milk protein milk replacer, B: 33% plasma protein addition, C: 33% plasma protein addition plus isoleucine, D: 67% plasma protein addition, E: 67% plasma protein addition plus isoleucine, F: 100% plasma protein addition, and G: 100% plasma protein addition plus isoleucine. Calves were fed milk replacer only, twice daily for 5 wk. During wk 1, calves were fed at a rate of 10% of BW (reconstituted to 12.5% solids). During wk 2 to 5, calves were fed at a rate of 12% of BW (12.5% solids). Body weight, body length, heart girth, withers height, hip height, and hip width were measured once weekly. Blood was sampled during wk 4 and serum was analyzed for urea N, total protein, total globulins, and albumin.

Calf growth decreased with increasing addition of plasma protein in the diet. Supplementation with isoleucine lessened the negative effects of increasing plasma protein. We observed no negative health effects with the addition of plasma protein in the diet, but neither did plasma protein improve measures of health status. Provided that amino acid balance is maintained, porcine plasma protein can replace substantial amounts of whey protein in calf milk replacer with minimal effects on calf growth.

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LIST OF ABBREVIATIONS

ADG	average daily gain
BUN	blood urea nitrogen
BW	body weight
cm	centimeter(s)
CP	crude protein
ºC	degree Celsius
DM	dry matter
g	gram(s)
Ile	isoleucine
kg	kilogram(s)
L	liter(s)
Mcal	megacalorie(s)
ME	metabolizable energy
NRC	National Research Council
%	percent
wk	week(s)

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Replacement heifers are one of the most costly sectors of dairy production. A recent survey from the USDA reported that mortality of pre-weaned heifers in 2006 was 7.8% (USDA, 2007). Although this represents a decrease from 8.7% in 2001 (USDA, 2002), to most producers and advisers this is still too high. Increases in mortality and morbidity increase the cost to the replacement heifer sector. Although many factors impact mortality and morbidity, adequate nutrition in the first few weeks of the young calf's life plays a large role. Giving the young calf the best possible start in life through adequate nutrition will pay off to the producer through decreased mortality and morbidity, in addition to potential decreases in age at first calving and increased future milk production.

Milk replacer has long been a standard calf feed in the dairy industry. There are a wide variety of liquid feed sources on the market today. There is also much controversy over the best formulation and ingredients to use to achieve maximum growth, health, and performance from dairy calves while minimizing costs of production.

Until the 1950s, most dairy calves were fed whole milk (Otterby and Linn,1981). Whole milk obviously is a great source of nutrition, but traditionally it has been the most expensive liquid diet option (Davis and Drackley, 1998). From an economic standpoint milk replacers have been the best choice for calf rearing. More than half of the dairy calves in the US are fed milk replacer for at least a portion of the pre-weaning period (Heinrichs et al., 1995).

PROTEIN AND AMINO ACID REQUIREMENTS

The nutrient profile of milk replacers should resemble whole milk as closely as possible. However, typical conventional (limit-fed) milk replacers contain 20% CP and 18 to 20% crude fat on a DM basis and are fed at 8 to 10% of BW. This contrasts with whole milk, which contains 25 to 26% CP and 28 to 30% fat on a solids basis. Ad libitum feeding rates for calves are in the range of 18 to >20% of BW daily (Davis and Drackley, 1998).

Proteins are an essential component of milk replacer because proteins supply amino acids for rapid growth and lean tissue disposition. Protein requirements are relatively low for maintenance and are determined mainly by the rate of growth. According to current recommendations, 188 g of protein are deposited for every kilogram of BW gain, which requires 250 to 280 g of dietary CP intake (Davis and Drackley, 1998; NRC, 2001). Researchers have demonstrated, however, that the current NRC (2001) overestimates energy accretion and underestimates protein requirements (Van Amburgh and Drackley, 2005).

Amino acid requirements must also be taken into account when considering protein requirements. The most recent dairy NRC (2001) does not consider individual amino acids for young calves. Limited research has been done on the amino acid requirements of the young calf. It has been generally assumed that, like swine and poultry, lysine and methionine are first and second limiting amino acids. This has been confirmed from a limited amount of research conducted by Williams and Hewitt (1979), Tzeng and Davis (1980) and Hill et al. (2008). The amino acid supply of the young calf, however, is much more complicated than that of young swine and poultry. The rumen is immature during the first few weeks of the calf's life. During this time milk replacer bypasses the rumen via the reticular (esophageal) groove, allowing the

composition of the milk to remain relatively unchanged until it reaches the abomasum. If the esophageal groove is not closed properly, milk will enter the rumen where it will be fermented by the developing microbial population, which in turn significantly changes the composition of amino acids reaching the lower digestive tract. As the rumen begins to develop, the amino acid requirements and supply of the young calf become much more complex. Researchers have begun looking at feeding solely a liquid diet to study amino acid requirements; this allows researchers to study amino acid requirements without the interference of rumen development and variable quantities of amino acids supplied from microbial protein.

Immune status of the calf also plays a role in amino acid requirements. Calves with an activated immune system will shuttle amino acids otherwise available for growth to the immune system to fight off infection. Requirements of the immune system for amino acids have not yet been established.

Recent interest has emerged in the use of the "ideal protein" concept in milk replacer formulation. This concept has been adopted in feed formulation in the swine and poultry industries. The theory behind this concept is described by the swine NRC (NRC, 1998) and considers that there is an optimal dietary pattern among essential amino acids that corresponds to the needs of the animal (NRC, 1998). Feeds are formulated using lysine as a reference set at 100%, and then the target concentrations for each of the other essential amino acids are expressed as a percentage of lysine. This ensures that all amino acids are present in the correct ratio to one another, which will allow the most rapid and efficient growth at the lowest total dietary CP supply.

MILK PROTEINS IN MILK REPLACER

Traditionally milk proteins make up the majority source of protein in milk replacers. Factors affecting the utilization of proteins are digestibility, amino acid balance, and the presence of antinutritional factors (Davis and Drackley, 1998). Milk proteins are highly digested by the young calf, have a desirable amino acid balance, and do not contain antinutritional factors.

Early milk replacer formulations contained primarily dried skim milk as the main source of protein. Dried skim milk is highly digestible; however, the quality can be questionable depending on the amounts of heat used in processing (Tanan, 2005). Caseins are the main protein source in dried skim milk. Early in the young calf's life chymosin activity is high. Chymosin coagulates casein in the abomasum, forming a clot, which modifies the rate of passage of proteins and the nature of proteins entering the small intestine.

Whey protein is another milk protein source that is highly digestible. Unlike dried skim milk whey proteins do not clot under chymosin activity in the abomasum. It was thought early on that whey proteins could not be added to milk replacers in excess amounts (>30%) without decreasing health and performance (Roy, 1980). More recent research has observed no negative effects on health or performance in calves fed milk replacer with protein from whey protein concentrate (Lammers et al.1998; Terosky et al. 1997). Today most "all-milk protein" products are composed of dried whey, whey protein concentrate, and delactosed whey (Drackley, 2008).

ALTERNATIVE PROTEINS

Economics play a large role in the choice to feed milk replacer, as well as what ingredients are used in milk replacers. Milk replacer was originally as a cheaper alternative to

feeding whole saleable milk. The protein source is one of the most expensive components of milk replacer. In recent years the price of the common milk proteins (mainly whey proteins) used in commercial milk replacers has risen due to demand for whey in the human market. This change in the price has raised the question of whether feeding milk replacer is still as economical. The increased price for whey proteins has led the search for alternative protein sources. There has been much research looking at various alternative protein sources over the last several decades. There are a number of factors that go into finding a suitable alternative protein source. The protein must 1) be easily digested and the amino acids used by the calf, especially during the first 3 wk of life when the digestive system is immature, 2) contain an optimal amino acid balance, 3) be highly palatable, and 4) possess acceptable mixing qualities.

Soy Protein

Soy protein sources have been extensively researched as an alternative protein source in milk replacers. Soy proteins are widely available, cheap, and have a favorable amino acid profile (Davis and Drackley, 1998). Soy flours, soy protein concentrate, and soy protein isolate have been widely studied. Soy flour (50% protein) is finely ground soybean meal. Soy protein concentrate (66% protein) is the protein portion of soybeans concentrated by the removal of soluble carbohydrates with hot aqueous ethanol. Soy isolate (85% protein) has the entire carbohydrate fraction removed and the protein precipitated.

The biggest concern with using soy proteins in milk replacer are the anti-nutritional factors present and the adverse responses associated with soy protein use. Soy proteins have been shown to cause allergenic reactions in calves. Dawson et al. (1988) reported elevated serum antibody responses as well as villous atrophy in the small intestine of calves fed soy products

(flour and concentrate). They also saw a decrease in growth and diet digestibility, as well as an increase in nitrogen retention. Calves fed soy flour had greater negative than those fed soy concentrate. Gardner et al. (1990) reported an elevation in heart rate, increased respiratory rate, and allergic sensitivity to soybean products.

Soy isolate is a more refined form of soy protein. Research comparing soy isolate and soy flour indicated that soy isolate has a high apparent digestibility and most importantly a lack of antibody synthesis (Lalles et al., 1995). Although a better source of protein than soy flour, soy isolate still was inferior to skim milk powder (Khorasani et al., 1989; Lalles et al., 1995).

There are a number of studies that have looked at various chemical and physical treatments to increase the digestibility and reduce the antibody activity of soy proteins. Colvin and Ramsey (1968) found that exposing soy flour to an acid environment increased the rate of calf growth nearly two times over that of untreated soy flour. A follow-up study by Colvin and Ramsey (1969) found that calves fed a milk replacer with soy flour treated with an alkali treatment grew as well as calves fed an acid treated soy, and both groups grew better than those fed an untreated soy.

Drackley et al. (2006) looked at supplementing soy protein concentrate with glutamine to help overcome the decreased ADG and altered intestinal morphology caused by milk replacers containing soy protein concentrate. Results indicated that glutamine did not improve ADG or intestinal morphology.

The amino acid profile of soy protein is deficient in methionine, threonine, and lysine relative to whole milk. Kanjanapruthipong (1998) looked at the addition of threonine, methionine, and lysine to milk replacers containing soy protein. Average daily gain, N retention,

and ileal digestibility of DM were higher in calves fed soy protein with the addition of amino acids verses those fed soy proteins without the addition of amino acids. However, ADG, N retention, and ileal digestibility of DM in calves fed soy protein with amino acids were still lower than those of calves fed skim milk protein.

Wheat Protein

Wheat protein is another alternative protein source that has been researched extensively. Wheat gluten is the protein derived from the wet milling process of wheat, after separation of the starch (Davis and Drackley, 1998). Native wheat gluten has been found to be well digested by the calf, at 92 to 99% of milk values (Branco-Pardel et al., 1995; Toullec and Grognet, 1990), and with an amino acid profile complementary to whey proteins (Davis and Drackley, 2005). However, Kilshaw and Slade (1982) found similar antigenic effects with wheat gluten as they saw in soy protein. The addition of wheat gluten caused villus atrophy in intestinal tissue. Toullec and Grognet (1990) reported an increase in antibodies; however, calves did not develop any allergy symptoms.

Native wheat gluten is unusually visocoelastic and insoluble (Tanan, 2005). Toullec and Grognet (1990) denatured the wheat protein by heating, which suppressed the viscoelasticity, but the product was still insoluble. As with soy protein, physical and chemical treatments of the wheat gluten have been researched as possible methods to increase digestibility. Toullec and Formal (1998) found that solubilized wheat gluten was slightly more digestible than native wheat gluten. Terui et al. (1996) showed that growth performance of calves fed spray-dried wheat gluten were the same as those fed solubilized wheat gluten or those fed soybean meal.

Soy and wheat protein have been shown to be adequate protein sources; however, additional processing is needed to overcome the antinutritional factors. Also, amino acids may need to be added in high amounts to the diet, which in turn increases the cost of the product.

Fish Protein

Various forms of fish protein have been studied: spray-dried fish, fish meal, fish protein concentrate, and soluble partially hydrolyzed fish protein. Calves fed fish protein concentrate had poor growth performance and digestibility. Fish protein concentrate is also deficient in vitamin E, which increased calf morbidity and mortality (Huber, 1975). Hydrolyzing the fish protein concentrate yielded a product that when fed resulted in similar digestibility and growth performance to calves fed milk protein (Jenkins et al., 1982). Spray-dried fish protein also produced inferior results to milk protein and soy protein concentrate. Diets containing spray-dried fish protein resulted in decreased ADG, increased fecal scores, increased rectal temperatures, and increased calf morbidity and mortality (Campos et al., 1982). Fish proteins have also yielded undesirable characteristics such as color, odor, and insolubility (Kolar and Wagner, 1991).

Egg Protein

There are large discrepancies when looking at performance data for egg protein as an alternative protein source in calf milk replacers. Research looking at liquid egg as an alternative protein source found that diets containing up to 10% liquid egg yielded similar results as diets containing only milk proteins (Touchette et al., 2003). In contrast, increasing spray-dried whole egg powder in milk replacers resulted in a large decrease in growth and feed efficiency (Quigley et al., 2002). As with many of the other sources of alternative proteins, spray-dried whole egg

powder is thought to contain antinutritional components. Quigley et al. (2002) looked at adding biotin to spray-dried whole egg powder to determine whether avidin inhibits growth; their results indicated no improvement with the addition of biotin.

Potato and Rice Protein

Research has also been conducted on use of potato and rice protein in milk replacers; however, neither has shown favorable results. Digestibility of potato protein has been found to be significantly lower than milk protein or wheat protein (Branco-Pardal et al., 1995). As rice protein concentrate increased in the diet, ADG and feed efficiency decreased (Hill et al., 2008). These two sources of protein also have poor amino acid profiles and are not economical.

Plasma, Serum, and Red Blood Cell Protein

The use of plasma proteins, serum proteins, and spray-dried red blood cells as an alternative protein source may be the most promising, not only from a nutrition standpoint but from a health standpoint as well. Health and nutrition go hand in hand in determining the success of calf rearing programs. Alternative protein sources previously studied were adequate from a nutrition standpoint, but few had any added health benefits; in fact, many compromised health when fed to young calves. For many years plasma protein, serum protein, and spray-dried red blood cells were not considered as potentially successful protein sources, as they were not economical. With the recent increase in the price of whey and potential increased supply of blood-derived proteins, along with the potential added health benefits, plasma proteins, serum proteins, and red blood cells now play an important role in the industry. All are high in protein and have a favorable amino acid profile, with the exception of methionine and isoleucine contents.

Plasma or serum protein is collected from bovine or porcine blood obtained from slaughter facilities. Blood is collected from the animal, centrifuged, and the plasma or serum is collected and processed to preserve the functional characteristics of the proteins, which include immunoglobulins, albumin, fibrinogen, growth factors, and other biologically active components. Spray-dried red blood cells are a co-product of plasma protein production.

From a nutritional standpoint, research has shown plasma proteins, serum proteins, and spray-dried red blood cells to be potential alternative sources of protein. Quigley and Bernard (1996) found no difference in BW gain, feed intake, feed efficiency, or fecal scores between milk replacers containing 25% of the total protein from plasma proteins compared to those containing all milk protein. Morrill et al. (1995) compared diets containing 25% of the protein from porcine plasma, 25% from bovine porcine plasma, and an all-milk protein milk replacer. Calves fed milk replacers containing either bovine or porcine plasma gained more weight than those fed milk replacer containing only milk proteins. In a two-part study by Quigley and Drew (2000), no differences in intake of MR and starter, BW gain, feed efficiency, or fecal scores were observed in calves fed milk replacers ranging in 0% to 43% CP from spray-dried red blood cells.

From a health standpoint, research has shown that plasma or serum proteins and spray-dried animal plasma may decrease mortality and morbidity. Quigley and Wolfe (2003) compared milk replacers containing only whey protein concentrate, whey protein concentrate with 5% porcine plasma, and whey protein concentrate with 5% bovine plasma. Mortality and morbidity were lower for calves fed milk replacers containing plasma compared to those fed milk replacer containing 100% whey protein concentrate. Quigley et al. (2002) found a decrease in mortality, improved fecal scores, fewer days with scours, and decreased days with antibiotics with the addition of 20% of the protein from spray-dried bovine plasma.

Spray-dried animal plasma and spray-dried red blood cells have been researched and used extensively in the swine and poultry industries. Researchers have reported a reduction in local inflammation of the small intestine (Jiang et al. 2000) as well as the large intestine (Nofrarias et al. 2006) of pigs when fed spray-dried plasma. When animals were challenged with pathogenic bacteria such as *E. coli* or *Salmonella*, beneficial effects were even more pronounced. Bosi et al. (2004) fed spray-dried plasma to pigs challenged with *E. coli* and reported reduced inflammation and pro-inflammatory cytokine expression in the gut, reduced IgA secretion, and decreased intestinal mucosa damage. Challenge studies in calves have shown similar results. Quigley and Drew (2000) challenged calves with *E. coli*. Calves were fed milk replacers containing no additive, an antibiotic, or spray-dried plasma at 3.3% of DM. Calves fed either the milk replacer containing spray-dried plasma or the milk replacer containing the antibiotic had lower morbidity and mortality than calves fed the control. Arthington et al. (2002) challenged calves with coronavirus; calves fed MR supplemented with dry bovine serum powder had a decrease in respiratory rate and an increase in feed intake after challenge, suggesting that the bovine serum powder decreased the severity of the disease.

Spray-dried serum has also been researched as an effective tool to increase the quality of colostrum. Arthington et al. (2000) found that adding spray-dried serum to poor-medium quality colostrum increased serum IgG concentrations in blood, thereby improving transfer of passive immunity.

Spray-dried animal plasma or serum and red blood cells have been shown to not only be an effective protein source but to have potentially important health benefits as well. Future research is needed to look at the range of inclusion, amino acid profile, health benefits, and

mechanism behind these benefits. In addition, research is needed on the use of these protein sources in accelerated feeding programs, as well as long-term effects of their use.

CHAPTER II

DEFINING THE RANGE FOR PLASMA PROTEIN INCORPORATION IN MILK REPLACERS FOR DAIRY CALVES

INTRODUCTION

More than half of the dairy heifers in the United States are fed milk replacer during at least some of the preweaning period (USDA, 2007). Most conventional (limit-fed) diets contain 20 to 22% protein and 20% fat. A number of different protein sources are used in milk replacer. Traditionally, diets are composed of milk protein sources because they are highly digestible, contain a nearly optimal amino acid profile, and are economical depending on the price of saleable milk and milk protein. Research in the last 20 yrs has been focused on finding alternative protein sources that perform similar to whey proteins, which have become the standard for all-milk protein milk replacers. Plasma proteins have emerged in the last 20 yrs as a potentially viable alternative protein source. Plasma proteins have been viewed as too expensive to incorporate into liquid diets as a substitute for milk protein. However, with the recent increases in the price of whey and predictions for even higher prices in the future, plasma proteins might become an economical choice. Plasma proteins are good sources of both CP and amino acids, with the exception of methionine and isoleucine.

Unlike previous research looking solely at CP replacement, our laboratory has researched alternative proteins with a focus on amino acid balance and availability. My objective here was to determine the effects of milk replacers containing from 0% to 100% of the total replaceable protein from porcine plasma protein (PPP), without or with amino acids balanced, on calf health and growth.

MATERIALS AND METHODS

Animals

All procedures were conducted under protocol #09116 approved by the University of Illinois Institutional Animal Care and Use Committee. Four groups of 31 male Holstein calves, less than 1 wk old, were purchased from sale barns in Wisconsin or New York and transported to the University of Illinois Nutrition Field Lab site. Upon arrival calves were fed 4 L of electrolyte solution (Land O' Lakes, Inc., Saint Paul, MN). Each calf was vaccinated with TSV-2 (Pfizer, Inc., New York, NY) and administered Excede (Pfizer) and MuSe (Intervet Shering Plough Animal Health, Union, NJ). Rectal temperatures were recorded and navels were sprayed with povidone iodine (Durvet, Inc., Blue Springs, MO). Initial measurements of BW, body length, heart girth, withers height, hip height, and hip width were taken. A blood sample was taken via jugular venipuncture into a 10-mL evacuated serum separation tube (Becton Dickenson, Rutherford, NJ). Blood was centrifuged at 1300 × *g* for 15 min. Serum was divided into aliquots and stored in polypropylene tubes at -20^0 C until analyzed later for total IgG. An additional blood sample was placed into a hematocrit tube and centrifuged at 1500 × *g* for 3 min. Plasma protein was determined using a refractometer. Ear notches were collected and placed into formalin to be analyzed later for presence of persistently infected bovine viral diarrhea (PI-BVD) virus.

Housing

Calves were housed in individual hutches (Calf-tel, Hampel Corp., Germantown, WI) placed 1.5 m apart from one another. Hutches were placed on crushed rock, covered by landscape cloth (DuPont) and a layer of straw. Straw was checked daily and added as needed.

Feeding and Management of Calves

Calves were blocked on the day of arrival (d 0) by BW and plasma protein and then randomly assigned within block to one of seven treatments. All treatments (Table 1) resembled a commercial 20% CP and 20% fat formula with linear increases of PPP until all whey proteins were replaced by PPP, with the exception of the whey proteins provided as part of the spray-dried fat in the formula. The maximum amount of whey proteins replaced in the "100%" PPP formulas was approximately 88%. Treatments were formulated to contain 1.75% lysine, 0.51% methionine, and were isonitrogenous. Amino acid balance was formulated using the ideal protein concept using unpublished estimates from M E. Van Amburgh as the standard (Table 4). Treatments were as follows: A: control-all milk protein milk replacer; B: 33% PPP addition (NutrPro B, APC, Inc., Ankeny, IA); C: 33% PPP addition plus isoleucine (Ile) to equalize to diet A; D: 67% PPP addition; E: 67% PPP addition plus Ile to equalize diet A; F: 100% PPP addition; and G: 100% PPP addition plus Ile to equalize diet A. Milk replacers were manufactured by Milk Specialties Company (Dundee, IL).

Calves were fed milk replacer twice daily at 0530 h and 1630 h for 5 wk. During wk 1, calves were fed at a rate of 10% of BW (reconstituted to 12.5% solids). During wk 2 to5, calves were fed at a rate of 12% of BW (at 12.5% solids). Amounts fed were updated weekly based on BW. Milk replacer intake was recorded daily. Water was offered for ad libitum consumption and intake was recorded daily. Starter was not offered until after the study ended after wk 5, because the study sought to observe the effects of amino acid balance and protein sources of the milk replacer only.

Health

Health checks were performed daily, after the morning feeding. Fecal scores were assigned on a 1 to 4 scale: 1=well formed, 2=slightly loose but still holds some form, 3=loose without form, 4=water. Respiratory scores were assigned on a 1 to 5 scale: 1=normal, 2=heavy breathing, 3=mucous, 4=dry cough, 5=wet cough. Overall appearance and behavior of calves was also recorded. Rectal temperatures were recorded for every calf daily during wk 1, thereafter for those calves showing signs of illness. FluMeglumine (Phoenix Pharmaceuticals, Inc., Burlingame, CA) was administered to calves with a rectal temperature of 40^0C or above. Navels were sprayed daily with povidone iodine (Durvet) until dry. Hydration status was assessed using fecal scores, skin tent test, eye appearance, and overall attitude. Electrolytes (Land O' Lakes, Inc., Saint Paul, MN) were administered as needed. Animals were monitored multiple times daily for illness and dehydration status and treated as needed. On d 1 and 14, calves received *Clostridium perfringens* types C & D bacterin-toxoid (Pfizer, Inc.) and *Clostridium perfringens* type A toxoid (Novartis Animal Health, Inc.,). On d 3, d 11, and during wk 6, calves received Bovi-Shield Gold 5 (Pfizer). On d 19 and during wk 5, calves received Draxxin (Pfizer). Uniprim (Macleod Pharmaceuticals, Inc., Fort Collins, CO) was added to milk replacer once daily during d 0 through 4, and BMD soluble powder (Alpharma King Pharmaceuticals, Inc., Bristol, TN) was added to milk replacer once daily during d 6 through 12.

Body Growth and Measurements

Body weight, body length, heart girth, withers height, hip height, and hip width were measured upon arrival and weekly every Friday for the duration of the trial. Body weight and measurements were taken at 0900 h following feeding.

Blood Collection and Analysis

Blood was sampled at arrival and during wk 4 at 0800 h after the morning feeding. Blood was obtained via jugular venipuncture into 10-mL evacuated serum separation tubes (Becton Dickenson, Rutherford, NJ). Blood was centrifuged at 1300 × *g* for 15 min. Serum was divided into aliquots in polypropylene tubes and stored at -20^0 C until analyzed.

Serum from the samples at arrival was analyzed for total IgG. Samples from wk 4 were analyzed for concentrations of urea N, total protein, and albumin. Total globulin was calculated as the difference between total protein and albumin. All analyses were conducted at the University of Illinois College of Veterinary Medicine diagnostic laboratory using automated analysis procedures.

Milk Replacer Sampling and Analysis

Milk replacers were sampled daily and stored at -20^0 C until analysis. Batch composites were prepared and analyzed by Dairy One (Ithaca, NY) for contents of DM, CP, soluble protein, crude fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, S, and Mb, by standard wet chemistry methods (www.dairyone.com/forage/default.htm). Samples were also sent to the University of Missouri Agricultural Experiment Station Laboratory for complete amino acid analysis by cation-exchange chromatography coupled with post-column ninhydrin derivatization and quantification. The laboratory used base-catalyzed hydrolysis so that tryptophan content could be determined.

Statistical Analysis

Analysis of variance was conducted using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). Calf, block, and batch were defined as random effects, whereas treatment was a fixed effect. For variables with repeated measurements, a covariate (initial measurement) was used and data were analyzed separately by week. Initial BW, body length, heart girth, withers

height, hip height, and hip width were used as covariates for the respective measurements. Treatment comparisons were made using six preplanned, non-orthogonal contrasts: 1) linear effect of increasing PPP without supplemental Ile; 2) quadratic effect of increasing PPP without supplemental Ile; 3) linear effect of increasing PPP with supplemental Ile; 4) quadratic effect of increasing PPP with supplemental Ile; 5) effect of addition of supplemental Ile; and 6) interaction of the linear effect of increasing PPP with addition of Ile. Significance was declared at $P < 0.05$ and trends discussed when $0.05 < P < 0.10$. Least square means were calculated and are presented with standard errors.

RESULTS

Nutrient Composition of Diets

Diet analyzed chemical composition of MR is shown in Table 2. All diets were formulated to contain 18.4% CP so that protein supply would be more limiting than energy supply to maximize the ability to detect differences in dietary protein utilization among treatments. The measured CP contents ranged from 20.4% for the control and 33% PPP diets to a low of 17.3% for the 100% PPP plus Ile diet.

Amino acid analyses of MR on a DM basis are shown in Table 3. Treatments were formulated to contain 1.75% lysine. Actual analyzed lysine content was 1.56% for control and ranged from a high of 1.76% for 66% PPP plus Ile to a low of 1.44% for 100% PPP plus Ile. The amino acid compositions relative to lysine content are shown in Table 4. Methionine was formulated at 29% of lysine (i.e., 0.51% of dietary DM). Analyzed methionine content was 24% of lysine for control and relative methionine contents ranged from there to a high of 30% of lysine for 100% PPP Analyzed Ile content of the control diet was 73% of lysine.

Supplementation of Ile increased relative Ile content over the respective unsupplemented PPP diets but did not achieve the same value as the control because of the low lysine content of the control diet.

Intakes

Overall and weekly mean intakes of DM, CP, ME, and lysine are shown in Table 5. There were no significant effects for overall DMI and ME intake of milk replacer, although the linear effect of increasing PPP in Ile-supplemented diets approached significance ($P = 0.07$). During wk 3, 4, and 5, there linear effect of increasing PPP in Ile-supplemented diets on DMI was significant, as DMI decreased with increasing PPP.

Differences in CP and lysine intakes reflect both small differences in DMI and differences in analyzed CP content among the diets. Overall mean CP intakes decreased in a quadratic manner as PPP increased either without or with supplemental Ile. The addition of isoleucine to plasma protein negatively affected average CP intake ($P = 0.0002$). The effect of increasing PPP without supplemental Ile on overall lysine intake was linear, although the quadratic effect approached significance ($P = 0.09$). The quadratic effect of increasing PPP with supplemental Ile was significant ($P < 0.0001$) on overall lysine intake. Diets supplemented with Ile had lower overall lysine intake (P=0.02), but the interaction of the linear effect of increasing PPP and Ile supplementation was significant, indicating that decreases in lysine intake for Ile-supplemented diets became larger as the amount of PPP increased.

Growth

Means for BW, ADG, and feed efficiency measurements are presented in Table 6. Initial BW did not differ among treatments. Final BW decreased linearly as PPP increased in diets without or with Ile supplementation. The overall ADG decreased linearly as PPP increased

regardless of Ile supplementation, and the quadratic effect of increasing PPP approached significance for both non-supplemented ($P = 0.07$) and Ile-supplemented ($P = 0.06$) diets. On average, Ile supplementation resulted in a weak trend ($P = 0.13$) for improved gain:feed.

Stature measurements are presented in Table 7. Initial stature measurements did not differ significantly among treatments. Final body length and daily gain of body length decreased linearly as PPP increased regardless of Ile supplementation. Final heart girth decreased linearly as PPP increased in either non-supplemented or Ile-supplemented diets. The effect of Ile supplementation approached significance ($P = 0.06$) for final heart girth. Average gain of heart girth decreased linearly in either supplemented or non-supplemented groups as PPP increased, although the effect tended ($P = 0.08$) to be quadratic in the non-supplemented diets.

Final withers height and hip height decreased linearly in both non-supplemented and Ile-supplemented calves as dietary PPP increased. Daily gains of withers height decreased quadratically as PPP increased in non-supplemented diets, and the decrease tended ($P = 0.06$) to be quadratic for hip height. The quadratic effects showed that decreases in height were greater at the higher levels of PPP inclusion. The significant interaction of the linear effects of PPP and Ile supplementation showed that the decreases in height as PPP increased were larger in the absence of supplemental Ile.

Finale hip width decreased linearly as PPP increased in diets without supplemental Ile, but was not affected significantly in Ile-supplemented diets. These differences in response resulted in a tendency ($P = 0.08$) for an interaction of linear effects of increasing PPP with Ile supplementation. The daily gain of hip width decreased quadratically as PPP increased in diets without Ile supplementation but the decrease was linear in diets with supplemental Ile. As a

result, the interaction of linear effects of increasing PPP with Ile supplementation approached significance ($P = 0.06$).

Blood Metabolites

Concentrations of urea N, total protein, albumin, and total globulins are presented in Table 8. The response of urea N in serum to increasing PPP was quadratic for diets supplemented with Ile, with the highest concentrations occurring in calves fed the 67% PPP diet and the lowest with the 100% diet. The interaction of the linear effect of increasing PPP and Ile supplementation was significant, because urea in the non-supplemented diets did not increase as much for the 67% PPP diet and did not decrease for the 100% diet.

In Ile-supplemented diets, increasing PPP resulted in a tendency ($P = 0.06$) for a quadratic increase of total protein in serum. Addition of Ile increased the total protein concentration. Serum albumin concentration tended ($P = 0.07$) to decrease linearly as PPP increased in non-supplemented diets, although changes were small. Increasing PPP in Ile-supplemented diets tended to increase total globulin concentrations in a quadratic manner, with largest concentrations for the 33% and 67% PPP diets. The addition of Ile increased total globulin concentration.

Health

Measurements related to health are presented in Table 9. The mean IgG concentrations on the day of arrival did not differ significantly among groups, although tendencies were present for quadratic effects of increasing PPP in non-supplemented diets ($P = 0.06$) and linear effects of increasing PPP in Ile-supplemented diets ($P = 0.07$). Because calves were blocked based on plasma protein determinations at arrival, these tendencies for pre-existing differences in IgG

occurred by chance. The lowest mean IgG was in the control calves, so we do not believe that the differences among groups confounded interpretation of results.

There were no significant effects of diets on body temperatures, number of antibiotics treatments, fecal scores, days of scours, or respiratory scores. The addition of Ile resulted in decreased average electrolyte intakes for diets containing PPP.

DISCUSSION

Previous research investigated PPP inclusion rates of < 33% of the dietary CP. The goal of the current trial was to determine response to inclusion levels higher than previous work. Quigley and Bernard (1996) observed that growth performance in calves fed milk replacer in which spray dried plasma provided 25% of the protein was similar to calves fed all whey proteins. Morrill et al. (1995) observed improved animal performance when calves were fed a diet providing 25% of the protein as spray dried plasma. In the current study we saw a decrease in BW, ADG, gain:feed, and stature measurements as the percentage of plasma protein increased in the diet. Although not a preplanned comparison, post-hoc contrasts showed that calves fed the diets containing 33% of the protein as PPP had growth performance at least equal to calves fed the all-milk-protein control diet. Therefore, our results agree with previous research for lower levels of PPP supplementation.

Plasma proteins are widely used in the swine industry to improve health of young piglets (van Dijk et al., 2001). The potential for PPP to improve health in young calves also has been of interest. Quigley et al. (2002) and Quigley and Wolfe (2003) observed decreased mortality and morbidity in calves fed low inclusion rates (5% of the diet) of spray dried animal plasma. In the current study we saw no treatment effects on scour scores, days of scours, antibiotic treatments,

body temperatures, or respiratory scores. Unexpectedly, we did observe a decrease in average amounts of electrolyte therapy when Ile was added to diets containing PPP.

There were few effects on blood metabolites related to N metabolism in this study. As predicted, calves fed the diet with 100% of the protein as PPP without Ile supplementation had the lowest ADG, final size measurements, and gain:feed. Despite this poor growth performance, however, serum protein concentrations did not differ from those of other groups.

CONCLUSIONS

Previous research has indicated that plasma protein addition at 25% of the dietary protein was a good alternative protein source. Our findings support that conclusion. Even replacing essentially all of the whey proteins in the diet with PPP and Ile supplementation resulted in overall ADG only 31% lower than calves fed the all-milk control diet. Although we saw few health improvements with the addition of PPP, neither did we see any negative effects even at the highest inclusion rate, which is in marked contrast to as results with most other alternative protein sources. Growth rates and gain:feed were decreased at inclusion rates > 33% of the protein, although additional titration studies would be needed to more closely determine at what inclusion rate performance begins to be compromised.

CHAPTER III

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TABLES

Table 1. Ingredient and formulated chemical composition of experimental milk replacers.

	Treatments[1]						
Component	A	B	C	D	E	F	G
Whey, 12.2%	53.82	53.41	53.38	45.32	43.46	2.56	-
WPC, 75.0%	12.94	5.82	5.67	-	-	-	-
7/60 MR	29.74	30.43	30.44	31.07	31.09	31.54	31.57
Lec/Lard/Peg blend	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dical Phos	0.62	0.42	0.42	0.41	0.47	1.30	1.38
Vitamin E 100,000 PRE	0.18	0.18	0.18	0.18	0.18	0.18	0.18
MR base mineral PR	0.38	0.38	0.38	0.38	0.38	0.38	0.38
MR base vitamin Plus	1.13	1.13	1.13	1.13	1.13	1.13	1.13
DL-Methionine	0.14	0.21	0.21	0.27	0.28	0.32	0.33
Dry MS Butter Flav	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Limestone	-	0.22	0.22	0.36	0.34	0.17	0.14
Nutr Pro B Plasma	-	6.63	6.63	13.27	13.27	19.90	19.90
L-Lysine (HCL) 98.5%	-	0.13	0.14	0.23	0.25	0.21	0.24
Isoleucine	-	-	0.15	-	0.30	-	0.41
Lactose	-	-	-	6.34	7.82	41.27	43.31
CP, %	18.4	18.4	18.4	18.4	18.4	18.4	18.4
Crude Fat, %	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Crude Fiber %	0.0	0.0	0.0	0.0	0.0	0.1	0.1
GE, Kcal/Kg	4716	4699	4698	4717	4723	4891	4900
Lactose %	45	45	45	45	46	50	50
Ca, %	1.04	1.04	1.04	1.04	1.04	1.04	1.04
P, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Vitamin A, KIU/Lb	30.1	30.1	30.1	30.1	30.1	30.1	30.1
Vitamin D, KIU/Lb	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Vitamin E, IU/Lb	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Lysine	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Methionine	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Isoleucine	1.12	0.98	1.12	0.84	1.12	0.73	1.12
Threonine	1.22	1.16	1.15	1.12	1.10	1.14	1.12
Histidine	0.30	0.40	0.40	0.50	0.50	0.60	0.60
Leucine	1.89	1.84	1.83	1.80	1.78	1.82	1.79
Valine	1.06	1.09	1.08	1.13	1.12	1.21	1.19
Phenylalanine	0.58	0.71	0.71	0.84	0.84	1.00	0.99
Tryptophan	0.31	0.32	0.32	0.33	0.33	0.34	0.34
Arginine	0.50	0.67	0.67	0.84	0.83	1.01	1
Cystine	0.54	0.53	0.53	0.54	0.54	0.62	0.61
Tyrosine	0.52	0.61	0.60	0.69	0.68	0.79	0.78

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

Table 2. Analyzed chemical composition of experimental milk replacers.

	Treatments[1]						
Component	A	B	C	D	E	F	G
DM, %	96.4	96.2	96.4	96.6	96.6	97.0	97.4
CP, % of DM	20.4	20.4	19.1	20.0	20.4	18.4	17.3
Soluble Protein, % of CP	93	96	95	94	93	93	92
Crude fat, % of DM	18.2	16.2	18.8	17.0	18.7	18.6	20.3
Ash, % of DM	7.62	7.59	7.45	7.42	7.54	5.52	5.10
Ca, % of DM	0.91	1.00	0.99	1.00	0.99	1.09	0.99
P, % of DM	0.68	0.73	0.71	0.72	0.74	0.74	0.70
Mg, % of DM	0.12	0.12	0.12	0.11	0.11	0.08	0.07
K, % of DM	1.54	1.60	1.58	1.37	1.32	0.51	0.42
Na, % of DM	0.575	0.669	0.643	0.674	0.694	0.603	0.558
S, % of DM	0.30	0.32	0.31	0.32	0.33	0.32	0.29
DE, Mcal/g of DM	4.67	4.57	4.69	4.61	4.70	4.75	4.84
ME, Mcal/g of DM	4.34	4.23	4.36	4.27	4.37	4.42	4.52
Fe, ppm	110	132	122	107	119	108	100
Zn, ppm	74	89	83	83	87	87	70
Cu, ppm	9	15	7	10	11	8	6
Mn, ppm	43	50	49	52	89	51	40
Mb, ppm	0.18	0.18	<0.1	<0.1	<0.1	0.28	<0.1

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

Table 3. Analyzed amino acid composition of experimental milk replacers.

	Treatments[1]						
Amino acid	A	B	C	D	E	F	G
Lysine	1.56	1.61	1.66	1.74	1.76	1.71	1.44
Methionine	0.37	0.40	0.40	0.48	0.43	0.52	0.39
Isoleucine	1.15	0.95	1.05	0.82	0.96	0.63	0.83
Threonine	1.12	1.13	1.08	1.10	1.07	1.01	0.97
Histidine	0.35	0.38	0.37	0.44	0.45	0.47	0.45
Leucine	1.87	1.81	1.73	1.78	1.75	1.61	1.55
Valine	1.06	1.04	1.00	1.10	1.11	1.08	1.04
Phenylalanine	0.61	0.66	0.64	0.76	0.78	0.81	0.78
Tryptophan	0.36	0.35	0.33	0.32	0.34	0.31	0.29
Arginine	0.52	0.59	0.57	0.72	0.75	0.83	0.80
Cysteine	0.39	0.43	0.44	0.50	0.51	0.53	0.52
Tyrosine	0.48	0.54	0.53	0.63	0.64	0.67	0.64
Methionine + Cysteine	0.76	0.84	0.84	0.98	0.94	1.05	0.91
Phenylalanine + Tyrosine	1.09	1.20	1.17	1.38	1.42	1.48	1.42
Taurine	0.06	0.07	0.07	0.07	0.06	0.07	0.06
Hydroxyproline	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspartic Acid	1.89	1.81	1.75	1.80	1.77	1.67	1.59
Serine	0.71	0.80	0.77	0.82	0.82	0.85	0.82
Glutamic Acid	2.92	2.71	2.60	2.56	2.52	2.28	2.18
Proline	1.04	0.98	0.95	0.95	0.92	0.85	0.82
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	0.40	0.42	0.41	0.49	0.50	0.53	0.51
Alanine	0.87	0.85	0.82	0.85	0.84	0.80	0.77
Hydroxylysine	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ornithine	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	17.73	17.55	17.18	17.92	18.00	17.12	16.46

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

Table 4. Amino acid profiles (% of lysine) compared with milk protein and proposed ideal amino acid profile.

	Treatments[1]								
Component	A	B	C	D	E	F	G	Whole Milk[2]	Ideal[3]
Lysine	100	100	100	100	100	100	100	100	100
Methionine	24	25	24	28	25	30	27	32	29
Isoleucine	73	59	63	47	55	37	58	66	47
Threonine	72	71	65	63	61	59	67	56	62
Histidine	22	24	22	25	26	27	31	47	39
Leucine	120	113	104	102	99	94	108	126	111
Valine	68	65	61	63	63	63	72	80	69
Phenylalanine	39	41	38	44	44	47	55	62	58
Tryptophan	23	22	20	18	19	18	20	16	18
Arginine	33	37	35	42	43	48	56	47	106
Cysteine	25	27	26	29	29	31	36	12	26
Tyrosine	31	34	32	36	37	39	45	62	41
Tyrosine + Phenylalanine	70	75	70	80	81	86	99	124	99
Cysteine + Methionine	49	52	51	56	54	61	44	44	55

1Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]From Milk Specialties Global database, courtesy S. Younker

[3]From M.E. Van Amburgh, unpublished results

Table 5. Intakes of DM, CP, ME, and lysine from milk replacer.

	Treatments[1]								Contrasts[2]					
Variable	A	B	C	D	E	F	G	SE[3]	1	2	3	4	5	6
DM, g/d														
Week 1	539	521	529	538	515	524	524	24.9	0.63	0.82	0.090	0.23	0.44	0.63
Week 2	631	654	660	663	627	659	646	36.0	0.28	0.48	0.94	0.81	0.35	0.63
Week 3	681	678	678	682	642	668	661	37.4	0.55	0.61	0.037	0.36	0.094	0.75
Week 4	715	720	713	706	684	692	683	40.5	0.17	0.55	0.042	0.94	0.30	0.95
Week 5	760	761	751	738	727	724	716	34.1	0.082	0.69	0.045	0.96	0.54	0.96
Overall	666	667	666	666	640	654	646	32.8	0.47	0.56	0.070	0.77	0.22	0.79
CP, g/d														
Week 1	110	106	101	108	104	96	90	4.9	<0.0001	0.020	<0.0001	0.083	0.0006	0.69
Week 2	129	133	126	133	127	121	111	6.9	0.19	0.037	0.005	0.076	0.018	0.68
Week 3	139	138	130	136	130	123	113	7.3	<0.0001	0.005	<0.0001	0.098	<0.0001	0.85
Week 4	146	146	136	141	139	127	117	8.0	<0.0001	0.013	0.0001	0.045	0.002	0.94
Week 5	155	155	144	148	147	133	123	7.6	<0.0001	0.040	<0.0001	0.061	0.016	0.87
Overall	136	136	127	133	130	120	111	6.4	<0.0001	0.004	<0.0001	0.021	0.0002	0.89
ME, kcal/d														
Week 1	2490	2356	2449	2459	2398	2454	2506	116.9	0.80	0.100	0.87	0.058	0.39	0.60
Week 2	2917	2956	3052	3027	2921	3083	3087	193.3	0.15	0.92	0.46	0.86	0.98	0.60
Week 3	3144	3066	3137	3115	2992	3127	3157	176.0	0.93	0.41	0.49	0.11	0.88	0.71
Week 4	3308	3252	3298	3224	3190	3239	3266	189.9	0.43	0.60	0.37	0.53	0.82	0.89
Week 5	3512	3435	3476	3367	3389	3385	3422	182.3	0.21	0.57	0.31	0.67	0.63	0.99
Overall	3074	3013	3081	3039	2981	3058	3089	154.0	0.97	0.45	0.68	0.34	0.76	0.74
Lysine, g/d														
Week 1	8.42	8.43	8.69	9.50	9.27	8.85	7.52	0.462	0.010	0.16	0.11	<0.0001	0.029	0.001
Week 2	9.85	10.58	10.82	11.71	11.33	11.13	9.26	0.642	0.004	0.105	0.59	0.0003	0.046	0.010
Week 3	10.63	10.97	11.12	12.06	11.61	11.32	9.47	0.683	0.018	0.103	0.081	0.0001	0.010	0.003
Week 4	11.19	11.64	11.69	12.48	12.37	11.75	9.80	0.738	0.093	0.12	0.077	<0.0001	0.033	0.009
Week 5	11.87	12.28	12.32	13.01	13.15	12.29	10.27	0.713	0.23	0.18	0.075	0.0001	0.078	0.016
Overall	10.39	10.78	10.92	11.76	11.56	11.07	9.273	0.591	0.017	0.086	0.104	<0.0001	0.017	0.002

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]Contrasts were 1=linear effect of increasing PPP without supplemental Ile 2=quadratic effect of increasing PPP without supplemental Ile 3=linear effect of increasing PPP with supplemental Ile 4= quadratic effect of increasing PPP with supplemental Ile 5=effect of addition of supplemental Ile 6=interaction of the linear effect of increasing PPP with addition of Ile

[3]SE=standard error of the mean

Table 6. Calf body weight (BW), average daily gain (ADG), and gain to feed ration.

	Treatments[1]								Contrasts[2]					
Variable	A	B	C	D	E	F	G	SE[3]	1	2	3	4	5	6
BW, kg														
Initial	43.2	43.0	42.6	43.0	42.7	42.5	42.3	2.320	0.47	0.80	0.41	0.85	0.59	0.85
Final	56.9	57.7	57.2	53.3	54.4	50.3	51.6	2.824	<0.0001	0.11	0.0004	0.20	0.51	0.44
ADG, g/d														
Week 1	474	518	524	283	318	348	356	100	0.030	0.88	0.051	0.9402	0.78	0.9882
Week 2	194	192	162	132	68	-55	62	76	0.010	0.18	0.100	0.8538	0.8892	0.2939
Week 3	312	397	403	266	427	133	229	52	0.003	0.039	0.39	0.007	0.045	0.3916
Week 4	483	463	428	369	404	319	306	68	0.004	0.74	0.010	0.64	0.9057	0.8089
Week 5	492	531	571	419	467	415	392	42	0.044	0.61	0.023	0.069	0.5268	0.4582
Overall	392	422	419	292	338	232	269	27	<0.0001	0.074	<0.0001	0.064	0.21	0.42
Gain/DMI														
Week 1	0.88	1.02	1.01	0.51	0.63	0.68	0.72	0.171	0.049	0.92	0.13	0.874	0.67	0.8792
Week 2	0.38	0.30	0.24	0.20	0.13	-0.17	0.07	0.147	0.002	0.25	0.060	0.7232	0.70	0.2251
Week 3	0.45	0.58	0.58	0.40	0.65	0.19	0.33	0.080	0.004	0.031	0.50	0.004	0.0308	0.35
Week 4	0.70	0.67	0.61	0.54	0.60	0.46	0.45	0.115	0.003	0.75	0.013	0.6507	0.9599	0.7265
Week 5	0.66	0.69	0.75	0.55	0.63	0.56	0.54	0.056	0.064	0.80	0.046	0.095	0.4107	0.4611
Overall	0.61	0.66	0.64	0.43	0.53	0.34	0.42	0.043	<0.0001	0.11	0.0004	0.1056	0.1249	0.2779

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]Contrasts were 1=linear effect of increasing PPP without supplemental Ile 2=quadratic effect of increasing PPP without supplemental Ile 3=linear effect of increasing PPP with supplemental Ile 4= quadratic effect of increasing PPP with supplemental Ile 5=effect of addition of supplemental Ile 6=interaction of the linear effect of increasing PPP with addition of Ile

[3]SE=standard error of the mean

Table 7. Stature measurements.

	Treatments[1]								Contrasts[2]					
Variable	A	B	C	D	E	F	G	SE[3]	1	2	3	4	5	6
Body length, cm														
Initial	65.3	64.6	65.1	64.3	64.3	65.0	65.6	1.30	0.55	0.13	0.89	0.11	0.31	0.95
Final	72.1	71.5	71.8	69.9	70.2	70.1	70.6	1.61	0.001	0.45	0.009	0.55	0.43	0.87
Overall daily gain	0.20	0.19	0.19	0.15	0.16	0.15	0.15	0.027	0.001	0.95	0.003	0.71	0.86	0.94
Heart girth, cm														
Initial	79.2	78.6	79.0	78.2	79.2	78.3	78.5	1.83	0.12	0.42	0.44	0.6208	0.1559	0.8673
Final	86.4	86.4	86.9	83.7	85.0	82.5	83.7	1.86	<0.0001	0.37	0.0006	0.1547	0.057	0.6065
Overall daily gain	0.21	0.22	0.23	0.15	0.17	0.12	0.15	0.015	<0.0001	0.076	0.0005	0.1746	0.2239	0.3926
Withers height, cm														
Initial	79.3	78.5	79.7	77.9	78.8	79.0	78.6	1.40	0.49	0.07	0.13	0.4812	0.206	0.100
Final	84.4	83.9	84.2	82.5	83.7	81.9	82.4	1.35	<0.0001	0.98	0.005	0.2807	0.1332	0.90
Overall daily gain	0.15	0.15	0.13	0.13	0.14	0.08	0.11	0.013	<0.0001	0.040	0.017	0.4455	0.4501	0.043
Hip height, cm														
Initial	82.5	82.1	82.9	81.9	82.5	82.7	82.4	1.50	0.89	0.26	0.75	0.62	0.40	0.31
Final	87.9	87.8	87.5	86.1	87.3	85.5	86.3	1.38	<0.0001	0.67	0.045	0.5714	0.1604	0.2744
Overall daily gain	0.15	0.16	0.13	0.11	0.14	0.08	0.11	0.016	<0.0001	0.057	0.008	0.7923	0.2673	0.006
Hip width, cm														
Initial	17.6	17.5	17.4	17.4	18.0	17.6	17.7	0.46	0.88	0.36	0.17	0.81	0.22	0.60
Final	19.4	19.7	19.3	18.9	19.4	18.8	19.0	0.39	0.0004	0.32	0.19	0.5249	0.681	0.080
Overall daily gain	0.05	0.06	0.05	0.04	0.04	0.04	0.04	0.013	<0.0001	0.039	0.006	0.5258	0.627	0.064

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]Contrasts were 1=linear effect of increasing PPP without supplemental Ile 2=quadratic effect of increasing PPP without supplemental Ile 3=linear effect of increasing PPP with supplemental Ile 4= quadratic effect of increasing PPP with supplemental Ile 5=effect of addition of supplemental Ile 6=interaction of the linear effect of increasing PPP with addition of Ile

[3]SE=standard error of the mean

Table 8. Concentrations of metabolites in blood serum from calves during week 4 of experiment.

	Treatments[1]								Contrasts[2]					
Variable	A	B	C	D	E	F	G	SE[3]	1	2	3	4	5	6
Total protein, g/dL	5.0	5.2	5.3	5.0	5.3	4.9	5.2	0.18	0.31	0.18	0.41	0.063	0.025	0.56
Urea N, mg/dL	6.1	5.8	5.9	6.6	6.9	6.4	5.0	0.38	0.19	0.88	0.26	0.017	0.22	0.034
Albumin, g/dL	3.0	3.0	3.0	2.9	3.0	2.9	2.9	0.07	0.066	0.61	0.49	0.37	0.32	0.63
Total globulin, g/dL	2.0	2.2	2.3	2.2	2.4	2.0	2.2	0.13	0.76	0.17	0.18	0.073	0.027	0.63

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]Contrasts were 1=linear effect of increasing PPP without supplemental Ile 2=quadratic effect of increasing PPP without supplemental Ile 3=linear effect of increasing PPP with supplemental Ile 4= quadratic effect of increasing PPP with supplemental Ile 5=effect of addition of supplemental Ile 6=interaction of the linear effect of increasing PPP with addition of Ile

[3]SE=standard error of the mean

Table 9. Arrival (d 0) concentration of IgG in serum, body temperatures, antibiotic treatments, fecal scores, days of scours, respiratory scores, and average electrolyte intake.

	Treatments[1]								Contrasts[2]					
Variable	A	B	C	D	E	F	G	SE[3]	1	2	3	4	5	6
IgG, mg/dL	1024	1524	1604	1465	1531	1113	1720	277.3	0.87	0.065	0.069	0.39	0.19	0.25
Temperature, C	38.6	38.6	38.6	38.1	38.6	38.6	38.7	0.19	0.45	0.13	0.93	0.79	0.26	0.99
Antibiotics, no. of treatments	0.89	0.40	0.29	0.81	0.53	0.65	0.25	0.217	0.92	0.46	0.13	0.47	0.15	0.50
Fecal scores	2.4	2.2	2.3	2.4	2.4	2.3	2.19	0.1171	0.59	0.83	0.36	0.41	0.43	0.25
Scours, d	13.8	11.2	12.6	15.7	15.4	13.9	11.2	2.35	0.29	0.80	0.60	0.29	0.65	0.16
Respiratory scores	1.0	1.0	1.0	1.2	1.0	1.0	1.01	0.007	0.92	0.46	0.13	0.47	0.15	0.50
Mean electrolyte intake, L	0.37	0.28	0.05	0.27	0.24	0.64	0.04	0.197	0.30	0.18	0.38	0.74	0.041	0.26

[1]Treatments were A=control B=33% PPP C=33% PPP + isoleucine D=66% PPP E=66% PPP + isoleucine F=100% PPP G=100% PPP + isoleucine

[2]Contrasts were 1=linear effect of increasing PPP without supplemental Ile 2=quadratic effect of increasing PPP without supplemental Ile 3=linear effect of increasing PPP with supplemental Ile 4= quadratic effect of increasing PPP with supplemental Ile 5=effect of addition of supplemental Ile 6=interaction of the linear effect of increasing PPP with addition of Ile

[3]SE=standard error of the mean