Effects of oral rehydration solutions in neonatal calves

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Effects of oral rehydration solutions in neonatal calves

by

Sylvia Inga Wawrzyniak

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Major: Animal Nutrition

Program of Study Committee:
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Ames, Iowa

2003
This is to certify that the master’s thesis of

Sylvia Inga Wawrzyniak

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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Lastly, I would like to thank my fiance. Thank you for always making me feel so beautiful and believing in me.
CHAPTER 1

GENERAL INTRODUCTION

Thesis organization

The following thesis is organized into 4 chapters and 2 appendices. Chapter 1 is a review of literature in the area of coronavirus and its effects on intestinal morphology and function in neonatal calves. It also reviews acid-base balance and vitamins A, E and C as well as growth factors IGF-I, TGF-β1 and TGF-β2. Chapter 2 is a summary of research conducted to determine effects of the addition of vitamins A and E or concentrated protein serum fraction to electrolytes on intestinal recovery from coronavirus challenge in calves. Chapter 3 is a summary of research conducted to determine effects of infusing glucose and electrolytes into the rumen of calves on feed intake, gain, and feed efficiency. Chapter 4 consists of general conclusions. Appendix A contains methodology of xylose and haptoglobin analyses. Appendix B describes vitamin A, E, and C functions as well as vitamin interactions. Research summarized in this thesis will ultimately be submitted for publication and co-authors will include H. D. Tyler and J. D. Quigley, III, M.L. O'Brien, K.J. Touchette, JA Coalson

Effects of oral rehydration solutions in neonates challenged with bovine coronavirus: A review

Introduction

Coronavirus is one of the most common causes of calf diarrhea. Infection with coronavirus disrupts absorption of electrolytes by enterocytes, causing sloughing of the intestinal lining. This alters electrolyte balance, thus affecting acid-base balance in the body.
Current oral rehydration solutions restore acid-base balance, but with the addition of specific vitamins or growth factors, they may restore enterocyte function and maintain the primary defense against pathogens. This review focuses primarily on coronavirus and its effects on acid-base balance and intestinal morphology, as well as effects of supplementation of vitamins A and E or growth factors to oral rehydration solutions.

**Causes of enteric disease**

Neonatal calf diarrhea is a major cause of death and economic loss in the dairy industry. Treatment of scouring calves is not only expensive, but it is also often difficult because of the numerous causative pathogens and the difficulty in diagnoses (Constable et al., 1996; Torres-Medina et al., 1985).

Enteric bacteria most commonly disrupt the absorptive and secretory mechanisms of the intestinal tract. For example, *Cryptosporidium parvum* causes inflammation by attaching to the epithelial cells and decreasing absorption. *Escherichia coli* secretes bacterial enterotoxins, disturbing the secretion and absorption of ions through the villi (Nappert et al., 2000; Torres-Medina et al., 1985). *Salmonella* produces enterotoxins, causing inflammation and intestinal disturbance of glucose-linked sodium absorption. Coronavirus infection results in the replacement of mature epithelial cells with immature cells, disrupting absorption (Torres-Medina et al., 1985). Coronavirus is one of the most common pathogens that causes calf scours, along with cryptosporidium and rotavirus (McDonough et al., 1994); anywhere from 15 to 70% of diarrheal outbreaks in calves may be due to coronavirus (Crouch et al., 1985).
Coronavirus

Coronavirus was named after its resemblance to the solar corona (Sharpee et al., 1976). It is a pneumoenteric virus in the Coronaviridae family of RNA viruses, infecting both the respiratory tract as well as the gastrointestinal tract (Saif et al., 1986; Sharpee et al., 1976; Torres-Medina et al., 1985). It is widely distributed and affects many species, including human, cattle, mice, pigs, rats, dogs, chickens, turkeys, and maybe even foals (Sharpee et al., 1976). Most calves are infected with bovine coronavirus (BCV) between 5 and 20 d of age, but adult cattle can also be infected (Kapil et al., 1990; Torres-Medina et al., 1985).

The virus infects the cytoplasm of cells (not the nuclei) and replicates in epithelial cells of the respiratory and gastrointestinal tracts (Kapil et al., 1990; Pensaert and Callebaut, 1994; Sharpee et al., 1976). It replicates in enterocytes of the small intestine as well as the colonic villous and crypt epithelium (Hoblet et al., 1992; Kapil et al., 1990; Pensaert and Callebaut, 1994). This can be demonstrated experimentally by injecting fluorescence-labeled coronavirus antisera to directly display the location of virions in the intestinal tract. Tips of the villi and villous cells fluoresce in the both jejunum and ileum, indicating attachment of coronavirus in the distal part of the small intestine (Bridger et al., 1978). Mebus (1975) also reported immunofluorescence in the villous epithelium of the duodenum.

Six properties characterize the coronavirus group. They have petal-like surface structures 20 nm long, their size averages 80 nm, they replicate within cytoplasmic vesicles, they have an essential lipid envelope (that makes them sensitive to temperature), they have low particle density, and they use ribonucleic acid to replicate (since inhibiting their DNA metabolism does not prevent replication). Enteric coronaviruses are acid stable at a pH as
low as 3.0, enabling them to safely pass through the stomach into the intestinal tract. Conversely, respiratory coronaviruses are acid labile, and are more dramatically affected by fluctuations in acidity (Sharpee et al., 1976).

Coronaviruses are able to infect other cells due to their surface glycoproteins. These glycoproteins interact with host cell membrane receptors and fuse the viral envelope of the coronavirus with the plasma envelope of host cells (Holmes and Williams, 1990). All coronaviruses contain the membrane glycoproteins M and S and some, especially BCV, contain the membrane glycoprotein HE (Holmes and Williams, 1990; Kienzle et al., 1990; Vlasak et al., 1988).

The HE glycoprotein enables BCV to infect many hosts and have an altered pathogenicity (Holmes and Williams, 1990). It enables the virus to enter the cell by destroying cell receptors that would prevent entry under normal circumstances. The HE glycoproteins that are not incorporated into virions migrate to cell surfaces and adsorb to erythrocytes causing hemagglutination (Clark, 1993; Holmes and Williams, 1990; Kienzle et al., 1990; Vlasak et al., 1988).

The M glycoprotein determines the site of virus budding in host cells and interacts with the nucleocapsid. The glycoprotein S is essential for infectivity and ensures that host cells fuse with viral cells (Holmes and Williams, 1990; Kienzle et al., 1990; Vlasak et al., 1988).

Replication occurs in the cytoplasm when viral RNA attaches to ribosomes and translates its own viral proteins. Viral proteins replace host proteins in the golgi apparatus and rough endoplasmic reticulum, thus pinching off whole virions and expelling them into
the intestinal lumen. Few virions are released through lysis of dying cells; most are released from normal, living cells (Clark, 1993).

**Small intestinal morphology**

Small intestinal cells are highly differentiated and morphologically complex. The surface area of the small intestine is greatly amplified by longitudinal and circular concentric folds (Barnwal and Yadava, 1975; Trier and Madara, 1981). These folds are mainly located in the distal duodenum and proximal jejunum and are less visible in the ileum (Trier and Madara, 1981).

The small intestine is comprised of three tissue layers; muscularis mucosa, lamina propria, and epithelium. The muscularis mucosa is a continuous sheet of smooth muscle 3 to 10 cells thick that separates the mucosa from the submucosa. Contractions of the muscularis may add to the movement of the villi, mixing the contents of the small intestinal lumen and increasing absorptive function. This may also facilitate emptying of the crypt luminal contents by causing compression of the lumen (Trier and Madara, 1981).

The lamina propria lies between the epithelium and the muscularis mucosa. It surrounds the crypt epithelium and contains cells that are important for immune function as well as blood vessels that provide a nutrient supply (Trier and Madara, 1981).

The third layer is a single cell layer of epithelial cells that line the villi and the crypts. The crypt epithelium contains undifferentiated cells in the process of mitosis and proliferation. Although the villus epithelium absorbs many substances, it also secretes numerous substances that act to maintain homeostasis. The brush border includes long, thin microvilli that line columnar epithelial cells and increase the absorptive surface 7- to 14-fold (Trier and Madara, 1981).
Differentiation of enterocytes occurs along the villus, starting out as undifferentiated cells at the crypt base and migrating into specified cell types (Leblond and Stevens, 1948; Sassier and Bergeron, 1978; Trier and Madara, 1981; Zile et al., 1977). The replacement of cells maintains a steady state in the population of epithelial cells (Sassier and Bergeron, 1978). Dividing cells that are labeled in the crypts either migrate up the villus or remain in the crypts to divide again (Zile et al., 1977).

**Effects of coronavirus on intestinal functions**

Normal small intestinal villous function includes secretion of enzymes on the apical villous membrane that digest carbohydrates. Solutes, including sodium, chloride and glucose, are transported by carriers or passive diffusion through the villous cell membrane (Reifen et al., 1998; Torres-Medina et al., 1985). Crypt cells, which are too immature to either digest or absorb, secrete bicarbonate and chloride ions into the lumen of the small intestine, directing water movement through passive diffusion. During normal conditions, absorption of substances through the villi outweighs the crypt secretion, resulting in net absorption of water, substrates, and ions. When these enzymes or transport processes are negatively affected, substrates, ions and water are excreted out of the digestive tract at a faster rate than absorption can occur (Torres-Medina et al., 1985).

Diseases that affect mucosal function of the intestine destroy villus architecture and decrease absorptive surface area of the small intestine (Bridger et al., 1978; Clark, 1993; Holland et al., 1992; Saif et al., 1986). Infection with coronavirus frequently causes lesions to appear on the intestinal surface, decreasing the villus to crypt ratio. Villi become thick and lined with cuboidal epithelial cells (Bridger et al., 1978; Clark, 1993; Mebus et al., 1975; Pensaert and Callebaut, 1994; Saif et al., 1986). Some villi become ragged at the luminal
surface causing the lamina propria to be exposed at the villous tips (Bridger et al., 1978; Mebus et al., 1975). Similarly, large intestinal cells become cuboidal and squamous and goblet cell numbers decrease although no defined lesions appear (Bridger et al., 1978; Clark, 1993).

This loss of epithelial function results in malabsorption of water, sodium, and chloride (Torres-Medina et al., 1985). These poorly absorbed solutes, in addition to undigested lactose remaining in the small intestinal lumen, can cause osmotic retention of fluid. This results in less absorption of solutes and further draws water from the enterocytes (Clark, 1993; Menzies et al., 1990). As excretion of electrolytes and fluids continues, losses of electrolytes from extracellular fluid (ECF) cause water to move out of the hypo-osmotic ECF (Constable et al., 1996; Nappert et al., 2000). Decreased intestinal absorption and prolonged diarrhea occur due to cells sloughing off and being replaced with immature cells that are unable to perform normal functions and are redirected to function only for viral production (Clark, 1993; Holland et al., 1992; Mebus et al., 1975). Continued diarrhea increases the risk of dehydration, lactic acidemia and hypoglycemia and may eventually lead to death (Clark, 1993; Pensaert and Callebaut, 1994).

Although the incubation period of coronavirus is only 24 to 48 hours, clinical signs of the infection usually take 36-60 hours (Clark, 1993; Pensaert and Callebaut, 1994; Torres-Medina et al., 1985). Forty to ninety hours after infection, loss of enterocytes from the small intestinal villi is extensive. Alteration in cellular potassium levels and hypoglycemia cause calves to become lethargic. Water loss decreases total circulatory fluid volume stimulating vasoconstriction to maintain blood pressure. Blood flow to extremities is decreased, causing them to cool and atrophy (Torres-Medina et al., 1985). Lack of blood flow to the tips of the
intestinal villi decreases absorption of ingested nutrients and impairs villous function (Nappert et al., 2000). Calves become depressed, anorexic and their feces may include mucus and milk curds. If diarrhea is not treated, calves can become too weak to stand after 2-4 days and death may occur (Pensaert and Callebaut, 1994; Torres-Medina et al., 1985).

**Acid-base balance**

Once a calf develops diarrhea, it needs to be treated with fluid therapy either orally or intravenously to avoid problems such as metabolic acidosis (Booth and Naylor, 1987; Groutides and Michell, 1990). If rehydration therapy does not occur, mortality rate increases significantly (up to 80%) especially if blood pH drops below 7.2 (Booth and Naylor, 1987).

One primary function of therapy with oral rehydration solutions (ORS) is to restore acid-base balance. Conventionally, this is assumed to be accomplished by affecting the concentration of hydrogen ions in the various fluid compartments of the body. Traditionally, the concentration of hydrogen ions is monitored by analyzing the pH of body fluids. The pH is the inverse of the concentration of hydrogen ions and the Henderson-Hasselbalch equation is used to describe the relationship between dissociable acids and pH:

\[
\text{pH} = \text{pK} + \log([A^-]/[HA])
\]

where concentration of A- represents any anion, concentration of HA represents the undissociated acid, and pK represents the dissociation constant of the acid. The Henderson-Hasselbalch equation is used to understand changes in pH when \([H^+]\) or \([OH^-]\) are changed (Ganong, 1999).

The carbonic acid-bicarbonate system is a major buffer system in the body that regulates acid-base in blood. Bicarbonate dissociates into hydrogen ions and carbonic acid:

\[
\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-
\]
In the body, bicarbonate and carbonic acid also equilibrate with dissolved carbon dioxide:

\[ \text{H}_2\text{CO}_3 \leftrightarrow \text{CO}_2 + \text{H}_2\text{O} \]

This buffer system is easily regulated as dissolved carbon dioxide concentrations are regulated by respiration and plasma \([\text{HCO}_3^-]\) is conventionally assumed to be regulated by the kidneys. The enzyme that regulates the dissociation of bicarbonate is carbonic anhydrase. When activated, carbonic anhydrase increases the conversion of bicarbonate to water and carbon dioxide, helping to regulate blood acid-base balance (Ganong, 1999).

This conventional paradigm of acid-base balance is disputed by Stewart (1981). Instead of looking at pH in the entire body, Stewart (1981) focuses on individual systems in the body and how specific variables act to affect acid-base balance.

Stewart (1981) describes two types of variables that exist in all systems of the body and influence acid-base balance. The first are independent variables that are controlled from outside the system and do not affect each other. The second are dependent variables that are affected only by independent variables. The three main independent variables that exist in biological systems are \(p\text{CO}_2\), strong ion difference ([SID]), and the concentration of weak non-volatile acids ([A\text{TOT}]) (usually serum proteins and inorganic phosphates). Strong ion difference is defined as the net charge resulting from the combination of all strong cations and strong anions within that system (Stewart, 1981).

Stewart’s approach uses equations to demonstrate that kidneys do not control bicarbonate concentration, but instead regulate one of the independent variables, [SID], that determines whether strong ions are added or removed in the nephron. Since \(p\text{CO}_2\) is an independent variable as well, both \(p\text{CO}_2\) and [SID] affect the ratio of \(p\text{CO}_2\) to \([\text{HCO}_3^-]\) (a dependent variable), thus affecting \([\text{H}^+]\) (Stewart, 1981). An increase in anions results in a
negative [SID]. The increased concentration of anions causes an increase in the concentration of $H^+$, thereby lowering pH (metabolic acidosis) (Stocker et al., 1999). The only way to buffer this would be to increase the [SID], lowering the anion gap and ultimately decreasing the concentration of $H^+$ (increasing pH) which would alleviate the metabolic acidosis (Stewart, 1981).

**Components of oral rehydration solutions**

There are many oral rehydration solutions available for treating diarrheic calves (Table 1). All oral rehydration solutions contain water, sodium, potassium, chloride, and an energy source, among other things. Most oral rehydration solutions are suitable for rehydration although not all have the ability to successfully correct acidosis in calves (Naylor, 1990).

The main goal of an oral rehydration solution (ORS) should be to increase extracellular fluid volume (ECF) by replacing water and electrolytes, thereby decreasing dehydration and hyponatremia (Clark, 1993; Groutides and Michell, 1990). An effective ORS should also reduce metabolic acidosis and hyperkalaemia (Groutides and Michell, 1990).

A key component of ORS should be glucose, as it enhances the absorption of sodium and provides an energy source (Nappert et al., 2000). Most calves become hypoglycemic due to diarrhea and decreased glucose absorption, developing a higher dietary requirement for energy (Cleek et al., 1979). Both glucose and glycine are usually added to ORS to enhance sodium absorption, but glucose should not be added at more than 200 mmol/L of ORS due to changes in the osmolarity of the ORS. As more glucose is added to ORS, the
<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Glucose mmol/L</th>
<th>Glycine mmol/L</th>
<th>Sodium mmol/L</th>
<th>Chloride mmol/L</th>
<th>Potassium mmol/L</th>
<th>Bicarbonate mmol/L</th>
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<td>0.0</td>
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Table 1. Components of a few commercially available calf oral rehydration solutions (Naylor; 1992, 1996, 1999)
ORS becomes hyperosmolar and is absorbed more slowly in the small intestine than an isotonic ORS (Naylor, 1996).

Sources of energy added to ORS include glucose, lactate, citrate, and acetate (Naylor and Forsyth, 1986). The daily energy requirement for maintenance of a young calf is about 2000 kcal. The daily energy requirement for maintenance with an additional weight gain of 0.5 kg/day is 3500 kcal (Naylor, 1996). This energy requirement can be met by providing the calf with milk while the calf is receiving fluid therapy.

If milk is not fed, a higher energy ORS should be fed to provide the calf with at least enough energy to maintain weight (Naylor, 1996). As energy content of the ORS decreases, weight loss is inversely proportional and increases (Fettman et al., 1986). Acetate, along with glucose, is a good source of energy and facilitates the absorption of water and sodium from the jejunum (Demigne et al., 1981). Acetate is easily metabolized in the cells of muscle and other tissues, requiring less oxygen than lactate, as demonstrated by the efficient disappearance of acetate from the blood after intravenous infusion (Park and Arieff, 1980):

\[
\text{CH}_3\text{Coo}^- + \text{H}^+ + \text{O}_2 \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O}
\]

Bicarbonate infused intravenously immediately alkalinizes the blood when it is catalyzed by carbonic anhydrase to water and carbon dioxide. Addition of sodium citrate may be a possible replacement for sodium bicarbonate (Hunt et al., 1992). Citrate is a disodium salt that is more stable during storage and may take the place of bicarbonate in ORS as a sodium carrier as it is a precursor to bicarbonate.

Lactate and propionate are oxidized by liver and peripheral tissue or are converted to glucose (Ballard, 1972):

\[
2\text{CH}_3\text{CHOHCOO}^- + 2\text{H}^+ + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}
\]
\[2\text{CH}_3\text{CHOHCOO}^- + 2\text{H}^+ \rightarrow \text{C}_6\text{H}_{12}\text{O}_6\]

Sodium salts, such as sodium bicarbonate, sodium acetate, sodium lactate and sodium propionate, are buffers and should be added at 50 to 80 mmol/L of ORS (Naylor, 1996). The best alkalinizing ability is provided by sodium acetate as it is easily metabolized (Naylor and Forsyth, 1986). Most research has concluded that solutions with added sodium bicarbonate are the answer to correcting metabolic acidosis in the calf. These solutions increase pH more effectively than solutions without sodium bicarbonate (Booth and Naylor, 1987; Michell et al., 1992; Naylor and Forsyth, 1986). Oral rehydration solutions without added sodium bicarbonate reduce mortality and dehydration as well as solutions with sodium bicarbonate, but do not increase pH or reduce depression as effectively (Booth and Naylor, 1987). When sodium bicarbonate enters the blood, it dissociates and the bicarbonate combines directly with hydrogen ions and is quickly exhaled through respiration. This decreases metabolic acidosis, according to conventional acid-base theory (Naylor and Forsyth, 1986).

This is not possible according to Stewart’s equations. It is actually the sodium in sodium bicarbonate that plays the main role in decreasing metabolic acidosis. The bicarbonate rapidly equilibrates with pCO2 and is exhaled, having no effect on acid-base balance. Sodium, on the other hand, enters the blood and lowers [SID], increasing the concentration of \(\text{OH}^-\) and decreasing the concentration of \(\text{H}^+\), thereby increasing the pH of the blood and alleviating metabolic acidosis. Bicarbonate, although added to ORS, is only a carrier for sodium. It does not participate in altering \([\text{H}^+]\) since it is not an independent variable, but a dependent one itself (Stewart, 1981).

Another function of sodium in ORS is to allow water to return to the extracellular fluid from the cells (Michell et al., 1992). Sodium concentrations in ORS should not exceed
145 mmol/L, as this may induce hypernatremia in calves that are unable to drink more water than the ORS provides (Naylor, 1999). Sodium should be added to ORS at molar concentrations equal to the molar concentrations of glucose and glycine provided in the ORS. Glucose and glycine drive sodium absorption through enterocytes in a mole-to-mole ratio, thus if sodium concentrations exceed the combined concentration of glucose and glycine, then sodium is only partially absorbed in the small intestine (Constable et al., 1996; Hellier et al., 1973).

Providing the calf with milk and ORS within a short time can be detrimental when the ORS contains bicarbonate or citrate, both of which prevent or reduce milk clotting. If the ORS contains acetate or lactate instead of bicarbonate or citrate, there are no detrimental effects on clotting and milk can continue to be fed to calves while they are receiving ORS. If milk is fed, sodium acetate is the best alkalinizing agent provided in ORS as it does not interfere with milk clotting in the abomasum (Naylor and Forsyth, 1986).

Feeding milk while feeding ORS without bicarbonate does not affect fecal scores, indicating there is no extra fermentation of milk by bacteria in the gut (Garthwaite et al., 1994). Other research indicates that feeding milk along with ORS provides enough energy to the calf to maintain growth and develop better fat stores, while also regenerating the intestinal mucosa for improved absorption (Heath et al., 1989).

Other possible additions to ORS

Carbohydrates

As an alternative source of energy, dextran is added to ORS for animals that become hypoglycemic due to diarrhea. Dextran added to a hypertonic saline solution causes the expansion of plasma volume by altering the osmotic gradient and provides a sustained source
of glucose (Constable et al., 1996). Commercial pectins are also adequate as an alternative energy source to glucose, although sucrose and corn syrup are not utilized because they are less digestible (Cleek et al., 1979).

**Amino Acids**

Glutamine is added to some oral rehydration solutions because of its effect on maintaining villous integrity and increasing sodium uptake. The addition of glycine at 40 mmol/L improves nitrogen balance as well as enhancing absorption of sodium and water from the intestine. Improved results are obtained when glycine is added to ORS containing enough energy to improve the negative energy balance caused by diarrhea. Glycine added to low caloric density ORS is not as effective as when it is added to a high caloric density ORS (Fettman et al., 1986).

**Vitamins**

A viral challenge may cause the development of nutritional deficiencies and, in turn, accentuate the effects of the disease by suppressing immune response and resistance (Wang et al., 1994). This suggests that supplementing vitamins may be beneficial during a viral challenge.

**Vitamin A**

Although most domestic animals are fed amounts of vitamins that should be sufficient in their diet, there are many factors that may cause vitamin depletion and deficiency (Uni et al., 1998). Vitamin A deficiency is one of the most common vitamin deficiencies around the world and is reported to increase the incidence of diarrhea in vitamin A deficient children (Ahmed et al., 1990; Fawzi et al., 1995). Many researchers have reported that supplementation of vitamin A to vitamin A deficient children decreases the incidence of
diarrhea and reduces mortality (Ahmed et al., 1990; Alvarez et al., 1995; Fawzi et al., 1995). Calves may be at particular risk because they are born with low reserves of fat-soluble vitamins as they do not cross the placental membrane (Branstetter et al., 1973). Colostrum provides large amounts of vitamin A, however, stressors and pathogens may quickly deplete stores of vitamin A in the neonate.

Morphology of the small intestine changes during vitamin A deficiency. Mucosal thickness and villus height are reduced and enterocyte size decreases (Uni et al., 1998). Overall protein synthesis and enterocyte proliferation are impaired (Uni et al., 2000; Uni et al., 1998). There are also decreases in goblet cell numbers in the small intestinal villi (Ahmed et al., 1990; Rojanapo et al., 1980; Uni et al., 2000).

Jejunal enterocytes may undergo hypertrophy during vitamin A deficiency; there is a decreased ratio of RNA to DNA and protein to DNA during these periods of deficiency (Reifen et al., 1998; Uni et al., 1998). There is also an increase in the length of the cell cycle of jejunal crypt cells indicating a decrease in the proliferation of cells and a decreased rate of cell sloughing from the villus tip. As the DNA-synthesis cycle increases, cells are not able to migrate out of the crypts as quickly because of feedback regulation from differentiated cells not yet sloughed off the villus (Zile et al., 1977).

Deficiency of vitamin A may also alter the biochemical and morphological characteristics of the small intestine by altering disaccharidase activities (Reifen et al., 1998; Uni et al., 2000; West et al., 1992). In vitamin A deficient animals, ribosomal capacity is decreased and jejunal brush border enzyme activity is low. If vitamin A is once again added to the diet, enzyme activity returns to normal confirming that vitamin A deficiency alters enzyme activity in the brush border (Reifen et al., 1998).
Others have shown vitamin A deficiency does not alter morphology of the intestinal lining (Ahmed et al., 1990; Reifen et al., 1998; Rojanapo et al., 1980; West et al., 1992; Zile et al., 1977). This may be a result of animals not being truly deficient in vitamin A due to the presence of extrahepatic reserves of vitamin A (Green and Green, 1994).

Some research has reported a lowered resistance to specific diseases as a result of vitamin A deficiency (Uni et al., 2000; Uni et al., 1998). There is an impairment of mucosal defense and a defect in the integrity of the epithelium when vitamin A is deficient (Warden et al., 1997). This may cause an increased systemic exposure to a viral challenge, decreasing the ability of the enterocytes to defend themselves (Ahmed et al., 1990; Wang et al., 1994). This is demonstrated by an increase in the weight of the spleen and an increase in the B-cell: T-cell ratio, indicating an increase in activity of the immune system (Ahmed et al., 1990).

Calves challenged with Cryptosporidium parvum have decreased retinol uptake due to increases in the number of undifferentiated enterocytes. These immature enterocytes may not be able to synthesize adequate numbers of chylomicrons or they may not esterify retinol to the same degree as differentiated enterocytes (Holland et al., 1992).

**Function**

Vitamin A is needed to maintain normal proliferation and differentiation of cells in a variety of epithelial tissues (Uni et al., 1998; Zile et al., 1977). The small intestinal lining has a high cell turnover rate, increasing the requirement for vitamin A. Cells that may become adversely affected by frequent exposure to environmental stressors and pathogens, such as enterocytes, need a rapid turnover rate to maintain normal function (Zile et al., 1977). Vitamin A is an essential part of this cell replacement cycle and animals that are deficient in
vitamin A may be less capable in protecting their epithelial linings from such stressors (Filteau et al., 2001; Zile et al., 1977).

**Absorption**

Vitamin A is picked up by micelles in the lumen of the small intestine for transport, along with fatty acids, bile salts and other fat-soluble vitamins (Leeson and Summers, 2001). These micelles are secreted with nonpolar hydrophobic ends pointing in and polar hydrophilic ends pointing out, allowing for the solubilization of fat-soluble substances within the nonpolar environment (Hollander, 1981).

Hydrolases located in the brush border and produced by the pancreas hydrolyze retinyl esters to retinol (Combs and Gerald, 1992; Hollander, 1981). There are different enzymes that hydrolyze retinyl esters to free retinol. This hydrolysis determines whether retinol is absorbed or excreted from the body (Schindler et al., 2002).

Under normal conditions, the efficiency of vitamin A absorption through the intestinal wall is high, about 80 to 95 percent. Of this, 30 to 60 percent is esterified and stored in the liver.

**Transport**

Once retinol enters the enterocyte, it is attached to a carrier protein for transport through the aqueous cytosol to cellular organelles (Hollander, 1981). Retinol, formed by hydrolysis of retinyl esters or by the reduction of retinal from the cleavage of beta-carotene, is re-esterified with long chain fatty acids in the intestinal mucosa (Combs and Gerald, 1992).

Rats maintain stores of up to 300 nmol of retinol in extravascular and extrahepatic tissues after liver retinol is depleted (Green and Green, 1994). This is confirmed by the presence of lecithin: retinol acyl-transferase (LRAT) in nonhepatic tissues. This enzyme
converts retinol to retinyl esters for storage in extrahepatic tissues, such as the intestinal wall (Herr et al., 1993). Cellular retinol binding protein (CRBP) is also found in the intestinal wall and serves as a carrier of retinol and retinal for LRAT (Herr et al., 1993). Extrahepatic stores of retinol may help explain why no differences are seen between vitamin A deficient and vitamin A sufficient groups in some trials (Warden et al., 1997). Deficient animals tend to conserve their extrahepatic stores, using them only when hepatic stores are depleted (Green and Green, 1994).

When a stressor is introduced, such as Newcastle disease virus in chickens, plasma concentrations of retinol decrease dramatically not only because of impairments in absorption of vitamin A from the intestine, but also because of a more rapid utilization of vitamin A by tissues (West et al., 1992). For more vitamin A review please refer to Appendix B.

**Vitamin E**

Any situation that is highly stressful to an animal increases the requirement for vitamin E. Vitamin E deficiency in swine and cattle is characterized by dermatitis, alopecia, and muscular dystrophy, or white muscle disease. Vitamin E-deficient calves become depressed and inactive and may appear weak. Dietary intakes of polyunsaturated fatty acids (PUFA) and selenium affect the need for vitamin E, as do deficiencies of amino acids and other vitamins (Combs and Gerald, 1992). Calf milk replacers may not provide enough vitamin E to meet the needs of calves, especially under stressful conditions due to weaning, transport, disease, and confinement. Calves injected intramuscularly with supplemental vitamin E tend to exhibit higher growth rates and improved feed intakes (Reddy et al., 1985).
Vitamin E acts as an antioxidant, inhibiting oxidation and reducing free radicals, that are generated from covalent bonds that are cleaved (Combs and Gerald, 1992; Gonzalez et al., 2001; Sato et al., 1990). In diseases that are characterized by a high production of free radicals, such as inflammatory bowel disease, supplementation of vitamin E decreases the amount of oxidative damage (Gonzalez et al., 2001). Supplementing dietary alpha-tocopherol helps maintain membrane integrity (Burton et al., 1983; Combs and Gerald, 1992; Gonzalez et al., 2001).

Absorption

Vitamin E absorption is dependant on the absorption of other lipids because it is a fat-soluble vitamin (Combs and Gerald, 1992; Hollander, 1981). The first site of absorption is the proximal small intestine where alpha-tocopherol is hydrolyzed by bile salts and pancreatic juices and absorbed as a free alcohol via passive diffusion (Combs and Gerald, 1992; Hollander, 1981). Once through the membrane, it is transported to the liver by chylomicrons, very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) (Bjorneboe et al., 1986; Bjorneboe et al., 1987; Cohn et al., 1988; Kostner et al., 1995; Massey, 1984). Alpha-tocopherol is then absorbed into the intestinal lymph and transported unchanged to the liver (Bjorneboe et al., 1986; Burton et al., 1983).

Storage

There are two storage pools of vitamin E in tissues. A fixed pool that turns over slowly is located in adipose tissue, and a labile pool that turns over rapidly is usually located in cellular membranes of other tissues, such as small intestine or liver. During alpha-tocopherol deficiency, stored alpha-tocopherol is rapidly depleted from the labile pool,
making plasma and liver most susceptible to deficiency (Combs and Gerald, 1992). For more review of vitamin E please refer to Appendix B.

**Vitamin A and E interactions**

Several researchers have reported interactions between vitamin A and vitamin E in vivo. High dietary vitamin A decreases plasma alpha-tocopherol (Bieri and Tolliver, 1982; Franklin *et al.*, 1998) as well as liver alpha-tocopherol levels (Combs, 1976; Dicks *et al.*, 1959; Eicher *et al.*, 1997; Sklan and Donoghue, 1982). Conversely, deficiencies in retinol increase hepatic alpha-tocopherol levels (Roels *et al.*, 1964).

The form of retinol provided in the diet also affects plasma and hepatic concentrations of vitamin E. Increases in dietary retinoic acid decrease absorption of free and esterified alpha-tocopherol independently of triglyceride absorption. It is theorized that feeding this form of vitamin A may alter the physical or chemical conditions present in the intestinal wall, preventing or decreasing the absorption of alpha-tocopherol (Bieri and Tolliver, 1982).

Plasma vitamin A concentrations are also affected by the level of intake of vitamin E. Plasma vitamin A concentrations are decreased during high or moderate dietary vitamin E intakes (Abawi *et al.*, 1985; Eicher *et al.*, 1997; Napoli *et al.*, 1984). However, although plasma vitamin A concentrations are decreased, hepatic vitamin A stores are increased (Combs and Gerald, 1992; Napoli *et al.*, 1984; Roels *et al.*, 1964).

In the small intestine, high concentrations of fat-soluble vitamins compete for hydrolysis by the brush border enzymes (Abawi *et al.*, 1985; Franklin *et al.*, 1998). Once in the enterocytes, high vitamin concentrations compete for transfer by fat-soluble carriers.
(Eicher et al., 1997). For more review of vitamin A and E interactions, please refer to Appendix B.

**Vitamin C**

Vitamin C is a water-soluble antioxidant vitamin found in many food sources, although almost all animals are capable of synthesizing it by way of the glucuronic acid pathway. Animals that completely rely on dietary sources include humans, primates, guinea pigs, and fruit bats (Combs and Gerald, 1992). Although cattle are generally able to synthesize their own ascorbic acid, active synthesis does not begin until about 3 weeks of age (Combs and Gerald, 1992; Cummins and Brunner, 1991).

Young calves therefore require an exogenous source of ascorbic acid, usually provided by colostrum and milk, to be able to reduce radicals in the aqueous portion of the cell (Black and Hidiroglou, 1996; Bouda et al., 1980). Although deficiency of vitamin C is rare, animals may become deficient during stressful situations or in high production environments (McDowell, 1989).

A decrease in plasma ascorbate levels occurs in calves during infections in either the enteric or respiratory systems (Bouda et al., 1980; Cummins and Brunner, 1991). Stressful conditions alter the rate of endogenous synthesis. In addition, calves that are housed in colder environments are older when endogenous ascorbate synthesis is initiated than calves that are housed in more protected environments (Cummins and Brunner, 1991).

**Absorption**

Species that can synthesize their own ascorbic acid absorb it via passive diffusion through the wall of the small intestine into enterocytes. Species that cannot synthesize ascorbic acid utilize two different methods of absorption through small intestinal membranes.
When dietary intake is low, sodium-dependant active transport is the primary absorptive mechanism. When dietary intake is adequate or high, passive diffusion becomes the primary absorptive mechanism (Combs and Gerald, 1992; McDowell, 1989).

*Functions*

The primary functions of ascorbic acid are based on its reduction and oxidation capabilities (Black and Hidiroglou, 1996; McDowell, 1989; Sato *et al.*, 1990). It is able to scavenge free radicals in aqueous portions of a cell (Niki, 1991; Sato *et al.*, 1990). Once it scavenges a free radical, it becomes oxidized to dehydroascorbate, which is then reduced back to ascorbate by GSH-dehydroascorbate reductase (Niki, 1991). For more review of vitamin C please refer to Appendix B.

*Vitamin C and E interactions*

When vitamin E recycles itself from alpha-tocopheroxyl back to alpha-tocopherol, it requires a reducing agent such as ascorbic acid (Combs and Gerald, 1992; Doha *et al.*, 1985; Ginter *et al.*, 1982; Niki, 1991; Sato *et al.*, 1990). Some research indicates that *in vivo* recycling of vitamin E by vitamin C may not happen due to compartmentalization of vitamin C in the aqueous cytosol and tocopheroxyl in the lipid membrane (Combs and Gerald, 1992; Sato *et al.*, 1990). Others reported that, although compartmentalization exists, alpha-tocopheroxyl radicals are available to vitamin C because the polar alpha-tocopherol head is closer to the membrane surface (Doha *et al.*, 1985; Niki, 1991; Sato *et al.*, 1990). In support of this theory, Niki (1991) reported that the efficiency with which ascorbic acid reduces tocopherol decreases as the tocopheroxyl radical is located deeper in the membrane.

The spatial problems associated with compartmentalization may also be overcome by the glutathione peroxidase system which actively shuttles electrons from soluble phases of
the cell to radicals in cell membranes (Chen and Chang, 1978; Combs and Gerald, 1992). Vitamin C interacts with the glutathione peroxidase system, which interacts synergistically with alpha-tocopherol, thereby sparing alpha-tocopherol (Chen and Chang, 1978).

Vitamin C deficient guinea pigs tend to have low concentrations of alpha-tocopherol in liver, lungs and kidneys resulting from an increased susceptibility of vitamin E to oxidation without the presence of the reducing ability of vitamin C (Hruba et al., 1982). For more review of vitamin E and C interactions please refer to Appendix B.

**Vitamin A and C interactions**

Although interactions between vitamins A and C do exist, there are no interactions during absorption or transport in enterocytes. For other interactions of vitamins A and C please refer to Appendix B.

**Serum protein fraction**

Serum protein fraction consists of elevated levels of growth factors, such as insulin-like growth factors and transforming growth factor-β. These growth factors stimulate enterocyte differentiation and intestinal growth, especially after viral damage. Growth factors play a role in re-establishing the mucosal barrier that is affected by viral infection or damage, increasing primary defense in the gut.

**Insulin-like growth factor-I**

Insulin-like growth factors (IGF) are part of the insulin family of hormones and growth factors, including IGF-I, IGF-II and relaxin. Insulin-like growth factors are heat and acid stable allowing them to be ingested and to reach the small intestine intact (Baumrucker and Blum, 1993).
In the small intestine, IGF-I binds to receptors located throughout in crypt and villus areas, as well as in the muscularis and submucosal layers of the small intestine (Morgan et al., 1996; Young et al., 1990). The IGF-I receptors are located on basolateral and apical membranes of the jejunum (Morgan et al., 1996). Although IGF receptors are located all along the length of the small intestinal, the highest concentration of IGF-I binding is in the jejunum due to highest receptor concentration, a higher concentration of binding proteins or an altered response in the jejunum to IGF-I (Young et al., 1990).

Type I IGF receptors have greater affinity for IGF-I than IGF-II and the least affinity for insulin (Baumrucker et al., 1994; Schober et al., 1990). The affinity of IGF-I for type I receptors is about five times more potent than IGF-II and one thousand times more potent than insulin (Baumrucker et al., 1994). Type II IGF receptors have greater affinity for IGF-II than IGF-I, but do not bind insulin (Baumrucker et al., 1994; Schober et al., 1990).

The presence of IGF-I receptors in differentiated villus cells and proliferating crypt cells may indicate a role for IGF-I in cellular maturation (Morgan et al., 1996). Levels of IGF-I receptors are maintained while concentrations of IGF-II receptors decrease during suckling (Young et al., 1990). When IGF-I is added to the diet, type I receptors are upregulated due to the increased concentration of IGF-I (Baumrucker et al., 1994; Schober et al., 1990). The highest concentration of IGF-I binding to receptors occurs at a pH of 7.8 to 8.2, indicating that the small intestine is a prime site of IGF-I binding (Schober et al., 1990). Decreases in binding are usually due to a decrease in receptor numbers and not a decrease in affinity (Schober et al., 1990). This may be a way for cells to regulate the effects of IGF-I on intestinal epithelium (Schober et al., 1990).
Insulin-like growth factor binding proteins (IGFBP) are present in plasma and milk and function to bind IGF (Clemmons, 1991). Four specific IGFBPs (IGFBP 1, 2, 3, and 4) have been identified in plasma (Clemmons, 1991). The binding protein IGFBP-3 has the greatest affinity to binding IGF-I and is present in the highest concentrations in plasma (Clemmons, 1991). Infusion of IGF-I stimulates an increase in the serum concentration of IGFBP-3, indicating that serum concentrations of IGFBP-3 are responsive to serum concentrations of IGF-I, thus tightly regulating IGF-I availability to peripheral tissues (Clemmons et al., 1989). Any excess IGF-I that is not bound to IGFBP-3 binds to IGFBP-1, IGFBP-2, or IGFBP-4 (Clemmons, 1991).

Binding of IGFs by IGFBPs affects the utilization of IGFs in the neonate. Cells secrete different concentrations of each IGFBP, regulating the amount of specific IGFs that are needed locally (Clemmons, 1991; Philipps et al., 1995). These binding proteins partition IGFs between receptors and the vascular compartment (Clemmons, 1991). Binding of an IGFBP to IGF-I or IGF-II in plasma forms a complex that, although capable of crossing the endothelium, does not pass through capillary membranes. This provides a reservoir of these growth factors for use by peripheral tissues (Clemmons, 1991). It is also possible that once the complex passes through the capillary endothelium, the IGFBPs direct IGFs to specific tissues (Clemmons, 1991).

The IGFBP have a greater affinity for binding IGFs than IGF receptors (Clemmons, 1991). Although IGFBPs bind IGFs, IGFBPs themselves are also able to bind to type I receptors (Clemmons, 1991). Once the complex is bound to a receptor, it is not known whether IGFs are then transferred off the IGFBP or whether they are transferred as a complex through the membrane (Clemmons, 1991).
Neonatal rats fed IGF-I result in hyperplasia of the small intestine, indicated by an increase in crypt length, villus height and number of cells emerging from the crypts, resulting in a new steady-state equilibrium in the small intestinal epithelium (Steeb et al., 1994). This increase in villus cell density increases intestinal surface area which then increases absorptive capacity of the small intestine in neonatal rats (Burrin et al., 1995; Steeb et al., 1994). This improvement in absorptive capacity leads to an improved feed efficiency in rats subcutaneously infused with rhIGF-I compared to control rats (Steeb et al., 1994). The increase in this cell population is also reflected in an increase in tissue weight and protein and RNA content of the duodenum in IGF-treated rats (Burrin et al., 1995; Steeb et al., 1994).

Absorption of IGF-I is low in the small intestine of the neonatal rat (Burrin et al., 1995). This may also be true in other species. Plasma concentrations of IGF-I in calves fed six feedings of colostrum containing high concentrations of IGF-I were not different than calves fed diluted colostrum (Rauprich et al., 2000).

Concentrations of plasma IGF-I were not altered in pigs infected with rotavirus, however, they were affected by malnourishment. Lowered plasma concentrations of IGF-I in malnourished, rotavirus-infected pigs may contribute to slower recovery of the small intestine. Concentrations of intestinal IGF-I binding proteins (IGFBP) are increased following rotavirus challenge in pigs, indicating that IGFBP may help regulate IGF effects on the small intestine (Zijlstra et al., 1997). The mRNA concentration of IGF-I was decreased in protein-depleted rats. When rats were allowed a protein-rich diet, jejunal concentrations of IGF-I increased and IGF-I receptors decreased (Qu et al., 1998).

Rats treated with dexamethasone, which disrupts intestinal morphology, benefit from IGF-I infusion with an increase in gut mass by 60% when compared with rats treated with
dexamethasone but not infused with IGF-I. This indicates the gut is one of the most responsive target tissues for IGF-I due to either increased receptor number, increased receptor sensitivity, or increased delivery of IGF-I from the circulation to the gut. The increase in gut mass increases surface area due to increased villus height and mucosal area, leading to an increase in nutrient uptake even if gut morphology is negatively affected (Read et al., 1992).

**Transforming growth factor-β1 and -β2**

Transforming growth factor-βs (TGF-β) have a molecular mass of 25,000 kDa and consist of polypeptide dimers linked by sulfur bonds (Ruscetti et al., 1998). There are three different genes that encode for TGF-βs in the mammalian genome, producing TGF-β1, TGF-β2, and TGF-β3 (Roche et al., 2000; Ruscetti et al., 1998). Most mature cells are able to produce one of the isoforms of TGF-βs during tissue repair and inflammation (Roche et al., 2000). Cells have three receptor types (I, II, and III) that are capable of binding all three TGF-βs in mammals (Letterio and Roberts, 1998).

Cell adhesiveness is regulated by TGF-β1 through increased expression of extracellular matrix proteins as well as increased cell surface receptors. The increase in matrix protein expression may strengthen tight junctions between cells, maintaining the mucosal barrier in the intestine (Roche et al., 2000). In response to addition of TGF-β into cell culture there is an increase in the incorporation of matrix proteins, such as fibronectin and collagen-related proteins, into cells, changing the extracellular matrix composition. This increase in extracellular proteins occurs in the extracellular matrix, the intracellular matrix and the medium of all cellular compartments, with the largest increase in the extracellular
matrix. The ability of cells to incorporate these proteins is also stimulated by TGF-β (Ignotz and Massague, 1986).

Transforming growth factor-β is a potent inhibitor of cellular proliferation. The addition of TGF-β does not increase [³H]-thymidine incorporation into small intestinal cells in vitro (Ciacci et al., 1993; Dignass and Podolsky, 1993; Kurokowa et al., 1987). However, there are no decreases in cell number when compared to control cells indicating that TGF-β is not cytotoxic (Dignass and Podolsky, 1993; Kurokowa et al., 1987). The presence of growth factors that stimulate cell proliferation, such as IGF-I, insulin or EGF, do not override the inhibition of cell proliferation by TGF-β, indicating that TGF-β affects cell proliferation through the same pathway as other growth factors. The addition of TGF-β either blocks an earlier step in the pathway (Kurokowa et al., 1987) or it may have a higher affinity to the same receptors as other growth factors (Dignass and Podolsky, 1993). Interestingly, the addition of TGF-β to cell culture also stimulates sucrase activity, indicating TGF-β has a role in promoting the differentiation of epithelial cells (Kurokowa et al., 1987).

Detrimental effects of viral challenge with *Cryptosporidium parvum* on colonic cells are ameliorated when the cells are treated prior to viral challenge with TGF-β1. This is not the case with cells that are TGF-β1 treated during or after challenge. Cells pretreated with TGF-β1 maintain their mucosal barrier and cell necrosis that results from *C. parvum* infection is reduced accordingly (Roche et al., 2000).

Any type of epithelial injury causes an upregulation of TGF-β1, stimulating restitution, which is when epithelial cells surrounding the injury migrate across and take the place of injured and sloughed epithelial cells (Ciacci et al., 1993; Dignass and Podolsky,
Although TGF-β1 is secreted as a latent complex, cells produce plasmin-like protease activity that activates TGF-β1 (Dignass and Podolsky, 1993).

Colostrum also contains many growth factors and growth hormones, such as insulin-like growth factors and transforming growth factors. Small intestinal villus development is changed by the prolonged feeding of colostrum for 6 feedings to neonatal calves compared to calves fed colostrum only once or calves fed only milk replacer (Buhler et al., 1998). Hyperplasia of the intestinal epithelium occurs, resulting in a decrease in crypt depth: villus height ratios in calves (Buhler et al., 1998) and rats (Berseth et al., 1983) fed colostrum, indicating an increase in differentiated cells moving up the villus and shedding off.

Xylose absorption is higher in calves fed colostrum for 6 feedings than in calves fed colostrum only once or calves fed only milk replacer, suggesting an increase in absorptive capacity due to increased numbers of differentiated enterocytes and greater intestinal surface area (Buhler et al., 1998).

**Immunoglobulin G**

A method to prevent colonization of bacteria in the small intestine is to block the binding sites and receptors that are available to bacterial lectins by binding the sites with receptor analogues, such as glycoproteins or immunoglobulins (Nollet et al., 1999). Immunoglobulin G (IgG) can be fed even after the neonatal intestine cannot absorb the immunoglobulins for passive immunity, approximately after the calf is 24 hours of age. The IgG is also partially resistant to digestion in the stomach and has the ability to reach and affect the small intestine (Brock et al., 1977). Calves treated orally with immunoglobulins have a less severe response to *E. coli* than calves not treated with immunoglobulins (Quigley
Treatment with immunoglobulins may also benefit calves if infection with other enteric pathogens occurs (Quigley and Drew, 2000).

Calves challenged with cryptosporidium have improved diarrhea and reduced gut permeability when orally fed with bovine serum concentrate, containing concentrated amounts of IgG, IGF-I, and TGF-βI (Hunt et al., 2002). The potential mechanism may be due to the prevention of attachment of cryptosporidial organisms, reducing replication and invasion of the gut (Hunt et al., 2002).

**Serum protein separation**

Blood is collected from cows that passed veterinary inspection before slaughter. Blood is freshly obtained from abattoirs under USDA inspection. Anticoagulant is added to blood and centrifuged to separate plasma and red blood cells. Plasma is defibrinated and resulting plasma is concentrated by ultrafiltration. This plasma is then spray-dried to produce powder containing elevated levels of growth factors and IgG.

**Summary**

The epithelium of the gastrointestinal tract is the first line of defense to disease and infection. The ability to diminish effects of gastrointestinal injury and preserve normal homeostasis is important for health and performance of neonates. Current oral rehydration solutions restore acid-base balance, but with the addition of specific vitamins or growth factors, they may also have the ability to help restore the integrity of the epithelial barrier.

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CHAPTER 2

EFFECTS OF AN ORAL REHYDRATION SOLUTION WITH ADDED FAT-SOLUBLE VITAMINS OR BOVINE SERUM PROTEINS ON SMALL INTESTINAL ABSORPTIVE CAPACITY

A paper, a portion of which is to be submitted to the Journal of Dairy Science


ABSTRACT

Young calves commonly become infected with viruses and bacteria that damage the intestinal lining. Deficiencies in fat-soluble vitamins also occur in young calves and may impair the rate of differentiation of intestinal epithelial tissues, especially following enteric infections. To enhance recovery of small intestinal function following a coronavirus challenge, either fat-soluble vitamins or bovine serum proteins, containing IgG, TGF-β and other growth factors, were added to an oral rehydration solution (ORS) for 32 Holstein and Jersey calves. Calves were housed individually and offered water ad libitum and milk replacer at 10% of BW daily. Treatment one consisted of a commercial ORS (COM) and treatment two consisted of a control ORS (CON). Treatment three consisted of CON with added antioxidants, to provide 70,000 IU of vitamin A and 300 IU of vitamin E per day (VIT). Treatment four consisted of CON with added bovine serum proteins (GFR). After a 2 d adjustment, calves were orally challenged with 5x10^6 plaque-forming units of a moderately virulent bovine coronavirus isolate. Xylose (0.5 g/kg of BW) was administered orally once daily for 6 d and jugular blood was sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 h post-dosing. Hematocrits, fecal dry matter, rectal temperatures, attitude scores and dehydration scores were recorded once daily. Concentrations of serum xylose increased with
time post-dosing, but did not vary by treatment. Hematocrits and other clinical scores were not significantly different ($P > 0.05$) between treatments. In this model, antioxidants or bovine serum proteins did not appear to enhance intestinal recovery from a coronavirus challenge when added to ORS.

**INTRODUCTION**

Neonatal calf diarrhea is a major cause of death and economic loss in the dairy industry (Constable *et al.*, 1996; Torres-Medina *et al.*, 1985). Coronavirus is one of the more common pathogens causing calf scours (McDonough *et al.*, 1994). Pathogens that affect mucosal function of the intestinal tissues, such as coronavirus, destroy villus architecture and decrease absorptive surface area of the small intestine (Bridger *et al.*, 1978; Clark, 1993; Holland *et al.*, 1992; Saif *et al.*, 1986). Loss of epithelial function results in malabsorption of water, sodium, and chloride (Torres-Medina *et al.*, 1985). Continued diarrhea increases the risk of dehydration and hypoglycemia and, if not treated, eventually leads to death (Clark, 1993; Pensaert and Callebaut, 1994).

When calves develop diarrhea, treatment with oral or intravenous fluid therapy may aid in avoiding problems such as metabolic acidosis (Booth and Naylor, 1987; Groutides and Michell, 1990). In addition, oral rehydration solutions (ORS) are formulated to maintain hydration, replace lost electrolytes, and provide an energy source for the diarrheic calf (Nappert *et al.*, 2000).

Some additions to ORS can include specific fat soluble and antioxidant vitamins. Calves may be at particular risk of deficiency in fat-soluble vitamins and are born with low reserves because fat-soluble vitamins do not cross the placental membrane (Branstetter *et al.*, 1973). Deficiencies in fat-soluble vitamins, such as vitamin A, induce impairments of
mucosal defense and defects in the integrity of the small intestinal epithelium (Warden et al., 1997). This may cause an increased systemic exposure to a viral challenge, decreasing the ability of enterocytes to defend themselves (Ahmed et al., 1990; Wang et al., 1994).

Morphology of the small intestine changes during vitamin A deficiency (Uni et al., 1998). Changes such as increased permeability of the small intestine and decreased solute absorption may lead to diarrhea (Filteau et al., 2001). Supplementation of vitamin A to vitamin A deficient children decreases incidence of diarrhea and reduces mortality (Ahmed et al., 1990; Alvarez et al., 1995; Fawzi et al., 1995). Vitamin A supplementation maintains tight junctions in the small intestine of infants born to HIV-infected women, decreasing intestinal permeability and maintaining membrane integrity (Burton et al., 1983; Combs and Gerald, 1992; Filteau et al., 2001; Gonzalez et al., 2001).

In conditions that are characterized by a high production of free radicals, such as inflammatory bowel disease, supplementation with vitamin E (Gonzalez et al., 2001; Srigiridhar and Nair, 2000) decreases oxidative damage to the colon. This may also occur in the small intestine.

Small intestinal epithelium that has been damaged due to pathogens is one of the most sensitive organs to additions of growth factors (Read et al., 1992). Calves challenged with Cryptosporidium parvum have reduced gut permeability when dosed orally with bovine serum concentrate containing elevated amounts of immunoglobulin G (IgG), insulin-like growth factor-I (IGF-I), transforming growth factor-β1 (TGF-β1) and transforming growth factor-β2 (TGF-β2) (Hunt et al., 2002). Rats treated with dexamethasone, which disrupts intestinal morphology, benefit from IGF-I infusion with a 60% increase in gut mass as well as increased intestinal surface area when compared to control rats (Read et al., 1992).
Colonic cells treated with TGF-β1 before challenge with *Cryptosporidium parvum* maintain their mucosal barrier as well as membrane integrity (Roche *et al.*, 2000).

Therefore, the objective of this study was to enhance the rate of recovery of small intestinal absorptive function following a coronavirus challenge by supplementing an oral rehydration solution with either vitamins A and E or a bovine serum fraction containing elevated levels of growth factors.

**MATERIALS AND METHODS**

The trial utilized 32 Jersey and Holstein male calves (3-5 d of age) purchased from sale barns during the month of March 2001. Following arrival, calves were randomized into 8 blocks of 4 calves and weighed before being placed in individual plastic hutches bedded with wood shavings. Four pints of commercial oral rehydration solution (ORS) were fed on arrival. Calves were offered water ad libitum and fed a complete (20% fat and 20% protein) commercial milk replacer at a rate of 5% of bodyweight, twice daily. Feed refusals were monitored and recorded. No hay or starter was offered.

**Treatments**

Treatments began approximately 12 h after viral challenge, and were provided at 10% of initial bodyweight, twice daily, for 3 d. Treatment one (COM) calves were fed a commercial oral rehydration solution (Table 1). Treatment two (CON) calves were fed an oral rehydration solution formulated to include 4g/lb of ascorbic acid (Table 2). Treatment three (VIT) calves were fed CON with the addition of 70,000 IU of retinyl acetate and 300 IU of d-α-tocopherol acetate per day. Treatment four (GFR) calves were fed CON with the addition of a serum protein fraction consisting of elevated levels of IgG, IGF-I, TGF-β1 and
TGF-β2 (Table 3). Batches were mixed daily by treatment order to minimize risk of contamination.

Table 2. Ingredient composition of commercial oral rehydration solution\(^1\).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>145.87</td>
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<td>Chloride</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Sodium Bicarbonate</td>
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</tr>
<tr>
<td>Citric Acid</td>
<td>3.69</td>
</tr>
</tbody>
</table>

\(^1\) Merrick’s, Inc. Blue Ribbon Electrolyte Formula, Union Center, WI

Table 3. Ingredient composition of control oral rehydration solution\(^2\).

<table>
<thead>
<tr>
<th>Ingredient</th>
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<td>Calcium Lactate</td>
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<tr>
<td>Ascorbic Acid</td>
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</table>

\(^2\) Merrick’s, Inc., Union Center, WI
Table 4. Ingredient composition of plasma serum fraction with elevated levels of growth factors$^2$.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>Total protein</td>
<td>93.24%</td>
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<tr>
<td>Albumin</td>
<td>56.64%</td>
</tr>
<tr>
<td>IgG</td>
<td>19.70%</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.36%</td>
</tr>
<tr>
<td>Ash</td>
<td>0.61%</td>
</tr>
<tr>
<td>Standard Platelet Count</td>
<td>600 CFU/g</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>0.311 EU/g</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>765 ng/g</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>9 ng/g</td>
</tr>
<tr>
<td>IGF-1</td>
<td>1368 ng/g</td>
</tr>
<tr>
<td>Salmonella</td>
<td>negative</td>
</tr>
</tbody>
</table>

$^2$ APC, Inc. Ames, IA.

Scoring, blood sampling and analysis

Fecal samples were obtained and analyzed daily for dry matter content. Starting on the second day, xylose was fed orally at 0.5 g/kg of BW. Jugular blood samples were obtained prior to xylose feeding to provide baseline values at time 0 and then after xylose feeding at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h. An additional 3 cc sample of venous blood was obtained into a heparinized tube at time 0 during collection days and immediately analyzed for hematocrit. Serum was separated from the sample by centrifugation at 3600 x g for 15 minutes, collected, and frozen at -20 F for later analysis of xylose and haptoglobin concentrations. Xylose concentrations were determined spectrophotometrically by the method described by Eberts et al. (1979). Haptoglobin was determined spectrophotometrically using a commercial kit (TriDelta LTD., Morris Plains). Xylose was dosed and serial blood samples collected on d 1, 2, 3, 4, 5, and 6. Calves were challenged orally with bovine coronavirus ($5 \times 10^6$ plaque forming units of a moderately virulent...
coronavirus isolate) following the 4 h blood collection period on d 2. Treatments were provided on d 3, 4, and 5.

All performance scores were evaluated and recorded daily by a single person; these scores included fecal scores on a four-point scale (1=firm, 2=soft, 3=runny, 4=watery), attitude scores on a four-point scale (1=alert, 2=slightly depressed, 3=depressed, 4=laterally recumbent), dehydration scores using skin tent time based on a three-point scale (1=<1s, 2=2-3s, 3=>3s), and a score based on rectal temperature (1=100-102.5, 2=<99.9, 3=>102.6).

Statistical analysis

Analysis of variance was performed using the mixed procedures of SAS 8.1 (SAS, 1999) and a model for a completely randomized design. The xylose data were log transformed and sorted by treatment. Mean values of data from days 1 and 2 prior to viral challenge were used as a covariate in the model. Least squares means were used to evaluate treatment differences for xylose concentrations, performance parameters and haptoglobin values. Significance was declared at $P < 0.05$ and trends at $P < 0.15$. Treatment means were compared using the method of least significant differences.

RESULTS

Xylose concentrations

Treatment values for xylose concentrations in all figures represent covariate-adjusted least squares means. Changes in xylose concentrations (mg/dl) for calves grouped by treatments over time are shown in Figure 1. Changes in xylose concentrations (mg/dl) for calves grouped by day are shown in Figure 2. There were no significant differences ($P > 0.05$) in xylose concentrations between treatment groups for any day post-viral challenge.
There were time effects evident for xylose concentrations within each day ($P < 0.05$). Figure 3 shows changes in xylose concentrations (mg/dl) over sampling times for d 3, the first day of ORS treatment for the various treatment groups. Although xylose concentrations increased over time for all treatment groups, xylose concentrations for GFR tended to be higher at 120 and 210 minutes when compared to CON ($P < 0.15$). Figure 4 shows changes in xylose concentrations (mg/dl) over sampling times for d 4, the second day of ORS treatment for the various treatment groups. Xylose concentrations again increased in all treatment groups over time. Xylose concentrations tended to be higher for GFR than VIT at 90 and 150 minutes ($P < 0.15$), and for GFR than CON at 120 and 210 minutes ($P < 0.15$). Figure 5 shows changes in xylose concentrations (mg/dl) over sampling time for d 5, the last day of ORS treatment for the various treatment groups. Xylose concentrations increased in all treatment groups over time. Xylose concentrations tended to be higher for GFR than CON at 180 and 210 minutes ($P < 0.16$). Figure 6 shows changes in xylose concentrations (mg/dl) over sampling time for d 6.

Although there were no significant differences between any treatment groups, peak xylose concentrations tended to increase over time for CON and VIT treatment groups ($P < 0.15$). First day post-challenge peak concentrations of xylose tended to be higher for CON calves than peak xylose concentrations of 3 consecutive days pre-challenge. Peak xylose concentrations for treatment CON for the second and third day post-challenge tended to be higher than peak xylose concentrations for the first day post-challenge. For VIT treatment calves, peak xylose concentrations for two and three days pre-challenge and one day post-challenge tended to be higher than peak concentrations of xylose for three days pre-challenge (first day of the trial).
Figure 1. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Figure 2. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Figure 3. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations for the first day of challenge and first day of electrolyte treatment. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Figure 4. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations for the second day post-challenge and second day of ORS treatment. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Figure 5. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations for the third day post-challenge and third day of ORS treatment. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Figure 6. Least squares means (using baseline values as a covariate) of xylose concentrations for calves receiving different electrolyte formulations for the fourth day post-challenge and no ORS treatment. A, commercial; B, control; C, added antioxidants; D, added serum protein fraction.
Performance parameters

Least squares means of fecal scores are shown in Figure 7. There were no significant differences between treatment groups, although all treatment groups increased numerically on day 2, before viral challenge. Least squares means of fecal dry matter scores are shown in Figure 8. There were no significant differences between treatment groups, although dry matter was numerically lower for all treatment groups on day 2, correlating to the observed increase in scour scores. Least squares means of dehydration scores are shown in Figure 9. There were no significant differences in dehydration scores between treatments, although dehydration scores for all treatment groups were numerically higher on day 2, correlating to both the increase in fecal scores and decrease in fecal dry matter. Least squares means of attitude scores are shown in Figure 10. There were no significant differences in attitude scores between treatment groups. Attitude scores numerically increased on day 2, indicating a decline in attitude, corresponding to an increase in fecal scores and dehydration that day. Least squares means for temperature are shown in Figure 11. There were no significant differences in temperature between treatment groups. Least squares means for hematocrit are shown in Figure 12. There were no significant differences in hematocrit between treatment groups. In addition, there were also no significant differences in feed refusals between treatments (data not shown).

Haptoglobin concentrations

Least squares means of haptoglobin concentration (mg/ml) are shown in Figure 13 for day 2, one day pre-challenge, day 4, one day post-challenge and day 6, three days post-challenge. There were no significant differences in haptoglobin concentrations between treatments for any time periods measured.
Figure 7. Least squares means of fecal scores for calves receiving different ORS formulations throughout sampling days. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 8. Least squares means of fecal dry matter throughout sampling days for calves receiving different ORS formulations. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 9. Least squares means of dehydration scores throughout sampling days for calves receiving different ORS formulations. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 10. Least squares means of attitude scores throughout sampling days for calves receiving different ORS formulations. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 11. Least squares means of temperature values throughout sampling days for calves receiving different ORS formulations. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 12. Least squares means of hematocrit values throughout sampling days for calves receiving different ORS formulations. A, Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
Figure 13. Least squares means of haptoglobin values throughout sampling days for calves receiving different ORS formulations. Haptoglobin was analyzed on d 2 prechallenge, d 4, one day post-challenge, and d 6, 3 days post-challenge. Commercial; B, Control; C, Added antioxidants; D, Added serum fraction.
DISCUSSION

In the present study, there were no significant differences in xylose uptake from the small intestine between treatment groups. Mean peak xylose concentrations in the current study ranged from 23 to 40 mg/dl throughout all sampling times and numerically increased following treatments post-challenge, indicating some improvement in gut function. In other studies, xylose concentrations in calves with intestinal damage peaks at 45 to 75 mg/dl (Holland, 1986; Nappert et al., 1993). In healthy dogs, peak xylose concentrations range from 45 to 75 mg/dl. Peak xylose concentrations in dogs suffering from bacterial overgrowth in the small intestine are 30 mg/dl (Kirk, 1986). Peak xylose concentrations in the present study may indicate the presence of bacterial overgrowth in the small intestine, suggesting these calves were infected with multiple pathogens prior to coronavirus challenge. Necropsy of one calf revealed the presence of multiple pathogens, including coronavirus, E. coli, salmonella and clostridium. Salmonella did not cause significant infection as there was no increase in rectal temperature. Another indicator of pathogen overload was a calf mortality rate of 24% in this study. Although calves were challenged with coronavirus after arrival, they were purchased from sale barns and exposed to many pathogens prior to arrival; infection with multiple pathogens may have severely disrupted the absorptive and secretory mechanisms of the intestinal tract in ways that were not compatible with the design of the study.

Decreased intestinal absorption and prolonged diarrhea in coronavirus challenged calves occurs as cells are sloughed off and replaced with immature cells unable to perform normal functions. These immature cells are redirected due to coronaviral infection to function only for viral production (Clark, 1993; Holland et al., 1992; Mebus et al., 1975).
Insulin-like growth factor-I receptors are present in differentiated villus cells and proliferating crypt cells (Morgan et al., 1996). Differentiated villus cells also contain TGF-β receptors on their surface (Letterio and Roberts, 1998). Decreases in binding of growth factors are usually due to a decrease in number of receptors and not a decrease in receptor affinity (Schober et al., 1990). Enterocytes that are undifferentiated due to pathogen overload may have decreased numbers of IGF-I and TGF-β receptors. Due to decreased receptor concentrations, growth factor binding would also be decreased, thus anticipated effects on maturation or proliferation of enterocytes would be decreased as well.

Additionally, differences between treatments may have been more evident if calves were pretreated with concentrated serum protein fraction. Roche et al. (2000) reported that effects of viral challenge with Cryptosporidium parvum were ameliorated when colonic cells were pre-treated before viral challenge with TGF-β1 versus cells that were treated with TGF-β1 during or after challenge. Cells pretreated with TGF-β1 were not as permeable as control cells and were able to better maintain their mucosal barrier (Roche et al., 2000).

Serum protein fraction used in the present study included elevated concentrations of IGF-I (1368 ng/ml), TGF-β1 (765 ng/ml), TGF-β2 (9 ng/ml) and IgG (19.7%) fractions. Levels of IGF-I found in first-milking colostrum range from 248 to 1850 ng/ml, while TFG-β1 concentrations range from 12.4 to 42.6 ng/ml (Ginjala and Pakkanen, 1998). The serum protein fractions were fed at 1% of BW, with total concentration of TGF-β1, for example, fed at .2754 mg versus TGF-β1 in colostrum fed at birth at .015336 mg for a calf with an average bodyweight of 36 kg. Quigley and Drew (2000) reported calves that were treated orally with immunoglobulins had a less severe response to E. coli than calves not treated with immunoglobulins. Immunoglobulin G may defend the intestine by preventing the attachment
of organisms, reducing their ability to replicate and invade the gut. Treatment with immunoglobulins may also affect calves favorably if infection with other enteric pathogens occurs (Quigley and Drew, 2000).

In addition, other researchers have reported that calves fed bovine serum concentrate containing concentrated amounts of IgG, IGF-I, and TGF-β1 have reduced diarrhea and gut permeability when challenged with cryptosporidium. This may be due to a decreased concentration of cryptosporidial organisms able to attach to the small intestinal wall, thereby reducing replication of the virus and enterocytes infection (Hunt et al., 2002).

In the present study, responses to treatments were not significantly different when vitamins were supplemented. Once again, calves may not have been able to absorb the supplemented vitamins due to extensive epithelial damage caused by infection with multiple pathogens. Holland et al. (1992) reported that calves challenged with Cryptosporidium parvum have decreased retinol uptake due to increases in the number of undifferentiated enterocytes. These immature enterocytes may not be able to synthesize adequate numbers of chylomicrons or they may not be able to esterify retinol to the same degree as differentiated enterocytes (Holland et al., 1992).

The optimal ratio of supplementation of vitamins A and E has not yet been determined. High dietary vitamin A decreases plasma concentrations of alpha-tocopherol (Bieri and Tolliver, 1982; Franklin et al., 1998) as well as liver alpha-tocopherol levels (Combs, 1976; Dicks et al., 1959; Eicher et al., 1997; Sklan and Donoghue, 1982). In the small intestine, high concentrations of fat-soluble vitamins compete for hydrolysis by brush border enzymes (Abawi et al., 1985; Franklin et al., 1998). Once in enterocytes, fat-soluble vitamins compete for transfer by fat-soluble carriers (Eicher et al., 1997). In the present
study, the ratio of supplemented fat-soluble vitamins may have not allowed for optimal absorption and transport of vitamins to tissues under the damaged conditions present in the gut.

Although no significant differences were seen between treatments in the present study, many unforeseen factors may have affected these data. Further research needs to be conducted to clarify vitamin supplementation ratios as well as effects of growth factor in response to controlled damage to intestinal epithelium.

**LITERATURE CITED**


CHAPTER 3
EFFECTS OF RUMINAL INFUSION OF ELECTROLYTE SOLUTIONS ON Calf PERFORMANCE

A paper, a portion of which is to be submitted to the Journal of Dairy Science

S. I. Wawrzyniak, H.D. Tyler, J.D. Quigley, III

ABSTRACT

To treat dehydration, diarrheic calves are often ruminally drenched with electrolyte solutions. To determine the effects of these solutions on feed intake and average daily gain, 43 bull calves were ruminally infused with either a commercial electrolyte solution or a 9% saline solution at the rate of 10% of body weight per day for three consecutive days. Fifteen control calves were sham-infused by inserting the esophageal feeder down the esophagus without infusing any fluids. Calf starter and water were offered ad libitum and starter intake was recorded daily. Body weights were determined at day -1 (pre-infusion) and day 8 (post-infusion). Feed intake was significantly lower in calves infused with fluids than in control calves ($P < .007$). Average daily gain was significantly lower in calves infused with fluids than in control calves ($P < .002$). Feed intake ($P < .023$) and average daily gain ($P < .007$) were decreased in calves infused with electrolytes as compared to calves infused with saline solution or control calves. Overall, the infusion of either electrolytes or saline solution into the rumen was detrimental to calf performance.

INTRODUCTION

Although calves are born as functional nonruminants, microbial populations are quickly established and nutritional strategies become dependent on the efficiency of rumen
fermentation. When treating calf scours and dehydration using oral rehydration solutions (ORS), a common technique is to use an orogastric tube. However, this approach delivers ORS directly into the rumen environment. Unfortunately, there is little research on the effects of ruminally infusing calves with large amounts of glucose and electrolytes.

Giduck et al. (1988) reported that infusion of glucose into the rumen of sheep resulted in an increase in volatile fatty acids and a decrease in the pH of rumen contents. Although rumen contents are well buffered, large quantities of starch added into the rumen overwhelm the buffering system, decreasing pH and reducing feed intake (Russell and Hino, 1985). A decrease in pH due to the addition of large amounts of starch increases lactic acid-producing bacteria, such as Streptococcus bovis, increasing lactate production and further decreasing pH. Infusion of large quantities of glucose into the rumen contents also results in extreme changes in microbial populations, quickly leading to the death of the animal (Hungate et al., 1952).

In addition, ORS contain elevated concentrations of sodium chloride, which may induce changes in rumen osmolality. Added amounts of sodium chloride need to be evaluated because changes in rumen osmolality decrease voluntary feed consumption when osmolality increases over 400 mOsm/kg (Bergen, 1972). The influx of large quantities of fluid into the rumen also increases the rate of outflow of organic matter from the rumen, decreasing efficiency of microbial digestion (Godwin and Williams, 1986).

There are no published data on changes in feed intake of calves due to ruminal infusion of commercial oral rehydration solutions (ORS) containing high concentrations of glucose and electrolytes. The purpose of this study was to evaluate the effects of infusing
commercial ORS directly into the rumen on feed intake and body weight gain of young calves.

MATERIALS AND METHODS

Forty three male Holstein calves between 8 and 10 weeks were housed in individual plastic hutches. Calves were offered water and grain starter ad libitum and feed intake was monitored starting -3 days before the start of the treatments through 7 days after initiation of treatments. Calves were weighed on day 1 and day 10.

Treatments

For calves in the control group (n=15) orogastric tubes were inserted for 5 seconds and withdrawn. Calves in the saline group (n=15) were infused with a standard 9% NaCl solution and calves in the electrolyte group (n=13) were infused with a commercial electrolyte solution. All calves in the latter two groups were infused with their respective treatments at the rate of 10% of BW for 3 days. For the first treatment day, they received treatments at the rate of 10% of BW in the morning only, the second treatment day they received treatments at the rate of 5% of BW in the morning and the rate of 5% of BW at night and the third treatment day they received treatments at the rate of 5% of BW in the morning and the rate of 5% of BW at night.
Table 5. Ingredient composition of commercial oral rehydration solution

<table>
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<tr>
<th>Ingredient</th>
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<td>Citric Acid</td>
<td>3.69</td>
</tr>
</tbody>
</table>

1 Merrick’s, Inc. Blue Ribbon Electrolyte Formula, Union Center, WI

Statistical analysis

Analysis of variance was performed using the mixed procedures of SAS 8.1 (SAS, 1999) using a model for a completely randomized design. Mean values of feed intake from days -1, -2 and -3 pre-infusion were used as a covariate in the model. Calf weight from day -1 pre-infusion was also used as a covariate in the model. The error term in the model was calf within treatment. Orthogonal contrasts were used to compare differences between calves sham-infused and calves infused with saline, calves sham-infused and calves infused with ORS and calves infused with saline and calves infused with ORS. Treatment means were compared using the method of least squares means in the general linear model procedure of SAS 8.1 (SAS, 1999). The error term in the model was calf within treatment.

RESULTS

Least squares means for feed intake for all groups are shown in Figure 14. Calves infused with fluids had lower feed intake than control calves \((P < 0.007)\) during infusion and post-infusion. Calves infused with electrolytes had lower feed intake than calves infused with saline or control calves \((P < 0.023)\) during infusion and post-infusion.
Figure 14. Least squares means for calf starter intake. Performance parameters were measured pre-infusion on days -3, -2, and -1. Infusion of calves with respective treatments occurred from day 0 to 2. Performance parameters were measured post-infusion from day 3 to 6.
Figure 15. Least squares means of calf average daily gain (kg) from days -3 to 6. Performance parameters were measured pre-infusion on days -3, -2, and -1. Infusion of calves with respective treatments occurred from day 0 to 2. Performance parameters were measured post-infusion from day 3 to 6.
Figure 16. Least squares means of feed efficiency (kilograms of calf starter: kilograms of gain). Performance parameters were measured pre-infusion on days -3, -2, and -1. Infusion of calves with respective treatments occurred from day 0 to 2. Performance parameters were measured post-infusion from day 3 to 6.
Least squares means for average daily gain for all groups are shown in Figure 15. Calves infused with fluids had lower average daily gain than control calves during infusion and post-infusion (\(P < 0.002\)). Calves infused with electrolytes had lower average daily gain than calves infused with saline or control calves during infusion and post-infusion (\(P < 0.007\)).

Results for feed efficiency for all groups are shown in Figure 16. Calves infused with fluids had higher feed to gain ratio than control calves, indicating lower feed efficiency for these calves than for calves not infused with fluids during infusion and post-infusion (\(P < 0.011\)). Calves infused with electrolytes had higher feed to gain ratio than calves infused with saline or control calves, indicating lower feed efficiency during infusion and post-infusion (\(P < 0.0024\)). However, feed efficiency for calves infused with saline was not significantly different from feed efficiency for control calves during infusion and post-infusion (\(P > 0.05\)).

**DISCUSSION**

Infusion of large amounts of fermentable carbohydrates into the rumen of an adult animal increases lactic acid production and decreases rumen pH (Eadie et al., 1967; Giduck et al., 1988; Irwin et al., 1972). In the present trial, calves infused with fluids into the rumen had lower feed intake than control calves that were sham infused. Feed intake was even further decreased in calves infused with electrolytes compared to calves infused with saline or control calves.

In the present study, calves infused with a standard electrolyte solution containing 2000 mmol/L glucose received a large amount of highly fermentable carbohydrate in a single dose. It is well documented that abrupt additions of large amounts of concentrates to diets of
ruminants results in acidosis. Giduck et al. (1988) reported that infusing whethers with 40% (w/v) glucose resulted in a drop in rumen pH, which then normalized within two hours. This decrease in pH is due to an increase in the production of lactic acid, which accumulates when its rate of production exceeds its rate of metabolism (Giduck et al., 1988; Slyter, 1976). This causes a shift in microbial populations in the rumen, inhibiting microbial cellulose-degrading activity and decreasing feed intake (Irwin et al., 1972). An increase in concentrations of starch-digesting microbes, as well as high amounts of concentrates fed, can lead to sub-acute or acute acidosis. Acidosis, defined as a digestive disturbance, leads to reduced performance of the animal (Eadie et al., 1967; Slyter, 1976).

In the present study, average daily gain decreased in calves that were infused with fluids as compared to control calves, possibly due to sub-acute acidosis. This decrease in weight gain may be a result of a lower digestibility of feed in conjunction with a decreased feed intake. In sheep, ruminal infusion of 30 g NaCl per day resulted in an increased osmotic pressure, decreasing dry matter digestibility, nitrogen digestibility, and rumen ammonia (Bergen, 1972; Godwin and Williams, 1986).

In conclusion, infusing ORS into the rumen of calves may induce adverse effects on calf performance, even in healthy calves. Average daily gain was reduced in calves infused with fluids as a result of decreased feed intake and reduced feed efficiency. Fluid infusion in this trial may have negatively affected rumen microbial populations and increased production of acids, adversely affecting rumen environment. Further investigation into both short and long term effects of ruminal infusion of fluids in young calves is warranted.
LITERATURE CITED


CHAPTER 4

GENERAL CONCLUSIONS

This research represents an effort to further the knowledge and understanding of oral rehydration solutions and their effects on neonatal calves. Neonatal calf diarrhea is a major cause of death and economic loss from calves in the dairy industry (Constable et al., 1996; Torres-Medina et al., 1985). Once a calf develops diarrhea, it is often treated with an oral rehydration solution, to increase hydration and replace extracellular fluid volume (Clark, 1993; Groutides and Michell, 1990). An effective oral rehydration solution should reduce dehydration, metabolic acidosis and hyperkalaemia (Groutides and Michell, 1990).

Coronavirus is one of the most common pathogens that causes calf scours, along with cryptosporidium and rotavirus (McDonough et al., 1994). Anywhere from 15 to 70% of diarrheal outbreaks in calves may be due to coronavirus (Crouch et al., 1985). A viral challenge may cause the development of nutritional deficiencies and, in turn, accentuate the effects of the disease by suppressing immune response and resistance (Wang et al., 1994). Calves challenged with viral disease benefit from the supplementation of vitamins and serum proteins (Arthington et al., 2002; Reddy et al., 1985).

In the current study supplementation of vitamins or serum proteins into oral rehydration solutions had no effect on return of intestinal function or performance parameters in neonatal calves. Young pigs show an improvement in small intestinal absorption when fed a serum fraction during challenge with disease-causing pathogens (Cain and Zimmerman, 1997). During E. coli challenge, oral administration of immunoglobulins protects the calf against harmful effects (Quigley and Drew, 2000) and may also protect during infection with bovine coronavirus. Tight junctions are maintained in the small intestine with vitamin A
supplementation in infants born to HIV-infected women, decreasing intestinal permeability and maintaining membrane integrity (Burton et al., 1983; Combs and Gerald, 1992; Filteau et al., 2001; Gonzalez et al., 2001).

Although no significant differences were seen between treatments in the present study, many unforeseen factors may have affected these results. Further research needs to be conducted to clarify vitamin supplementation ratios as well as effects of growth factor in damaged intestinal epithelium.

When infusing calves with either an oral rehydration solution or saline solution into the rumen, calf performance is decreased and this practice should be avoided if possible. Although esophageal feeding may be a necessary treatment in extreme cases, all calves still capable of suckling should be bottle-fed to avoid a decline in rumen function that may adversely affect feed intake and average daily gain.

In conclusion, formulations of oral rehydration solutions need to be developed that not only correct acid-base balance in the body but repair gut damage in infected calves. These oral rehydration solution formulations need to have less adverse effects when ruminally infused as not to further deteriorate the performance and health of infected calves.

LITERATURE CITED


APPENDIX A

XYLOSE AND HAPTOGLOBIN METHODOLOGY

Protocol for assay of haptoglobin concentrations

1. Label 5 tubes C1, C2, C3, C4, and C5. These correspond to haptoglobin standards 2, 1, 0.5, 0.25 and 0 mg/ml.
2. C1: Add 50 µL of stock (volume calibrator)
3. C2: Add 50 µL of stock (volume calibrator) and 50 µL of volume diluent
4. C3: Add 50 µL of C2 and 50 µL of volume diluent
5. C4: Add 50 µL of C3 and 50 µL of volume diluent
6. C5: Add 50 µL of volume diluent
7. Reagent 1: Premix equal volumes of hemoglobin and hemoglobin diluent (half of each)
8. Reagent 2: Mix 7.2 ml chromogen and 4.0 ml substrate
9. Transfer 7.5 µL of C1, C2, C3, C4, and C5 to plate
10. Transfer 7.5 µL of calf specimens, in duplicate, to plate
11. Add 100 µL of Reagent 1 to each microwell with multi-channel pipette
12. Tap gently to mix
13. Add 140 µL of Reagent 2 to each microwell with multi-channel pipette
14. Incubate for 5 minutes at room temperature
15. Read IMMEDIATELY at 630 nm.
16. Generate a curve.

Protocol for assay of xylose concentrations

1. Place 50 µL of serum in 16 x 100mm disposable test tube
2. Add 5 mL of phloroglucinol color reagent (consisting of 0.5 g phloroglucinol, 100 mL glacial acetic acid, and 10 mL concentrated hydrochloric acid)
3. Heat all tubes exactly 4 min at 100°C, and cool to room temperature in cool water
4. Adjust spectrophotometer to zero absorbance with a reagent blank containing water (50µL) and phloroglucinol reagent (5.0 mL) before reading standard solutions, and with a serum blank (50 µL of xylose-free serum plus 5.0 mL of phloroglucinol reagent) before reading absorbance of serum samples. These “blank” solutions are heated and cooled along with the other solutions before reading absorbance. A single serum blank is used for the entire analytical run.
5. After mixing each serum sample, read absorbance at 554 nm.
Appendix B
ADDITIONAL REVIEW INFORMATION OF SPECIFIC VITAMINS

Vitamin A

Vitamin A includes many compounds that display retinoid activity. These are hydrophobic and insoluble in aqueous environments, such as intestinal lumen, plasma, interstitial fluid, and cytosol, and soluble in alcohols and fats. Although vitamin A is most widely known for its function in visual processes, it has many other systemic functions in the body (Combs and Gerald, 1992).

Clinical signs of vitamin A deficiency consist of keratinization of epithelial membranes leading to rough, scaly skin (Combs and Gerald, 1992; Rojanapo et al., 1980; Uni et al., 2000; Uni et al., 1998). Other clinical signs include xerophthalmia, weight loss (Combs and Gerald, 1992; Zile et al., 1977) and disturbances in the central nervous system (Zile et al., 1977). These clinical symptoms may be caused by lesions and morphological changes at the cellular level (West et al., 1992; Zile et al., 1977).

Retinol is secreted from enterocytes into hydrophobic cores of chylomicron particles (Combs and Gerald, 1992). There, retinol is transported to the liver as retinyl esters, attached to fatty acids that were present during absorption (Leeson and Summers, 2001). These chylomicra are hydrolyzed, causing them to become chylomicron remnants, retain all the retinyl esters and, as a result, have a higher concentration of vitamin A than chylomicra themselves. The retinyl esters are transported through the lymphatic system and enter the plasma compartment through the thoracic duct (Combs and Gerald, 1992). Once at the liver, chylomicron remnants are taken up by parenchymal cells through receptor-mediated endocytosis (Combs and Gerald, 1992; Matsuura et al., 1997).
When chylomicron remnants enter the liver they are degraded by lysosomal enzymes and retinol is re-esterified with long chain fatty acids to retinyl esters in the parenchymal cells (Combs and Gerald, 1992; Matsuura et al., 1997). This re-esterification in the liver is accomplished by retinol binding to cellular retinol-binding protein type II [CRBP(II)] and being catalyzed by lecithin:retinol acyl-transferase (LRAT) (Combs and Gerald, 1992; MacDonald and Ong, 1988). When retinal binds to CRBP(II), forming retinal-CRBP(II) complex, it is reduced by retinal reductase to retinol. When retinol binds to CRBP(II) forming retinol-CRBP(II) complex, it is reduced by LRAT to retinyl esters (Herr et al., 1993). This binding protein serves to increase the efficiency of vitamin A absorption by shuttling retinoids to their respective enzymes (Herr et al., 1993; MacDonald and Ong, 1988).

Retinyl esters are transferred to stellate cells where they are stored; thus, the liver contains more than 90% of the total vitamin A stores in the body (Combs and Gerald, 1992; Matsuura et al., 1997). Stellate cells are nonparenchymal cells that are in the perisinusoidal space and include fat-storing cells, Ito cells, and lipocytes (Blomhoff et al., 1982; Matsuura et al., 1997).

Retinyl ester hydrolase mobilizes retinyl esters from hepatic cells (Combs and Gerald, 1992). Once mobilized in the liver, plasma retinol binding protein (RBP) transports retinol to peripheral tissues (Alvarez et al., 1995; Combs and Gerald, 1992; Nonnecke et al., 2001). Retinol binding proteins are secreted from the liver attached to retinol, forming complexes that attach to transthyretin (formerly known as prealbumin) in the plasma. This attachment to transthyretin decreases the chances of retinol being filtered out and catabolized in the kidney (Alvarez et al., 1995; Combs and Gerald, 1992; Nonnecke et al., 2001).
Secretion of this transporter is regulated by several factors, including estrogen levels, vitamin A intake, vitamin A hepatic stores, and zinc status (Combs and Gerald, 1992; Nonnecke et al., 2001). If animals become vitamin A deficient, retinol-binding protein (RBP) production is reduced, decreasing levels of retinol in the bloodstream (Combs and Gerald, 1992).

Once retinol enters target cells, it is bound by cellular retinol-binding protein (CRBP), which transports retinol within and between cells and allows the hydrophobic moiety to be presented to enzymes (Combs and Gerald, 1992).

Storage

There is an initial rapid uptake of retinyl esters from blood into parenchymal cells of the liver. Some of the retinyl esters are directed back through the blood to other nonparenchymal cells that include adipocytes, lipocytes, perisinusoidal stellate cells, Ito cells, and vitamin-A storing cells (Blomhoff et al., 1982).

Studies in rats show stores of up to 300 nmol of retinol in extravascular and extrahepatic tissues when liver retinol is depleted (Green and Green, 1994). This is confirmed by the presence of LRAT in nonhepatic tissues, which can convert retinol to retinyl esters for storage in extrahepatic tissues, such as the intestinal wall (Herr et al., 1993). Cellular retinol binding protein is also found in the intestinal wall and serves as a carrier of retinol and retinal for LRAT (Herr et al., 1993). This may help explain why no differences are seen in some studies comparing vitamin A deficient and vitamin A sufficient groups, if stores of retinol are present in extrahepatic tissues (Warden et al., 1997). Deficient animals tend to conserve their extrahepatic stores, using them only when hepatic stores are low (Green and Green, 1994).
When there is a stressor introduced, such as Newcastle disease virus in chickens, plasma concentrations of retinol decrease dramatically not only because of an impairment of absorption from the intestine, but also because of an increased rate of utilization by the tissues (West et al., 1992).

Literature Cited


**Vitamin E**

Vitamin E is the primary antioxidant capable of breaking the chain of lipid peroxidation in plasma and red blood cells (Burton et al., 1983). During transport of compounds that are easily oxidized, such as polyunsaturated fatty acids (PUFAs) or cholesterol, vitamin E inhibits oxidation, keeping fatty acids viable (Traber and Kayden, 1984). To effectively reduce radicals, vitamin E also maintains the glutathione antioxidant system. This system is not limited to lipid membranes and therefore allows alpha-tocopherol to reduce radicals in aqueous portions of cells (Gonzalez et al., 2001).

A single free radical in one PUFA can begin a chain reaction affecting other PUFAs until the integrity of the entire membrane is compromised, as seen during the oxidation of low-density lipoproteins (LDL) (Combs and Gerald, 1992; Sato et al., 1990).

By donating a hydrogen off the phenol group located on the phytic tail of alpha-tocopherol, unpaired electrons are stabilized and peroxidative damage is terminated.
This results in the production of alpha-tocopheroxyl, a radical that is stable and much safer than membrane free radicals. Donation of a second hydrogen from alpha-tocopheroxyl will form alpha-tocopherylquinone. This second step is irreversible causing this vitamin E metabolite to be excreted in urine or feces (Combs and Gerald, 1992).

When alpha-tocopherol-rich lipoproteins are incubated with other lipoproteins that are devoid of alpha-tocopherol, there is transfer and equilibration of the vitamin (Massey, 1984). However, Clevidence and Lehmann (1989) reported that the distribution of alphatocopherol among lipoproteins was not solely based on nonspecific diffusion. High-density lipoproteins (HDL) have a high affinity for alpha-tocopherol, indicating that diffusion of alpha-tocopherol between lipoproteins may be regulated (Clevidence and Lehmann, 1989). Using affinity chromatography, Clevidence and Lehmann (1989) showed that most alpha-tocopherol that is bound to HDL remained bound even when low-density lipoprotein (LDL) concentration was increased to ten times that of HDL concentration. This may provide an advantage for tissues that have a high utilization and storage requirement for alpha-tocopherol and also have a high affinity to HDL. Although alpha-tocopherol reaches many tissues through LDL receptor pathways, other tissues receive alpha-tocopherol because of their affinity for HDL (Clevidence and Lehmann, 1989). Certain peripheral tissues may have a high affinity for HDL because of its size and ability to move easily from plasma to tissues (Clevidence and Lehmann, 1989).

Once alpha-tocopherol is delivered to the liver, human plasma lipid transfer protein was found to accelerate the transfer between lipoprotein classes, especially HDL and cell membranes (Kostner et al., 1995). About 90% of alpha-tocopherol is secreted from the liver in VLDL; when VLDL secretion is inhibited, plasma alpha-tocopherol is decreased
(Bjorneboe et al., 1987; Cohn et al., 1988). Less than 1% of alpha-tocopherol is secreted in HDL from hepatic stores (Bjorneboe et al., 1987). Because LDL are precursors to VLDL, the amount of alpha-tocopherol found in VLDL is directly related to amounts found in LDL (Cohn et al., 1988).

Although cellular uptake of vitamin E is not well understood, lipoprotein lipase may be involved (Combs and Gerald, 1992; Traber and Kayden, 1984; Traber et al., 1985). Traber et al. (1985) reported that addition of lipoprotein lipase to chylomicrons in cell cultures hydrolyzed triglycerides and transferred both fatty acids and alpha-tocopherol into the cells. When lipoprotein lipase was not present in chylomicrons, cellular tocopherol did not increase, indicating that lipoprotein lipase must bind to cell membranes in order to transfer tocopherol to cells from lipid droplets. Research conducted with human subjects suffering from disorders involving low levels of lipoprotein lipase suggests that lipoprotein lipase is involved in transferring dietary tocopherol to cells (Traber et al., 1985).

Another method of delivery of alpha-tocopherol to cells may be through LDL binding to high affinity receptors (Traber and Kayden, 1984; Traber et al., 1985). The presence of LDL receptors causes an increased uptake of LDL into cells increasing cellular alphatocopherol concentrations when compared to cells that do not contain LDL receptors (Traber and Kayden, 1984). Although both pathways are important, the pathway utilizing lipoprotein lipase seems to be 10-fold more effective than the LDL pathway (Traber et al., 1985).

Once in cells, there are tocopherol-binding proteins (TBPs) which then serve to transport alpha-tocopherol within the cell. Alpha-tocopherol also binds to retinol-binding proteins for transport, although it is displaced by the presence of retinol (Combs and Gerald, 1992).
Ninety percent of alpha-tocopherol is stored in skeletal muscle, liver and adipose tissue. Adrenal glands have the highest amount of alpha-tocopherol per gram of tissue due to their high affinity for HDL (Bjorneboe et al., 1986). In the liver, 25% of total alpha-tocopherol stores are found in nonparenchymal cells (as compared to 44% of hepatic retinoids) (Bjorneboe et al., 1986; Blomhoff et al., 1982).

Literature Cited


Interactions Between Vitamins A and E

Many researchers have reported an interaction between vitamin A and vitamin E in vivo. High dietary vitamin A decreases plasma alpha-tocopherol (Bieri and Tolliver, 1982; Franklin et al., 1998) as well as liver alpha-tocopherol levels (Combs, 1976; Dicks et al., 1959; Eicher et al., 1997; Sklan and Donoghue, 1982). Conversely, if there is a deficiency in retinol, this increases concentrations of hepatic alpha-tocopherol levels (Roels et al., 1964).

Plasma vitamin A concentrations are also affected by the level of intake of vitamin E. Plasma vitamin A concentrations are decreased when dietary vitamin E intakes are high or moderate (Abawi et al., 1985; Eicher et al., 1997; Napoli et al., 1984). However, although plasma vitamin A concentrations are decreased, hepatic vitamin A stores are increased (Combs and Gerald, 1992; Napoli et al., 1984; Roels et al., 1964).

In the liver, retinyl ester hydrolase releases vitamin A from storage in the stellate cells (Combs and Gerald, 1992). This enzyme hydrolyzes vitamin E and K as well, and when they are present in higher concentrations, less hepatic retinol may be released due to competitive inhibition (Combs and Gerald, 1992). Napoli et al. (1984) reported that high
dietary vitamin E may also decrease retinol stores by altering retinyl palmitate hydrolase activity.

Literature Cited


Vitamin C

Ascorbic acid is a water-soluble antioxidant vitamin capable of easily diffusing into blood vessels and extravascular fluid (Black and Hidiroglou, 1996). Because of this, ascorbic acid is involved in electron transfer reactions in every tissue throughout the body.

Ascorbic acid has a role in collagen synthesis due to its ability to hydroxylate two amino acids, proline and lysine (McDowell, 1989). Ascorbic acid plays an important role in reproductive function because there are high levels of collagen synthesis in corpus luteum and ovarian stroma. Porcine corpus luteum, when fully developed, contains the second highest concentration of ascorbic acid in the body. As the corpus luteum develops, concentrations of ascorbic acid steadily increase within the corpus luteum. As the corpus luteum regresses, ascorbic acid concentrations decrease as well. The ratio of dehydroascorbate to total ascorbate are high when the corpus luteum is forming indicating significant collagen synthesis and increased capacity of antioxidant function (Petroff et al., 1997).

Ascorbic acid also modifies bone mineral content by hydroxylating proline and lysine, increasing collagen in bone. Animals deficient in vitamin C may suffer bone abnormalities due to lack of hydroxylation of collagen residues resulting in decreased stability of bone matrix. This alters cell function and increases mineralization, affecting the growth plate in bone. When vitamin C is added to chondrocyte cultures, the chondrocytes secrete different collagens and upregulate alkaline phosphatase, increasing cellular maturation of bone and cartilage (Shapiro et al., 1991).

Animals that are deficient in vitamin C have decreased uptake of vitamin B12 through the intestinal wall, which may contribute to the development of anemia in scorbutic
animals (Dulloo et al., 1981). Ascorbic acid increases the uptake of selenium in the gut. This increases glutathione peroxidase function, thereby decreasing lipid peroxidation in chicken capillary cell walls (Combs and Pesti, 1976).

**Literature Cited**


**Interactions Between Vitamins C and E**

The conversion from alpha-tocopheroxyl back to alpha-tocopherol requires a reducing agent such as ascorbic acid (Combs and Gerald, 1992; Doba et al., 1985; Ginter et al., 1982; Niki, 1991; Sato et al., 1990). Some research indicates that in vivo recycling of vitamin E by vitamin C may not happen due to compartmentalization of vitamin C in the aqueous cytosol and tocopheroxyl in lipid membranes (Combs and Gerald, 1992; Sato et al., 1990). Others reported that, although there is compartmentalization, alpha-tocopheroxyl radicals are available to vitamin C due to exposure of the polar alpha-tocopherol head at the
membrane surface (Doba et al., 1985; Niki, 1991; Sato et al., 1990). Niki (1991) showed that the efficiency with which ascorbic acid reduces tocopherol decreases when the tocopheroxyl radical is located deeper in the membrane.

The problem of compartmentalization may also be overcome by the glutathione peroxidase system which actively shuttles electrons from the soluble phase of cells to radicals in membranes (Chen and Chang, 1978; Combs and Gerald, 1992). Vitamin C favors the glutathione peroxidase system, which interacts synergistically with alpha-tocopherol, thereby sparing alpha-tocopherol in the process (Chen and Chang, 1978).

When vitamin C regenerates alpha-tocopherol, a vitamin C radical is formed (Doba et al., 1985; Ginter et al., 1982; Packer et al., 1979; Sato et al., 1990). When vitamin C was added to diets of animals fed low dietary alpha-tocopherol, tissue antioxidant status was improved by increasing mitochondrial respiration, resulting in a decrease in liver peroxidation (Chen and Chang, 1978).

Vitamin C deficient guinea pigs tend to have low concentrations of alpha-tocopherol resulting from an increased susceptibility of vitamin E to oxidation without the presence of the reducing ability of vitamin C (Hruba et al., 1982). Vitamin C deficient animals have low concentrations of alpha-tocopherol in liver, lungs and kidneys, due to alpha-tocopherol replacing the missing ascorbic acid in some oxidation-reduction processes (Hruba et al., 1982).

Enriching a vitamin E deficient diet with vitamin C results in less lipid peroxidation (Doba et al., 1985; Ginter et al., 1982; Sato et al., 1990). The addition of vitamin C into aqueous parts of a mixture in the process of LDL oxidation decreases alpha-tocopherol
utilization and oxygen uptake, thereby increasing concentrations of vitamin E (Sato et al., 1990).

**Literature Cited**


