EVALUATION OF SERUM PROTEIN-BASED ARRIVAL FORMULA AND SERUM PROTEIN (GAMMULIN) ON PERFORMANCE, MORBIDITY, AND MORTALITY OF STRESSED (TRANSPORT AND COLD) MALE DAIRY CALVES

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THESIS
Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana- Champaign, 2011

Urbana, Illinois

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ABSTRACT

Adequate nutrition is important to provide all nutrients for proper health and growth of young calves, especially in the presence of stressors such as cold and transport. Serum protein products have been proposed to improve health and diminish effects of stress in dairy calves. The aim of this study was to assess a serum protein-based arrival formula (AF) and use of a serum protein supplement (G; Gammulin, APC Inc.) in milk replacer for neonatal male calves stressed by transport and cold on performance, morbidity, and mortality.

Ninety-three male Holstein calves were stratified by arrival BW and plasma protein concentration, and then randomly assigned to 1 of 4 treatment groups. Treatments were 1 = control electrolyte, milk replacer without G (E; n = 25); 2 = AF, milk replacer without G (AF; n = 22); 3 = control electrolyte, milk replacer with G (EG; n = 24); and 4 = AF, milk replacer with G (AFG; n = 22). At arrival, calves were fed either AF or a control electrolyte solution. At the next feeding, all calves received either a commercial calf milk replacer (20% CP, and 20% fat; no G supplementation) or the same milk replacer supplemented with G. The G-supplemented group received 50 g/d of G in the milk replacer during the first 14 d of feeding only. Feed offered and refused was recorded daily. Calf health was assessed by daily assignment of scour and respiratory scores. Body weight, withers height, body length, heart girth, hip height, and hip width were measured weekly. Blood samples were taken at d 0 (before treatments), d 2, d 7, d 14, and d 28. Calves were weaned at d 42 and remained in the experiment until d 56. Data were analyzed using the MIXED, GLIMMIX, LIFETEST, and LOGISTIC procedure of SAS (v. 9.2).

The environmental temperature during all periods was below the lower critical temperature. During the first 2 wk of dietary treatment, calves fed AF had significantly longer

body length ($P = 0.05$); greater starter DM and CP intakes ($P = 0.0007$); superior total DM, CP, and ME intakes ($P < 0.05$); and greater cortisol concentration ($P = 0.02$) in blood. Supplementation with G resulted in greater BW ($P = 0.06$); greater milk replacer DM and CP intakes ($P < 0.0001$); higher total CP intake ($P < 0.0001$); lower mean respiratory score, less odds for days ($P = 0.08$) and number of calves ($P = 0.0008$) with high respiratory score; and greater concentrations of total protein ($P = 0.04$) and urea N ($P = 0.01$) in plasma. In addition, during the 8-wk experiment G supplementation resulted in improved mean fecal score ($P = 0.02$) and less antibiotic treatments per calf ($P = 0.05$). Mortality was greater ($P = 0.02$) for calves that did not receive G.

Results indicated that a serum protein-supplemented arrival formula improved early starter and total nutrient intakes. Addition of a serum protein product also improved early growth, and decreased morbidity and mortality in transported male calves. Despite the marked reduction in mortality of transported cold-stressed male calves fed the serum protein product, indicators of acute-phase response were not affected; however, protein status of calves may have been improved by early serum protein supplementation.

DEDICATION

In loving memory of my father Victor Pineda, who always motivated me to look for the highest and best education. To my mother, brothers, and sisters for all the support that they have given to help me achieve my goals.

ACKNOWLEDGEMENTS

First, I want to thank to Dr. James K. Drackley for giving me the opportunity to work with him; it has truly been an honor. I always have admired his enormous professionalism, patience, wisdom, and kindness, which make for me a great model to follow.

I thank American Proteins Corporation (APC, Inc.) for the sponsorship of this research, and especially to Dr. Joy Campbell. I thank the University of Illinois and Department of Animal Sciences for providing the resources and environment for me to obtain my M.S. degree.

I would like to extend thanks to my committee members, Dr. Michael Murphy and Dr. Michael Hutjens, for their time and help reviewing this document. Also, great thanks to Dr. Michael Ballou for his collaboration in some of the laboratory analysis.

Finally, I express gratitude to Johan Osorio, Felipe Cardoso, Shaoyu Luan, undergraduate students, veterinary and dairy farm staff for all their assistance in this project.

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ASP	acid-soluble protein
ADP	apparently digestible protein
AF	arrival formula
AFG	arrival formula, milk replacer with Gammulin treatment
ADG	average daily gain
BV	biological value
BW	body weight
BVD	bovine viral diarrhea
cm	centimeter(s)
EG	control electrolyte, milk replacer with Gammulin treatment
CP	crude protein
d	day(s)
dL	deciliter(s)
°C	degrees Celsius
DM	dry matter
E	electrolyte treatment
FS	fecal score
G	Gammulin
g	gram(s)
h	hour(h)
Ig	immunoglobulins
IgA	immunoglobulin A

IgG	immunoglobulin G
IgM	immunoglobulin M
kg	kilogram(s)
L	liter(s)
Mcal	megacalorie(s)
ME	metabolizable energy
µg	microgram(s)
mg	milligram(s)
mL	milliliter(s)
mo	month(s)
ng	nanogram(s)
NE_G	net energy for gain
NE_L	net energy for lactation
NE_M	net energy for maintenance
N	nitrogen
OD	optical density
OR	0dds ratio
ppm	parts per million
%	percent
P	probability
RS	respiratory score
SEM	standard error of means
TDN	total digestible nutrients

v	version
VFA	volatile fatty acid
wk	week(s)

CHAPTER I

INTRODUCTION

According to the National Animal Health Monitoring System in its publication "Biosecurity Practices on U.S. Dairy Operations, 1991-2007" (National Animal Health Monitoring System, 2010), the percentage of pre-weaned heifer deaths decreased from 10.8% in 1996 to 7.8% in 2007. Similarly, weaned heifer-calf deaths decreased from 2.8% in 2002 to 1.8% in 2007. Diarrhea and respiratory problems are the major causes of death for pre-weaned heifers. Diarrhea accounted for more than 50% of deaths since 1991, while respiratory problems accounted for 21 to 25% of mortality during the same period (National Animal Health Monitoring System, 2010). Although mortality of heifers during the pre-weaned period has decreased slightly, it remains a considerable problem and the risk of death is greater than in the post-weaned period.

Infectious disease of the intestine is the main cause of calf diarrhea (von Buenau et al., 2005) and is most likely to be a problem in calves < 1 mo-old. In contrast, pneumonia is the major cause of respiratory distress in calves (Ferrari et al., 2010) after 1 mo of age. High occurrence of these health problems is associated with the lack of adequate nutrition and management, plus the presence of environmental pathogens. For successful calf raising, producers must understand and manage the interactions among nutrition, disease, and environment (Davis and Drackley, 1998). A clear understanding of the factors associated with the incidence of scours and respiratory problems is fundamental to decrease morbidity and mortality during the pre-weaned period.

One of the factors associated with high morbidity and mortality during the pre-weaned phase is low quality and low intake of colostrum. Colostrum is a rich source of nutrients, containing higher amounts of solids than normal milk (Davis and Drackley, 1998). It provides the calf with antibodies in the form of immunoglobulins (Hammer et al., 2004). Colostrum is vital to the health and survival of the neonatal calf. Absorption of colostral antibodies starts to decrease right after birth and is completed by 36 h (Morin et al., 1997). A fundamental nutrition principle and management practice is to feed the newborn calf suitable amounts of high quality colostrum as soon as possible after birth in order to decrease disease susceptibility and neonatal mortality.

Stressors such as cold weather and transport, among others, may have a negative effect on morbidity and mortality of the young calf. An increased risk of health problems during winter might be caused by higher levels of infectious agents and lower ambient temperature (Gulliksen et al., 2009). Scours and respiratory problems are more likely to occur during this season. On the other hand, transport causes physiological changes in the animal resulting in increases in plasma cortisol concentrations (Johnston and Buckland, 1976), immune suppression, and increases in heart and respiration rates (Fike and Spire, 2006). Young calves, < 1 mo-old, are more susceptible to transport stress; hence, morbidity and mortality can be high (Knowles, 1995).

Adequate early nutrition is important to provide all nutrients for proper health and growth of young calves. However, under the presence of high environmental pathogen loads, unfavorable environmental conditions, stress from transport, and insufficient colostrum intake; proper nutrition is not enough to ensure adequate growth and health of young calves. Nutritional supplements and additives might help producers to improve nutrition and health status in young calves under adverse conditions.

Serum protein products have shown potential in maintaining or increasing growth and health while decreasing morbidity and mortality (Arthington et al., 2000; Arthington et al., 2002; Quigley et al., 2002). Our hypothesis in this study was that serum protein products might help stressed dairy calves at arrival and during the pivotal first 2 wk of life. Therefore, the objectives were: 1) to evaluate a serum protein-based arrival formula in the first feeding after the arrival in stressed (by transport and cold weather) male calves on performance, morbidity, and mortality, and 2) to evaluate the use of a commercial serum protein supplement (Gammulin) in milk replacer during the first 2 wk on performance, morbidity, and mortality of these calves.

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CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Adequate nutrition is crucially important for proper health and growth of young calves as future replacements for the dairy enterprise. Considerable knowledge has been gained throughout the last decade in calf nutrition and its effects on growth and health status. However, mortality and morbidity remain major problems for dairy calves (National Animal Health Monitoring System, 2010a), especially those colostrum-deprived and stressed by transport and cold weather. Greater morbidity and mortality is related to poor nutrition along with unfavorable management and environmental conditions (Davis and Drackley, 1998).

Raising healthy and productive replacements for the dairy enterprise is in large part a function of proper feeding and care of young calves. Intake of high quality and adequate amounts of colostrum followed by consumption of milk or milk replacer that provides adequate energy, protein, mineral, and vitamin contents is important to ensure adequate nutrition and high survival in newborn calves (National Research Council, 2001). Offering a palatable calf starter, as soon as possible after birth, helps to stimulate rumen development and may allow early weaning (Hill et al., 2005).

Nutrition and management are not the only factors affecting calf performance; environmental conditions and other stressors also have a significant impact. Presence of stressors along with unfavorable conditions increases risk and susceptibility of calves to high morbidity and mortality. Under such circumstances adequate nutrition is compromised and proper calf

performance may not be achieved. Nutritional supplements may help to overcome negative effects of adverse conditions and improve nutrition in such circumstances. Recently, serum protein and plasma protein-based products have shown potential to improve health and nutrition in young pigs and calves.

Calf's Digestive System Development

Although the digestive system of the calf starts to develop early in the embryonic stage it is immature at birth (Davis and Drackley, 1998). At this time, the stomach contains the same four compartments found in adult ruminants. However, the reticulum, rumen, and omasum are not active and fully developed. From birth to about 2 wk of age, the calf is basically monogastric, because the abomasum is the only stomach compartment actively involved in digestion (Drackley, 2008). During this time, nutrients are acquired mainly from milk or milk replacer. Development of the other compartments occurs later and is determined by the diet consumed. As the calf begins to eat dry feeds, particularly grains containing readily fermentable carbohydrates, the other stomach compartments start to develop in size and function until the calf becomes a functional ruminant.

While the digestive system is developing in the process of becoming a true ruminant, , the calf goes through three phases: pre-ruminant, transition, and ruminant (National Research Council, 2001). In the pre-ruminant phase the digestive tract is more similar to humans than cows. During this time, in absence of a functional reticulo-rumen the calf relies on digestive enzymes to break down nutrients present in milk or milk replacer. Milk replacer passes directly to the abomasum through the reticular groove, which consists of muscular folds that make up a

duct that prevents liquid feed from entering into the undeveloped reticulo-rumen (Davis and Drackley, 1998). Once in the abomasum, digestive enzymes released primarily from the pancreas, abomasum, and small intestine carry out the digestion process. Chymosin and pepsin activated by hydrochloric acid act on casein of the milk in the abomasum (Guilloteau and Zabielski, 2005). A curd that binds casein and fat is formed and slowly digested in the abomasum. The fraction of milk that does not form a curd is called whey, which goes directly into the small intestine for digestion and end-product absorption (Drackley, 2008).

During the pre-ruminant stage, digestive enzymes are unable to digest complex feeds other than milk (Guilloteau and Zabielski, 2005).Iif good quality starter is offered as early as possible, by 3 wk of age the digestive enzymes of the calf become more active. As the calf's ability to eat and digest starch increases it moves to the transition phase (National Research Council, 2001). During this phase the calf is generally fed milk replacer and grain with or without forage. Greater intake of starter over forage during the transition period allows better development in function, but perhaps not size, of the rumen (Heinrichs and Lesmeister, 2005). High quality starter, high in fiber and readily fermentable carbohydrates, allows development of rumen papillae and increases the rumen microbial population (Drackley, 2008). The developing rumen microbial population ferments carbohydrates in starter leading to greater production of volatile fatty acids (VFA), mainly propionate and butyrate. Volatile fatty acids, especially butyrate and to a lesser extent propionate, stimulate rumen papillae development and functionality (Davis and Drackley, 1998). As the rumen papillae become functional their ability to absorb VFA increases, which drives changes in rumen environment. Rumen pH begins to increase and with it the cellulolytic bacteria population increases; however, physical size of the rumen limits forage intake at this time. Despite capacity limitation in the rumen, if good quality

starter is offered and intake is acceptable, nutrient requirements for maintenance and growth can be satisfied.

Development of a functional rumen as soon as possible, from an efficiency and economic standpoint, is crucially important in heifer raising programs. Successful replacement programs removed calves from liquid diets to lower feed cost, reducing the risk of digestive upsets while developing a functional rumen (Hutjens, 2003). In this way, when the calf is eating enough starter to support its growth, 0.9 kg or more calf starter (Hutjens, 2003), it can be weaned and considered a true ruminant.

Calf's Nutritional Requirements

As a future replacement the newborn calf must be fed highly digestible feeds containing appropriate levels of high-quality nutrients. Like other animals, the young calf has requirements for energy, protein, vitamins, and minerals for maintenance and to support adequate growth and health.

Energy. Requirements for energy in young calves are stated depending on the feeding system and/or digestive system development phase: calves fed only milk or milk replacer, milk/milk replacer and starter feed, and weaned calves (National Research Council, 2001). Energy requirement is expressed in units of metabolizable energy (ME), which is total energy intake minus energy loss in feces, urine, and gases (Drackley, 2008). In addition, energy requirements for growing calves are partitioned into ME for maintenance and ME for growth. Energy required for maintenance refers to the amount of energy necessary to support essential body functions (body temperature regulation, essential metabolic processes, and physical

activity) (National Research Council, 2001). Energy required for growth refers to energy needed for deposition of new skeletal and muscle tissue (National Research Council, 2001). The ME requirements would vary depending on BW, rates of gain, environmental conditions, health status, and stress factors. The National Research Council establishes energy requirements for maintenance for a 45-kg calf fed milk/milk replacer and starter, under thermoneutral conditions, to be 1.81 Mcal of ME/d. The thermoneutral zone would vary depending on many factors: calf's age, feed intake, and amount of subcutaneous fat and hair coat (National Research Council, 2001). From birth to 3 wk of age the thermoneutral zone for calves is defined as 15 to 25 °C (National Animal Health Monitoring System, 2010a). As the calf gets older the thermoneutral zone range increases. When the ambient temperature drops below the lower critical temperature (15°C) ME requirements for maintenance are increased. The NRC states that ME for maintenance increases approximately 14% for every 5 °C that ambient temperature decreases below the lower critical temperature (National Research Council, 2001).

Protein. Requirement for protein is expressed in terms of apparently digestible protein (ADP) (National Research Council, 2001). The requirement for ADP is calculated from the formula $ADP\ (g/d) = 6.25\ [1/BV\ \ (E + G + M * D) - M * D]$ where 6.25 is the factor to convert N to protein; BV = biological value of proteins; E = endogenous N excretion (g/d); G = the N (g/d) stored in liveweight gain; M = metabolic fecal N (g/kg); and D = DM intake (kg/d) (Davis and Drackley, 1998).

Protein requirements vary with the rate of BW gain expected; greater average daily gains (ADG) will require greater amounts of protein intake. Similar to energy, requirement for protein is partitioned into components of maintenance and gain. Requirement for maintenance is assessed from metabolic fecal N and endogenous urinary N losses. Metabolic fecal N is the non-

dietary N found in feces, which originates from gut tissue, bacterial debris, and digestive secretions. Endogenous urinary N, expressed on the basis of metabolic body size ($kg^{0.75}$), originates from tissue metabolism (Davis and Drackley, 1998). For gain, the NRC assumes a constant of 30 g of N per kg of BW gain, which is equivalent to 187.5 g of protein per kg of BW gain (National Research Council, 2001).

An important concept in the formula for requirement of ADP is the biological value (BV) of proteins. According to (National Research Council, 2001), BV refers to how adequately balanced is the amino acid profile of the diet being fed, and assumes that energy intake is enough to support protein synthesis. This relates to the importance of energy – protein interrelationships in the diet. Feeding higher amounts of protein without enough energy will result in waste of protein and excretion of N. Conversely, higher energy intake with inadequate intake of protein will result in fat, rather than lean tissue, deposition (Drackley, 2008). The BV for milk is considered to be 80 to 90% (Davis and Drackley, 1998) while BV for starter is assumed to be 70% (National Research Council, 2001).

Protein content in feeds is expressed as crude protein (CP). Requirements also are expressed in terms of CP. Conversion of ADP to CP is a function of digestibility and BV of feeds. For milk proteins conversion of CP to ADP is assumed to be 93% while for starter and grower feeds the conversion of CP to ADP is 75% (National Research Council, 2001).

Vitamins and Minerals. Requirements for vitamins and minerals in young calves are not as well defined as they are for pigs, chickens, and older cattle (Drackley, 2008). Calves have requirements for many of the same minerals and vitamins as other animals. Required vitamins are: vitamin K, water-soluble B vitamins (thiamine, riboflavin, niacin, choline, biotin,

pyridoxine, folic acid, B12, and pantothenic acid), and fat-soluble vitamins (A, D, and E) (Davis and Drackley, 1998). Most of the minerals and vitamins are found in colostrum, fermented colostrum, milk/milk replacers, and starter grain fed to calves during younger age. Once the rumen becomes functional microorganisms in the rumen produce some of the B-vitamins and supplementation may not be needed. Vitamin C, synthesized in the calf's tissues, is not required in the diet.

Water. Although not always seen as important water is an essential and critically important nutrient. Water is important to carry out metabolic process and is deposited in greatest amount in calf tissue during growth (Drackley, 2008). Fresh and clean water should be available at all times, especially in growing calves. Water and starter intake are highly related; as starter intake increases water intake increases as well (Davis and Drackley, 1998). Scouring calves lose considerable amount of water and electrolytes resulting in dehydration and electrolyte imbalance (National Research Council, 2001). Resent research demonstrated that calves that were offered warm water had greater intake than calves offered cold water (Huuskonen et al., 2011). This may be important to encourage water intake, particularly in dehydrated calves, in winter where water intake could be low.

Calf's Feeds

Colostrum. The first milk produced after a normal dry period contains antibodies in the form of immunoglobulins (Ig), which are critical in providing the calf with passive immunity from infectious diseases (Godden, 2008). Colostrum provides essential nutrients, contains twice as much DM, three times as many minerals, and five times as much protein as whole milk (Davis

and Drackley, 1998). It is also higher in energy and vitamins (National Research Council, 2001). The high content of fat and vitamins A, D, and E in colostrum is especially important because the newborn calf has low reserves of these nutrients. In addition, the relatively low lactose content of true colostrum may reduce the incidence of diarrhea.

Antibodies in bovines cannot cross the placental barrier from the cow to the fetus (Hutjens, 2003) . Different from many other species, calves are born with no disease protection. They receive Ig through passive transfer by consuming adequate amounts of colostrum within the first few hours after birth (Davis and Drackley, 1998). Absorption of antibodies directly from the gut into the bloodstream starts to decrease right after birth and ceases by 36 h (Morin et al., 1997). Consequently, timing of colostrum feeding is critically important because of the limited time to absorb Ig and the potential for pathogenic bacterial colonization of the intestine (Godden et al., 2009). The primary colostrum antibodies are immunoglobulins G (IgG), A (IgA), and M (IgM). The IgG fraction constitutes 80 to 85% of all Ig in colostrum and provides immunity against a wide variety of systemic infections and disease. The IgM makes up 5 to 12%, is an important contributor to early immunity especially to enteric pathogens, and is most important in prevention of septicemia. Secretory production of IgA in bovine is less than in many other species, constituting 8 to 10% of the immunoglobulins. In the calf, many of the functions of IgA in other species are assumed by IgM and IgG (Davis and Drackley, 2008; Godden, 2008).

Important factors affecting calf morbidity and mortality during the pre-weaning period are the quality, quantity, and timing of colostrum fed (Gulliksen et al., 2009c). Colostrum quality is a function of Ig concentration and the presence or absence of bacteria. Quality depends on several factors, including immune status of the cow, age of the cow, volume of colostrum produced, cow nutrition, breed, and season of the year (Godden, 2008). An easy and practical

way to estimate quality of colostrum in the field is through a colostrometer. The colostrometer, a hydrometer, estimates IgG concentration by measuring specific gravity of colostrum. Colored areas on the scale indicate whether the colostrum is superior, acceptable, or unacceptable for feeding newborn calves (Hutjens, 2003). Good quality colostrum contains at least 50 g/L of IgG (Davis and Drackley, 1998)

Another important factor when feeding colostrum is quantity. Calves should receive 2 to 3 L of undiluted colostrum as soon as possible after birth, and another 2 to 3 L within 8 h (Jaster, 2005). If the second feeding is not possible 4 L of colostrum should be fed in a single feeding. Newborn calves generally will not drink a large amount of colostrum by themselves at one time; in this case an esophageal feeder may be used to feed all or part of the colostrum.

Colostrum supplements and colostrum replacers are another option when high quality colostrum is not available. These products can be added to marginal colostrum when no other source of colostrum is available. Several studies indicate that calves fed colostrum supplements and colostrum replacers have performed as well as calves fed maternal colostrum with no differences in IgG concentrations, incidence of scours, or growth rates (Quigley III et al., 1998; Jones et al., 2004). Colostrum replacers have more Ig than colostrum supplement products and provide more antibodies than poor or moderate quality colostrum (Morin et al., 1997).

Liquid Feed. Consumption of adequate amounts of high quality colostrum is the first step to ensure calf survival. Next, the calf needs to be fed with liquid feed containing adequate amounts of highly digestible nutrients. There are several liquid feed options available to feed the young calf. These include milk replacers, waste milk, fresh or fermented colostrum, or whole milk (Drackley, 2008). Any of these is excellent feed when available and if it fits into the calf-

raising program. Research indicates that a variety of liquid feeds fed at correct dilution rates can achieve satisfactory results.

Generally, milk replacers are fed on most farms in United States. Nutrient levels, composition, and quality vary considerably among milk replacers. Composition and quality of milk replacer influence growth, health, and overall performance of calves (National Research Council, 2001). Milk replacers generally contain 18 to 28% CP and 15 to 22% crude fat. In addition, they should contain and be well-balanced for major minerals (including calcium, phosphorus, and magnesium), trace elements, and vitamins. In milk replacers, milk proteins are typically more digestible and contain a favorable profile of amino acids (Tanan, 2005). However, they are the most expensive source of protein. In an effort to reduce cost, extensive research has been conducted in search for alternative protein sources.

Plant proteins have been extensively evaluated. Plant protein sources often contain more crude protein than milk proteins; however, protein quality or amino acid balance is slightly inferior (Drackley, 2008). Soy proteins have been evaluated and can provide an economic alternative to milk proteins. These vegetable proteins can be substituted for a portion of the milk proteins in milk replacers, providing acceptable calf growth and performance. Some vegetable proteins contain anti-nutritional compounds that cause allergic reactions, poor digestion, or diarrhea (Drackley, 2005; Tanan, 2005).

Another protein source for calf milk replacer that has received attention by the scientific community is protein from animal origin (Drackley, 2005; Drackley, 2008). Egg proteins have been incorporated into commercial milk replacers with variable results. Calves fed milk replacers containing animal plasma proteins also have shown to have similar or improved performance

than calves fed milk proteins (Quigley and Bernard, 1996; Quigley et al., 2000; Quigley and Wolfe, 2003).

Carbohydrates and fat are important as a source of energy in milk replacers. During the pre-ruminant phase, the calf's digestive system is not well-developed (Guilloteau and Zabielski, 2005). At this time, the major sources of energy are lactose (milk sugar) and highly digestible fat. Lactose is a carbohydrate and is the other major energy source in milk replacers (Davis and Drackley, 1998). A typical milk replacer formulation contains about 40 to 45% lactose. Fats and oils provide a concentrated energy source for animal feeds. Fat levels in milk replacers typically range from 10 to 24%, with 15 to 20% being the most common. Calves can digest saturated fats including milk fat, coconut oil, lard, and tallow; however, they have limited ability digesting unsaturated fats such as corn and soybean oils (Drackley, 2008). Higher fat milk replacers are often selected for cold climates while low-fat formulas are more often used in hot climates and in formulations designed for intensive milk replacer feeding programs (Drackley, 2008).

Dry Feed. The pre-weaned calf requires both liquid and dry feeds. Conventional feeding programs typically rely on dry feed intake to meet large portions of the calf's requirement. Early intake of dry feed in young calves is important to stimulate development of the reticulo-rumen (Hill et al., 2005). Developing of the rumen as a fermentation chamber in the young calf is crucially important from an efficiency and economy standpoint as it decreases feed costs, lessens labor needs, and can overcome the calf's stress associated with the transition from pre-weaned to weaned stage (Hutjens, 2003).

Rumen development in the young calf is separated into two components: physical size (volume) and the development of rumen papillae (functionality) (Heinrichs and Lesmeister,

2005). When dry feed is offered beginning in the first week of age, the calf increases intake as it ages, which increases the size of the rumen (Drackley, 2008). The development of the rumen papillae (elongation and absorptive function) is a function of the diet ingested. Limitation in size of the rumen, fewer cellulolytic bacteria, and low digestibility of forages makes starters a better option to feed calves at younger age. In addition, extensive research has demonstrated that starter is more effective than forage in developing the absorptive epithelium of the rumen. Fermentation of starter in the rumen leads to high production of VFA, especially propionate and butyrate (National Research Council, 2001). Butyrate and to a lesser extent propionate stimulate growth and differentiation of rumen papillae (Heinrichs and Lesmeister, 2005). High quality starter, high in fiber and readily fermentable carbohydrates, should be offered from a very early age (Drackley, 2008)

There are many types of calf starters available on the market. Starters are a good source of energy, protein, minerals, and vitamins, and may include antibiotics that improve efficiency of nutrient use and offer protection against pathogens. In general, calf starter should be palatable and provide nutrients required for rumen development and adequate calf growth (Drackley, 2008). Small amounts of forage are also important to promote muscular growth and maintain health of the rumen epithelium (Hill et al., 2005). At an early age rumen pH is low and the population of cellulolytic bacteria is not sufficiently high to digest forages. As starter intake increases rumen pH rises; this favors development of the population of cellulolytic bacteria. Despite the increase in cellulolytic bacteria, digestibility of forages by the increased bacteria population is still limited. When the calf is consuming around 2.0 kg of starter, generally after weaning, good quality forage (hay or haylage) can be offered (Hutjens, 2003).

Calf's Health and Stressors

Health is as important as nutrition to ensure proper growth and high survival of the young calf. Considerable knowledge has been gained during the last decade in calf nutrition and its impact on health status. Morbidity and mortality during the pre-weaned period still remain high in some dairy farms in United States (National Animal Health Monitoring System, 2010a). According to (National Animal Health Monitoring System, 2010a) diarrhea accounted for more than 50% of pre-weaned heifer deaths while respiratory problems accounted for 25% during the same period.

Causes of diarrhea in pre-weaned calves can be grouped in three areas: mechanical, nutritional, and environmental causes. Mechanical causes include, for example, overfeeding and drastic changes in liquid feed source. Nutritional causes are related with feeding milk replacers of poor quality or containing poorly digestible ingredients; inclusion of soy flour in milk replacers is a typical example. Mechanical and nutritional causes are not considered a disease problem and will not respond to antibiotic treatments. Environmental causes are associated with presence of pathogens causing enteric disease. Bacteria such as *Escherichia coli*, *Salmonella* sp, and *Clostridium perfringens*; protozoa such as *Coccidia* and *Cryptosporidia*; and rota- and coronavirus are common pathogens causing calf scours (Gulliksen et al., 2009a). Respiratory problems, the second leading cause of death in pre-weaned heifer and principal cause in weaned heifer (National Animal Health Monitoring System, 2010a), are associated with housing, season, and diarrhea incidence (Gulliksen et al., 2009b).

Clearly, enteric disease and respiratory issues are the major health problems associated with morbidity and mortality during the pre-weaned period. Presence of pathogens along with

poor nutrition and management increase stress on calves, resulting in greater incidence of disease problems (Davis and Drackley, 1998). The principal nutritional and management factor associated with high morbidity and mortality in pre-weaned calves is colostrum intake. Colostrum-deprived calves present weak immunity and greater susceptibility to diseases. Higher levels of infectious agents, lower ambient temperature, and higher humidity of inside air might increase susceptibility to disease in winter (Gulliksen et al., 2009a). Although reasons were not determined, research done by Olson et al. (1980b) demonstrated that cold stress decreased rate of absorption of colostral immunoglobulins, compromising immunity of calves born in winter.

Several research reports point out that stressors negatively affect the immune response of pre-weaned calves. As measured by increases in plasma cortisol transportation was found to be the greatest stressor in male Holstein calves (Johnston and Buckland, 1976) , compared to water withdrawal, castration, and dehorning. According to Mackenzie et al. (1997), stressors such as transportation, weaning, and poor housing affected the immune response of pre-weaned calves and the effects persisted for several weeks. Cold, another important stressor in young calves, affected the clinical condition of newborn calves and represented the major contributor of disease and death of young calves in cold climate areas (Olson et al., 1980a). Also, Kelley et al. (1982) reported that chronic heat and cold caused greater mortality and affected immune response by altering both antibody- and cell-mediated immunity in young calves.

Calves may react to stressors either by behavioral or physiological responses, but most often present a combination of both. Physiological reactions to stress have been investigated more than behavioral responses. Physiological changes result in activation of the hypothalamic-pituitary-adrenal axis, resulting in increases in cortisol concentrations, immune suppression, and increased heart and respiration rates (Fike and Spire, 2006). Changes in hormones such as

cortisol, heart and respiration rates, and fluctuations in white blood cell counts have been utilized to quantify stress responses. In the short term, stressors can increase heart rate, increase respiration rate, and decrease feed intake. On the other hand, in the long term stressors can influence essential hormones involved in growth, energy metabolism, immune system, and reproduction (Knowles, 1995).

A series of reactions known as the acute-phase response begins in response to infection or physical trauma. The objective of these reactions is to prevent ongoing tissue damage, isolate and destroy the infective organism, and activate the repair processes necessary to restore normal functions (Baumann and Gauldie, 1994; Ballou et al., 2011). Acute-phase response is characterized by increases in white blood cells, fever, and changes in the plasma concentrations of various acute-phase proteins (Kushner, 1993; Ballou et al., 2011). Acute-phase proteins have been defined as any protein whose plasma concentrations increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) in response to an inflammatory disorder. Positive acute-phase proteins include fibrinogen, serum amyloid A, C-reactive protein, and haptoglobin, while negative acute-phase proteins include albumin, transferrin, insulin, and insulin-like growth factor I (Kushner, 1982).

Extensive research reports that products containing coccidiostats, probiotics, or plasma proteins may improve performance and health of the young calf (Morrill et al., 1995; Quigley et al., 1997; Quigley et al., 2002). According to Nonnecke et al., 2009) pre-ruminant calves provided with adequate nutrition exhibit a remarkable degree of adaptability to sustained moderate cold. However, extreme environmental conditions along with poor management may impair nutrition enough to reduce calf performance. In an effort to overcome negative effects of adverse conditions and poor management, researchers and industry are constantly looking for

possible alternatives. Recently, research in pigs and also in calves (Arthington et al., 2000b; Quigley et al., 2002) suggested that serum protein products may improve health and decrease morbidity and mortality (Quigley and Wolfe, 2003), while maintaining or increasing growth. These results are promising; however, to validate the effectiveness of serum protein products it is necessary to evaluate them under unfavorable conditions.

Animal Protein Products (Serum and Plasma Protein)

Commercial calf milk replacer formulations are widely used in the US dairy industry to provide nutrients to calves prior to weaning (Quigley and Bernard, 1996). According to the National Animal Health Monitoring System (2010b), 57.5% of the dairy operations fed medicated milk replacer while 12.7% fed non-medicated milk replacer. These formulations have traditionally contained ingredients based on milk, including whey, dried skim milk, and lactose. Most protein in milk replacer is provided from these ingredients (Quigley and Bernard, 1996). The value of milk as a human food makes milk proteins the most expensive in milk replacer formulas (Morrill et al., 1995). As the use of milk components in human food increases the availability of milk proteins may decline in the future (Quigley et al., 2000). Alternative sources of protein in milk replacer have attracted intense interest, as they may decrease cost and improve profitability of the heifer rearing program. Many alternative proteins have been evaluated in milk replacer formulations, including soy protein (Dawson et al., 1988; Lalles et al., 1995; Kanjanapruthipong, 1998), wheat protein (Terui et al., 1996), egg protein (Touchette et al., 2003), and fish protein (Huber, 1975; Opstvedt et al., 1978; Jenkins et al., 1982). Many of these

alternative protein sources have reduced calf performance (Huber and Slade, 1967; Colvin and Ramsey, 1968; Seegraber and Morrill, 1986; Quigley et al., 2002; Drackley et al., 2006).

More recently, blood proteins from porcine or bovine origin have shown potential as alternative sources of protein in milk replacer (Morrill et al., 1995). Blood consists of two components: a fluid portion called plasma, and formed elements, which include cells (erythrocytes or red blood cells, and leukocytes or white blood cells) and cell fragments called platelets. Plasma is an aqueous solution in which a great variety of proteins, small nutrients (such as glucose, lipids, and amino acids), metabolic waste products (such as urea and lactic acid), gases (oxygen, carbon dioxide, nitrogen, and others), and electrolytes (such as sodium, potassium, and chloride) are dissolved. Plasma proteins are categorized in three main groups: albumins (the most abundant plasma proteins), globulins (transport lipids, hormones, and other substances in the blood; important and defending the body against foreign substances), and fibrinogen (key substance in the formation of blood clots) (Germann and Stanfiled, 2002). Erythrocytes (red blood cells) are the most abundant cells in the blood, in which the major function is the transport of oxygen and carbon dioxide in blood. Leukocytes (white blood cells) are far less numerous than red blood cells in blood. White cells assist in defending the body against invading microorganisms and other foreign materials (Tortora and Derrickson, 2009).

Blood is collected at slaughter plants from animals determined to be fit for slaughter by veterinary inspection. The blood is transferred to stainless steel tanks and treated with an anticoagulant, then transported to a processing plant. The plasma is then separated from blood cells by centrifugation and chilling to 4 to 5°C. In the manufacturing of blood meal, blood has traditionally been collected and heated to high temperature, which destroys the functional components of the proteins. Conversely, spray-dried plasma proteins are processed to preserve

the functional characteristics of the proteins, including biologically active peptides such as albumin and IgG (Quigley and Wolfe, 2003).

Extensive research has demonstrated that calves fed milk replacer containing animal plasma have performed similar to or better than calves fed proteins derived from milk. Body weight gain and starter consumption were greater in calves fed milk replacer containing 25% of protein from porcine or bovine origin compared to calves fed milk replacer containing all milk protein or all milk protein plus probiotic (Morrill et al., 1995). Plasma proteins fed at 25% of CP in milk replacer also supported intake and rates of BW gain similar to milk replacer containing milk proteins (Quigley and Bernard, 1996). In another experiment Quigley and Wolfe (2003) compared milk replacer formulated with whey protein concentrate as the primary source of protein with milk replacer containing whey protein concentrate plus 5% (of total DM) spray-dried bovine or porcine plasma. It was concluded that dietary plasma either from bovine or porcine origin is a potential source of biologically active proteins, including immunoglobulins, and reduced morbidity and mortality when included in commercial milk replacer.

In pigs, the use of animal plasma has given favorable results. Faster growth and greater feed intake were observed by Pierce et al., (2005) when early weaned pigs were fed spray-dried porcine or bovine plasma. According to Pierce et al. (2005), the IgG fraction of plasma is the component that is responsible for enhanced growth rate and feed intake in early weaned pigs. In an effort to replace dried skim milk or dried whey in diets of starting pigs, Hansen et al. (1993) evaluated spray-dried porcine plasma, spray-dried porcine blood, spray-dried bovine plasma, and spray-dried extracted meat protein. Porcine plasma protein supplementation was superior to skim milk, and positively influenced growth performance of starting pigs (Hansen et al., 1993).

Bovine serum, which is plasma from which the coagulation factor fibrinogen has been removed (Germann and Stanfiled, 2002), also has been used in nutrition of young calves. Dried bovine serum was effectively used as a supplement for marginal or low quality colostrum to improve passive transfer of IgG in newborn calves (Arthington et al., 2000a; Arthington et al., 2000b). Milk replacer containing spray-dried animal plasma with the addition of spray-dried serum was evaluated by (Quigley et al. (2002) in a 56-d trial. Results indicated that calves fed additives containing bovine serum or milk replacer containing spray-dried plasma had lower mortality and improved indices of enteric health (Quigley et al., 2002). No differences in calf performance were reported by Jones et al. (2004) when feeding maternal colostrum and replacer colostrum derived from bovine serum with milk replacer with or without animal plasma. It was concluded by Jones et al. (2004) that colostrum replacement from bovine serum offered equal protection as maternal colostrum and that animal plasma is an acceptable replacement for up to 20% of milk proteins in milk replacer.

Spray-dried red blood cells are a co-product of plasma production (Duarte et al., 1999) and have shown potential as an alternative protein in milk replacer. Quigley et al. (2000) concluded that performance was similar among calves fed milk replacer containing different amounts of CP as spray-dried hydrolyzed red blood cells and indicated that spray-dried hydrolyzed red blood cells can replace up to 43% of CP from whey proteins without detrimental effects on calf performance. In pigs, DeRouchey et al. (2002) compared spray-dried blood meal and blood cells with a control diet without added blood products; they concluded that improved BW gain and feed efficiency were observed in diets containing blood product compared to those fed the control diet without added blood products.

The mechanisms through which dietary plasma improves animal performance are not completely clear. The complex mixture of physiological components in dietary plasma including immunoglobulins (Arthington et al., 2000b), antibodies, growth factors (Hansen et al., 1993), glycoproteins, and modulators of the immune system appears to increase disease resistance (Pettigrew et al., 2006). In addition, plasma proteins are highly digestible and soluble, and possess a reasonable amino acid profile with the exception of very low isoleucine and methionine (Tybor et al., 1975; Penteado et al., 1979; Drackley, 2008). Properties and components of plasma may make it a valuable source of dietary components for young calves especially during periods of stress.

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CHAPTER III

EVALUATION OF SERUM PROTEIN-BASED ARRIVAL FORMULA AND SERUM PROTEIN (GAMMULIN) IN STRESSED (TRANSPORT AND COLD) MALE DAIRY CALVES ON PERFORMANCE, MORBIDITY, AND MORTALITY

INTRODUCTION

The most challenging period for the young calf is from birth until weaning; during this time the calf experiences remarkable physiological, metabolic, and environmental changes (Davis and Drackley, 1998). Despite the advances in calf nutrition and health, higher morbidity and mortality occur during this time (Quigley et al., 2005; National Animal Health Monitoring System, 2010). For the dairy enterprise, decreased morbidity and mortality is important because of the economic losses associated with treating illnesses, decreased calf performance, and death. Factors associated with poor performance are morbidity, poor management during the pre-weaned period, nutrition, and the presence of stressors such as cold weather and transport (Wells et al., 1996; Svensson et al., 2006b; Gulliksen et al., 2009; Vasseur et al., 2010).

Young calves represent future replacements for the dairy enterprise. Successful heifer raising programs result in healthy calves of adequate frame size and with good rumen development. Success in calf rearing is a function of excellent nutrition and management

practices that start as soon as the calf is born. Adequate colostrum intake and provision of a dry, comfortable, and clean environment to minimize exposure to pathogens is the first step to provide better adaptation of the newborn calf to the new environment (Quigley et al., 1995; Godden, 2008). Colostrum-deprived calves exposed to unfavorable conditions after birth exhibit greater risk for high morbidity and mortality (Quigley et al., 2005; Godden, 2008; Gulliksen et al., 2009). Subsequent nutrition is crucially important to provide all the nutrients for proper growth and health. However, adequate nutrition is not enough to ensure proper survival, growth, and health of the young calf in circumstances where the calf is immunologically disadvantaged, facing unfavorable environmental conditions, or subjected to other stressors. Transport (Johnston and Buckland, 1976; Odore et al., 2004) and cold weather represent stressors for the young calf, affecting its immune system (Odore et al., 2004; Fike and Spire, 2006) and increasing the susceptibility to enteric and respiratory problems (Fike and Spire, 2006; Svensson et al., 2006a), which are the principal causes of high morbidity and mortality during the pre-weaned period (National Animal Health Monitoring System, 2010).

In an effort to improve nutrition and diminish the negative effects of poor management and unfavorable conditions, several dietary supplements and additives have been proposed. Extensive research reports that products containing coccidiostats, prebiotics, probiotics, or plasma proteins may improve performance and health of the young calf. Research in pigs and also in calves (Arthington et al., 2000b; Quigley III et al., 2002) suggested that serum protein products improve health and decrease morbidity and mortality (Quigley and Wolfe, 2003), while maintaining or increasing growth. Based on these data, our prediction was that serum protein products would improve performance, morbidity, and mortality of dairy calves subjected to the stressors of winter cold weather and transport, especially during their first 2 wk of life.

MATERIALS AND METHODS

Animal Management

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol number 06132). Ninety-three male Holstein calves, < 1-wk old, were purchased from dairy farms in New York by a buyer in three blocks of 30 to 33 and shipped to the University of Illinois Nutrition Field Laboratory. Calves were transported in a livestock trailer and subjected to a ~14-h trip. The first group was acquired in February 2009 (32 calves), the second group in November 2009 (31 calves), and the third in January 2010 (30 calves).

Each group of calves was evaluated by the veterinary staff upon arrival. The evaluation included heart and lung auscultation, hydration status, alertness, mobility, navel status, and body temperature. Each calf received an ear tag in the left ear for identification purposes. Calves were subjected to the normal arrival and post-arrival processing scheme, which consisted of an intramuscular injection of 1.0 mL of Mu-Se (Shering-Plough Animal Health Corp., Union, NJ), an intranasal vaccination with TVS-2® (Pfizer, Inc., New York, NY), and a prophylactic subcutaneous injection of 1.5 mL of Excede (Pfizer, Inc., New York, NY). Ear notches were taken from each calf, placed in formalin, and analyzed for persistently infected bovine viral diarrhea (PI-BVD) status. Navels were coated with 7% tincture of iodine solution (First Priority, Inc., Elgin, IL) and rectal temperatures were recorded. A blood sample was obtained by jugular venipuncture and plasma protein was determined by refractometry; calves then were weighed.

Calves were stratified by arrival BW and plasma protein, blocked, and randomly assigned within blocks to one of four treatments. Treatments were assigned in a 2 × 2 factorial

arrangement of arrival formula (AF) and Gammulin (G) supplementation as follows: 1) control electrolyte, milk replacer without G (E); 2) AF, milk replacer without G (AF); 3) control electrolyte, milk replacer with G (EG); and 4) AF, milk replacer with G (AFG).

Housing

Calves were housed in individual hutches (Calf-tel, Hampel Corp., Germanton, WI) bedded deeply with straw over crushed rock covered with landscape cloth. Straw was added to each hutch daily. Calves remained in hutches from arrival until d 56 when they were sold.

Feeds and Feeding Program

The AF (APC, Inc., Ankeny, IA) nutritional supplement was administrated via nipple feeder or esophageal feeder upon arrival, and was prepared by mixing 250 g of AF in 2 L of warm (40 °C) water. The ingredient composition of AF is shown in Table 3.3 and contained spray-dried bovine serum in addition to milk products (dried whey and dried whey protein concentrate), minerals, electrolytes, and vitamins. Administration of AF was compared with administration of a commercial electrolyte solution (Land O'Lakes® electrolyte system; Land O'Lakes Animal Milk Products Co., Arden Hills, MN), which was prepared by mixing 77 g in 2 L of warm (45 °C) water.

Commercial non-medicated milk replacer (Sav-A-Caf; Milk Products LLC, Chilton, WI), containing all milk proteins, 20% CP and 20% fat, was fed to all calves. Gammulin (APC, Inc., Ankeny, IA) nutritional supplement was added to reconstituted milk replacer. Ingredients in Gammulin (Table 3.3) included bovine serum, fructooligosacharide (inulin), dried whey, maltodextrin, minerals, and vitamins. Commercial starter (20% MomentaCalf Starter – RUM;

Vita Plus Corporation, Madison, WI) containing 20% CP was provided to all calves. The ingredients are listed in Table 3.2.

At arrival, calves were fed either AF or a control electrolyte solution (E). At the next feeding, all calves received the commercial calf milk replacer. The milk replacer contained either no G supplementation or was supplemented with G. Gammulin was supplemented twice daily by adding 25 g each feeding (50 g/d) to the reconstituted milk replacer. Milk replacers were reconstituted to 12.5% solids and fed at a rate of 10% of arrival BW in two feedings daily. Beginning on d 3 after arrival, milk replacers were fed at a rate of 12% of arrival BW for 2 wk, followed by 10% of arrival BW during wk 3 to 5. The G-supplemented group received 50 g/d of G in the milk replacer during the first 14 d of feeding only. During wk 6, the afternoon milk replacer feeding was eliminated, and calves were weaned at d 42 after arrival. Commercial calf starter was provided daily for ad libitum intake from d 4 until d 56. Calves were fed at 0530 and 1630 h each day; milk replacer, starter, and water intakes were recorded daily. Clean, fresh water was available at all times.

Health

Calf health status was monitored throughout each day and assessed by daily assignment of scour scores and respiratory scores, as well as recording all medical treatments and elevated body temperatures. Fecal scores were recorded using the following guidelines: 1 = firm, well formed (not hard); 2 = soft, pudding-like; 3 = runny, pancake batter; 4 = liquid, splatters. Calves with high fecal scores (fecal score $\geq$ 3) that appeared depressed and dehydrated were treated with electrolytes during the day until fecal score decreased to 2 and behavior returned to normal. Calves with scours (fecal score $\geq$ 3) received an electrolyte solution (Land O'Lakes® electrolyte

system; Land O'Lakes Animal Milk Products Co.) 2 h post-feeding. Severely dehydrated calves received lactated Ringer's solution intravenously and antibiotics as recommended by veterinary staff. Respiratory scores were also recorded once daily using the following guidelines: 1 = normal; 2 = runny nose; 3 = heavy breathing; 4 = cough-moist; 5 = cough-dry.

Body temperatures were recorded daily until d 7, and at any time when a calf was depressed or off-feed. As post-arrival protocol, all calves received 2.0 mL of *Clostridium perfringens* types C & D antitoxin (Boehringer Ingelheim, St. Joseph, MO) and 2.0 mL of *Clostridium perfringens* type A toxoid (Novartis Animal Health Inc., Larchwood, IA) subcutaneously 24 h and 14 d after arrival. At d 3, d 11, and wk 6 after arrival, 2.0 mL of Bovishield® Gold 5 (Pfizer Animal Health, Exton, PA) were administrated intramuscularly. All calves received a second dose of TVS-2 vaccine at d 14 after arrival.

Data Collection

Intakes and Body Growth Measurements. Individual intakes of milk replacer, starter, and water were measured and recorded daily. Calves were measured for BW, withers height, body length, heart girth, hip height, and hip width at arrival and after the morning feeding on the same day each week until wk 8.

Blood Sampling and Analysis. Samples of blood were obtained at arrival (day 0) before the initial feeding and on d 2, 7, 14, and wk 4. Blood samples were collected via jugular venipuncture using 20 G × 2.5-cm needles (Nipro Medical Corporation, Miami, FL), and placed into 10-mL Vacutainer serum tubes (Becton Dickinson; Franklin Lakes, NJ) containing clot activator. Samples were then centrifuged within 1 h of collection at 1300 rpm for 15 min in a centrifuge (Eppendorf model 5804; Brinkmann Instruments; Westbury, NY). After

centrifugation, the serum was removed, placed into 5-mL Falcon tubes (Becton Dickinson; Franklin Lakes, NJ), and stored at -20 °C until analysis.

Serum from d 0, 2, 7, and 14 was analyzed for concentrations of IgG (d 0 only) and acid-soluble protein, haptoglobin, cortisol, zinc, and albumin to determine evidence of stress response. Serum from wk 4 was analyzed for concentrations of urea N and total protein. Concentrations of albumin, urea N, and total protein were determined at the University of Illinois College of Veterinary Medicine clinical pathology laboratory using commercially available kits (Olympus America Inc., Center Valley, PA). Concentration of IgG was determined by radial immonodifusion (RID kit, VRMD Inc., Pullman, WA) by APC, Inc. Acid-soluble protein, haptoglobin, cortisol, and zinc were analyzed at Texas Tech University (courtesy of Dr. M. Ballou). Haptoglobin and zinc were determined as described by Makimura and Suzuki (1982) and Ballou et al. (2011) respectively. Cortisol was quantified using an enzyme immunoassay kit (Arbor Assays, Ann Arbor, MI) http://www.arborassays.com/products/inserts/K003-H5.pdf. Acid-soluble protein was determined using perchloric acid to precipitate most of the protein. The remaining protein concentration was measured; most of the acid soluble protein was alpha-1 acid glycoprotein.

Sampling and Analysis of Milk Replacers, Starter Grain, Arrival Formula, and Gammulin. For each replicate, milk replacer and starter grain were sampled weekly from wk 1 to 8, while G was sampled during the supplementation period (wk 1 and 2). The AF was only offered and sampled on the arrival day for each replicate of calves. All samples were stored at -20 °C and then composited by period and replicate. Composited samples were sent to a commercial laboratory (Dairy One Corporative Inc., Ithaca, NY), where they were analyzed for

concentrations of dry matter (DM), crude protein (CP), fat, and minerals by wet chemistry methods.

Statistical Analysis

Daily and weekly statistical analysis was performed using the GLIMMIX, MIXED, LOGISTIC and LIFETEST procedures of SAS (SAS v9.2 Institute Inc., Cary, NC). Growth, feed intakes, and health data were analyzed in in two periods: the first 2 wk (G supplementation period) and wk 1 through wk 8 (entire research period). A linear mixed model (MIXED procedure) was constructed to analyze growth, feed intakes, and blood metabolites data. The model contained the fixed effects of AF and G, the interaction of AF and G, time (day or week), and interactions of time with AF and G. Replicate and calf were considered random effects; calf was nested within replicate, AF, and G. The covariance structures considered for repeated measures analysis were compound symmetric, autoregressive order one, and unstructured. The covariance structure autoregressive order one yielded the lowest AICC value and was used in the models (Littell et al., 1998). Initial measurements of BW, withers height, body length, heart girth, hip height, hip width, and concentrations of plasma albumin, acid soluble protein, cortisol, haptoglobin, and zinc at d 0 were used as covariates when analyzing the respective data. Least squares means were calculated and are presented with standard errors of means (SEM). Degrees of freedom were estimated by using the Kenward-Roger method in the model statement. Residual distribution was evaluated for normality and homoscedasticity.

Daily health data was analyzed using a multivariable logistic mixed models (GLIMMIX procedure) considering the count outcome variables number of calves having high fecal score (fecal score ≥ 3 in a 1 to 4 scale), number of days with high fecal score, percentage of calves

with high fecal score, number of calves with high respiratory score (respiratory score ≥ 3 in a 1 to 5 scale), number of days with high respiratory score, and percentage of calves with high respiratory score. The model contained the fixed effects of AF and G, the interaction of AF and G, time (week), and interactions of time with AF and G. When the variance and mean of fecal and respiratory scores were evaluated, the data were found to be over-dispersed. To compensate for this, a negative binomial regression analysis was used to establish relationships of the fixed effects AF and G, and the interaction of AF and G. The coefficients in the log scale from negative binomial regression analysis are presented, as well as the odds ratios.

Calf survival analysis was assessed using a Cox proportional hazard model (LIFETEST procedure). The fixed effects of AF, G, and the interaction of AF and G were treated as strata and forced in to the model. The assumption of the proportionality of hazard of the model was assessed graphically by plotting the logarithm of the hazard function versus the logarithm of time. Residuals were evaluated for homogeneous distribution. Finally, a logistic regression model (LOGISTIC procedure) considering the binary outcome variable MORTALITY was constructed. The odds ratio from main effects and treatments is described.

In all statistical procedures significant differences were declared when $P \leq 0.05$, and trends toward significant effects were noted when $0.06 \leq P \leq 0.10$.

RESULTS

Temperature

Mean low and high ambient temperatures ranged from −5.2 to 16.2, −17.1 to 11.4, and −10.8 to 12.4 °C for replicates 1, 2, and 3, respectively. Ambient temperature was below the

lower critical temperature (Figure 3.1) most of the time throughout the study, indicating that calves would need to expend energy for thermoregulation (National Research Council, 2001).

Nutrient Composition of Diets

Nutrient composition of milk replacer and starter are listed in Table 3.1 and Table 3.2, respectively. Commercial milk replacer was declared to contain 20% CP on an as-fed basis and the actual analyzed CP (DM basis) was 22.0, 21.3, and 21.7% for replicates 1, 2, and 3, respectively. Similarly, commercial starter declared to contain 20% CP on an as-fed basis had measured CP content (DM basis) of 22.9, 23.3, and 24.6% for replicates 1, 2, and 3, respectively. Chemical analysis of the serum protein-based products, AF and G, indicated that CP content on a DM basis was 47.5% and 75.3%, respectively (Table 3.3). Although nutrient composition of milk replacer and starter were similar, G supplementation allowed greater CP intake in supplemented calves.

Intakes

Calves were fed a limited amount of milk replacer (10 to 12% of arrival BW) as described previously. Calves supplemented with G had greater ($P < 0.0001$) intake of milk replacer DM during the supplementation period because of the addition of G. Greater intake of milk replacer DM and supplementation with G resulted in higher ($P < 0.0001$) intake of milk replacer CP in G-supplemented calves (Table 3.4). As part of limited liquid feeding, calf starter was offered ad libitum from d 4 to help meet nutrient requirements and support growth. Although the amount of starter intake during the first 2 wk was not large, calves fed AF had greater ($P = 0.0007$) intake of DM and CP from starter. As a result of greater intake of DM and CP from milk replacer and starter, calves fed AF ($P = 0.04$) and those supplemented with G ($P <$

0.0002) had greater intakes of total DM and total CP at 2 wk (Table 3.4). Greater starter intake in AF-fed calves led to greater starter ME intake ($P = 0.0007$) and greater ($P = 0.04$) total ME intake (Table 3.4). Trends toward significance were observed in the interaction AF × G for intakes of milk replacer DM ($P = 0.08$), CP ($P = 0.07$), and ME ($P = 0.08$). As discussed previously, supplementation of the high-CP G supplement resulted in calves fed AFG or EG having superior milk replacer DM ($P \leq 0.002$) and CP ($P < 0.0001$) intakes compared with calves fed AF or E. Additionally, calves fed the EG treatment had greater ($P = 0.04$) intake of ME from milk replacer than calves receiving the E treatment (Table 3.4).

Water intake was analyzed in different components: free water, water in milk replacer, water in feed, and total water intake. Water freely available at all times was considered as free water. Water in milk replacer was the water used to reconstitute the milk replacer. Water in feed was the water contained in the as-fed starter and milk replacer powder. Total water was the sum of all sources of water. There was no difference in intakes of free water, water in milk replacer, or total water; however, there was statistical difference in water in feed for calves fed AF ($P = 0.008$) or G ($P = 0.02$) (Table 3.4). These differences were small and likely were not biologically significant.

Through 8 wk, intakes were not affected by the main effects of AF or G except for milk replacer DM and CP intake (Table 3.5). Greater mean intake ($P < 0.0001$) of milk replacer DM and CP at 8 wk was the result of increased intake of milk replacer DM and CP at 2 wk (Table 3.5) as designed. At 8 wk, significant interactions of G × week were observed for milk replacer DM ($P < 0.0001$), milk replacer CP ($P < 0.0001$), and total CP intake ($P = 0.002$). Milk replacer DM and CP intake increased from wk 1 to wk 2 and were greater for G-supplemented calves as designed ($P < 0.0001$). After the second week, intakes of G-supplemented calves decreased and

became similar to intakes of non-G-supplemented calves because of the removal of G from the diet (Figure 3.2, Panels A and B, respectively). Total CP intake was higher ($P = 0.06$) at 2 wk for calves supplemented with G; from the second week total CP intake was similar for supplemented and non-supplemented calves (Figure 3.2, Panel C).

Growth

Initial calf BW and body conformation measures did not differ among treatment groups (Table 3.6). Differences at 2 wk were not evident; neither AF nor G had a great effect in most growth parameters at this time. For instance, withers height and hip height was not affected by G supplementation. However, calves fed AF had greater ($P = 0.05$) body length and tended to have greater ($P = 0.07$) heart girth; whereas, calves supplemented with G tended to have greater ($P = 0.06$) BW (Table 3.6).

Through 2 wk, the G × week interaction was significant for average daily gain (ADG, $P = 0.03$) and feed efficiency (gain: feed, $P = 0.05$) (Table 3.6). The G-supplemented calves had greater ADG ($P = 0.01$) and tended to have superior ($P = 0.06$) feed efficiency in the first week (Figure 3.3). At wk 2, differences were not significant between G-supplemented and non-G-supplemented calves (Figure 3.3). The interaction AF × G × week was significant for body length ($P = 0.02$), heart girth ($P = 0.01$), and hip width ($P = 0.01$) (Table 3.6). In the first week, calves fed AFG treatment had longer ($P = 0.02$) body length (Figure 3.4, Panel A) and tended to have greater ($P = 0.08$) heart girth (Figure 3.4, Panel B) than EG-fed calves. In the second week, AF-fed calves had greater heart girth ($P = 0.02$; Figure 3.3, Panel B) and hip width ($P = 0.01$; Figure 3.3, Panel C), and tended to have bigger hip width ($P = 0.06$, Figure 3.3, Panel C) than calves on the other treatments.

At 8 wk, BW and body conformation parameters, except for hip width, were similar for all groups of calves. Calves fed AF exhibited greater ($P = 0.04$) hip width (Table 3.7) but differences were numerically small. The interaction AF × G was significant for hip height. Calves fed AFG or E treatments had greater ($P = 0.05$) hip height than EG-fed calves (Table 3.7).

The G × week interaction was significant for ADG ($P = 0.02$), gain: feed ($P < 0.0001$), and tended to be significant ($P = 0.06$) for withers height. Through the entire experiment, differences in ADG were not evident, except in the first week where G- supplemented calves had superior ($P = 0.03$) ADG compared to non-supplemented calves (Figure 3.5, Panel A). Feed efficiency was greater ($P = 0.0006$) for G-supplemented calves in the first week only, then became lower ($P \leq 0.02$) until wk 4 for these same calves. After wk 5, differences in feed efficiency among G-supplemented and non-G-supplemented calves were not detected (Figure 3.5, Panel B). Finally, withers height was greater ($P = 0.02$) for calves supplemented with G at wk 8 only (Figure 3.5, Panel C).

The interaction AF × G × week tended ($P = 0.08$) to be significant for body length (Table 3.7). Differences were not significant among treatments through the 8 wk of the study except for calves fed the EG treatment, which exhibited smaller ($P < 0.05$) body length from wk 5 (Figure 3.6).

Health

Supplementation with G influenced health during the supplementation period. Supplemented calves had lower ($P = 0.01$) mean respiratory score (Table 3.8), less odds for days (OR = 0.33, $P = 0.008$) with high respiratory score, and lower odds for both number (OR = 0.32,

$P = 0.0008$) and percentage (OR = 0.30, $P = 0.001$) of calves with high respiratory score (Table 3.10). Calves fed treatment AFG also presented smaller (OR = 0.18, $P = 0.008$) odds for percentage of calves with high respiratory score (Table 3.10). The interaction of the main effect AF and week was significant ($P = 0.01$) for fecal score (Table 3.8); calves fed AF had decreased ($P = 0.04$) mean fecal score during the first 2 wk (Figure 3.7).

Over the entire 8 wk, G supplementation also influenced health status. Thus, G-supplemented calves had lower ($P = 0.02$) mean fecal scores (Table 3.9). Although there was no difference in electrolyte treatments, calves supplemented with G received fewer ($P = 0.05$) antibiotic treatments than calves without G supplementation (Table 3.11). Throughout the study, mortality occurred during the first 3 wk and was higher ($P = 0.02$) for non-G-supplemented calves (Figure 3.8). Accordingly, G-supplemented calves had lower mortality odds (OR = 0.12, $P = 0.04$) during the 56 d of the experiment (Table 3.10). Analysis of plasma IgG revealed that concentration of IgG was significantly lower ($P = 0.008$) in calves that died compared to those that survived (Figure 3.9).

Blood Metabolites

Total plasma protein measured by refractometry upon arrival, to stratify and block the calves; total plasma protein analyzed by cobas, and plasma IgG determined by radial immonodifusion several weeks after arrival were not different among treatments (Figure 3.10). Although blood metabolite concentrations were statistically different ($P < 0.05$) in time, the main effects and interactions were not significant for plasma concentrations of albumin, haptoglobin, zinc, and ASP (Table 3.12). Plasma cortisol, on the other hand, was affected by the main effect of AF. Calves fed AF exhibited higher ($P = 0.02$) concentrations of cortisol in plasma than E-fed

calves. Interactions of main effects and time also were not significant for plasma cortisol. Likewise, the main effect of G was significant for urea N and total protein. As a result, calves supplemented with G had greater concentrations of total protein ($P = 0.04$) and urea N ($P = 0.01$) in plasma at wk 4 (Table 3.12).

DISCUSSION

Serum protein products had only small effects on growth and intakes in this study, but health status, morbidity, and mortality were significantly improved. Fecal score, incidence of scours, and respiratory problems decreased during the supplementation period. Improved health in serum protein-supplemented calves led to less antibiotic use, decreased morbidity, and less mortality. Arthington et al. (2000a) reported fewer treatments for illness when calves were fed bovine serum as an IgG source at birth and 12 h later. Lower mortality and improved indices of enteric health (improved fecal scores, fewer days with diarrhea, and lower use of electrolyte) were reported by Quigley et al. (2002) when additives containing bovine serum or milk replacer containing spray-dried plasma were fed to calves. Similarly, Arthington et al. (2002) reported improvement in average respiratory rate of calves infected with coronavirus when bovine serum was supplemented. Reduced calf morbidity and mortality also were reported by Quigley and Wolfe (2003) with inclusion of spray-dried bovine or porcine plasma in milk replacer.

Serum protein-based AF contained spray-dried bovine serum, minerals, and vitamins. Gammulin contains the same components as AF plus fructooligosaccharides (Quigley et al., 2002). Bovine serum is a source of immunoglobulins that might provide intestinal protection against enteric pathogens (Arthington et al., 2000a; Arthington et al., 2002; Quigley et al., 2002; Quigley and Wolfe, 2003). Fructooligosaccharides may increase the growth and population of

beneficial intestinal microorganisms, improving intestinal health and decreasing incidence of diseases (Grizard and Barthomeuf, 1999; Menne et al., 2000).

Improvements in health for calves supplemented with G might be related to greater nutrient intake in those calves. Supplementation with G led to greater intake of milk replacer DM and CP during the first 2 wk. Supplemental G resulted in G × week interactions for ADG during the first 2 wk, showing that growth was improved. These results agree with Quigley et al. (2002) and Quigley and Wolfe (2003) who reported, even though minimal, an increase in milk replacer intake when spray-dried serum or plasma (either from bovine or porcine origin) were fed to calves. In other species similar results were described; Pierce et al. (2005) reported enhanced feed intakes when spray-dried plasma was fed to early weaned pigs. Supplementation with serum protein and greater intakes of DM and CP during the first 2 wk improved BW, ADG, and feed efficiency during early serum protein supplementation. In a 56-d experiment, Quigley et al. (2002) found a 12.9% increase in BW gain from d 29 to 56 when serum protein was supplemented to calves. Morrill et al. (1995) also reported greater BW gain when calves were fed milk replacer containing plasma protein of bovine or porcine origin. However, Quigley et al. (2000) did not see a change in calf performance when spray-dried red blood cells were tested in milk replacer.

Although all measures were not significant, the single dose of AF given at arrival resulted in weak tendencies for greater growth during wk 1 to 2, as shown by small increases in final BW ($P = 0.17$), heart girth ($P = 0.07$), body length ($P = 0.05$), hip height ($P = 0.19$), hip width ($P = 0.13$), and ADG ($P = 0.15$). These tendencies may be related to the stimulation of starter intake during the first 2 wk. Greater early starter intake in stressed calves would be a benefit in terms

of growth and resistance to disease. The improved early growth is in agreement with Jones et al. (2004) who fed a colostrum replacement derived from bovine serum at 1.5 and 13.5 h of age.

Although concentrations of albumin, haptoglobin, zinc, and ASP were statistically different by day they remained within the considered normal ranges. Plasma cortisol concentrations in AF-fed calves were greater than those in calves fed E, but concentrations were still very much lower than values of 38.0 ng/mL and 79.0 ng/mL reported by Khan et al. (1970) and Willett and Erb (1972), respectively. Thus, these increases in our study were small and likely cannot be considered biologically elevated. Increases may be related to the greater protein intake during the first electrolyte feeding.

Increased concentrations of urea N and total protein in plasma at wk 4 may have been the result of greater intake of CP during G supplementation. Tendency toward greater plasma protein status was observed by Quigley et al. (2002) and Jones et al. (2004) when feeding milk replacer containing spray-dried bovine plasma. However, it must be noted that the wk 4 samples were taken 2 wk after G supplementation was discontinued, indicating that the improved protein status was in some way a residual effect of early G supplementation.

CONCLUSIONS

Feeding a serum protein product to pre-weaned dairy calves that were exposed to stressors of transport and cold weather resulted in little improvement of early feed intakes and growth but significantly decrease morbidity and mortality. The serum protein-based AF promoted starter intake, growth, and improved fecal score during the first 2 wk. Similarly, G supplementation stimulated early milk replacer intake and growth, decreased incidence of respiratory problems at 2 wk, and significantly decreased mortality. Indicators of acute-phase

response were not affected by AF or G; however, protein status of calves assessed at 4 wk after arrival may have been improved by G supplementation during the first 2 wk.

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Table 3.1. Analyzed chemical composition of milk replacer by group (replicate) of calves[1].

Item	Group of Calves			Mean
	1	2	3	
DM, %	94.3	93.0	94.1	93.8
CP, % of DM	22.0	21.3	21.7	21.7
Crude fat, % of DM	18.7	18.8	19.1	18.9
Ash, % of DM	10.1	9.8	10.2	10.0
TDN, % of DM	105	105	105	105
NE_L, Mcal/kg	2.71	2.72	2.72	2.72
NE_M, Mcal/kg	2.66	2.67	2.67	2.67
NE_G, Mcal/kg	1.90	1.90	1.90	1.90
Ca, % of DM	0.74	0.74	0.74	0.74
P, % of DM	0.72	0.70	0.75	0.72
Mg, % of DM	0.14	0.14	0.14	0.14
K, % of DM	2.30	2.19	2.32	2.27
Na, % of DM	0.99	0.97	1.00	0.99
S, % of DM	0.31	0.32	0.32	0.32
Fe, ppm	59	115	133	102
Zn, ppm	52	59	60	57
Cu, ppm	9	10	3	7
Mn, ppm	37	34	42	38
Mo, ppm	0.7	0.8	1.0	0.8

[1] Manufacturer's indicated list of ingredients: Dried whey, dried whey protein concentrate, animal fat (preserved with BHA, BHT, citric acid, and ethoxyquin), dried whey product, dried skimmed milk, calcium carbonate, L-lysine, sodium silico aluminate, DL-methionine, ferrous sulfate, magnesium sulfate, choline chloride, artificial flavor, vitamin E supplement, maltodextrin, selenium yeast, brewer's dried yeast, vitamin A supplement, zinc sulfate, lecithin, ethoxylated mono-diglycerides, propylene glycol, manganese sulfate, copper sulfate, ascorbic acid, niacin supplement, vitamin D3 supplement, calcium pantothenate, menadione sodium bisulfite complex, biotin, riboflavin supplement, thiamine mononitrate, pyridoxine hydrochloride, vitamin B12 supplement, folic acid, cobalt sulfate.

Table 3.2. Analyzed chemical composition of starter grain by group (replicate) of calves[1].

	Group of Calves			
Item	1	2	3	Mean
DM, %	87.5	88.0	88.2	87.9
CP, % of DM	22.9	23.3	24.6	23.6
ADF, % of DM	6.0	6.2	8.0	6.7
NDF, % of DM	12.1	13.9	14.0	13.3
TDN, % of DM	83	83	83	83
NE_L, Mcal/kg	2.06	2.04	2.05	2.05
NE_M, Mcal/kg	2.15	2.13	2.14	2.14
NE_G, Mcal/kg	1.47	1.46	1.47	1.47
Ca, % of DM	1.23	1.15	1.20	1.19
P, % of DM	0.60	0.61	0.64	0.62
Mg, % of DM	0.27	0.29	0.30	0.29
K, % of DM	1.14	1.20	1.22	1.19
Na, % of DM	0.25	0.23	0.31	0.26
Fe, ppm	293	301	322	305
Zn, ppm	147	135	125	136
Cu, ppm	25	22	23	23
Mn, ppm	105	97	119	107
Mo, ppm	1.5	1.3	1.3	1.4

[1] Manufacturer's indicated list of ingredients: Steam flaked corn, soybean meal, oats, cane molasses, fat product feed grade, heat processed soybeans, canola meal, wheat middlings, corn destillers dried grains, calcium carbonate, monocalcium phosphate, dicalcium phosphate, salt, dried whey solubles, magnesium sulfate, potassium sulfate, propionic acid, ammonium hydroxide, acetic acid, benzoic acid, sorbic acid, tartaric acid, sodium sesquicarbonate, zinc sulfate, manganese sulfate, copper sulfate, calcium iodate, cobalt carbonate, vitamin E supplement, selenium yeast, brewer's dried yeast, artificial flavor, dextrose, saccharine sodium, yeast culture, choline chloride, vitamin A supplement, vitamin D3 supplement, ferrous sulfate, niacin supplement, calcium pantothenate, vitamin B12 supplement, riboflavin, supplement, thiamine mononitrate, menadione sodium bisulfite complex, pyridoxine hydrochloride.

Table 3.3. Analyzed chemical composition of Gammulin[1] and Arrival Formula[2].

Item	Gammulin	Arrival Formula
DM, %	93.5	90.6
CP, % of DM	75.3	47.5
Crude fat, % of DM	1.6	4.5
Ash, % of DM	6.1	12.3
Ca, % of DM	0.87	0.81
P, % of DM	0.26	0.17
Mg, % of DM	0.07	0.10
K, % of DM	0.18	0.85
Na, % of DM	1.11	2.32
S, % of DM	1.11	0.67
Fe, ppm	131	121
Zn, ppm	358	121
Cu, ppm	89	5
Mn, ppm	160	57
Mo, ppm	0.8	0.6

[1] Manufacturer's indicated list of ingredients: Bovine serum, inulin, dried whey, maltodextrin, sorbitol, calcium carbonate, DL-methionine, magnesium sulfate, lecithin, mineral oil, silicon dioxide, choline chloride, polyoxyethylene glycol (400) mono and dioleates, dried whey protein concentrate, dicalcium phosphate, natural and artificial flavors, zinc sulfate, vitamin E supplement, manganese sulfate, vitamin A acetate, copper sulfate, ascorbic acid, calcium iodate, vitamin D3 supplement, vitamin B12 supplement, sodium selenite, niacinamide, calcium pantothenate, biotin, menadione sodium bisulfite complex, sodium bicarbonate, folic acid, riboflavin, pyridoxine hydrochloride, cobalt sulfate, thiamine mononitrate.

[2] Manufacturer's indicated list of ingredients: Spray dried bovine serum, dextrose, animal and vegetable fat (preserved with BHA and BHT), sodium citrate, glycine, dried whey, salt, potassium chloride, dried whey protein concentrate, calcium carbonate, sodium acetate, calcium lactate, fructose, lecithin, magnesium sulfate, choline chloride, natural and artificial flavors, vitamin E supplement, silicon dioxide, mineral oil, emulsifier, zinc proteinate, dicalcium phosphate, ferrous sulfate, manganese proteinate, vitamin A acetate, selenium yeast, brewer's dried yeast, iron proteinate, ascorbic acid, calcium pantothenate, vitamin B12 supplement, vitamin D3 supplement, niacinamide, riboflavin, biotin, pyridoxine hydrochloride, thiamine mononitrate, folic acid, calcium iodate, menadione sodium bisulfite complex, cobalt sulfate.

Table 3.4. Mean daily intakes of dry matter (DM), crude protein (CP), and metabolizable energy (ME) from milk replacer and starter, and water intake from wk 1 to wk 2 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					***P***					
Variable	**E**	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**AF*wk**	**G*wk**	**AF*G*wk**
Total DM, g/d	555.6	599.3	624.3	638.8	19.7	0.03	0.0002	0.28	0.24	0.91	0.41
Milk replacer DM, g/d	522.6	540.6	594.3	579.8	20.2	0.85	<.0001	0.08	0.32	0.62	0.16
Starter DM, g/d	34.2	58.3	30.1	58.7	7.4	0.0007	0.80	0.76	0.41	0.55	0.94
Total CP, g/d	121.1	131.1	160.9	163.8	5.2	0.04	<.0001	0.27	0.26	0.71	0.42
Milk replacer CP, g/d	113.3	117.2	153.8	150.1	5.4	0.97	<.0001	0.07	0.37	0.20	0.18
Starter CP, g/d	8.05	13.80	7.08	13.72	1.74	0.0007	0.76	0.79	0.42	0.54	0.95
Total ME, Mcal/d	2.31	2.48	2.41	2.45	0.08	0.05	0.49	0.24	0.22	0.76	0.35
Milk replacer ME, Mcal/d	2.20	2.28	2.31	2.25	0.08	0.79	0.29	0.08	0.30	0.90	0.15
Starter ME, Mcal/d	0.11	0.19	0.10	0.20	0.02	0.0007	0.80	0.76	0.41	0.55	0.94
Total water intake, kg/d	4.56	4.81	4.73	4.60	0.22	0.63	0.88	0.12	0.46	0.77	0.61
Free water, kg/d	0.60	0.99	0.60	0.56	0.29	0.29	0.20	0.21	0.27	0.48	0.84
Water in milk replacer, kg/d	3.90	4.03	4.09	3.99	0.13	0.79	0.29	0.08	0.30	0.90	0.15
Water in feed[1], kg/d	0.04	0.04	0.04	0.05	0.002	0.008	0.02	0.60	0.26	0.95	0.76

[1] Water in starter and milk replacer powder.

Table 3.5. Mean daily intakes of dry matter (DM), crude protein (CP), and metabolizable energy (ME) from milk replacer and starter, and water intake from wk 1 to wk 8 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

Variable	Treatment					*P*					
	E	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**AF*wk**	**G*wk**	**AF*G*wk**
Total DM, g/d	1251.0	1255.1	1203.6	1265.1	40.4	0.41	0.64	0.47	0.64	0.59	0.33
Milk replacer DM, g/d	480.7	478.9	504.0	500.0	19.3	0.23	<.0001	0.64	0.94	<.0001	0.16
Starter DM, g/d	891.9	895.8	824.9	889.4	43.7	0.38	0.34	0.43	0.67	0.87	0.36
Total CP, g/d	287.8	289.3	285.8	300.1	9.1	0.38	0.63	0.48	0.65	0.002	0.34
Milk replacer CP, g/d	104.2	103.8	121.9	120.8	5.1	0.22	<.0001	0.55	0.92	<.0001	0.17
Starter CP, g/d	210.8	211.1	194.3	209.3	9.4	0.35	0.18	0.47	0.67	0.85	0.38
Total ME, Mcal/d	4.55	4.57	4.34	4.54	0.13	0.40	0.37	0.48	0.65	0.86	0.33
Milk replacer ME, Mcal/d	2.02	2.02	2.02	2.01	0.08	0.23	0.57	0.68	0.95	0.79	0.16
Starter ME, Mcal/d	3.04	3.05	2.81	3.03	0.14	0.38	0.34	0.43	0.67	0.87	0.37
Total water intake, kg/d	4.88	5.08	4.67	4.83	0.18	0.20	0.10	0.89	0.70	0.68	0.75
Free water, kg/d	2.06	2.25	1.85	2.02	0.13	0.17	0.10	0.93	0.71	0.76	0.93
Water in milk replacer, kg/d	3.59	3.57	3.58	3.56	0.13	0.22	0.56	0.68	0.95	0.79	0.16
Water in feed[1], kg/d	0.15	0.15	0.14	0.15	0.01	0.39	0.26	0.45	0.74	0.08	0.88

[1] Water in starter and milk replacer powder.

Table 3.6. Initial and final body weight (BW), final body conformation measurements, average daily gain (ADG), and feed efficiency from wk 1 to wk 2 for calves electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					***P***					
Variable	**E**	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**AF*wk**	**G*wk**	**AF*G*wk**
Initial BW, kg	42.7	42.7	42.6	42.6	0.40	--	--	--	--	--	--
Final BW, kg	45.6	46.5	46.4	47.0	0.70	0.17	0.06	0.96	0.39	0.52	0.35
Heart girth, cm	81.2	82.2	81.5	81.7	0.59	0.07	0.72	0.94	0.52	0.67	0.01
Body length, cm	66.6	67.0	66.5	66.8	0.37	0.05	0.82	0.17	0.48	0.66	0.02
Withers height, cm	79.9	79.9	79.8	79.9	0.20	0.65	0.38	0.49	0.76	0.24	0.47
Hip height, cm	84.0	84.1	84.0	84.4	0.22	0.19	0.97	0.40	0.96	0.19	0.76
Hip width, cm	17.6	17.9	17.6	17.7	0.11	0.13	0.85	0.72	0.19	0.20	0.01
ADG, kg/d	0.20	0.27	0.26	0.30	0.05	0.15	0.24	0.72	0.98	0.03	0.29
G:F	0.36	0.45	0.43	0.45	0.08	0.32	0.54	0.61	0.83	0.05	0.30

Table 3.7. Initial and final body weight (BW), final body conformation measurements, average daily gain (ADG), and feed efficiency from wk 1 to wk 8 for calves electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					***P***					
Variable	**E**	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**AF*wk**	**G*wk**	**AF*G*wk**
Initial BW, kg	42.7	42.6	42.6	42.6	1.2	--	--	--	--	--	--
Final BW, kg	82.2	81.0	79.0	81.0	1.2	0.52	0.50	0.55	0.78	0.13	0.77
Heart girth, cm	100.4	100.0	99.4	99.6	0.57	0.29	0.26	0.85	0.82	0.87	0.19
Body length, cm	78.8	78.2	77.6	78.7	0.66	0.20	0.26	0.12	0.78	0.93	0.08
Withers height, cm	89.2	88.5	88.0	88.2	0.35	0.85	0.35	0.19	0.57	0.06	0.52
Hip height, cm	93.1	92.2	91.6	92.6	0.32	0.69	0.55	0.03	0.65	0.32	0.25
Hip width, cm	21.9	22.0	21.4	21.9	0.16	0.04	0.30	0.94	0.43	0.18	0.14
ADG, kg/d	0.71	0.69	0.65	0.69	0.03	0.78	0.36	0.37	0.62	0.02	0.85
Gain:feed	0.56	0.55	0.53	0.53	0.02	0.82	0.21	0.83	0.60	<.0001	0.96

Table 3.8. Mean daily fecal score (FS) and respiratory score (RS), coefficients for days with high FS and RS, coefficients for number of calves with high FS and RS, and coefficients for percentage of calves with high FS[1] and RS[2] from wk 1 to wk 2 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

Variable	Treatment					*P*					
	E	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**AF*wk**	**G*wk**	**AF*G*wk**
Days in trial	14	14	14	14	--	--	--	--	--	--	--
Calves per treatment	25	24	22	22	--	--	--	--	--	--	--
Fecal score											
Mean fecal score	2.7	2.7	2.6	2.6	0.14	0.72	0.25	0.82	0.01	0.14	0.65
Days with high FS*	1.8	1.8	1.7	1.8	0.11	0.78	0.61	0.65	--	--	--
High FS, no. of calves*	2.4	2.3	2.2	2.3	0.09	0.75	0.25	0.28	0.05	0.27	0.94
Calves with high FS*, %	3.8	3.8	3.7	3.8	0.11	0.87	0.59	0.75	0.11	0.21	0.68
Respiratory score											
Mean respiratory score	1.1	1.2	1.1	1.0	0.06	0.63	0.01	0.47	0.44	0.87	0.92
Days with high RS*	-0.2	0.0	-0.7	-1.7	0.40	0.31	0.008	0.15	--	--	--
High RS, no. of calves*	0.3	0.6	-0.3	-1.1	0.30	0.43	0.0008	0.10	0.26	0.84	0.89
Calves with high RS*, %	1.8	2.0	1.3	0.1	0.34	0.17	0.001	0.05	0.19	0.64	0.54

[1] High FS: calves with fecal score ≥ 3 (1-4 scale).

[2] High RS: calves with respiratory score ≥ 3 (1-5 scale).

* Coefficients in the log scale from negative binomial regression analysis.

Table 3.9. Mean daily fecal score (FS) and respiratory score (RS), coefficients for days with high FS and RS, coefficients for number of calves with high FS and RS, and coefficients for percentage of calves with high FS[1] and RS[2] from wk 1 through 8 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					*P*					
Variable	E	AF	EG	AFG	SEM	AF	G	AF*G	AF*wk	G*wk	AF*G*wk
Days in trial	56	56	56	56	--	--	--	--	--	--	--
Calves per treatment	25	24	22	22	--	--	--	--	--	--	--
Fecal score											
Mean fecal score	1.7	1.7	1.6	1.6	0.09	0.92	0.02	0.80	0.20	0.66	0.16
Days with high FS*	2.2	2.1	1.9	1.9	0.12	0.86	0.16	0.55	--	--	--
High FS*, no. of calves	0.7	0.5	-1.5	0.1	0.16	0.97	0.96	0.97	0.43	0.11	0.44
Calves with high FS*, %	2.2	2.1	-0.1	1.7	0.13	0.97	0.95	0.96	0.52	0.01	0.18
Respiratory score											
Mean respiratory score	1.0	1.0	1.0	1.0	0.04	0.64	0.96	0.62	0.68	0.16	0.78
Days with high RS*	-0.1	0.0	-0.7	0.0	0.44	0.34	0.51	0.45	--	--	--
High RS, no. of calves*	-1.4	-1.5	-1.6	-1.1	0.30	0.48	0.91	0.33	0.81	0.29	0.75
Calves with high RS*, %	-7.0	-10.6	-10.8	-1.5	148.4	0.98	0.98	0.96	0.96	1.00	0.99

[1] High FS: calves with fecal score ≥ 3 (1-4 scale).

[2] High RS: calves with respiratory score ≥ 3 (1-5 scale).

* Coefficients in the log scale from negative binomial regression analysis.

Table 3.10. Odds ratio of high respiratory score (RS[1]) from wk 1 to wk 2 and mortality from wk 1 through 8 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

Variable	**Main Effect**	**Treatment**	**Level**	**Odds Ratio**	**95% CI[2]**	***P***
High RS, no. of calves	Gammulin	--	G/NG	0.32	0.17 – 0.60	0.0008
Calves with high RS, %	Gammulin	--	G/NG	0.30	0.15 – 0.59	0.001
	--	EG	EG/E	1.24	0.50 – 3.07	0.63
	--	AF	AF/E	0.59	0.23 – 1.50	0.26
	--	AFG	AFG/E	0.18	0.06 – 0.52	0.001
Days with high RS	Gammulin		G/NG	0.33	0.14 – 0.75	0.008
Mortality	Gammulin	--	G/NG	0.12	0.01 – 1.00	0.04
	Arrival formula	--	AF/E	0.80	0.20 – 3.19	0.07
	--	AF	AF/E	1.05	0.23 – 0.48	0.93
	--	EG	EG/E	0.25	0.03 – 2.43	0.96
	--	AFG	AFG/E	0.00	0.00 – 999	0.94

[1] High RS: calves with respiratory score ≥ 3 (1-5 scale).

[2] CI: confidence interval derived from a negative binomial regression.

Table 3.11. Mortality, serum IgG, plasma protein, electrolyte[3] and antibiotic[4] treatment from wk 1 through 8 for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					***P***		
Variable	**E**	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**
Calves per treatment	25	24	22	22	--	--	--	--
Calves died per treatment	4	4	1	0	--	--	--	--
Mortality, %	16	16	5	0	--	--	--	--
Mean age at mortality, d	16	7	8	--	--	--	--	--
IgG[1], g/dL	9.8	14.2	13.8	13.5	2.1	0.23	0.31	0.17
Plasma protein[2], g/dL	5.60	5.71	5.59	5.67	0.19	0.82	0.60	0.76
Electrolyte, # treatments/calf	9.6	7.4	6.0	4.3	2.7	0.97	0.32	0.11
Antibiotic, # treatments/calf	2.8	2.3	1.0	2.1	0.64	0.59	0.05	0.10

[1] Measured on day of arrival (d 0). SD: E = ±6.53, AF = ±11.91, EG = ± 7.72, and AFG = ±5.45.

[2] Measured on day of arrival (d 0) by refractometry.

[3] Electrolyte: 77 g of Land O'Lakes electrolyte mixed in 2 L of water.

[4] Antibiotics: individual administration of tetracycline, penicillin, ceftiofur, or tulathromycin as recommended by manufacturer.

Table 3.12. Blood metabolites for calves fed electrolyte plus milk replacer without Gammulin (E), arrival formula plus milk replacer without Gammulin (AF); electrolyte plus milk replacer with Gammulin (EG); or arrival formula plus milk replacer with Gammulin (AFG).

	Treatment					***P***						
Variable	**E**	**AF**	**EG**	**AFG**	**SEM**	**AF**	**G**	**AF*G**	**d**	**AF*d**	**G*d**	**AF*G*d**
Albumin[1], g/dL	2.58	2.33	2.48	2.55	0.10	0.34	0.55	0.11	<0.0001	0.60	0.25	0.29
Cortisol[1], ng/mL	13.3	17.8	14.5	16.2	1.37	0.02	0.89	0.29	<0.0001	0.62	0.61	0.52
Haptoglobin[1], OD*100	2.83	2.56	2.29	2.54	0.32	0.97	0.23	0.27	0.008	0.14	0.94	0.64
Zinc[1], ppm	1.21	1.20	1.18	1.20	0.07	0.94	0.66	0.77	<0.0001	0.36	0.87	0.17
ASP[1], µg/mL	251	261	238	251	8.50	0.18	0.21	0.86	<0.0001	0.81	0.28	0.87
Urea N[2], mg/dL	6.04	6.37	7.59	7.36	0.52	0.91	0.01	0.59	--	--	--	--
Total protein[2], g/dL	4.01	4.16	4.60	4.90	0.32	0.48	0.04	0.80	--	--	--	--

[1] Measured on day of arrival (d 0) and d 2, 7, and 14.

[2] Measured on wk 4.

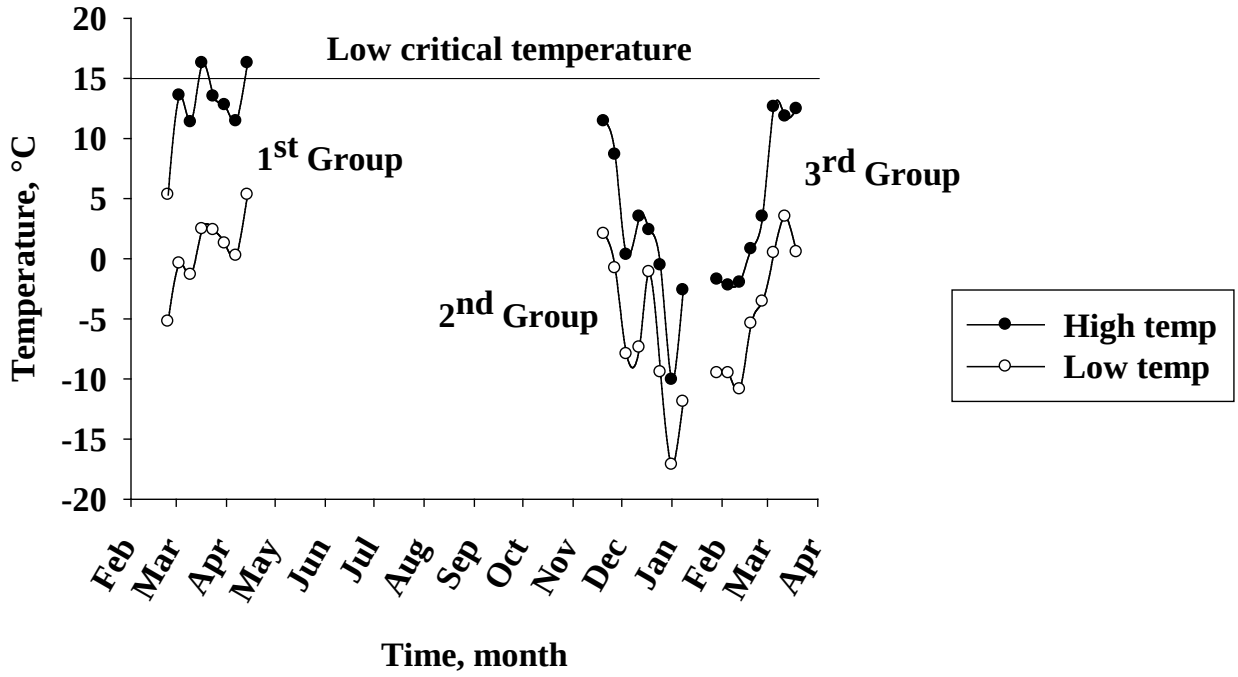

Figure 3.1. Mean high and low ambient temperature during research period by group of calves.

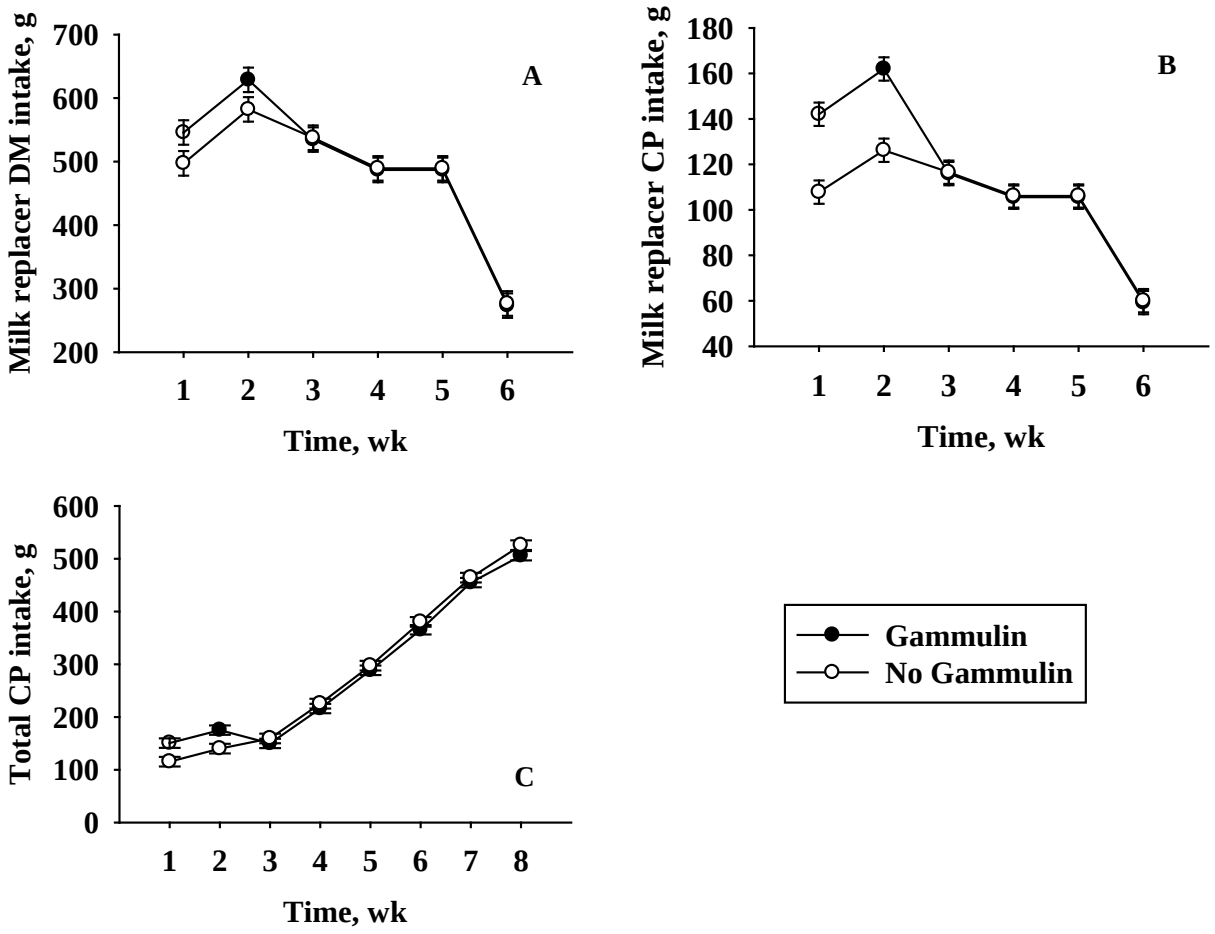

Figure 3.2. Mean daily intake of milk replacer DM, milk replacer CP, and total CP for calves supplemented with Gammulin (—●—) or not supplemented with Gammulin (—○—). **Panel A:** milk replacer DM intake from wk 1 to wk 6. G x wk: $P < 0.0001$. **Panel B:** milk replacer CP intake from wk 1 to wk 6. G x wk: $P < 0.0001$. **Panel C:** total CP intake from wk 1 to wk 8. G x wk: $P = 0.002$.

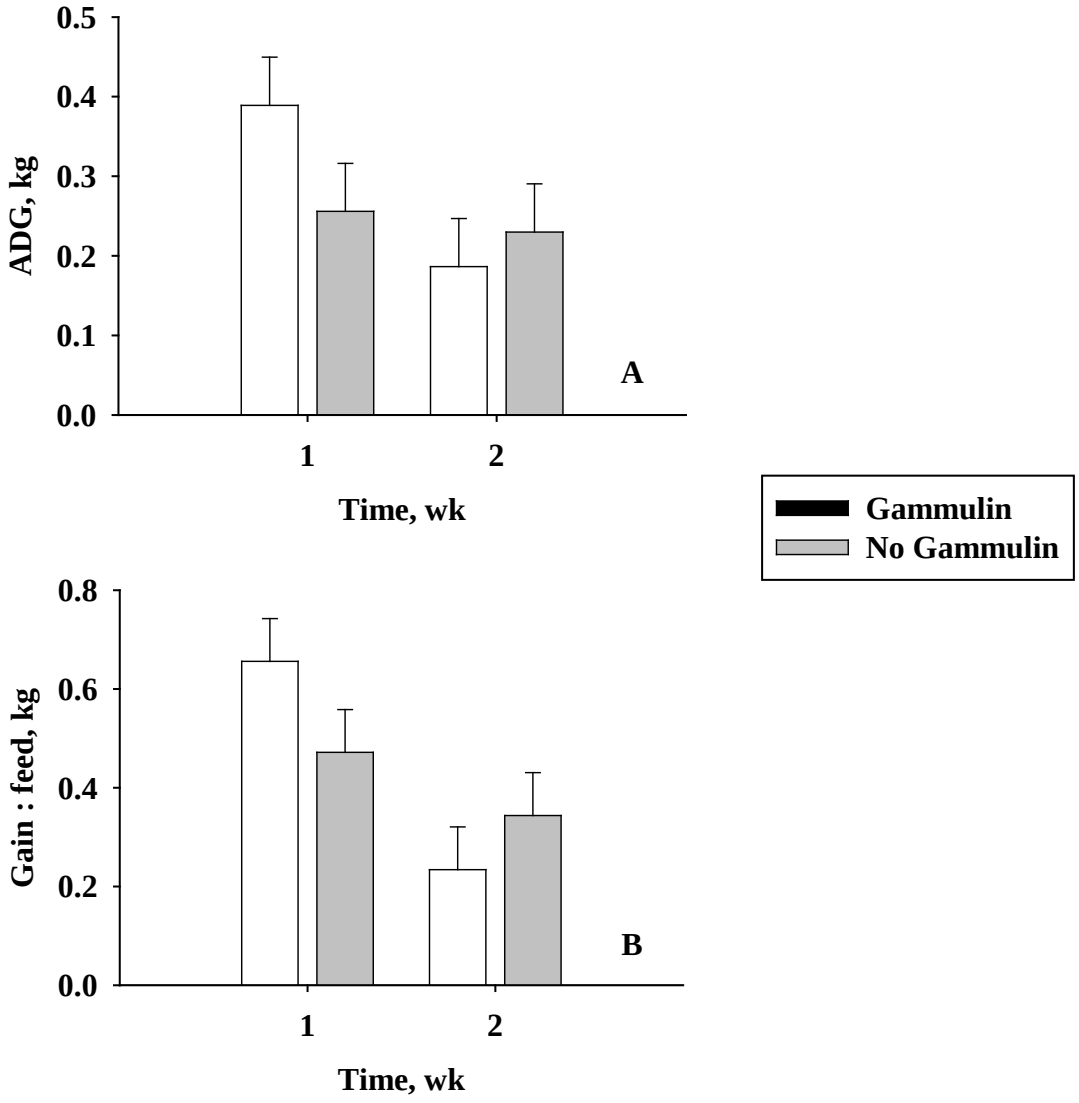

Figure 3.3. Average daily gain (ADG) and feed efficiency (gain: feed) from wk 1 to wk 2 for calves supplemented with Gammulin (▬) or not supplemented with Gammulin (▭). **Panel A:** ADG, G x wk: $P = 0.03$. **Panel B:** gain: feed, G x wk: $P = 0.05$.

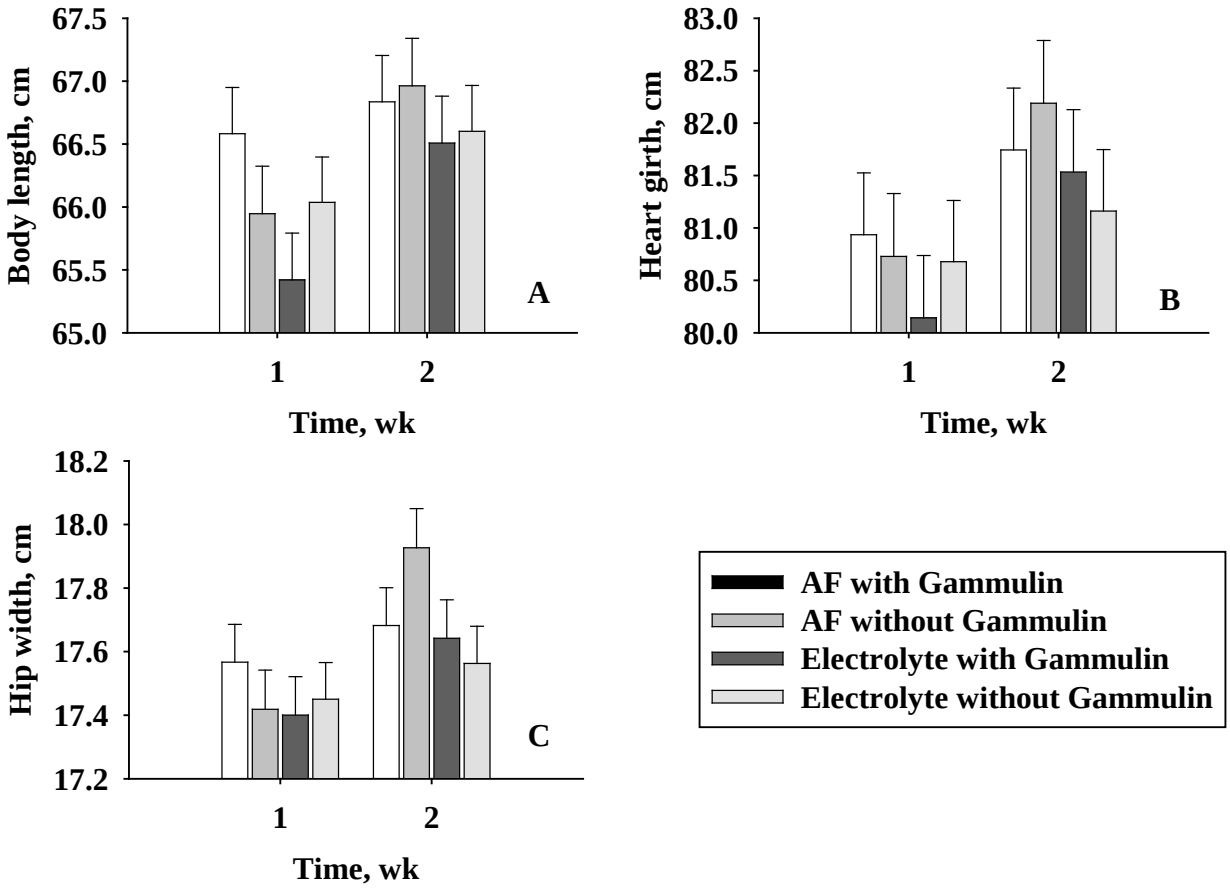

Figure 3.4. Body length, heart girth, and hip width from wk 1 to wk 2 for calves fed arrival formula plus milk replacer with Gammulin (▬); arrival formula plus milk replacer without Gammulin (▭); electrolyte plus milk replacer with Gammulin (▬); or electrolyte plus milk replacer without Gammulin (▭). **Panel A:** body length, AF x G x wk: $P = 0.02$. **Panel B:** heart girth, AF x G x wk: $P = 0.01$. **Panel C:** hip width, AF x G x wk: $P = 0.01$.

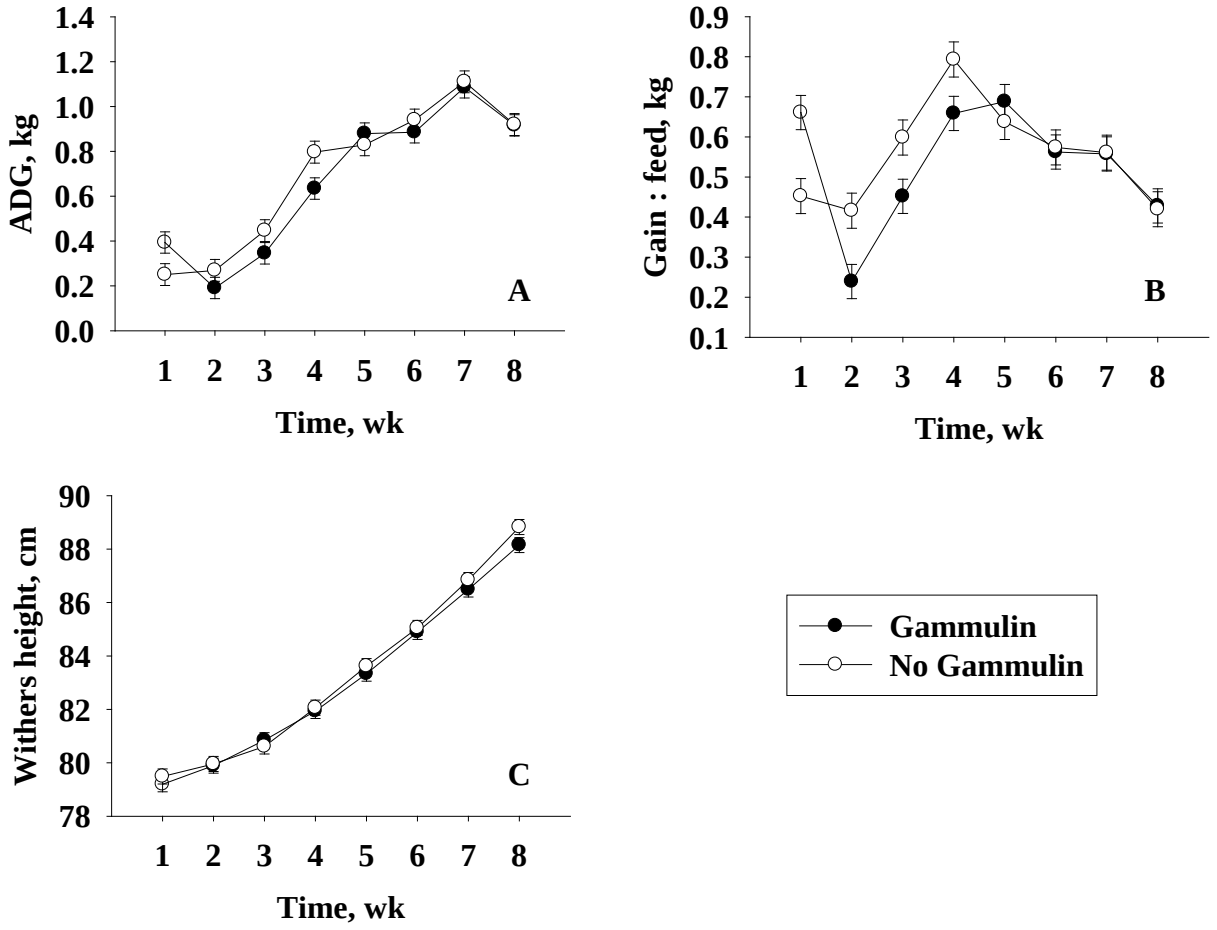

Figure 3.5. Average daily gain (ADG), feed efficiency (gain: feed), and withers height from wk 1 to wk 8 for calves supplemented with Gammulin (—●—) or not supplemented with Gammulin (—○—). **Panel A:** ADG, G x wk: $P = 0.02$. **Panel B:** gain: feed, G x wk: $P < 0.0001$. **Panel C:** withers height, G x wk: $P = 0.06$.

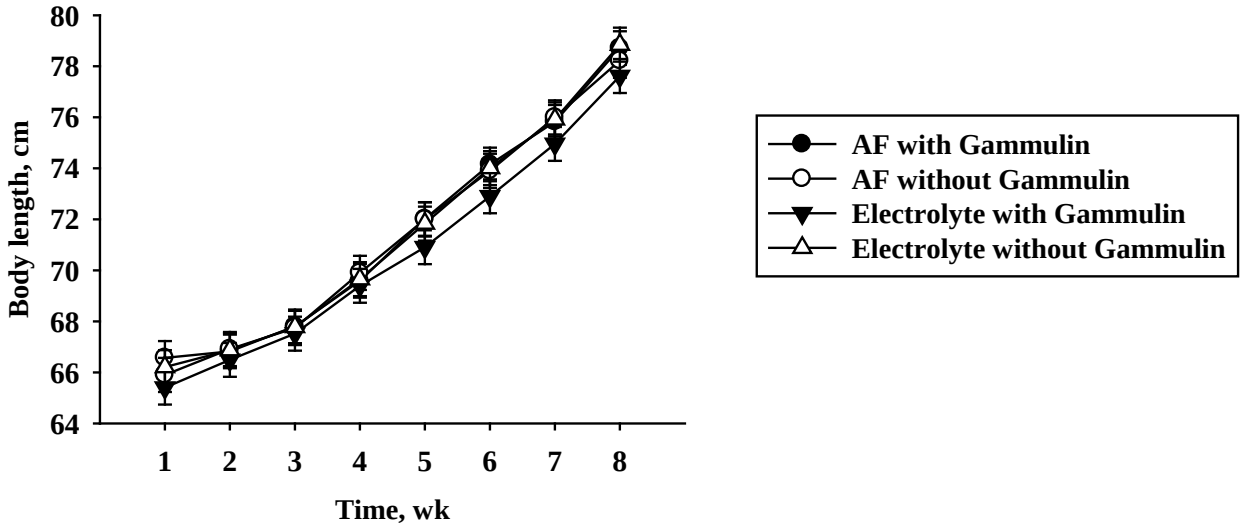

Figure 3.6. Body length from wk 1 to wk 8 for calves fed arrival formula plus milk replacer with Gammulin (—●—); arrival formula plus milk replacer without Gammulin (—○—); electrolyte plus milk replacer with Gammulin (—▼—); or electrolyte plus milk replacer without Gammulin (—△—); AF x G x wk: $P = 0.08$.

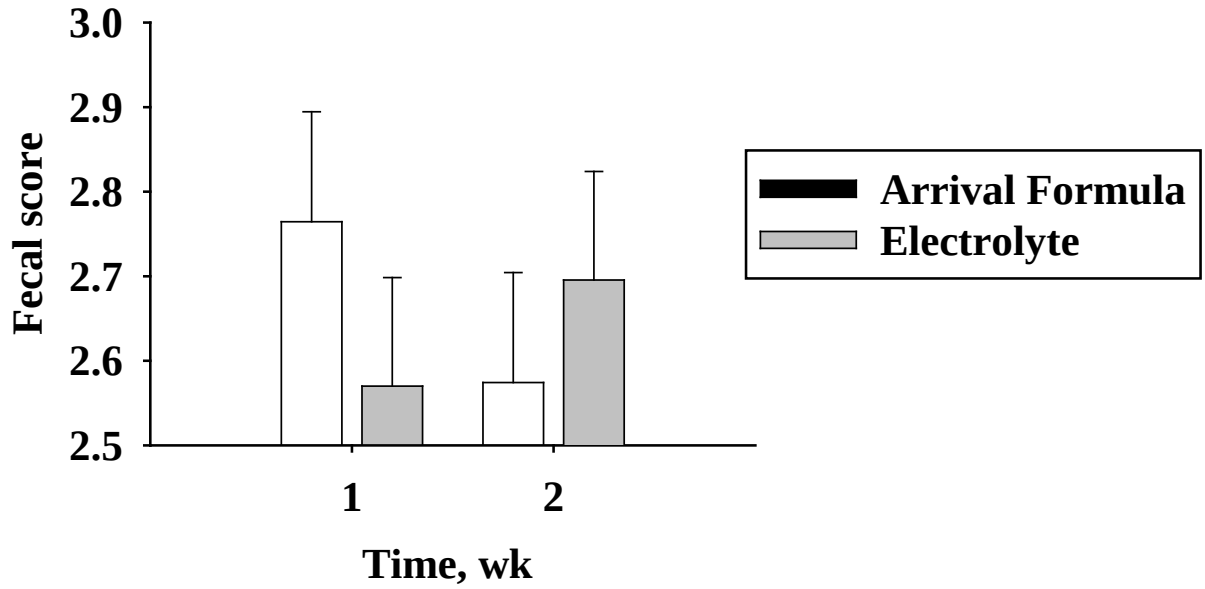

Figure 3.7. Fecal score from wk 1 to wk 2 for calves fed arrival formula (▬) or electrolyte (▭). AF x wk: $P = 0.01$.

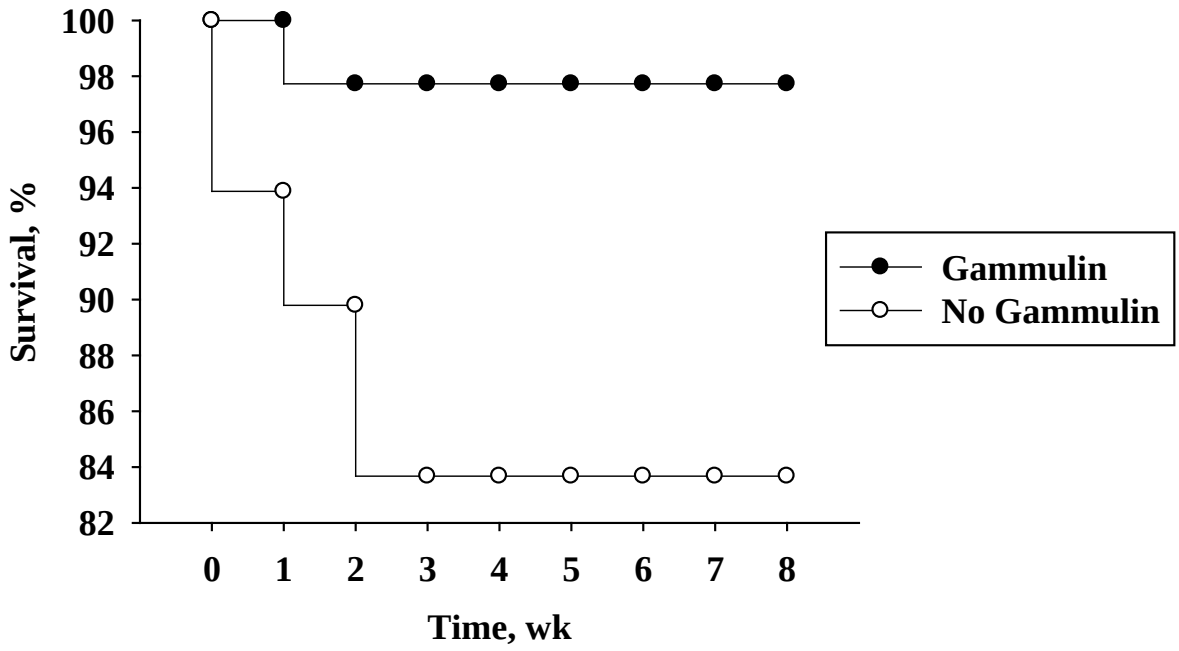

Figure 3.8. Survival percentage from wk 1 to wk 8 for calves supplemented with Gammulin (—●—) or not supplemented with Gammulin (—○—). *P* = 0.02.

Figure 3.9. Mean plasma IgG, determined by radial immonodifusion, at arrival day before treatment assignment for calves that survived (▬) or died (▭) during the 56-d experiment. *P* = 0.008.

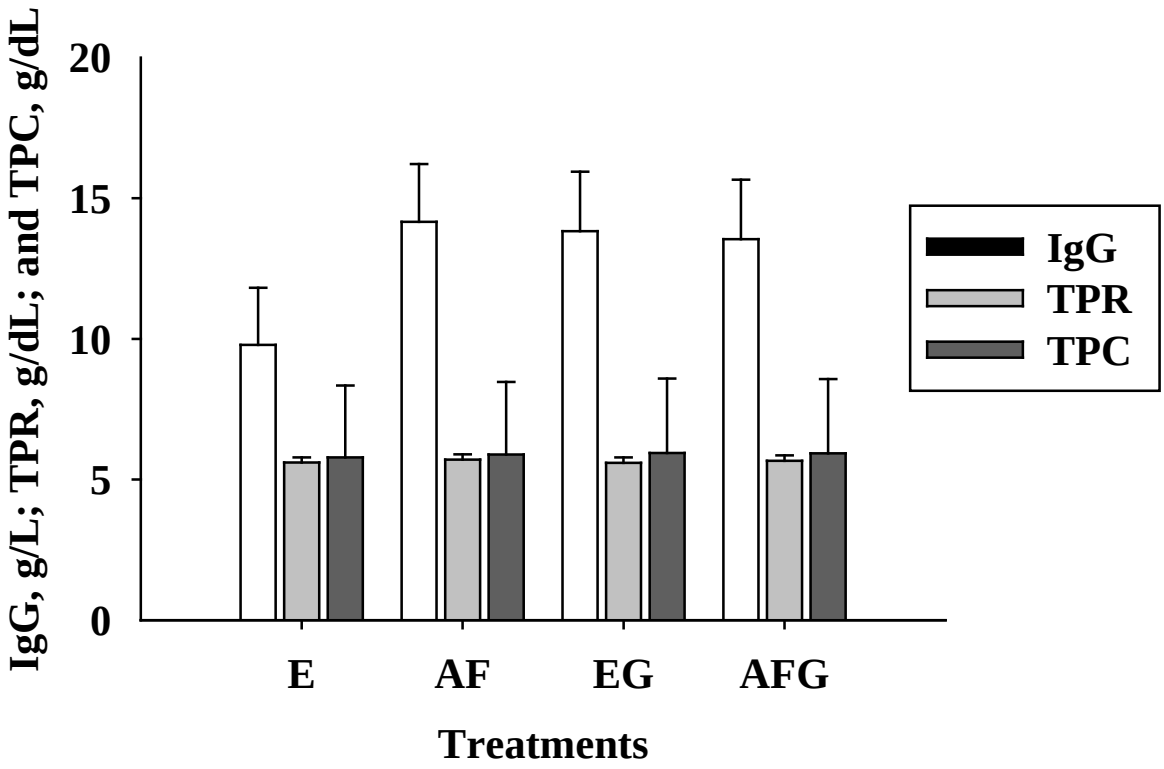

Figure 3.10. Plasma IgG (■), plasma total protein by refractometry (TPR,▭), and plasma total protein by cobas (TPC,▭) before treatment assignment. PlasmaTPR measured upon arrival was usded to stratify and block the calves; IgG determined by radial immonodifusion and TPC were analyzed after the experiment. $P > 0.05$.