Effect of forage addition to the diet on rumen development in calves

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT

The consumption of solid feed is essential for successful transition from a preruminant to a functional ruminant animal. Calves fed starter rations containing highly fermentable carbohydrates often experience dramatic changes in concentrations of rumen and blood metabolites. The optimal amount of roughage required in the diet of young calves is still unclear. The objective of these studies was to determine the effect of form of diet (coarse versus ground) and the inclusion of various levels of hay of consistent particle size on rumen development and immune function in young calves. In experiments 1 and 2, calves were fed one of four treatments. Diets consisted of coarse starter (C), ground starter (G), coarse starter with 7.5% grass hay of consistent particle size (H1), and coarse starter with 15% grass hay of consistent particle size (H2). When intake was restricted prior to weaning, calves consuming H1 and H2 tended to have greater body weight ($P < 0.07$). Calves receiving H1 and H2 also had greater average daily gain (ADG) ($P < 0.04$) and feed efficiency (GF) ($P < 0.12$) postweaning. Intake of dry matter did not differ by treatment. Total ruminal VFA concentrations were higher in calves consuming G versus C ($P < 0.01$). Acetate to propionate ratio was higher in calves fed H2 than H1 ($P < 0.03$). In experiment 2, calves received access to feed ad libitum. There was a tendency for calves offered H1 and H2 to consume more starter and total dry matter than those fed C ($P < 0.11$ and $P < 0.15$ respectively). There were no differences in body weight, ADG, GF, or age at weaning. In experiment 3 calves were offered spray-dried animal plasma (SDAP) or red blood cells and whey protein concentrate (RBCW) in addition to coarse and ground diets in experiment 1. Calves receiving diets supplemented with SDAP were heavier ($P < 0.11$). Changes in white

blood cell populations were also observed, although it is doubtful that these changes are of physiological significance because the values did not differ from normal ranges. It appears that the use of forage of a consistent particle size does encourage intake of solid feed and promotes more efficient gain. The use of hay in the starter diet appears to create a more stable rumen environment for the calf.

CHAPTER ONE

GENERAL INTRODUCTION

Dissertation Organization

The following dissertation is divided into five chapters. Chapter one includes a review of the literature pertaining to development of the rumen and the effects of forage on rumen development. Chapter two presents a summary of research conducted to determine effects of various levels of forage in the diets of young calves on metabolic reactions and changes in rumen environment. Chapter three presents a summary of the research conducted to determine the effect of various levels of crude fiber in the diet at ad libitum intakes. This trial concentrated on growth parameters and age at weaning in calves. Chapter four presents a summary of research conducted to determine the effect of form of diet and the addition of plasma protein to meal fed calves. Chapter five includes the general conclusions that can be drawn from this research in relation to previous work in this field. Portions of this thesis will be submitted for publication; co-authors will include H.D. Tyler, J.D. Quigley III, T.M. Wolfe, and J.A. Brumm.

The Development of the Bovine Rumen and the Effects of Dietary Crude Fiber Addition: A Review

Introduction

Calves have unique dietary considerations in contrast to older heifers and mature cows. In order to formulate rations for the preruminant calf, one must consider many factors. First, the combination of both liquid and solid feed in the diet markedly influences intake. It is relatively easy to meet the nutrient needs of a young calf with only liquid feed, however this diet does not promote rumen development. In addition, consumption of large amounts of

liquid feed delays the consumption of solid feed. Furthermore, liquid feed does not provide the most economic rate of gain. The use of milk as a feedstuff requires additional labor and cost when compared to solid feed. Digestibility and utilization of solid feed also changes with age of the calf and development of the rumen. Digestibility of solid feed depends largely on fermentation in the rumen. This ability to ferment solid feed changes rapidly in the calf. To utilize feed effectively the rumen must have a dense and stable microbial population and developed epithelial surfaces to absorb the products of fermentation.

Young calves must make a dramatic transition in the first few weeks of life. Calves are born with digestive systems that function in a manner similar to a monogastric animal. In a short period of time calves must develop a fully functional rumen capable of providing nutrient requirements. This transition from monogastric to ruminant animal is vital to the survival of the calf. Additionally, the success of this transition benefits the producer greatly. It is more economical to feed a young calf a dry versus liquid diet. The use of liquid diets is more expensive due to the cost of the milk or milk replacer itself and the large amount of labor involved in feeding this type of diet. Once a calf is weaned from milk, the cost of production dramatically decreases. Dry feed intake provides the most cost effective gain. Therefore, many management schemes and dietary adjustments have been attempted in an effort to promote early weaning. However, early weaning is only economical and successful if the rumen is fully developed.

Development of the rumen is critical for the utilization of dry feed. The organ must have sufficient physical capacity, a functional microbial population, and an efficient absorptive surface to effectively utilize starter rations. The proper weaning strategy and optimal starter ration will contribute to reduced stress after weaning. The optimal

composition of calf rations is not well defined. Roughage or forage feedstuffs promote muscular development of the rumen and also maintain the integrity of the rumen epithelium. However, these feeds do not provide sufficient amounts of the volatile fatty acids (VFA) needed for development of the rumen mucosa. In addition, these feeds may not contain sufficient energy and protein to meet the requirements of a rapidly growing calf. Concentrate feeds provide necessary readily fermentable carbohydrates for production of VFA. However, these feedstuffs do not promote muscularity of the rumen wall, integrity of the rumen epithelium, or stimulate rumination or saliva production. The optimal combination of forage and concentrates that should be included in the diets of young calves is unclear.

Gastrointestinal Structure and Physiology in the Calf

Calves are born with all the stomach compartments of an adult ruminant animal. The reticulum, rumen, omasum, and abomasum are distinct pouches in the digestive system. However, at birth the relative size of these compartments and their contribution to the digestive function of the animal differ from an adult (Huber, 1969). It is during the transition period from milk feeding to consumption that the calf's stomach compartments must develop in relative size and metabolic activity.

Stomach Compartment

Development of the stomach compartment begins during gestation. A primitive stomach is visible at 28 days of gestation (Warner, 1958). This primitive pouch soon begins to differentiate into 4 distinct compartments. During this development, there is no esophageal tissue contribution (Warner and Flatt, 1965). The reticulum, rumen, omasum, and abomasum are visibly separate at 56 days of gestation (Warner, 1958). Tissue differentiation also begins in utero. Fetal rumen samples have stratified cuboidal epithelium.

However, rumen epithelial samples taken at birth shows a transition from stratified cuboidal to keratinized, stratified squamous epithelium. The basal layer then begins to fold to form papillae (Wardrop, 1961).

In early gestation, the stomach compartment comprises more than 1.8% of the fetal weight. This percentage decreases with gestation, so that at birth the stomach compartment comprises approximately 1.3% of the calf's body weight (Becker et al., 1951). During the latter part of gestation (8 to 9 months) the compartments are in their final position (Warner and Flatt, 1965). During early calf development prior to weaning, growth of the stomach compartments are greater than the empty body growth (Bailey, 1986). Development of the individual compartments after birth varies greatly. Each organ grows at a different rate and begins to function according to substrate available.

Reticulum

Development of the reticular structure begins late in gestation. Differentiation of tissue into the characteristic honeycomb surface begins at 72-100 days in utero (Becker et al., 1951). The outline of the reticular ribs can also be clearly viewed during the third trimester. At birth, the honeycomb structure is clearly visible and small papillae are present on the reticular ribs, which are conical and rounded in shape. During postnatal development, honeycomb cells and individual papillae increase in size. As an adult, papillae on the reticular ribs are more pointed and conical in shape in contrast to the rounded papillae of the neonate (Wardrop, 1961).

During fetal life, the reticulum is the lighter of the four compartments. It is four times lighter than the rumen beyond the eighth month of gestation and until full term (Becker et al., 1951). The reticulum is of little importance to the young calf. Due to the action of the

esophageal groove, liquid feed bypasses the reticulum. However, once consumption of solid feed begins, the reticulum becomes functional. In the mature ruminant animal this compartment assists with the microbial fermentation of feed. It stores and delays the passage of solid food. The reticulum is also necessary for eructation in adult ruminants (Hofmann, 1988).

Rumen

During the first 4 months of fetal development, the rumen is the predominant stomach compartment and is approximately 2 to 3 times the weight of the abomasum. After 4 months of gestation the abomasum begins to increase in weight more rapidly than the rumen (Becker et al., 1951). After birth the rumen compartment increases in size at a faster rate than the abomasum. At this time it is the fastest growing stomach compartment. At 4 months of age, it is approximately 4 times the size of the abomasum and 8 times the weight at birth when calves are offered access to solid feed (Kesler et al., 1951; Wardrop and Coombe, 1960; Godfrey, 1961a). Calves offered only milk have relatively small increases in the capacity of the rumen in proportion to body weight (Tamate et al., 1962). As the weight of the rumen compartment increases, the capacity also increases (Godfrey, 1961b).

This increase in rumen weight during development is linked to gain of body weight, intake of solid feed, and final body weight of the animal (Hamada et al., 1976). Growth of the rumen compartment continues to exceed the other stomach compartments after weaning (Bailey, 1986). Rumen development is highly dependent on access to solid feed. Adult rumen size, expressed as a percentage of body weight, is obtained at approximately 5 weeks of age (Godfrey, 1961a). Rumen epithelial tissue also develops with solid feed consumption in addition to the increase in rumen size.

During the first two trimesters of fetal life rumen mucosa is smooth and has virtually no visible papillae. Papillae begin to develop on the rumen surface towards the end of gestation (Wardrop, 1961). Changes that occur in the rumen follow changes occurring in the connective tissue during fetal development (Arias et al., 1980). Small papillae are clearly present at birth. These small papillae are approximately 1 to 2.6 mm in length (Tamate et al., 1962; Huber, 1969), and are short, densely packed, and have a conical shape. Papillae become longer, irregular and more tongue-shaped in appearance with exposure to solid feed. The inter-papillary spaces are also visible at this time (Wardrop, 1961). Physical development of papillae is essential to the absorption of fermentation products from the rumen.

The rumen mucosa is a complex structure composed of several layers (Figure 1), with specific functions. The rumen mucosal surface is a mucoploysaccharide-rich, stratified squamous epithelium. Cells of the substratum spinosum and stratum granulosum can simultaneously produce keratohyalin granules needed for keratinization and mucus production. Basal cells are undifferentiated cells involved in metabolism and assimilation of absorbed microbial end products (Lavker et al., 1969). This complex layer of cells is essential for proper nutrient absorption and utilization in the adult ruminant animal.

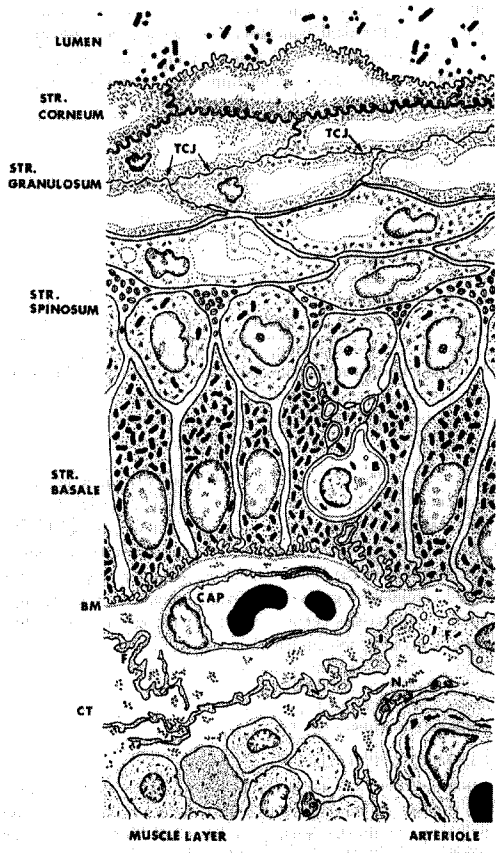

Figure 1. Cell layers of the rumen wall (Steven and Marshall, 1969).

In addition to the changes in rumen size and mucosal surface, the position of the rumen compartment in the abdominal cavity changes during development in the presence of solid feed. In the newborn calf, the posterior point of the rumen dorsal blind sac reaches to the third lumbar vertebrae, and the ventral floor of the rumen is positioned halfway between the dorsal and ventral surfaces of the abdominal cavity. At approximately 8 weeks of age, the posterior point of the dorsal blind sac reaches further towards the 5th lumbar vertebrae. Finally, by 12 weeks of age, the ventral floor of the rumen reaches the floor of the abdominal cavity. The rumen will remain in this position throughout the adult life of the animal (Warner and Flatt, 1965). If solid feed is not available, the rumen will extend down towards

the lower one-fourth of the abdominal cavity, but never reaches the cavity floor by 12 weeks of age (Tamate et al., 1962)

The rumen remains the largest of the stomach compartments during adult life. The rumen is of little importance to the liquid-fed calf due to the function of the esophageal groove. Rumen development is rapid with the consumption of solid feed. The rumen is primary site of microbial fermentation in the adult animal. Once fully functional, the rumen is the primary compartment for the production and absorption of volatile fatty acids (VFA) (Hofmann, 1988). Volatile fatty acids (VFA) will serve as the primary source of energy for the mature ruminant animal. Remaining products of digestion in the rumen continue to move through the stomach compartment to the omasum.

Omasum

The omasum develops rapidly in early gestation. At this time, it is second only to the rumen in total weight. However, by 8 months of gestation, the omasum is much less prominent (Becker at al., 1951). Structural features of the omasum also develop in early fetal life. Fetal tissue samples from early gestation show characteristic omasal folds. During gestation the normal leaf structure of the compartment is also observed (Wardrop, 1961). The laminae of the omasum develop rapidly between days 72 and 100 of fetal life (Becker et al., 1951). The organ increases in size (length) and small papillae develop on the surface of the laminae after birth (Wardrop, 1961).

Similar to the reticulum and rumen, the omasum is non-function in the preruminant calf. With closure of the esophageal grove, liquid feed also by-passes this stomach compartment. The omasum becomes more functional with consumption of solid feed. The omasum serves as an area for further VFA absorption in the adult ruminant animal

(Hofmann, 1988). Both butyrate and acetate are readily absorbed from the omasum mucosa of the calf (Joyner et al., 1963).

Abomasum

The most distal stomach compartment is the abomasum or "true stomach". This organ has an essential function in the preruminant calf. The abomasum is smaller than both the rumen and omasum during early fetal life. After 4 months of gestation, the abomasum begins to develop more rapidly than other compartments. It is the heaviest and most functionally developed stomach compartment at birth (Becker et al., 1951; Tamate et al., 1962). In contrast to the other compartments, there is little development of the abomasum after birth. The relative volume of abomasal content differs little with age of the animal (Godfrey, 1961b).

The abomasum functions as the only glandular portion of the bovine stomach. The main function of this compartment in the preruminant calf is to digest milk by the secretion of rennin and pepsin. It functions similar to the simple stomach of a monogastric animal in adult ruminants. The abomasum plays an important role in protein digestion in both the calf and adult bovine (Hofmann, 1988).

The following table presents a summary of changes that occur with respect to organ weight as the calf ages in the presence of solid feed consumption:

Table 1. The changes in weight of the stomach compartments from birth to adult as a percentage of the total stomach (adapted from Godfrey, 1961a).

Age (weeks)	Ruminoreticulum (%)	Omasum (%)	Abomasum (%)
Birth	35	14	51
2	40	15	45
4	55	11	34
8	65	14	21
12	66	15	19
17	68	18	14
Adult	62	24	14

Esophageal Groove

The diet of a young calf consists solely of liquid feed immediately after birth. Whether whole milk or milk replacer is offered, the liquid must pass through the esophageal groove unless it is administered via an esophageal feeder (Chapman et al., 1986). The esophageal groove is an anatomical structure that allows for direct entry of milk into the abomasum from the esophagus. This groove closes in response to chemical stimulus provided by the action of suckling composition of the fluid. The groove shunts only milk directly to the abomasum for digestion. The suckling reflex allows for more effective use of the groove. When calves suck instead of drink, it takes longer to complete the first swallow and less milk is consumed per swallow. This slower rate of milk intake causes fewer openings of the groove, resulting in less spillage of milk into the reticulum (Wise et al., 1984). It is the action of this groove that causes the preruminant calf to digest liquid feed

similar to a monogastric animal. However, the functional ability of this groove decreases with age and the transition to solid feed (Huber, 1969).

Enzyme Activity in the Pre-ruminant Calf

Secretion of abomasal and intestinal enzymes change in conjunction with both development of the stomach compartments and the animal itself. Enzymatic concentrations and activities of a preruminant calf do not closely resemble that of an adult ruminant animal. Secretion of intestinal enzymes must adapt as the sources of food changes from milk to solid feed. The activity of many digestive enzymes increases with age and available substrate, however the activity of several enzymes decreases.

Saliva

Digestion of liquid feed begins immediately upon entry into the mouth. In the preruminant, calf salivary enzymes play a small role in digestion. Unlike some monogastric species, the calf does not have salivary amylase. This enzyme also does not exist in the adult bovine (Huber at al., 1961). However, the preruminant calf does have an active lipolytic enzyme in saliva. Pregastric esterase is a complex composed of at least 6 different enzymes and is secreted from the palatine glands of the oral cavity (Young et al., 1960; Ramsey, 1962). Pregastric esterase has a high specificity for butyrate and other short chain fatty acids commonly found in milk. The enzyme functions best at pH of 6 (Ramsey and Young, 1961). It was first thought that a gastric lipase existed, however it was later discovered that pregastric esterase is solely responsible for all lipid digestion prior to the small intestine (Ramsey, 1962; Otterby et al., 1964a and 1964b). The output and activity of pregastric esterase is highly linked to the type of feedstuff consumed by the animal. When the calf is consuming liquid feed the concentration and activity of pregastric esterase is high. However,

the output of pregastric esterase decreases as the intake of solid feed increases. Lipid digestion after weaning is provided primarily by lipase activity in the small intestine (Young et al., 1960).

Abomasum

Enzymatic activity in the abomasum is critical for digestion of feed in the young calf and adult ruminant. Due to the action of the esophageal groove, milk bypasses the first 3 compartments of the stomach and enters the abomasum of the calf. Therefore, the abomasum is an important area for milk digestion. Secretions in the preruminant abomasum include rennin (chymosin), pepsin, and hydrochloric acid (HCl). Hydrochloric acid is secreted by the parietal cells of the abomasal wall. It is secreted in small amounts at birth, but this concentration increases as the calf ages. Secretion of HCl serves to activate both rennin and pepsin secreted into the abomasum in an inactive (zymogen) form (Garnot et al., 1977). Active forms of rennin and pepsin play vital roles in protein digestion for the young calf.

Rennin, also known as chymosin, is responsible for clot or curd formation in the abomasum. It is a proteolytic enzyme that aids in milk protein digestion. Rennin causes the formation of casein and fat clots (Garnot et al., 1977). Starches also may become associated with the clot (Coombe and Smith, 1974). Clot-forming activity slows the passage of milk allowing more complete digestion of protein (Garnot et al., 1977). Activity of rennin is swift and the clotting process is complete within one minute after finishing the meal (Porter, 1969). However, formation of a clot in the abomasum depends on the available substrate. Milk replacer made with whey proteins or milk proteins subjected to excessive heat will not clot in the abomasum (Garnot et al., 1977). This lack of clot formation does not necessarily indicate a decrease in digestibility of the product. The activity of rennin is high at birth and declines

with age of the calf and consumption of solid feed. There is little or no rennin secretion present in the calf after weaning (Garnot et al., 1977).

Pepsin is also a proteolytic enzyme that is active at birth in the abomasum. This enzyme is secreted from the chief cells of the abomasum (Huber, 1969). Pepsin secretion increases with age of the calf and remains active into adult life. In general, pepsin has greater proteolytic activity than rennin (Garnot et al., 1977). Pepsin begins digestion of microbial crude protein and undegraded intake protein from the rumen in the adult animal. Further digestion of protein and other nutrients occurs in the small intestine.

Small Intestine

Digestion of a variety of substrates occurs in the small intestine. This organ receives secretions from the liver and pancreas and also produces enzymes at the mucosal level. Bile and pancreatic secretions enter the small intestine via the common bile duct. The pancreatic secretions provide proteolytic, amylolytic, and lipolytic enzymes that are essential for digestion in the adult ruminant animal. The flow rate of these enzymes into the small intestine increases with age. Adult levels are reached at approximately 100 days of age (McCormick and Stewart, 1967).

Activity of proteolytic enzymes also increases with age along with pancreas flow rate. Proteolytic enzymes originating in the pancreas include trypsin, chymotrypsin, and carboxypeptidase. These enzymes are similar to rennin and pepsin in that they are secreted into the small intestine in inactive forms (McCormick and Stewart, 1967). Limited proteolytic activity in the preruminant small intestine is thought to be responsible for the inability to utilize soy proteins in the diet of young calves (Ramsey and Willard, 1975 and 1976). Furthermore, soy protein sources also contain antinutritional factors that may hinder

digestion, absorption, or metabolism (Ramsey and Willard, 1975 and 1976). Once adult concentrations of proteolytic enzymes are achieved, plant protein sources can be effectively utilized.

Secretion of enzymes from intestinal mucosa also varies with age of the animal. Virtually all the enzymes are found in relatively low concentrations in the small intestine of the young calf. The exception to this is the enzyme lactase (Huber et al., 1969). The disaccharidase lactase is vital for the digestion of the primary milk sugar lactose. All bovines lack sucrase activity. The secretion of pancreatic amylases and brush border enzymes (maltase, isomaltase, etc.) increase as the calf ages. In contrast, the activity of lactase decreases with age and the availability of solid feed (Huber et al., 1961). This change in enzyme activity reflects the changing substrates available in the diet as the calf makes the transition from preruminant to ruminant animal. In turn, this alteration of enzyme activity determines substrate digestibility and utilization.

The young calf utilizes glucose, galactose, and lactose well due to the high activity of lactase in the small intestine. Lactase activity is highest at day one and decreases as the calf ages. Calves maintained on liquid diets maintain lactase activity. After weaning lactase activity declines dramatically (Huber et al., 1961). Maltase activity is low at birth and changes little with age (Huber et al., 1961). No intestinal sucrase is ever detected in the bovine intestine (Huber et al., 1961). Pancreatic amylase, lipase, and proteolytic activity are also lowest at day 1, increase during the first week of life, and then change little as the calf continues to age (Huber et al., 1961). Due to the low activity of various enzymes at birth, the calf is unable to utilize sucrose, maltose, or starch well. Utilization of maltose and starch increases with increasing activity of amylolytic enzymes (Porter, 1969). In the young calf,

97%of lactose is absorbed, 43% of maltose is digested and absorbed, and 60% of starch is utilized (Coombe and Smith, 1974).

Changes in Metabolic Activity with Calf Development

Glucose

Glucose metabolism in the young calf closely resembles that of a monogastric animal. In the preruminant calf, glucose serves as the primary source of energy. Plasma glucose concentrations in the calf (approximately 100 mg/100ml) are similar to those of a monogastric animal (Young et al., 1970). Rumen epithelial cells in vitro express low glucose oxidation (expressed as nmol/(1 x 10^6 ruminal cells •120 min) rates at birth (14.2). However, by 14 days of age, glucose oxidation by the rumen epithelial cells is elevated (71.38) and remains high until approximately 42 days of age. After 42 days, the rate of glucose oxidation begins to decline. In adult ruminants rates of glucose oxidation remain lower than what is observed at birth (6.11) (Baldwin and Jesse, 1992). This decline in glucose oxidation by rumen epithelial cells causes blood glucose levels to decline as the calf ages (McCarthy and Kesler, 1956; Young et al., 1970). However, insulin concentrations remain constant with age of the animal (Young et al., 1970).

It has been suggested that this decline in blood glucose is related to both age of the calf and, more importantly, the diet. It was believed that rumen development causes the decline in blood glucose concentrations due to the production of VFA (Attebery and Colvin, 1963). However, when the forestomach (reticulum, rumen, and omasum) is removed from the young calf, a decrease in blood glucose still occurs as the calf ages. This suggests that the rumen tissue is not responsible for the decline in blood glucose levels, and that the decline in blood glucose is independent of rumen development and increased levels of VFA

(Lupien, 1962). The decline in glucose utilization by the young calf does occur during the transition from liquid to solid diet, but the form of the diet itself has little effort on the decline in glucose utilization.

Volatile Fatty Acids

Metabolism of VFA becomes of greater importance as the age of the calf increases. Although glucose is an important source of energy for the preruminant animal, volatile fatty acids are the primary energy source for adult ruminants. During development there is an obvious change from glucose metabolism to VFA metabolism in the young calf. Volatile fatty acids are produced by the fermentation of plant carbohydrates by rumen microbes (Figure 2). The common intermediate in the metabolism of most plant carbohydrates is pyruvate. Pyruvate is then converted to the individual volatile fatty acids (Figure 3). Once produced in the rumen, VFA are then absorbed via passive diffusion into the rumen wall. The passive diffusion of VFA across the rumen wall leads to eventual accumulation in plasma (Figure 4). Relative accumulation of individual VFA is dependent on diet (Leng, 1969).

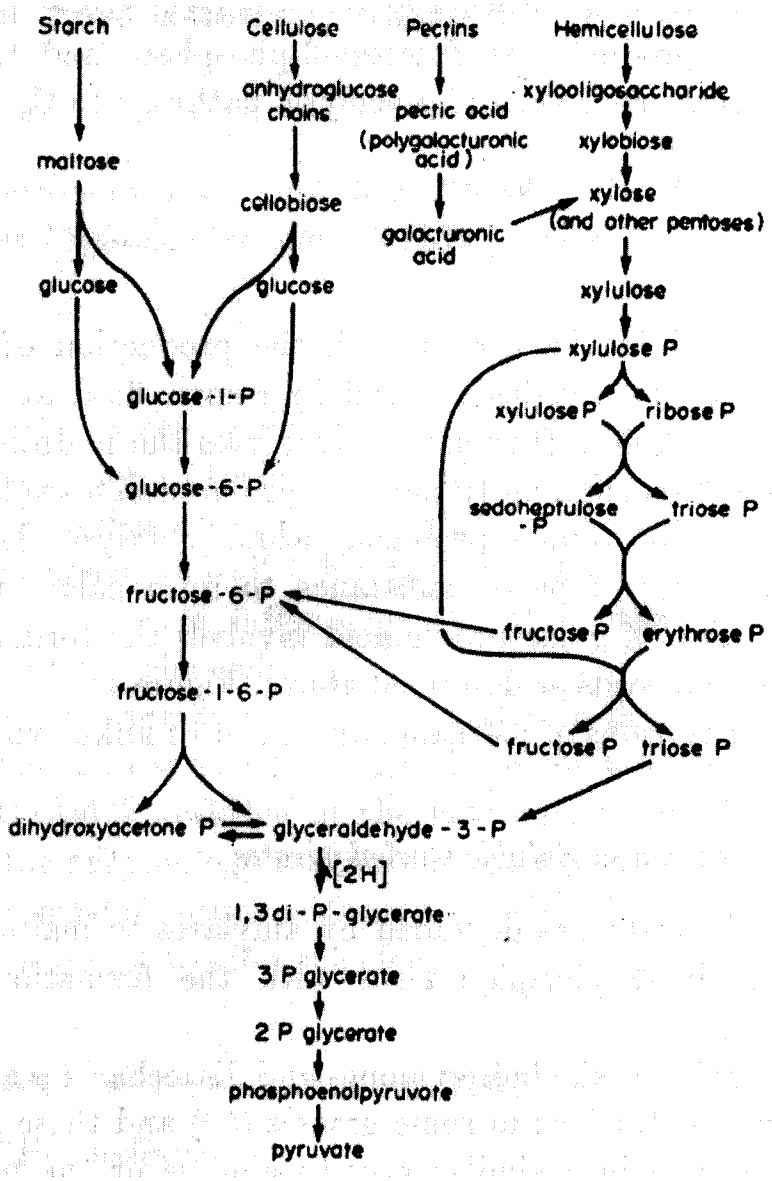

Figure 2. The fermentation of common plant carbohydrates to pyruvate (Leng, 1969).

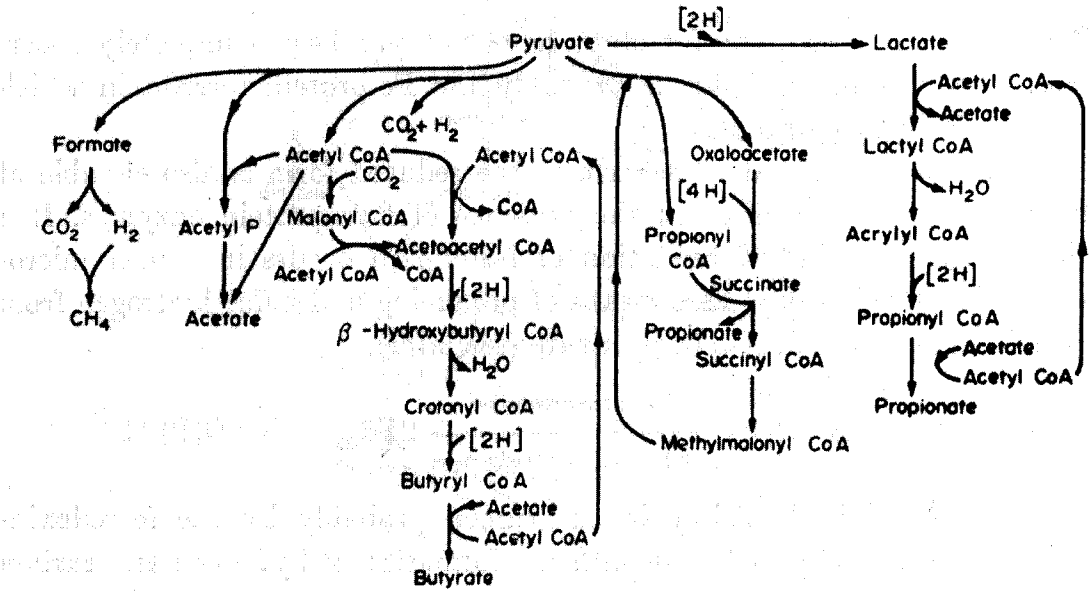

Figure 3. Conversion of pyruvate to volatile fatty acids in the rumen (Leng, 1969).

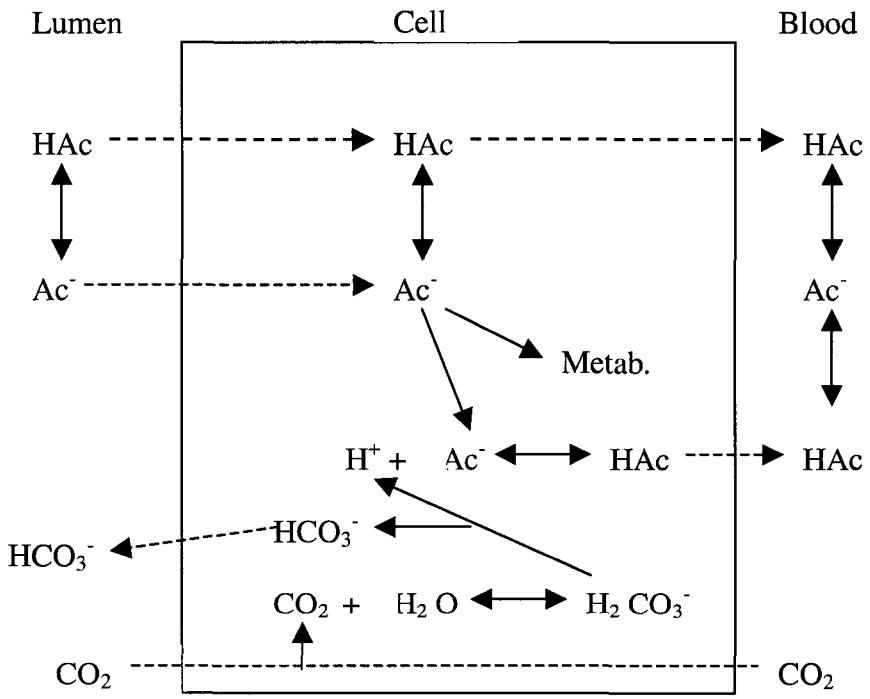

Figure 4. Volatile fatty acid absorption (adapted from Stevens, 1969) (HAc = free form of VFA; Ac = anion form of VFA).

Young et al. (1965) infused calves with labeled acetate, propionate, and butyrate in amounts sufficient to provide half of maintenance energy for 8 hours. Researchers reported the acids were not metabolized in large quantities. This particular investigation saw no differences in metabolism of individual acids or a change with age up to 80 days. Acetate and butyrate contributed to ketone production when metabolized. Infusion of acids increased blood lactate and decreased glucose indicating that VFA influence rumen epithelial metabolism (Young et al., 1965). The presence of butyrate inhibits glucose oxidation by rumen epithelial cells in vitro (Baldwin and Jesse, 1992). Young calves have the ability to absorb and metabolize VFA regardless of whether acids are generated in the forestomach or large intestine (Liang et al., 1967).

Ketogenesis

Ketogenesis is the conversion of acetyl-CoA to ketone bodies acetoacetate and β-hydroxybutyrate (BHBA). This process indicates oxidation of VFA by tissue. Ketone bodies generated are normal intermediates in the process of VFA oxidation. The developing and adult rumen epithelium is capable of ketogenesis (Lane et al., 2002). The source of acetyl-CoA in rumen epithelium is mitochondrial oxidation of butyrate. Butyrate is a VFA generated from the microbial fermentation of feed in the rumen (Lane et al., 2002). β-hydroxybutyrate is a normal intermediate of metabolism of butyrate in rumen epithelium of the adult animals (Ramsey and Davis, 1965). Consequently, plasma BHBA is often used to indicate VFA metabolism by the rumen wall and can serve as a marker for rumen development in the calf.

Neonatal rumen epithelium does not have the key enzymes responsible for ketogenesis (Lane et al., 2002). However, the rumen epithelial cells do develop ketogenic

activity and BHBA production increases with age in the preweaning period (Baldwin and Jesse, 1992). Development of ketogenic activity in the rumen epithelium occurs as the animal ages and is not affected by dietary treatment. Expression of genes encoding ketogenic enzymes are not activated by the presence of VFA in the rumen (Lane et al., 2002).

Gluconeogenesis

As stated previously, the preruminant calf relies heavily on glucose for energy. As the calf ages, reliance on glucose decreases and is replaced by VFA as the primary source of energy. As indicated by decreased concentration of glucose and increases concentration of acetate ketones in the blood. Despite this shift in energy sources, the adult ruminant animal still has a need for glucose. Glucose is the primary energy source for specific tissue types, such as red blood corpuscles and the central nervous system, that do not utilize VFA well. Adult animals obtain glucose from two sources, absorption from the gastrointestinal tract and gluconeogenesis. The amount of glucose absorbed from the small intestine of an adult ruminant animal is minimal. In a forage-fed adult ruminant, glucose is derived almost entirely from non-dietary sources (Van Soest, 1982). Rumen fermentation rapidly converts plant carbohydrates into VFA allowing little soluble carbohydrate to reach the small intestine. Therefore, the adult ruminant relies heavily on gluconeogensis to provide the necessary glucose (Young et al., 1974).

Precursors for gluconeogenesis include propionate, lactate, and some amino acids. Propionate is the primary precursor for the formation of glucose. The calf must develop the ability to effectively convert these precursors to glucose to survive as an adult ruminant. Gluconeogenic capability of these animals increases with age allowing for a successful transition (Young et al., 1974). However, solid feed must be offered to produce rumen VFA.

Rumen Development in the Calf

Physical Stimulus

Rumen development can be linked to consumption of dry feed (Gilliland et al., 1962; Warner et al., 1955 and 1956). However, originally it was thought that the development of the rumen could be linked only to the physical stimulus of dry feed. Eventually it was determined that dry feed does indeed promote the development of rumen capacity and musculature (Brownlee, 1956; Warner et al., 1956; Smith, 1961). However, this physical stimulation of the rumen does not provide the stimulus for epithelial development of the rumen. Calves consuming poor quality roughage (wood shavings) or inert physical objects (plastic sponges) had increased development of rumen capacity and musculature, but little mucosal development compared to calves receiving only milk (Flatt et al., 1958; Smith, 1961; Tamate et al., 1962). Therefore, it was determined that muscular development of the rumen does not necessarily correspond to epithelial development and investigations into the cause of epithelial development began (Brownlee, 1956).

Chemical Stimulus - Role of Volatile Fatty Acids

When inert objects (plastic sponges) are paired with a solution of sodium salts of VFA infused into the rumen, papillae development occurs (Flatt et al., 1958; Harrison et al., 1960; Tamate et al., 1962). Solutions of butyric and propionic acid promote the greatest development of the rumen papillae (Sander et al., 1959; Tamate et al., 1962). Other sodium salts, such as acetate and chloride, and glucose have little effect on mucosal development (Sander et al., 1959). All three volatile fatty acids exhibit mitogenic effects when infused into the rumen. However, butyrate has a greater mitogenic effect than propionate and acetate

(Sakata and Tamate, 1978 and 1979). This evidence suggests that VFA, in particular butyrate, stimulate epithelial development.

Young ruminant animals infused with VFA solutions have longer rumen papillae and oxidize less glucose, indicating that VFA are indeed responsible for mucosal development (Lane and Jesse, 1997). Feeding propionic and butyric salts increases papillae length from approximately 1.0 mm in control calves to 2.2 mm in calves receiving propionic acid and 4 mm in calves receiving butyric acid (Mentschel et al., 2001). The response is different for the two acids because of differences in the stimulation of mitotic rate (Mentschel et al., 2001). The rate of apoptosis is lower for calves receiving butyric acid than propionic acid. It appears that butyric acid is an inhibitor of apoptosis in rumen epithelial cells cultured in vivo (Mentschel et al., 2001). Butyric acid, in particular, plays a vital role in rumen mucosal development and is critical for the process of rumen development (Tamate et al., 1962).

Butyric, propionic, and acetic acid are all metabolized by the rumen epithelium. However, it appears that butyrate is metabolized preferentially. The activity of the enzyme acyl-CoA synthetase in rumen epithelial tissue is higher in the presence of butyrate (Cook et al., 1969). This enzyme is less active in the presence of propionate and least active in the presence of acetate (Cook et al., 1969). Absorption from the rumen also favors butyrate, then propionate, and finally acetate. (Weigand et al., 1972). The presence of butyrate lowers uptake of propionate by rumen epithelial cells (Pennington and Pfander, 1957). Butyrate metabolism is low at birth and increases considerably with age (Walker and Simmonds, 1962; Hird and Weidemann, 1964a). Formation of ketone bodies occurs in conjunction with metabolism of butyrate, primarily acetoacetate and β-hydroxybutyrate (BHBA) (Pennington, 1952; Walker and Simmonds, 1962; Koundakjian and Snoswell, 1970).

More than one half of the butyric acid removed from the rumen can be accounted for as ketone bodies (Pennington, 1952; Weigand et al., 1972). The increase in ketone bodies is greater in blood draining from the portal vein of rumen than from arterial blood indicating the ketones are produced within the rumen wall (Pennington, 1952). Acetoacetate and BHBA appear in the portal blood soon after butyrate is infused into the rumen (Annison et al., 1957). BHBA appears in the blood approximately 2 hours after feeding (Quigley and Bernard, 1992). Ketone bodies produced are easily oxidized by tissue from the liver, kidney, heart, spleen, muscle, brain, and lungs (Leng and Annison, 1964) (Figure 5).

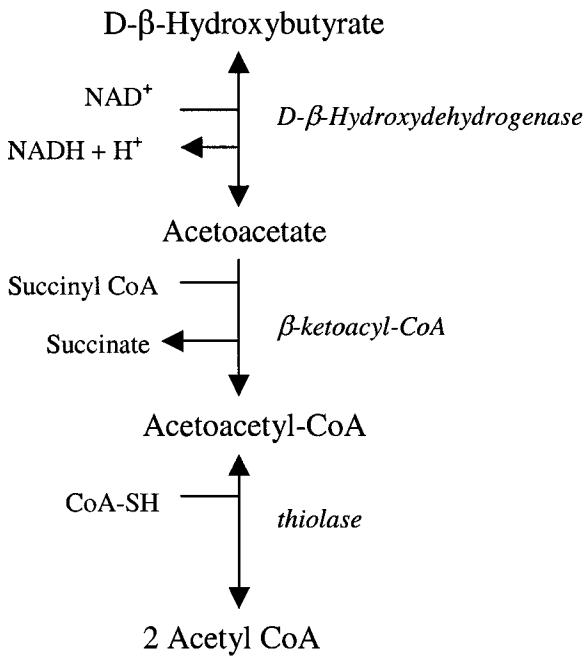

Figure 5. Oxidation of ketone bodies by tissue in the ruminant body (adapted from Lehninger et al., 1993).

Although the rumen epithelial tissue can produce ketone bodies from oxidation of acetate and butyrate, the liver also produces ketone bodies from the oxidation of fatty acids (Koundakjian and Snoswell, 1970). Metabolism of butyrate increases with age of the calf

and also is dependent on the size of rumen papillae. Total ketone production is larger and more rapid in large papillae versus small papillae (Hird and Weidemann, 1964b).

Ruminal VFA concentrations, including butyrate, increase with consumption of solid feed (Hibbs et al., 1956; McCarthy and Kesler, 1956; Godfrey, 1961b; Bomba and Zitnan, 1992; Zitnan et al., 1993; Lane et al., 2000). There is a rapid rise in ruminal VFA concentrations from 12.7 mmoles/l at 1 week of age to 67.4 mmoles/l at 5 weeks (Godfrey, 1961b). Adult concentrations are reached by approximately 5 to 9 weeks of age if solid feed is offered (Godfrey, 1961b; Bomba and Zitnan, 1992). This increase in rumen VFA corresponds to increases in plasma VFA levels (Quigley et al., 1992a). Calves that are weaned based on intake have higher rumen VFA concentrations than those weaned based on age (Anderson et al., 1987).

Either the concentration of VFA in the rumen or length of exposure to rumen VFA induces genes responsible for rumen metabolic development (Lane et al., 2000). Rumen tissue from calves fed milk, hay, and grain have greater uptake of VFA and conversion to ketones than calves fed only milk (Sutton et al., 1963b). When compared to calves receiving only milk, tissue from calves receiving solid feed produced ketones at a similar rate as mature animals at 60 days of age (Sutton et al., 1963b). In contrast, tissue from milk fed calves at 60 days of age produced only 10-15% the amount of ketones of an adult animal (Sutton et al., 1963b). This validates the role of VFA in mucosal function.

Total ruminal VFA concentrations are highly linked to consumption of solid feed, and the type of feed consumed affects proportions of individual VFA produced. Rations containing hay produce lower concentrations of total VFA, but greater proportions of acetate. Calves fed concentrates have higher total VFA concentrations and higher proportions of

valerate, propionate, and butyrate (Stobo et al., 1966b; Nocek and Polan, 1984). Other investigations have shown little influence of the hay to grain ratio on total VFA concentrations (Hibbs et al., 1956). Despite the composition of the diet, VFA formation in the rumen is relatively rapid and the concentration of total VFA peaks approximately 3 hours after feeding (Stobo et al., 1966b).

pH

Ruminal pH is a rumen parameter influenced both by age and diet in the young calf when solid feed is offered. Several investigations have observed a quadratic relationship between age of the calf and ruminal pH. Anderson et al. (1987) observed a decline in ruminal pH from birth to 4-5 weeks of age. Calves receiving access to a prestarter containing milk products had even lower ruminal pH than those consuming a conventional starter. Ruminal pH in all calves began to increase after 5 weeks of age. Beharka et al. (1998) also observed this quadratic relationship. A decline in rumen pH occurred after 14 days of age and then increased at 10 weeks. Calves on this trial received identical rations (25% alfalfa hay and 75% grain mix) of different particle size. Calves receiving the ground diet had even lower ruminal pH than those receiving the unground diet. Hibbs et al. (1956) also observed a gradual increase in ruminal pH as calves aged from 4 to 9 weeks. However, others have seen only a gradual increase in ruminal pH from 5.15 at one week of age increasing to 6.55 at 17 weeks of age in calves receiving conventional starter (Godfrey, 1961b). It is difficult to assess average ruminal pH values for developing calves due to the large variations in diet composition.

In adult ruminants, it has been established that diet can greatly influence ruminal pH. Ruminal pH can vary from approximately 7.0 to less than 5.0 depending on composition and

form of diet offered. Diets containing large amounts of rapidly fermentable carbohydrates tend to reduce ruminal pH while rations containing slower fermenting roughages tend to increase pH (Russell and Dombrowski, 1980). Calf starter rations often contain feedstuffs high in starch content. These types of rations ferment rapidly in the adult ruminant animal causing rumen pH to decrease. These conventional starter diets that utilize rolled grains cause decreases in ruminal pH that may reach acidotic levels (pH 4.5) (Williams et al., 1985a). However, Hibbs et al. (1956) determined that ruminal pH in the calf was not affected by the hay to grain ratio of the diet. In contrast, other investigations have seen an effect of diet composition and physical form on ruminal pH (Anderson et al., 1987; Beharka et al., 1998).

Microbial Populations

Similar to ruminal pH, the microbial populations in the rumen of the calf are influenced both by age and diet. The rumen environment is rapidly colonized by microflora after birth (Ziolecki and Briggs, 1961). Populations of aerobic bacteria are first to colonize. However, after 2-3 days anaerobic species begin to dominate (Anderson et al., 1987; Fonty et al., 1987). These populations of anaerobic bacteria continue to increase with age of the animal (Anderson et al., 1987; Fonty et al., 1987). The populations of both amylolytic and cellulolytic bacteria also increase with age.

Cellulolytic and methanogenic bacteria appear by 3 days of age regardless of diet (Bryant et al., 1958; Anderson et al., 1987; Fonty et al., 1987). These bacteria are established quickly and remain stable in calves fed pelleted concentrates (Fonty et al., 1991). The cellulolytic population is similar to adult values at 3 weeks of age in calves offered alfalfa hay and a grain mix (Bryant et al., 1958). Lactate-fermenting bacteria quickly establish

themselves in the rumen environment by 1 week of age, exceed adult values in early life, and then decline to populations similar to adult values at 9 to 13 weeks (Bryant et al., 1958). A functional rumen population of bacteria is established in the calf at 10 to 14 days of age when offered access to a conventional creep ration (Poe et al., 1971a).

In addition to an active bacterial population, protozoa also inhabit the rumen. These microbes will assist with cellulose digestion in the adult animal. The establishment of an active ciliate population requires contact with mature ruminant animals. Ciliate-free calves have been raised successfully when isolated from adult ruminants. These animals do not perform differently than those with active protozoal populations (Eadie, 1962). If contact with adult ruminant is established, protozoa populations develop at 15 to 20 days of age in calves fed hay and pelleted concentrate (Fonty et al., 1987). This development of a diverse microflora in the young ruminant establishes the ecosystem and begins the process of fermentative digestion in the animal (Fonty et al., 1991) (Table 2).

Table 2. Common microbes of the rumen (adapted from Baldwin and Allison, 1983).

Microbial Species	% of total isolates	Secondary substrates[a]	Net products in mixed culture[b]	Nutrient requirements[c]
Cellulolytic				
Bacteriodes succinogenes (Fibrobacter succinogenes)	5-9	ST, P	A, PS, CO_2	BFA, V, NH_3, biotin, PAB
Ruminococcus albus	3-5		A, H_2, CO_2	BFA, NH_3, biotin, PAB
Ruminococcus flavefaciens	3-5		A, PS, H_2, CO_2	BFA, NH_3, biotin, PAB
Amlyo- and Dextrinolytic				
Bacteriodes amylophilus (Ruminobacter amylophillus)	1-10	P, SR	A, PS, CO_2	NH_3
Streptococcus bovis	0-20	SS, PR	A, L, CO_2 (H_2)	AA, biotin
Succinimonas amlyolytica	1-3		A, PS, CO_2	BFA
Succinivibrio dextrinosolvens	1-13	P	A, PS, L, CO_2 (H_2)	AA
Saccharolytic				
Bacteroides ruminicola	10-19	ST, P, PR	A, P, CO_2 (H_2)	BFA
Butyrivibrio fibrisolvens	8-12	C, ST, PR	A, B, L, CO_2 (H_2)	BFA, NH_3, AA, biotin, folic acid, pyridoxal
Megasphaera elsdenii	0-1	L, PR	A, P, B, V, H_2, CO_2	AA
Selenomonas ruminantium	4-12	ST, L	A, P, L, H_2, CO_2	BFA, MET
Hydrogen utilizers				
Methanobrebibacter ruminantium (Methanobreuibacter)	<1		CH_4	BFA, NH_3
Vibrio succinogenes (Wolinella succinogenes)	Minimal		PS, NH_3	NH_3
Protozoa				
Isotricha, Epidinium, Diplodinium sp.		ST, SS	A, B, L, H_2, CO_2	
Dasytricha, Diplodinium sp.		ST, SS	A, B, L, H_2, CO_2	(bacterial cells)
Entodinium sp.		ST	A, P, B, L, CO_2 (H_2)	

[a]Coding for substrates is C for holocellulose, ST for starch, SS for soluble sugars, P for pectin, PR for protein, and L for lactate.

[b]Coding for products is A for acetate, P for propionate, B for butyrate, V for valerate and longer chained fatty acids and L for lactate. Listing H_2 and CO_2 indicates organism has a hydrogenase and produces hydrogen while CO_2 (H_2) indicates organisms producing formate that is converted to CO_2 + H_2 by another organism. PS codes for succinate that is converted to propionate and CO_2 by other organisms in mixed culture.

[c]Nutrient requirements in BFA is C4-C5 (branched chain) VFA, V = valerate, AA = amino acids, MET = methionine, PAB = para-aminobenzoate.

Composition of starter rations also influences establishment of microbial populations in the rumen. Consumption of solid feed causes a further increase of anaerobic populations (Fonty et al., 1987). Calves receiving solid feed have rapid decreases in aerobic bacterial populations, an increase in total bacterial counts, and an establishment of protozoa (Lengemann and Allen, 1959). Calves receiving access to milk for an extended period of time experience a delay in establishment of normal rumen populations until the consumption of solid feed begins (Lengemann and Allen, 1955). Consequently, calves that are weaned based on intake have greater microbial activity at the same age than those weaned based on age (Anderson et al., 1987). Starter rations commonly contain sources of both starch and cellulose. Young ruminants that receive conventional starter from birth in addition to milk have a greater ability to digest cellulose and starch compared to animals that receive only milk (Poe et al., 1971a). Consumption of solid feed increases development of microbial populations resulting in increased feed utilization.

Ingredient composition of starter rations varies greatly. The ratio of concentrate to hay in the ration influences the establishment of specific microbial populations (Pounden and Hibbs, 1948a). Streptococci populations flourish rapidly with the consumption of concentrates, and later populations of Lactobacilli increase with consumption of grain. These substrates markedly influence rumen pH causing the change in bacterial populations. Concentrate feeds promote a lower rumen pH (5.5) than forage (6.8) (Ziolecki and Briggs, 1961). Cellulolytic bacteria, such as Butyrivibrio fibrisolvens, Bacteroides succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens, are extremely sensitive to changes in pH. A decline in pH as little as 0.25 units can make the difference between maximal cell yield and washout (Russell and Dombrowski, 1980).

The ratio of grain to forage has profound effects on rumen environment and thus specific bacterial populations. Hibbs et al. (1956) observed a reduction in cellulolytic populations as the ratio of hay to grain decreased from 4:1 to 2:3. The protozoal populations were unaffected. In a similar study, the concentration of protozoa increased with larger proportions of grain in the diet until the ratio of grain to hay reached 3:2, then the populations declined. Celluloytic bacteria decreased as the ratio of grain to hay approached 1:1 and were virtually absent when the ratio was 3:1 (Pounden and Hibbs, 1948b). These variations in microflora with relation to diet are more severe in calves. Because of the relatively small capacity of the rumen at a young age the influence of a single feeding on the rumen microbial population is more profound (Pounden and Hibbs, 1948b).

Rumen Epithelial Development

Development of the rumen epithelium is critical for the absorption of fermentation products. Volatile fatty acids diffuse passively through the rumen epithelium into portal blood circulation. This area of the rumen is composed of structures known as papillae. These "finger-like" projections greatly increase surface area of the rumen mucosa and allow for efficient absorption of fermentation products. Once again, development of these structures is dependent on age and more importantly diet of the animal. Without the presence of VFA to serve as a chemical stimulus, papillae fail to develop fully (Warner et al., 1956).

Papillae are present at birth in the bovine rumen. At this time the structures are approximately 1 to 2.6 mm in length (Tamate et al., 1962; Huber, 1969). When compared to young ruminants that receive only milk, calves given access to solid feed have greater epithelial development with increasing age. From 1 to 4 weeks of age there is little remarkable papillae development in milk-fed calves. The surface of the rumen remains

relatively smooth with thin and flat epithelial cells (Warner et al., 1956; Zitnan et al., 1993). After 4 weeks of age papillae in calves receiving feed are 1.5 times longer than those receiving only milk. The rumen surface at this age is rough with developed papillae clearly visible (Warner et al., 1956; Tamate et al., 1962; Zitnan et al., 1993). By 8 weeks of age the difference in papillae length between milk and solid fed calves is 8-fold (Tamate et al., 1962). Papillae from young ruminants offered starter are longer, wider, and less dense than those from milk-fed animals (Zitnan et al., 1998; Zitnan et al., 1999; Lane et al., 2000). This difference in length and density continues to increase as consumption of starter increases (Stobo et al., 1966b).

The result of this increase in papillae length and width is greater absorptive surface area in the rumen (Zitnan et al., 1999). Absorption of VFA increases with age and access to starter. Calves consuming starter have greater absorption of VFA than milk-fed calves of the same age. Absorption of VFA does not increase with age in milk-fed calves (Sutton et al., 1963a). This increase in product absorption is correlated to the increase in papillae surface area. However, this development of papillae is not permanent. Papillae can easily regress back to smaller structures in the absence of solid feed and subsequent VFA production. Calves that have significant papillae development after access to solid feed show marked regression in papillae structure when switched to an all milk diet. Once developed, these epithelial structures must be maintained. Without sufficient concentrations of VFA, papillae will regress, even in adult animals. This regression in mucosal structures is more rapid than the regression of muscular development once it is established (Harrison et al., 1960).

Substrates available in solid feed also play a role in papillae development. Because the concentration of VFA is a critical stimulus for papillae development, the feedstuff offered

also effects mucosal development. Calves that are removed from all hay diets and placed on all concentrate diets show marked development of rumen papillae. Concentrate feeds promote greater production of total VFA, and produce high concentrations of butyrate. Butyrate serves as metabolic fuel for the epithelial cells promoting growth of the tissue. Therefore, diets that promote butyrate formation also support production of epithelial tissue. When calves are removed from all-concentrate diets and placed on all-hay diets a regression of the papillae is observed (Stobo et al., 1966b). Rations composed primarily of forage do not promote sufficient butyrate formation to support maximal epithelial development and maintenance. However, Warner et al. (1956) determined that there is little difference in papillae development on grain, hay, or hay-grain diets. There is a large amount of variation in papillae development with respect to diet composition.

Rumen Muscular Development

The consumption of dry feed does indeed promote the development of rumen capacity and musculature (Brownlee, 1956; Warner et al., 1956; Smith, 1961). Calves consuming poor quality roughage (wood shavings) or inert physical objects (plastic sponges) have increased development of rumen capacity and musculature (Flatt et al., 1958; Smith, 1961; Tamate et al., 1962). Presence of forage in the diet provides the bulk needed for development of rumen capacity and musculature. Young ruminants that are offered hay instead of milk replacer have larger rumino-reticulum capacity and more developed papillae. Although forages do not provide sufficient VFA to stimulate maximal mucosal development, they do provide enough VFA to stimulate minimal papillae development (Swan and Groenewald, 2000). Calves offered hay have larger rumen capacity than calves fed grain or milk only (Warner et al., 1956). This increase in capacity and musculature is less transient

than papillae development and is retained for a longer period of time if the animal is moved from a hay based diet to a milk only diet (Harrison et al., 1960).

Influence of Milk Feeding

As stated earlier the effect of prolonged milk feeding is profound. Calves receiving only milk replacer and no starter fail to develop successful microbial populations, produce sufficient VFA, and develop rumen papillae. The access to milk also affects intake of solid feed. Feeding large (>10% of BW) quantities of milk replacer results in greater weight gains and less starter intake. Calves receiving large amounts of milk and calves offered both milk and solid feed will have the same amount of total dry matter intake. This illustrates that calves will eat to meet their energy needs. If milk alone is not adequate to meet these needs, they will consume starter to further increase their energy intake (Huber et al., 1984). As the amount of milk offered decreases, the amount of voluntary intake of solid feed will increase, maintaining total dry matter intake (Broesder et al., 1990). However, quality of milk replacer can negatively impact feed intake. Calves receiving milk replacer with poor quality protein will consume less starter and in turn gain less weight preweaning (Kertz et al., 1979). The method of feeding and quality of milk replacer offered impacts the consumption of solid feed resulting in altered rumen development.

Criteria for Weaning

Historically, calves have been weaned based on age alone. This method of weaning is still practiced by many producers today. However, this method of weaning fails to take into account the impact of solid feed intake on rumen development and weight gain of the calf. The intake of starter alone accounts for 65% of variation in total body weight gain in calves (Kertz et al., 1979). Therefore, methods of weaning based on intake were developed

to account for variation associated with intake of solid feed. Leaver and Yarrow (1972) offered calves two levels of milk replacer (320 or 480 g) and weaned when calves were consuming 400, 650, or 900 g of starter. It was noted that calves of higher birth weight reached required levels of intake for weaning more rapidly than smaller calves. Calves receiving larger amount of milk replacer had reduced levels of starter intake, had increased total feed cost, and had largest costs per unit of gain. Increasing the amount of intake required for weaning also increased total feed costs and cost per unit of gain, but to a lesser extent than the larger volume of milk replacer. Although this method of weaning takes into account intake, it does not consider the large variation in the birth weights of dairy calves.

Due to the large variation in birth weights of dairy calves, particularly in mixed breed herds, weaning strategies were developed based on intake as a percentage of birth weight. Weaning calves when dry feed intake reaches 1% of birth weight for 3 consecutive days decreases variation in weaning age seen in the previously mentioned trial. This strategy for weaning has no negative effects on growth up to 20 weeks of age (Greenwood et al., 1997b). These types of early weaning programs are quite successful and the calves adapt well (Quigley et al., 1991).

Effect of Fiber Addition on Rumen Development

Epithelial Development.

Availability of substrates in concentrate feeds stimulates mucosal proliferation through the production of VFA. However, cellular growth produced by butyrate metabolism in epithelial cells with this type of diet is not controlled. Calves fed concentrate-based rations often have increased ruminal mucosa weights as a result of increased cellular growth. The concern is that concentrate rations do not contain adequate effective fiber to remove

degenerate cells that accumulate in the form of stratum corneum. These degenerate cells often become keratinized and act as a barrier to VFA absorption (Nocek et al., 1980).

Feed texture does influence papillae development. Diets without sufficient particle size cause thickening of the stratum corneum, including the keratin layer, and a decrease in size of stratum granulosum compared to normal papillae (Thompson et al., 1958; Hinders and Owen, 1965; Nocek et al., 1980). This increase in keratinization causes a decrease in VFA absorption from the rumen by decreasing the amount of metabolically active tissue available (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997a). In addition to the decrease in absorptive capacity, papillae that are not exposed to sufficient particle size in the diet also have an altered appearance.

Length, uniformity, and general appearance of papillae are greatly influenced by composition and particle size of the diet. Calves receiving concentrate diets have papillae that are longer, less uniform, and often have visible clumps and abnormal lesions compared to calves receiving ground or chopped hay (Nocek et al., 1980). Morphological abnormalities also occur more frequently in calves fed concentrate diets versus hay (Nocek et al., 1984).

Feeding of identical diets with different particle sizes also influences papillae development and maintenance. Diets that include finely ground hay have irregular papillae that are thicker. These papillae also begin to branch. However, despite branching and increased thickness, these papillae have less surface area than those found in rumen samples from calves receiving chopped hay. Papillae from diets containing chopped hay are more uniform and have a flattened and tongue-shaped appearance (Beharka et al., 1998). The keratin portion of the papillae is higher for finely ground diets, indicating that as particle size

decreases so does abrasive value of the feedstuff. This abrasive value is critical for maintenance of epithelial tissue and the prevention of keratinization (Greenwood et al., 1997a). Diets containing small particle size and high levels of concentrate promote keratinization of the rumen papillae (Hinders and Owen, 1965; Greenwood et al., 1997a).

Pelleted rations must contain feedstuffs of small particle size for successful processing. When compared to conventional diets (starter plus hay) complete pelleted rations (containing up to 50% roughage) result in abnormal papillae development. The papillae from complete pelleted rations are wider and longer. These papillae tend to branch and the tips become encrusted with feed. These long papillae are poorly attached and moderately keratinized (Thompson et al., 1958; Nocek and Kesler, 1980). Despite the presence of roughage in these pelleted diets, abnormal papillae development occurs. It is particle size of the ration that determines the abrasive value of the feedstuff rather than chemical composition, thus explaining the impact on papillae development and health.

VFA Production

The addition of forage to a high concentrate diet decreases the amount of total VFA produced in the rumen and increases the proportion of acetate produced (Quigley et al., 1992a; Zitnan et al., 1998). However, others have seen no alterations in VFA concentration when hay is added to the diet (Hibbs et al., 1956). Calves fed pelleted high roughage rations experience increased levels of VFA and low cellulolytic activity compared to diets with long hay (Conrad et al., 1958). This results in lower acetate production (Bull et al., 1965). The capacity of rumen cells to oxidize substrate in vitro is unaffected by the forage to concentrate ratio (Baldwin and McLeod, 2000). Ruminal and blood concentrations of butyrate are lower when hay is added to the diet (Quigley et al., 1992a).

Particle size of ration forage components may also influence VFA production. Rations that are identical in composition but with differing particle size influence VFA production in the rumen. As particle size of a ration, particularly the forage component, decreases, acetate to propionate ratios also decrease (Santini et al., 1983; Kerley et al., 1985). Complete and pelleted rations also produce less acetate than rations with starter plus hay. This difference in VFA production is due to the decrease in particle size of the components. This is required to make pelleted rations (Nocek and Kesler, 1980). However, several investigations have concluded that the particle size of the ration has little effect on molar concentrations of VFA and the acetate to propionate ratio (Greenwood et al., 1997a; Beharka et al., 1998). Investigations that were able to show an influence of particle size on VFA concentrations also observed changes in rumen environment (pH and microbial concentrations). It is the alteration of rumen environment that leads to changes in microbial populations and thus altered proportions of VFA.

pH

Several investigations have noted an increase in ruminal pH with increasing levels of forage in the diet (Conrad et al., 1958; Zitnan et al., 1998), but others have observed no change in rumen pH related to fiber addition in the diet (Hibbs et al., 1956; Quigley et al., 1992a). However, particle size of the forage component also influences ruminal pH. Sufficient particle size in the diet is required to promote rumination and saliva flow into the rumen.

Diets with decreased particle size (ground versus chopped hay) promote lowering of ruminal pH. Ruminal pH is lower for calves fed fine versus intermediate or coarsely ground diets (Greenwood et al., 1997a; Beharka et al., 1998). The same trend is also observed in

mature animals fed diets with differing particle size. Rumen pH is also lower in mature cows receiving decreasing levels of TMR particle size (0.31, 0.43, and 0.51 cm) (Santini et al., 1983). Thomas and Hinks (1982) showed that chopping roughage into 20-mm lengths does not alter rumen-buffering characteristics of long roughage. Pelleted diets with high roughage content also induce lower rumen pH due to small particle size of the ration components (Conrad et al., 1958 and Bull et al., 1965).

Therefore, roughage must be of a small particle size to lower pH of rumen contents. The influence of this change in rumen pH is critical to substrate utilization. Declines in rumen pH in turn alter microbial populations influencing the production of VFA from substrate. Calves with lower rumen pH have increased populations of amylolytic bacteria and fewer cellulolytic bacteria (Beharka et al., 1998). Cellulolytic bacteria are extremely sensitive to changes in rumen pH (Russell and Dombrowski, 1980). A decline in this population accounts for the decrease in acetate production from finely ground roughages (Nocek and Kesler, 1980; Kerley et al., 1985).

Physical Fill

Development of rumen capacity or fill is linked to the amount of roughage in the diet and the particle size of the ration. Calves and lambs receiving starter rations based on roughage have larger rumen capacity than animals receiving milk or grain (Warner, 1961; Strozinski and Chandler, 1971). Rumen volume can increase up to two fold with addition of chopped hay to the diet (Williams et al., 1985c). As the proportion of forage in the diet is increased, the rumen capacity increases accordingly (Strozinski and Chandler, 1971). However, mature rumen capacity will develop eventually even in the absence of grain or hay (Warner, 1961; Williams et al., 1985c).

Particle size of the dietary forage also may influence rumen capacity. Calves receiving chopped hay have greater rumen volume than those receiving ground hay. This investigation did not measure the specific particle size of each diet (Nocek et al., 1984). However, another investigation observed no change in rumen fill in relation to particle size (determined by sieve size) of the diet (Beharka et al., 1998). At this time the precise particle size required to stimulate development of rumen fill is unknown.

Flow of Digesta

Physical form of the ration has a large impact on the flow of digesta through the gastrointestinal system. As particle size decreases the flow of digesta increases (Kerley et al., 1985). The grinding and subsequent pelleting of a ration decreases retention time in the rumen. The same result occurs in finely ground forages of small particle size. This reduction in particle size often results in reduced digestibility of the diet due to insufficient time for fermentation of feed in the rumen (Hodgson, 1971).

Rumination and Saliva Production

Rumination and saliva production are highly related to amount of forage in the diet and particle size of the forage component. Rumination begins at approximately 2 weeks of age if the calf is offered solid feed. By 6 to 8 weeks of age most calves are actively ruminating solid feed of sufficient particle size (Conrad et al., 1958). A decrease in ration particle size is associated with a decrease in rumination and saliva flow into the rumen (Kellaway et al., 1977; Santini et al., 1983). Long roughage takes longer to eat than pellets and requires 7 times the amount of rumination. This increase in rumination promotes flow of saliva into the rumen, buffering the pH (Hodgson, 1971).

Complete pelleted diets often contain predetermined amounts of grain and roughage in small particle sizes. The decrease in particle size is necessary for processing. Pelleted diets may provide adequate amounts of forage to the calf, but it will not provide stimulus needed for rumination (Kellaway et al., 1977). However, the addition of buffers (sodium bicarbonate) to high grain diets successfully buffers pH of the rumen and promotes an increased acetate to propionate ratio (Kellaway et al., 1973b, Curnick et al., 1983, and Quigley et al., 1992b).

Evaluating Particle Size and Abrasive Value

There are numerous methods used to determine both particle size and abrasive value of a feedstuff. Particle size distribution of a ration can be determined in laboratories using a series of screens. A simple separator system is often used on the farm to determine the particle size of forages and total mixed rations (TMR). Lammers et al. (1996) developed this system after the more elaborate laboratory screens. This simple system uses a series of boxes or pans with varying holes in the bottom. The top box has holes 19 mm in diameter and is 12.2 mm thick. The middle box has holes 8 mm in diameter and 6.4 m thick. The box has solid bottom. The boxes are stacked and a known amount of sample (approximately 1.4 l) is placed in the top box. The box is then shaken horizontally 5 times in one direction. This is repeated by making one-quarter turns with the box until each side is shaken. The sample is then removed form each tray, weighed, and the proportion of the total sample weight is calculated. Particles greater than 19 mm remain in the top box, particles less than 19 mm and greater than 8mm remain in the middle box, and particles less than 8 mm are found in the bottom pan.

Particle size of the ration determines the ability of that feed to provide abrasion. Dietary abrasive value (DAV) determines the ability of a feed particle to wear, grind, or rub away at the surface of the rumen. As particle size decreases, the abrasive value also declines. Greenwood et al. (1997a) developed a system to measure abrasive value of feed using a mixer hook dipped in paraffin wax. A known weight of paraffin is added to the hook and placed in a mixer with 500 g of feed. The mixer is then run for 1.5 hours. After mixing the hook is weighed to determine the amount of paraffin displaced. Diet abrasion value is defined as the amount (grams x 100) of paraffin that is abraded during the mixing process. The greater the DAV value of a feed the greater the ability to abrade the epithelial surface. This abrasive value discourages keratinization of the rumen papillae maintaining integrity of the mucosal surface.

Fiber Inclusion - Intake and Feed Efficiency

The addition of forage to the diet of young calves influences both intake and feed efficiency. When forage is added to the ration, intake increases (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973a; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have found a negative correlation between the intake of starter and hay in the ration (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975). The intake of rations increase if it is in a pelleted form (Hodgson, 1971; Bartley, 1973; Kang and Leibholz, 1973; Stobo et al., 1985). Pelleting of forages yields inconsistent results. Some investigations determined that pelleting decreases weight gain and feed efficiency (Leibholz, 1975), although others saw increases in weight gain and feed efficiency (Hodgson, 1971; Borland and Kesler, 1979; Stobo et al., 1985).

The quality of the source of fiber added to the diet also determines the acceptability and efficiency. Grass and legume hay and straw are common sources of fiber in the diet of the young calf. The quality of these forage sources greatly impacts the palatability and the digestibility of the diet. Poor quality roughage such as straw bedding has little impact on grain consumption or weight gain (Morrill and Dayton, 1981). Further treatment of straw by ammoniation yields no improvement in intake or digestibility (Williams et al., 1985c). Hibbs et al. (1953) suggested that a high proportion of legume hay is desirable in mixes, but notes that immature grass hay is also readily consumed. This author also recommends a 2:1 ratio of grain to hay. Calves consume greater amounts of second cutting alfalfa-timothy hay than a first cutting from the same plot. The maturity of the forage also plays a role. Late cut hay is often inferior to early first and second cuttings (Hemken et al., 1958). However, legume and grass hay are not the only forage sources available for use in starter rations.

Alternative Fiber Sources

Alternate sources of fiber have been successful additions to starter diets for young calves. Cottonseed hulls added as additional fiber to complex starters increase intake and weight gain (Miller et al., 1969). Diets containing whole cottonseed are also readily consumed and promote adequate growth (Anderson et al., 1982). The addition of beet pulp to starter rations also increases dry matter intake (Williams et al., 1985b).

Fiber sources added to complete diet pellets include, alfalfa hay, cottonseed hulls, citrus or beet pulp, and soybran flakes. These fiber sources can be effectively utilized in complete starters. However, due to the small particle size, these sources provide little diet abrasion value (Addanki et al., 1966a and 1966b; Murdock and Wallenius, 1980; Williams et

al., 1987). However, it appears there is no advantage to feeding more complex or complete starters to young calves (Murley et al., 1958).

High quality silage also appears to be an acceptable forage source for calves. However, one must take care in assuring the product is fresh and safe for consumption by young ruminants (Porter and Kesler, 1957; Bush, 1991). Block and Shellenberger (1980) compared the feeding of corn silage or woodpulp fines along with starter in young calves from birth to 36 weeks of age. Both fiber sources were acceptable. Woodpulp yielded the most normal papillae, but gave the least amount of gain. The authors concluded that the optimal proportion of all these fiber sources in the diet of young calves should be further investigated (Block and Shellenberger, 1980a and 1980b).

Parakeratosis

The condition of rumen parakeratosis is characterized by long, dark, and unhealthy papillae. These papillae may also slough away from the rumen wall as they become more keratinized (Cullison, 1961). The papillae themselves have crusting of the tips and often become clumped with rumen content (Thompson et al., 1958). This condition is related to uncontrolled cell growth due to high levels of VFA production with insufficient particle size of the diet to provide abrasive value (Gilliland et al., 1962). Keratinized papillae experience lowered levels of VFA absorption (Bull et al., 1965; Hinders and Owen, 1965). When hay is added to a starter diet composed of barley and soybean meal the papillae are longer than when no hay is present. Papillae from the starter diet without hay experience strong proliferation with parakeratosis (Zitnan et al., 1998).

Particle size of the forage source is critical to abrasion value of the feed. Calves offered alfalfa in the form of a pellet versus long hay have changes in papillae structure (Bull

et al., 1965; Hinders and Owen, 1965). Papillae vary from reddening and hypertrophy to severely keratinized and atrophic. Severe keratinization often leads to sloughing (Hinders and Owen, 1965). Particle sizes of individual diets greatly influence papillae development. Calves fed pellets and hay have papillae that are flattened and tongue-shaped similar to normal papillae. Calves fed concentrate and ground hay have tongue-like and rounded finger-like papillae. Calves fed concentrate or concentrate and whey develop small, nodular papillae that are branched into a cauliflower shape. These papillae are classified as severely parakeratotic (McGavin and Morrill, 1976).

Summary and Conclusions

Research has yet to determine the optimal starter ration for the young calf that enhances healthy rumen development. Although many trials have tested the use of grain, pellets, and forage in many combinations, the conclusions of these trials are often conflicting. There are so many possible combinations of feedstuffs available that it makes comparisons between trials difficult. Therefore, the ideal ratio of forage to grain has yet to be determined.

It is clear that concentrate feeds are a necessary component of starter rations for the preruminant calf. However, the amount of forage, if any, required is unknown. Concentrate feeds provide energy and protein in sufficient amounts needed by the rapidly growing calf. In addition, concentrate feeds provide the necessary chemical stimulus to promote mucosal development. In particular, butyrate is necessary for papillae growth. However, this cellular growth lacks control. Uncontrolled growth of the rumen epithelium can lead to problems such as parakeratosis. This condition is characterized by keratinized papillae that have an abnormal appearance. These papillae lack the effective absorptive surface required for the passive diffusion of nutrients from the rumen.

Forage feedstuffs that are not finely ground do provide the abrasive value required to avoid keratinization of the papillae. These feedstuffs are important in maintaining integrity of the rumen epithelium. In addition, forages provide stimulus for rumination and the accompanying flow of saliva into the rumen. This increase in saliva flow provides an influx of buffers into the rumen environment. The flow of saliva stabilizes rumen pH providing an optimal environment for cellulolytic bacteria. These bacteria are extremely sensitive to changes in rumen pH. The presence of forage alone does not provide a chemical stimulus to promote rumination. The process is controlled entirely by particle size of the feedstuff. Forages will promote rumination only if particle size is sufficient. However, regardless of the particle size, forages are fermented more slowly in the rumen causing less rapid fluctuations in rumen pH. However, forages do not provide adequate VFA concentrations needed for optimal rumen development. The effectiveness of these feedstuffs is highly related to particle size.

Literature Cited

Addanki, S., J.W. Hibbs and H.R. Conrad. 1966. High-roughage system for raising calves based on the early development of rumen function. XI. Performance of calves fed alfalfa, beet pulp, or soybran flakes as the roughage in complete pelleted rations. J. Dairy Sci. 49:976-981.

Addanki, S., J.W. Hibbs and H.R. Conrad. 1966. High-roughage system for raising calves based on the early development of rumen function. XII. In vivo and in vitro changes in rumen contents of calves fed alfalfa, beet pulp, or soybran flakes as the roughage in complete pelleted rations. J. Dairy Sci. 49:982-985.

Anderson, K.L., T.G. Nagaraja, J.L. Morrill, T.B. Avery, S.J. Galitzer and J.E. Boyer. 1987. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215-1226.

Anderson, M.J., M. Khoyloo and J.L. Walters. 1982. Effect of feeding whole cottonseed on intake, body weight, and reticulorumen development in young Holstein calves. J Dairy Sci. 65:764-772.

Annison, E.F., K.J. Hill, and D. Lewis. 1957. Studies on the portal blood of sheep. 2. Absorption of volatile fatty acids from the rumen of the sheep. Biochem. J. 66:592-599.

Arias, J.L., E. Vial and R. Cabrera. 1980. Observations on the histogenesis of bovine ruminal papillae. Am. J. Vet. Res. 41:174-178.

Attebery, J.T. and H.W. Colvin. 1963. Effect of diet on fasting blood glucose levels in dairy calves 1 to 13 weeks old. J. Appl. Phy. 18:1221-1225.

Bailey, C.B. 1986. Growth of digestive organs and their contents in Holstein steers: Relation to body weight and diet. Can. J. Anim. Sci. 66:653-661.

Baldwin, R.L. and M.J. Allison. 1983. Rumen metabolism. J. Anim. Sci. 57:461-477.

Baldwin, R.L. and B.W. Jessee. 1992. Developmental changes in glucose and butyrate metabolism by isolated sheep ruminal cells. J. Nutr. 122:1149-1153.

Baldwin, R.L. and K.R. McLeod. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on isolated rumen epithelial cell metabolism in vitro. J. Anim. Sci. 78:771-783.

Bartley, E.E. 1973. Effects of a self-fed pelleted mixture of hay and calf starter on the performance of young dairy calves. J. Dairy Sci. 56:817-820.

Becker, R.B., P.T. Dix Arnold, and S.P. Marshall. 1951. Development of the bovine stomach during fetal life. J. Dairy Sci. 34:329-332.

Beharka, A.A., T.G. Nagaraja, J.L. Morrill, G.A. Kennedy and R.D. Klemm. 1998. Effect of form of diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946-1955.

Block, E. and P.R. Shellenberger. 1980. Woodpulp fines or corn silage as roughages in complete rations or a pelleted complete ration for young dairy replacements from birth through 18 weeks of age. J. Dairy Sci. 63:2060-2070.

Block, E. and P.R. Shellenberger. 1980. Woodpulp fines or corn silage as roughages in complete rations or a pelleted complete ration for young dairy replacements 18 through 36 weeks of age. J. Dairy Sci. 63:2071-2079.

Bomba, A. and R. Zitnan. 1992. Development of rumen fermentation in calves during milk feeding. Vet. Med. (Praha) 37:75-82.

Borland, K. and E.M. Kesler. 1979. Complete rations for Holstein calves 8 to 18 weeks of age. J. Dairy Sci. 62:304-309.

Broesder, J.T., M.B. Judkins, L.J. Kryst, S.A. Gunter and R.K. Barton. 1990. Thirty or sixty percent milk replacer reduction for calves: effects on alfalfa hay intake and digestibility kinetics and ruminal fermentation. J. Anim. Sci. 68:2974-2985.

Brownlee, A. 1956. The development of rumen papillae in cattle fed on different diets. Brit. Vet. J. 112:369.

Bryant, M.P., N. Small, C. Bouma and I. Robinson. 1958. Studies on the composition of the ruminal flora and fauna of young calves. J. Dairy Sci. 41:1747-1767.

Bull, L.S., L.J. Bush, J.D. Friend, B. Harris, and E.W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. J. Dairy Sci., 48:1459-1466.

Bush, R.S. 1991. The effects of hay and silage on growth and rumen function in young Holstein calves. Can. J. Anim. Sci. 71:145-153.

Chapman, H.W., D.G. Butler, and M. Newell. 1986. The route of liquids administered to calves by esophageal feeder. Can. J. Vet. Res. 50:84-87.

Conrad, H.R., J.W. Hibbs, and N. Frank. 1958. High roughage system for raising calves based on early development of rumen function. IX. Effect of rumen inoculations and chlortetracycline on rumen function of calves fed high roughage pellets. J. Dairy Sci. 41:1248-1261.

Cook, R.M., S.C.C. Liu, and S. Quraishi. 1969. Utilization of volatile fatty acids in ruminants . III. Comparison of mitochondrial acyl coenzyme A synthetase activity and substrate specificity in different tissues. Biochemistry. 8:2966-2971.

Coombe, N.B. and R.H. Smith. 1974. Digestion and absorption of starch, maltose and lactose by the preruminant calf. Br. J. Nutr. 31:227-235.

Cullison, A.E. 1961. Effect of physical form of the ration on steer performance and certain rumen phenomena. J. Anim. Sci. 20:478-483.

Curnick, K.E., L.D. Muller, J.A. Rogers, T.J. Snyder, and T.F. Sweeney. 1983. Addition of sodium bicarbonate to calf starter rations varying in protein percent. J. Dairy Sci. 66:2149-2160

Eadie, J.M. 1962. The development of rumen microbial populations in lambs and calves under various conditions of management. J. Gen. Microbiol. 29:563-578.

Flatt, W.P., R.G. Warner and J.K. Loosli. 1958. Influence of purified materials on the development of the ruminant stomach. J. Dairy Sci. 41:1593-1600.

Fonty, G., P. Gouet, J.P. Jouany and J. Senaud. 1987. Establishment of the microflora and anaerobic fungi in the rumen of lambs. J. Gen. Microbiol. 133:1835-1843.

Fonty, G., J.P. Jouany, M. Chavarot, F. Bonnemoy and P. Gouet. 1991. Development of the rumen digestive functions in lambs placed in a sterile isolator a few days after birth. Reprod. Nutr. Dev. 31:521-528.

Garnot, P.R., J.L. Toullec, J.L. Thapon, P. Martin, M.T. Hoang, C.M. Mthieu, and B. Ribadeau-Dumas. 1977. Influence of age, dietary protein and weaning on calf abomasal enzymatic secretion. J. Dairy Sci. 44:9-23.

Gilliland, R.L., L.J. Bush, and J.D. Friend. 1962. Relation of ration composition to rumen development in early-weaned dairy calves with observations on ruminal parakeratosis. 45:1211-1217.

Godfrey, N.W. 1961a. The functional development of the calf. I. Growth of the stomach of the calf. J. Agric. Sci. 57:173-175.

Godfrey, N.W. 1961b. The functional development of the calf. II. Development of rumen function in the calf. J. Agric. Sci. 57:177-183.

Greenwood, R.H., J.L. Morrill, E.C. Titgemeyer and G.A. Kennedy. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. J. Dairy Sci. 80:2534-2541.

Greenwood, R.H., J.L. Morrill and E.C. Titgemeyer. 1997. Using dry feed intake as a percentage of initial body weight as a weaning criterion. J. Dairy Sci. 80:2542-2546.

Hamada, T., S. Maeda and K. Kameoka. 1976. Factors influencing growth of rumen, liver, and other organs in kids weaned from milk replacers to solid foods. J. Dairy Sci. 59:1110-1118.

Harrison, H.N., R.G. Warner, E.G. Sander, and J.K. Loosli. 1960. Changes in the tissue and volume of the stomachs of calves following the removal of dry feed or consumption of inert bulk. J Dairy Sci. 43:1301-1312.

Hemken, R.W., G.W. Trimberger, and K.L. Turk. 1958. Growth rate of dairy calves fed on hay harvested at different stages of maturity and on two levels of grain. Bulletin #933 Cornell University Agricultural Experiment Station. Ithaca, N.Y.

Hibbs, J.W., W.D. Pounden and H.R. Conrad. 1953. A high roughage system for raising calves based on the early development of rumen function. I. Effect of variations in the ration on growth, feed consumption, and utilization. J. Dairy Sci. 36:717-727.

Hibbs, J.W., H.R. Conrad, W.D. Pounden, and N. Frank. 1956. A high roughage system for raising calves based on early development of rumen function. VI. Influence of hay to grain ration on calf performance, rumen development, and certain blood changes. J. Dairy Sci. 39:171-179.

Hinders, R.G. and F.G. Owen. 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. J. Dairy Sci. 48:1069-1078.

Hird, F.J.R. and M.J. Weidemann. 1964a. Transport and metabolism of butyrate by isolated rumen epithelium. Biochem. J. 92:585-589.

Hird, F.J.R. and M.J. Weidemann. 1964b. Ketone body synthesis in relation to age in lambs. Biochem. J. 93:423-430.

Hodgson, J. 1971. The development of solid food intake in calves. 1. The effect of previous experience of solid food, and the physical form of the diet, on the development of food intake after weaning. Anim. Prod. 13:15-24.

Hofmann, R.R. 1988. Anatomy of the Gastrointestinal Tract in The Ruminant Animal: Digestive Physiology and Nutrition. Page 14. Waveland Press, Inc. Prospect Heights, IL.

Huber, J.T., N.L. Jacobson, and R.S. Allen. 1961. Digestive enzyme activities in the young calf. J. Dairy Sci. 44:1494-1501.

Huber, J.T. 1969. Symposium: calf nutrition and rearing. Development of the digestive and metabolic apparatus of the calf. J. Dairy Sci. 52:1303-1315.

Huber, J.T., A.G. Silva, O.F. Campos and C.M. Mathieu. 1984. Influence of feeding different amounts of milk on performance, health, and absorption capability of baby calves. J. Dairy Sci. 67:2957-2963.

Jahn, E., P.T. Chandler, and C.E. Polan. 1970. Effects of fiber and ratios of starch to sugar upon the performance of young ruminating calves. J. Dairy Sci. 53:466-474.

Joyner, A.E., E.M. Kesler, and J.B. Holter. 1963. Absorption from the omasum and subsequent metabolism of butyrate and acetate. J. Dairy Sci. 46:1108-1113.

Kang, H.S. and J. Leibholz. 1973. The roughage requirement of the early-weaned calf. Anim. Prod. 16:195-203.

Kellaway, R.C., T. Grant, and J.W. Chudleigh. 1973a. The roughage requirement of early weaned calves. Aust. J. Exp. Agric. Anim. Husb. 13:9-12.

Kellaway, R.C., T. Grant, and J.W. Chudleigh. 1973b. The effect of roughage and buffers in the diet of early weaned calves. Aust. J. Exp. Agric. Anim. Husb. 13:225-228.

Kellaway, R.C., D.J. Thomaon, D.E. Beever, and D.F. Osbourn. 1977. Effects of NaCl And $NaHCO_3$ on food intake, growth rate, and acid-base balance in calves. J. Agric. Sci. (Camb) 88:1-9.

Kerley, M.S., A.R. Kinser, J.L. Firkins, G.C. Fahey and L.L. Berger. 1985. Effects of roughage particle size on site of nutrient digestion and digesta flow through the gastrointestinal tract of sheep fed corncob-concentrate diets. J. Anim. Sci. 61:504-513.

Kertz, A.F., L.R. Prewitt, and J.P. Everett. 1979. An early weaning calf program: Summarization and review. J. Dairy Sci. 62:1835-1843.

Kesler, E.M., M. Ronning and C.B. Knodt. 1951. Some physical characteristics of the tissue and contents of the rumen, abomasum, and intestines in male Holstein calves of various ages. J. Anim. Sci. 10:969-974.

Kincaid, R.L. 1980. Alternate methods of feeding alfalfa to calves. J. Dairy Sci. 63:91-94.

Klein, R.D., R.L. Kincaid, A.S. Hodgson, J.H. Harrison, J.K. Hillers, and J.D. Cronrath. 1987. Dietary fiber and early weaning on growth and rumen development in calves. J. Dairy Sci. 70:2095-2104.

Koundakjian, P. and A.M. Snoswell. 1970. Ketone body and fatty acid metabolism in sheep tissues. Biochem. J. 119:49-57.

Lammers, B.P., D.R. Buckmasters, and A.J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Lane, M.A. and B.W. Jesse. 1997. Effect of volatile fatty acid infusion on development of the rumen epithelium in neonatal sheep. J. Dairy Sci. 80:740-746.

Lane, M.A., R.L. Baldwin and B.W. Jesse. 2000. Sheep rumen metabolic development in response to age and dietary treatments. J. Anim. Sci. 78:1990-1996.

Lane, M.A., R.L. Baldwin, and B.W. Jesse. 2002. Developmental changes in ketogenic enzyme gene expression during sheep rumen development. J. Anim. Sci. 80:1538-1544.

Lavker, R., W. Chalupa, and P. Opliger. 1969. Histochemical observations on rumen mucosa structure and composition. J. Dairy Sci. 52:266-269.

Leaver, J.D. and N.H. Yarrow. 1972. Rearing of dairy cattle. 2. Weaning calves according to their concentrate intake. Anim. Prod. 14:161-165.

Lehninger, A.L., D.L. Nelson, and M.M. Cox. 1993. Principles of Biochemistry. 2nd ed. Worth Publishers, New York, N.Y.

Leibholz, J. 1975. Ground roughage in the diet of the early-weaned calf. Anim. Prod. 20:93-100.

Leng, R.A. and E.F Annison. 1964. The metabolism of D (-)-β-hydroxybutyrate in sheep. Biochem. J. 90:464-469.

Leng, R.A. 1969. Formation and production of volatile fatty acids in the rumen. Page 406 in Physiology of Digestion and Metabolism in the Ruminant. A.T. Phillipson ed. Oriel Press, Newcastle upon Tyne, England.

Lengemann, F.W. and N.N. Allen. 1955. The development of rumen function in the Dairy calf I. Some characteristics of the rumen contents of cattle of various ages. J. Dairy Sci. 38:651-656.

Lengemann, F.W. and N.N. Allen. 1959. Development of rumen function in the dairy calf. II. Effect of diet upon characteristics of the rumen flora and fauna of young calves. J. Dairy Sci. 42:1171-1181.

Liang, Y.T., J.L. Morrill and J.L. Noordsy. 1967. Absorption and utilization of volatile fatty acids by the young calf. J. Dairy Sci. 50:1153-1157.

Lupien, P.J., F. Sauer, and G.V. Hatina. 1962. Effects of removing the rumen, reticulum, omasum, and proximal third of the abomasum on digestion and blood changes in calves. J. Dairy Sci. 45:210-217.

McCarthy, R.D. and E.M. Kesler. 1956. Relation between age of calf, blood glucose, blood and rumen levels of volatile fatty acids, and in vitro cellulose digestion. J. Dairy Sci. 39:1280-1287.

McCormick, R.J. and W.E. Stewart. 1967. Pancreatic secretion in the bovine calf. J. Dairy Sci. 50:568.

McGavin, M.D. and J.L. Morrill. 1976. Scanning electron microscopy of ruminal papillae in calves fed various amounts and forms of roughage. Am. J. Vet. Res. 37:497-508.

Mentschel, J., R. Leiser, C. Mulling, C. Pfarrer, and R. Claus. 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction in apoptosis. Arch. Tierernahr. 55:85-102.

Miller, W.J., Y.G. Martin and P.R. Fowler. 1969. Effects of addition of fiber to simplified and to complex starters fed to young dairy calves. J. Dairy Sci. 52:672-676.

Morrill, J.L. and A.D. Dayton. 1981. Method of feeding milk and access to fiber source for young calves. J. Dairy Sci. 64:146-148.

Murdock, F.R. and R.W. Wallenius. 1980. Fiber sources for complete calf starter rations. J. Dairy Sci. 63:1869-1873.

Murley, W.R., R.D. Mochrie, J.R. Edwards and B.F. Holloy. 1958. Response of young dairy calves fed a simple versus complex starter with various kinds of hay. J. Dairy Sci. 41:982-986.

Nocek, J.E., J.H. Herbein and C.E. Polan. 1980. Influence of ration physical form, ruminal degradable nitrogen and age on rumen epithelial propionate and acetate transport and some enzymatic activities. J. Nutr. 110:2355-2364.

Nocek, J.E. and E.M. Kesler. 1980. Growth and rumen characteristics of Holstein steers fed pelleted or conventional diets. J. Dairy Sci. 63:249-254.

Nocek, J.E., C.W. Heald and C.E. Polan. 1984. Influence of ration physical form and nitrogen availability on ruminal morphology of growing bull calves. J. Dairy Sci. 67:334-343.

Otterby, D.E., H.A. Ramsey, and G.H. Wise. 1964a. Lipolysis of milk fat by pregastric esterase in the abomasum of the calf. J. Dairy Sci. 47:993-993.

Otterby, D.E., H.A. Ramsey, and G.H. Wise. 1964b. Source of lipolytic enzymes in the abomasum of the calf. J. Dairy Sci. 47:997-999.

Pennington, R.J. 1952. The metabolism of short-chain fatty acids in the sheep. 1. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. Biochem. J. 51:251-258.

Pennington, R.J. and W. H. Pfander. 1957. The metabolism of short-chain fatty acids in the sheep. 5. Some interrelationships in the metabolism of fatty acids and glucose by sheep rumen epithelial tissue. Biochem. J. 65:109-111.

Poe, S.E., D.G. Ely, G.E. Mitchell, W.P. Deweese and H. A. Glimp. 1971a. Rumen development in lambs. I. Microbial digestion of starch and cellulose. J. Anim. Sci. 32:740-743.

Poe, S.E., D.G. Ely, G.E. Mitchell, H.A. Glimp and W.P. Deweese. 1971b. Rumen development in lambs. II. Rumen metabolite changes. J. Anim. Sci. 32:989-993.

Porter, G.H. and E.M. Kesler. 1957. Value of alfalfa silage in the diet of the young dairy calf. J. Dairy Sci. 40:163-172.

Porter, J.W.G. 1969. Digestion in the pre-ruminant animal. Symposium of The Nutrition Society on Digestion in the Abomasum and Intestine of the Ruminant. Proc. Nutr. Soc. 28:115-121.

Pounden, W.D. and J.W. Hibbs. 1948a. The influence of the ration and rumen inoculation on the establishment of certain microorganisms in rumens of young calves. J. Dairy Sci. 31:1041-1050.

Pounden, W.D. and J.W. Hibbs. 1948b. The influence of the ration of grain to hay in the ration of dairy calves on certain rumen microorganisms. J. Dairy Sci. 31:1051-1054.

Quigley, J.D., Z.P. Smith and R.N. Heitmann. 1991. Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. J. Dairy Sci. 74:258-263.

Quigley, J.D., III and J.K. Bernard. 1992. Effects of nutrient source and time of feeding on changes in blood metabolites in young calves. J. Anim. Sci. 70:1543-1549.

Quigley, J.D., T.M. Steen and S.I. Boehms. 1992a. Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed with or without hay. J. Dairy Sci. 75:228-235.

Quigley, J.D., III, L.B. Wallis, H.H. Dowlen, and R.N. Heitmann. 1992b. Sodium bicarbonate and yeast culture effects on ruminal fermentation, growth, and intake in dairy calves. J. Dairy Sci. 75:3531-3538.

Ramsey, H.A. 1962. Electrophoretic characterization of pregastric esterase from the calf. J. Dairy Sci. 45:1479-1486.

Ramsey, H.A. and C.L. Davis. 1965. Metabolism of n-butyrate by the adult goat. J. Dairy Sci. 48:381-390.

Ramsey, H.A. and T.R. Willard. 1976. Utilization of soy protein by newborn mammals. World Soybean Research:977-989.

Ramsey, H.A. and T.R. Willard. 1975. Symposium: Recent advances in calf rearing: Soy protein for milk replacers. J. Dairy Sci. 58:436-441.

Ramsey, H.A. and J.W. Young. 1961. Substrate specificity of pregastric esterase from the calf. J. Dairy Sci. 44:2304-2306.

Russell, J.B. and D.B. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Micro. 39:604-610.

Russell, J.B. and R.B. Hespell. 1981. Microbial rumen fermentation. J. Dairy Sci. 64: 1153-1169.

Sakata, T. and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61:1109-1113.

Sakata, T. and H. Tamate. 1979. Rumen epithelium cell proliferation accelerated by propionate and acetate. J. Dairy Sci. 62:49-52.

Sander, E.G., R.G. Warner, H.N. Harrison and J.K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42:1600-1605.

Santini, F.J., A.R. Hardie, N.A. Jorgensen, and M.F. Finner. 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66:811-820.

Smith, R.H. 1961. The development and function of the rumen in milk-fed calves. II. Effect of wood shavings in the diet. J. Agric. Sci. 56:105-111.

Steven D.H. and A.B. Marshall. 1969. Organization of the rumen epithelium. Page 80 in Physiology of Digestion and Metabolism in the Ruminant. A.T. Phillipson ed. Oriel Press, Newcastle upon Tyne, England.

Stevens, C.E. 1969. Fatty acid transport through the rumen epithelium. Page 101 in Physiology of Digestion and Metabolism in the Ruminant. A.T. Phillipson ed. Oriel Press, Newcastle upon Tyne, England.

Stobo, I.J.F., J.H.B. Roy and H.J. Gaston. 1966a. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. Br. J. Nutr. 20:171-188.

Stobo, I.J.F., J.H.B. Roy and H.J. Gaston. 1966b. Rumen development in the calf. 2. The effect of diets containing different proportions of concentrates to hay on digestive efficiency. Br. J. Nutr. 20:189-215.

Stobo, I.J.F., C.S. Lucci, J.H.B. Roy, and M.W. Perfitt. 1985. Comparison of high-energy pellets containing processed fibre with a coarse concentrate mixture in relation to the development of solid food intake in the calf. Anim. Prod. 40:570.

Strozinski, L.L. and P.T. Chandler. 1971. Effects of dietary fiber and acid detergent lignin on body fill of ruminating calves. J. Dairy Sci. 54:1491-1495.

Sutton, J.D., A.D. McGilliard and N.L. Jacobson. 1963a. Functional development of rumen mucosa. I. Absorptive ability. J. Dairy Sci. 46:426-436.

Sutton, J.D., A.D. McGilliard, M. Richardson and N.L. Jacobson. 1963b. Functional development of rumen mucosa. II. Metabolic activity. J. Dairy Sci. 46:530-537.

Swan, G.E. and H.B. Groenewald. 2000. Morphological changes associated with the development of the rumino-reticulum in growing lambs fed different rations. Onderstepoort J. Vet. Res. 67:105-114.

Tamate, H., A.D. McGilliard, N.L. Jacobson and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Thomas, D.B. and C.E. Hinks. 1982. The effect of changing the physical form of roughage on the performance of the early-weaned calf. Anim. Prod. 35:375-384.

Thompson, G.B., L.D. Kintner and W.H. Pfander. 1958. Some effects of ration preparation on alterations of the rumen mucous membrane. J. Anim. Sci. 17:1220-1224.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Comstock Publishing Associates, Ithaca, NY.

Walker, D.M. and R.A. Simmonds. 1962. The development of the digestive system of the young animal. VI. The metabolism of short-chain fatty acids by the rumen and caecal wall of the young lamb. 59:375-379.

Wardrop, I.D. and J.B. Coombe. 1960. The post-natal growth of the visceral organs of the lamb. I. The growth of the visceral organs of the grazing lamb from birth to sixteen weeks of age. J. Agric. Sci. 54:140-143.

Wardrop, I.D. 1961. Some preliminary observations on the histological development of the fore-stomachs of the lamb. J. Agric. Sci. 57:335-341.

Warner, E.D. 1958. The organogenesis and early histogenesis of the bovine stomach. Amer. J. Anatomy. 102:33.

Warner, R.G. 1961. Is hay required to develop rumen capacity? J. Dairy Sci. 44:1177.

Warner, R.G. and W.P. Flatt. 1965. Anatomical development of the ruminant stomach. in Physiology of Digestion in the Ruminant. p.24. Butterworth Inc., Washington.

Warner, R.G., W.P. Flatt and J.K. Loosli. 1956. Dietary factors influencing the development of the ruminant stomach. J. Agric. Food Chem. 4:788-792.

Warner, R.G., C.H. Grippin, W.P. Flatt, and J.K. Loosli. 1955. Further studies on the influence of diet on the development of the ruminant stomach. J. Dairy Sci. 38:605.

Weigand, E., J.W. Young, and A.D. McGilliard. 1972. Extent of butyrate metabolism by bovine ruminoreticulum epithelium and the relationship to absorption rate. J. Dairy Sci. 55:589-597.

Whitaker, R.T., W.J. Miller, J.L. Carmon and H.L. Dalton. 1957. Influence of level and source of crude fiber in calf starters on weight and feed consumption. J. Dairy Sci. 40:887-892.

Williams, P.E.V., R.J. Fallon and G.M. Innes. 1985a. The effect of diet on the pH of the contents in the developing rumen of the calf. Anim. Prod. 40:571

Williams, P.E.V., R.J. Fallon and G.M. Innes. 1985b. The effect of diet on the degradation of barley and hay in the developing rumen of the calf. Anim. Prod. 40:571.

Williams, P.E.V., G.M. Innes, A. Brewer, and J.P. Magadi. 1985c. The effects of growth, food intake and rumen volume of including untreated or ammonia-treated barley straw in a complete diet for weaning calves. Anim. Prod. 41:63-74.

Williams, P.E.V., R.J. Fallon, G.M. Innes, and P. Garthwaite. 1987. Effect of food intake, rumen development and live weight of calves of replacing barley with sugar beet-citrus pulp in a starter diet. Anim. Prod. 44:65-73.

Wing, J.M., N.L. Jacobson, and R.S. allen. 1955. The effect of various restricted diets on the growth and on certain blood components of young dairy calves. J. Dairy Sci. 38:1006-1016.

Wing, J.M. 1959. Effect of a simple high-fiber feed on dairy calves. J. Dairy Sci. 42:1877-1879.

Wise, G.H., G.W. Anderson, and A.C. Linnerud. 1984. Relationship of milk intake by sucking and by drinking to reticular-groove reactions and ingestion behavior in calves. J. Dairy Sci. 67:1983-1992.

Young, J.W., H.A. Ramsey, and G.H. Wise. 1960. Effects of age and diet on the secretion of pregastric esterase in calves. J. Dairy Sci. 43:1068-1075.

Young, J.W., S.B. Tove, and H.A. Ramsey. 1965. Metabolism of acetate, propionate, and N-butyrate in young milk-fed calves. J. Dairy Sci. 48:1079-1087.

Young, J.W., E.O. Otchere, A. Trenkle, and N.L. Jacobson. 1970. Effect of age on Glucose, reducing sugars and plasma insulin in blood of milk-fed calves. J. Nutr. 100:1267-1274.

Young, J.W., D.R. Trott, P.J. Berger, S.P. Schmidt, and J.A. Smith. 1974. Gluconeogenesis in Ruminants: Glucose Kinetic Parameters in Calves under Standardized Conditions. J. Nutr. 104:1049-1055.

Ziolecki, A. and C.A.E. Briggs. 1961. The microflora of the rumen of the young calf: II. Source, nature and development. J. Appl. Bact. 24:148-163.

Zitnan, R., A. Bomba, A. Sommer and L. Kolodzieyski. 1993. Development of rumen metabolism and ruminal epithelium in lambs. Arch. Tierernahr. 44:227-233.

Zitnan, R., J. Voigt, U. Schomhusen, J. Wegner, M. Kokardova, H. Hagemeister, M. Levkut, S. Kuhla and A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Arch. Tierernahr. 51:279-291.

Zitnan, R., J. Voigt, J. Wegner, G. Breves, B. Shroder, C. Winckler, M. Levkut, M. Kokardova, U. Schomhusen, S. Kuhla, H. Hagemeister and A. Sommer. 1999. Morphological and functional development of the rumen in the calf: influence of the time of weaning. 1. Morphological development of rumen mucosa. Arch. Tierernahr. 52:351-362.

CHAPTER TWO

EFFECT OF VARIOUS LEVELS OF FORAGE AND FORM OF DIET ON RUMEN DEVELOPMENT IN CALVES

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

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ABSTRACT

Consumption of solid feed is essential for making the transition from preruminant animal to a functioning ruminant. The amount of roughage for inclusion in the diet to promote optimal rumen development of young calves is still unclear. The objective of this study was to determine the effect of form of diet (coarse versus ground) and inclusion of various levels of hay on rumen development in calves when intake is restricted. Holstein bull calves (n = 50) were randomly assigned to one of four treatments. Diets consisted of commercial coarse starter (C), a ground starter (G), coarse starter with 7.5% bromegrass hay of consistent particle size (8 - 19 mm) (H1), and coarse starter with 15% hay (H2). All diets were formulated to be isocaloric and isonitrogenous. Total ADF in diets were 6.39, 6.44, 6.47, and 7.43%, respectively. Intake was held constant across treatments until weaning, when feed was offered ad libitum. Jugular blood samples were obtained weekly and analyzed for β-hydroxybutyrate (BHBA). Body weight and rumen fluid samples were also obtained weekly. Daily scour scores and days receiving antibiotics and electrolytes did not differ with treatment. Calves receiving H1 and H2 tended to be heavier prior to weaning and were heavier postweaning than the calves receiving C. Calves fed the H1 and H2 also had higher BW gain (ADG) and feed efficiency (GF) postweaning than calves fed C. Calves fed

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H1 tended to have higher ADG and GF postweaning than calves fed H2. There were no differences in intake. Total VFA concentrations were higher for calves fed G diet versus C diet and tended to be higher for calves fed H1 versus H2. Calves fed H2 had greater acetate to propionate ratio than calves consuming H1. Calves receiving the G diet had lower proportions of acetate than calves fed C. Addition of controlled particle size hay to diets of young calves appears to favorably alter the rumen environment causing more efficient gain of body weight.

INTRODUCTION

Development of the rumen in the preruminant calf occurs on both physical and metabolic levels. Numerous factors influence rumen development in the young calf. The consumption of solid feed is essential to the physical development of the rumen and provides metabolites that direct the development of the ruminal mucosa. Solid feed is vital to the calf for making the transition from a preruminant animal to a functioning ruminant. However, there is still much controversy concerning the composition of starter that should be fed to preruminant calves especially regarding the level of forage those diets should contain.

Preruminant calves experience marked physical and metabolic changes when rumen development begins. Plasma glucose levels are high early in life and steadily decline as the calf ages (Young et al., 1970). This decline in glucose utilization along with a subsequent increase in concentrations of VFA is correlated with the development of the rumen and the consumption of solid feed. Measurement of ketones such as BHBA and acetoacetate reflect VFA utilization by the rumen epithelial cells (McCarthy et al., 1956).

Young calves have the ability to efficiently absorb and metabolize the VFA produced. Specifically, it is the utilization of butyrate by the rumen epithelium that stimulates papillae

development (Liang et al., 1967). Consumption of dry feed and the subsequent VFA production that follows also affects the microbial population of the rumen. After birth, the rumen is first populated by aerobic microbiota. Rumen development is marked by the increase in anaerobic populations. Specific changes in bacterial species are then influenced by dietary components (Eadie et al., 1962).

The amount of roughage necessary in the diet of young calves is unclear (Klein et al., 1987). Trials investigating the use of forage in starter rations have yielded inconsistent results. Several investigators concluded that forage addition to the diet increases starter intake (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have seen a negative impact of forage addition on the consumption of starter rations (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975). The inconsistent results may be influenced by variations in forage composition and quality between the individual investigations. Forage quality is highly dependent on plant maturity. Forages that are more mature when harvested have decreased nutrient content and lower palatability when compared to immature forages. This results in variable consumption of these forages by the animal (Hemken et al., 1958).

Forage consumption promotes muscular development of the rumen (Tamate et al., 1962; Hamada et al., 1976). However, it does not provide sufficient concentrations of rumen VFA, especially butyrate, required for optimal papillae development. Fermentation of concentrates provides the necessary butyrate to stimulate papillae development, but these feeds may promote keratinization of the papillae (Thompson et al., 1958; Hinders and Owen, 1965; Nocek et al., 1980). Ingestion of forage is also important in the process of rumination and the flow of saliva into the rumen. This flow of saliva serves to buffer rumen contents,

maintaining a stable pH (Hodgson, 1971). Therefore, the question remains unanswered as to how large of a role forage should play in the diet of preruminant animals.

Particle size and the associated abrasive value of the forage also have significant effects on rumen development. Ration particle size influences the rumen environment, volatile fatty acid production, and papillae structure and function. Forage that is chopped or ground to fine particle sizes decrease rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). This decrease in pH is caused by a lack of rumination and saliva flow into the rumen (Kellaway et al., 1977; Santini et al., 1983). Several investigations have observed decreases in the acetate to propionate ratio as a result of decreasing rumen pH and cellulolytic populations (Santini et al., 1983; Kerley et al., 1985). However, others have observed no changes in the acetate to propionate ratio (Greenwood et al., 1997; Beharka et al., 1998).

Rumen papillae are also influenced by the particle size of the ration. The length and branching of papillae tends to increase with decreases in forage particle size. Papillae of animals receiving small forage particles have increased keratinization; increases in keratinization are theorized to decrease the percentage of metabolically active tissue (McGavin and Morrill, 1976). This decrease in active tissue results in decreased VFA absorption (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997). The branching of papillae occurs to compensate for the loss in metabolically active tissue (Sander et al., 1959; Tamate et al., 1962). Small forage particles also do not increase the musculature of the rumen when compared to larger particle sizes (Greenwood et al., 1997; Beharka et al., 1998).

Determining the proper level of forage, if any, to include in starter diets for optimal rumen development will benefit the producer greatly. Optimal rumen development will benefit the producer by shortening the length of time the calf requires milk replacer and allow early weaning of a mature ruminant calf. There are several advantages of early weaning when compared to the prolonged feeding of milk replacer. Labor is greatly reduced when calves are fed starter rations when compared to feeding milk replacer. Calves that are weaned earlier have fewer digestive problems (Klein et al., 1987). Determining the proper level of forage necessary to develop the rumen properly will allow producers to wean younger calves that are more digestively mature. This should prevent the "slump" in intake and growth seen immediately after weaning.

The objective of this study was to determine the effect of form of diet and the inclusion of various levels of controlled particle size hay on rumen development in restricted-fed calves.

MATERIALS AND METHODS

The institutional Animal Care and Use Committee approved all procedures used in this experiment.

Holstein bull calves (n = 60) obtained from sale barns were utilized. The calves were approximately 2 to 5 days of age at arrival. Upon arrival (prior to initiation of the study), calves were randomly assigned to one of 4 treatment groups. On the day of arrival calves received one dose of a colostrum supplement (APC Lifeline Calf, Inc., Ames, IA). Calves were housed in individual hutches with wood shavings for bedding. Water was offered for ad libitum consumption. Eight calves died during the investigation and 2 more were removed

due to failure to consume adequate amounts of starter. Death losses did not differ according to treatment.

Calves were vaccinated based on the protocol of the research facility. On day 0 all calves received TSV-2 intranasal vaccine (Pfizer, New York, NY), 3 cc Naxcel IM (Pharmacia, Kalamazoo, MI), 1 cc Myosel (Vitamin E) IM (Schering Plough, Union, NJ), and Pasteurella vaccine SQ (Intervet, Millsboro, DE). In addition, on days 10 and 30 of the trial all calves were vaccinated with Bovishield (Pfizer, New York, NY) according to label directions.

Diets consisted of commercial coarse starter (Land O' Lakes, Ft. Dodge, IA) (C), a starter with ground grain processed with a hammermill and intact pellets (G), coarse starter with 7.5% bromegrass hay of consistent particle size (H1), and coarse starter with 15% grass hay of consistent particle size (H2). All diets were formulated to be isocaloric and isonitrogenous. The pellets in diets H1 and H2 were modified to account for the addition of hay (Table 1). Bromegrass hay used for the H1 and H2 diets was chopped and sorted by particle size using a Penn State Forage Separator described by Lammers et al. (1996). Hay particles were approximately 8 to 19 mm in length. Samples of diets were collected weekly and stored (-20°C) prior to analysis by a commercial laboratory (Silliker Laboratories of Iowa, Cedar Rapids, IA) (Table 2).

A commercial milk replacer (12.5% DM, 20% CP, 20% EE, Merricks Inc., Middleton, WI) was offered at 10% of the initial body weight daily (Appendix A) via bottles. Calves were offered milk replacer at this volume until weaned. Intake was held constant across treatments. Calves did not receive access to greater amounts of starter until greater than 80% of the calves were consuming all starter offered. Weaning occurred when all

calves were consuming 450 g of starter on day 52 of the trial (Table 3). Amounts of starter and milk replacer offered and refused were recorded daily. Daily fecal scores (Appendix B), administration of antibiotics and electrolytes were also noted. Jugular blood and rumen samples were obtained weekly (days 1, 8, 15, 22, 29, 36, 43, 50, 57, and 64 of the trial). Body weights were also measured weekly.

Table 1. Ingredients of dietary treatments (as fed basis)

	Concentration in Diet[1], %			
	C	G	H1	H2
Ingredient				
Grass hay	------	------	7.50	15.00
Steam rolled corn	31.85	------	32.05	31.96
Fine cracked corn	------	31.85	------	------
Whole oats	10.00	------	9.81	9.86
Ground oats	------	10.00	------	------
Molasses	8.00	8.00	8.00	8.00
Preservative	0.15	0.15	0.14	0.13
Mixer Pellet[2]	50.00	50.00	42.50	35.05
Soybean meal	41.00	41.00	55.50	69.00
Wheat middlings	33.00	33.00	15.00	8.10
Soybean hulls	10.00	10.00	10.00	2.30
Distillers dried grains	5.00	5.00	5.00	5.00
Choice white grease	------	------	3.50	4.30
Molasses	1.50	1.50	1.50	1.50
Microingredients	9.50	9.50	9.50	9.80

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay

[2]Ingredients of mixer pellet listed as a percentage of the pellet mix (as fed basis)

Table 2. Composition of diets (dry matter basis).

	Diet[1]				
Measurement	C	G	H1	H2	Grass Hay[2]
DM, %	87.9	87.58	88.37	88.71	86.23
ADF, %	6.39	6.44	6.47	7.43	37.57
CP, %	23.58	25.44	23.08	21.60	15.56
Ash, %	6.54	7.37	6.01	5.83	8.83
Ca, %	1.24	1.46	1.16	0.95	------
P, %	0.63	0.70	0.51	0.50	------
K, %	1.14	1.33	1.22	1.24	------
Mg, %	0.31	0.34	0.28	0.28	------

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay

[2]Grass hay = bromegrass hay used in diets H1 and H2

Table 3. Amount of starter offered (as fed basis).

Day of Trial	Starter offered, g
2-24	250
25-47	350
48-52	450
53-64	Ad Libitum

Collection of Jugular Blood Samples and BHBA Analysis

Blood samples were obtained approximately 3 hours after the a.m. meal. Blood was collected by jugular venipuncture in tubes containing ethylenediamine tetra-acetic acid (EDTA) (K_3) as an anticoagulant. After collection, plasma was harvested by centrifugation (3,000 x g for 15 min), placed in storage tubes and frozen (-20°C) until further analysis. Samples were then transported to the Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory. Plasma samples were allowed to thaw and then concentrations of BHBA were determined spectrophotometrically at 340 nm using a commercial reagent kit (Sigma Diagnostics, St. Louis, MO).

Collection of Rumen Fluid and VFA Analysis

Rumen samples were obtained via a stomach tube approximately 3 to 4 hours after the a.m. meal. Fluid was immediately frozen using dry ice and stored (-20°C) until later analysis. Samples were first prepared for VFA analysis by allowing them to thaw at room temperature. The sample tubes were then centrifuged at 3500 x g for 10 min. Samples containing large amounts of particulate matter were strained through 2 layers of cheesecloth. After centrifugation, samples were treated with 25% meta-phosphoric acid at a ratio of 5 parts rumen fluid to 1 part acid. Tubes were then covered, mixed, and allowed to stand for 30 minutes. Fluid was then centrifuged at 3500 x g for 10 minutes. The clear supernatant was removed and placed in 15 ml tubes and frozen (-20°C). Samples were than transported to North Carolina State University for VFA analysis.

Frozen supernatant thawed and centrifuged at 3500 x g for 10 min. A 1-ml aliquot of supernatant was added to 100 µl of internal standard (Appendix C) in a gas chromatogram vial. The mixture was placed in the autoanalyzer. Rumen fluid VFA concentrations were determined by a Varion 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) using a Nikol fused silica capillary column (15 m; 0.53 mm i.d.; 0.5 µm film thickness; Supleco, Bellefonete, P.A.). Samples were run with a run time of 6 min. Peaks from samples were then compared to 4 standard solutions (Appendix C).

Statistical Analysis

All data were analyzed using the mixed linear model (PROC MIXED) of SAS (1989). The covariance structure was modeled using an autoregression structure within animals and a random effect between animals (Littell et al., 1998). The model uses both RANDOM and REPEATED statements. This covariance structure specifies a random effect of differences

between animals and creates a correlation structure within animals that decreases with increasing amount of time between measurements (Littell et al., 1998). Least squares means and standard errors were obtained according to treatment and week/day by treatment. The data were analyzed for 4 treatments (coarse = C, ground = G, coarse with 7.5% hay = H1, and coarse with 15% hay = H2). Main treatment effects were analyzed using calf within treatment as the error term. Orthogonal contrasts were made: G vs C, C vs H1 + H2, and H1 vs H2. Data were analyzed for the entire trial (day 0-64), as well as, preweaning (day 0-52) and postweaning (day 53-64) time periods to account for different methods of feeding. Insufficient rumen fluid was obtained from calves on days 8 and 15. Therefore, data were omitted. Statistical significance was declared at probabilities < 0.05. Probability values between 0.05 and 0.15 were considered to be trends towards significance.

RESULTS AND DISCUSSION

Intake

The intake of starter and total DM intake increased with age of the calves ($P < 0.01$). There was also a tendency for calves offered diets H1 and H2 to have higher DM intake over the entire trial ($P< 0.13$). Mean total DM intake was 0.96, 0.99, 1.04 and 1.00 kg (SE = 0.03) for calves fed C, G, H1 and H2, respectively. However, there were no other differences with respect to the intake of milk replacer, starter, or total DM intake due to the restricted amounts offered prior to weaning. Using forage of a consistent particle size appeared to reduce variation in intake observed in other investigations using long roughage (Thomas and Hinks, 1982). Several investigators have reported an increase in intake with addition of forage to the diet (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973a; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have found a negative

correlation between intake of starter and hay in the ration (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975).

Body Weight Gain and Feed Efficiency

Entire Trial

Throughout the course of the trial, there was a tendency for body weight to differ with treatment ($P < 0.15$) and age ($P < 0.01$) (Table 5). Mean bodyweight of the treatment groups did not differ on day 0 of the trial ($P > 0.15$). Average daily gain (ADG) also was affected by treatment ($P < 0.02$) and increased with the use of the coarse diet and the addition of hay (Table 5).

Preweaning

No differences were observed in bodyweight, ADG, and gain to feed ratio (G/F) with treatment prior to weaning ($P = 0.70$, P = 0.40, and $P = 0.33$ respectively). However, calves fed diets H1 and H2 tended to have greater body weights than calves fed C ($P < 0.07$).

Postweaning

Differences were observed in bodyweight, ADG, and G/F with treatment ($P < 0.01$, $P < 0.01$, and $P < 0.01$ respectively) after weaning. Calves fed diet C were heavier ($P < 0.03$), had greater ADG ($P < 0.01$), and had greater feed efficiency ($P < 0.01$) than calves fed G. Calves fed diet H1 and H2 were heavier ($P < 0.01$), had greater ADG ($P < 0.04$), and greater G/F ($P < 0.03$) than calves fed diet C. Calves fed diet H1 tended to have higher ADG ($P < 0.07$) and greater feed efficiency ($P < 0 .12$) than calves fed diet H2.

These results conflict with those observed by Beharka et al. (1998) who observed greater body weight in calves fed a ground diet offered in restricted amounts versus an unground diet of identical composition. However, all diets in thir study included 25% alfalfa

hay. Others have shown no difference in body weight gain with respect to form of diet (fine, intermediate, and coarse). All diets used in their study included a source of bromegrass hay added at 15% of the diet (Greenwood et al., 1997). Due to differences in both form of diet and forage inclusion in the current trial, gut fill may have influenced body weight measurements.

Table 4. Effect of form of diet and inclusion of forage on BW, ADG and G/F.

Item	Diet[1]				SE	Contrast *P* Values		
	C	G	H1	H2		C vs G	C vs H1 + H2	H1 vs H2
BW, kg								
Entire trial	47.8	48.7	51.9	50.7	1.4	NS	*	NS
Preweaning	44.9	46.3	48.5	47.6	1.4	NS	†	NS
Postweaning	59.2	58.7	65.6	63.3	1.6	*	**	NS
ADG, kg								
Entire trial	0.33	0.29	0.41	0.37	0.03	**	†	NS
Preweaning	0.17	0.17	0.18	0.18	0.03	NS	NS	NS
Postweaning	0.91	0.74	1.22	1.02	0.08	**	*	†
G/F[2], kg								
Entire trial	0.25	0.23	0.30	0.29	0.03	NS	NS	NS
Preweaning	0.17	0.18	0.20	0.20	0.04	NS	NS	NS
Postweaning	0.52	0.42	0.66	0.58	0.03	**	*	NS

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay.
[2]Ratio of body weight gain (kg) to DM consumed (kg).
†$P \leq 0.10$
*$P \leq 0.05$
**$P \leq 0.01$

Calf Health

Daily fecal scores and days of electrolyte and antibiotic administration did not differ with treatment. Fecal scores were not different throughout the trial ($P = 0.64$), preweaning ($P = 0.66$), or postweaning ($P = 0.81$). Mean fecal scores were 1.38, 1.39, 1.39 and 1.41 (SE = 0.02) for calves fed C, G, HI and H2, respectively. There was no administration of

antibiotics or electrolytes after weaning. Prior to weaning there was no difference in antibiotic treatments ($P = 0.68$) or electrolyte administration ($P = 0.59$) with respect to treatment. Mean days given electrolytes were 0.003, 0.005, 0.008, 0.009 (SE = 0.003) for calves fed C, G, H1 and H2, respectively. Mean days treated with antibiotics were 0.03, 0.03, 0.02 and 0.04 (SE = 0.01) for calves fed C, G, H1 and H2, respectively.

Volatile Fatty Acid Concentrations

Total VFA concentrations (sum of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate) differed by treatment ($P < 0.01$) and age ($P < 0.01$) (Figure 1). Total VFA concentrations increased with increasing age of calves. Others have also observed this increase in total VFA concentrations with age (Greenwood et al., 1997; Beharka et al., 1998). Total ruminal VFA concentrations observed in this investigation are higher than those previously recorded from rumen fluid sampled via stomach tube (Greenwood et al., 1997). This most likely reflects the time of sampling and difference in feeding methods between the preweaning and postweaning periods. Samples of rumen fluid were obtained approximately 3 hours after the offering of starter when VFA concentrations typically peak (Stobo et al., 1966).

In particular, samples taken on week 8 have extremely high concentrations. This is most likely due to the change in feeding methods. Calves were offered starter ad libitum after weaning and intake of solid feed increased greatly in the first week postweaning. Increased ruminal VFA concentrations after weaning have been reported in other investigations (Anderson et al., 1987; Klein et al., 1987; Greenwood et al., 1997). This increase in feed intake may have caused the large increase in ruminal VFA concentrations. In addition, the restricted intake prior to weaning may have resulted in an immature rumen

epithelium that was not capable of rapid VFA absorption. By week 9, total VFA concentrations decreased to levels more commonly observed (Greenwood et al., 1997). At this time ad libitum intake of starter was stable for most calves and the increased access to solid feed may have resulted in a more mature rumen epithelium capable of absorbing large quantities of VFA. Molar concentrations of individual VFA also increased at 8 weeks and returned to normal values at 9 weeks.

Total ruminal VFA concentrations were higher in calves fed G diet versus C ($P < 0.01$) and tended to be higher for calves fed H1 versus H2 ($P < 0.10$) (Figure 1). Beharka et al. (1998) also observed an increase in total VFA concentrations with ground diets versus unground.

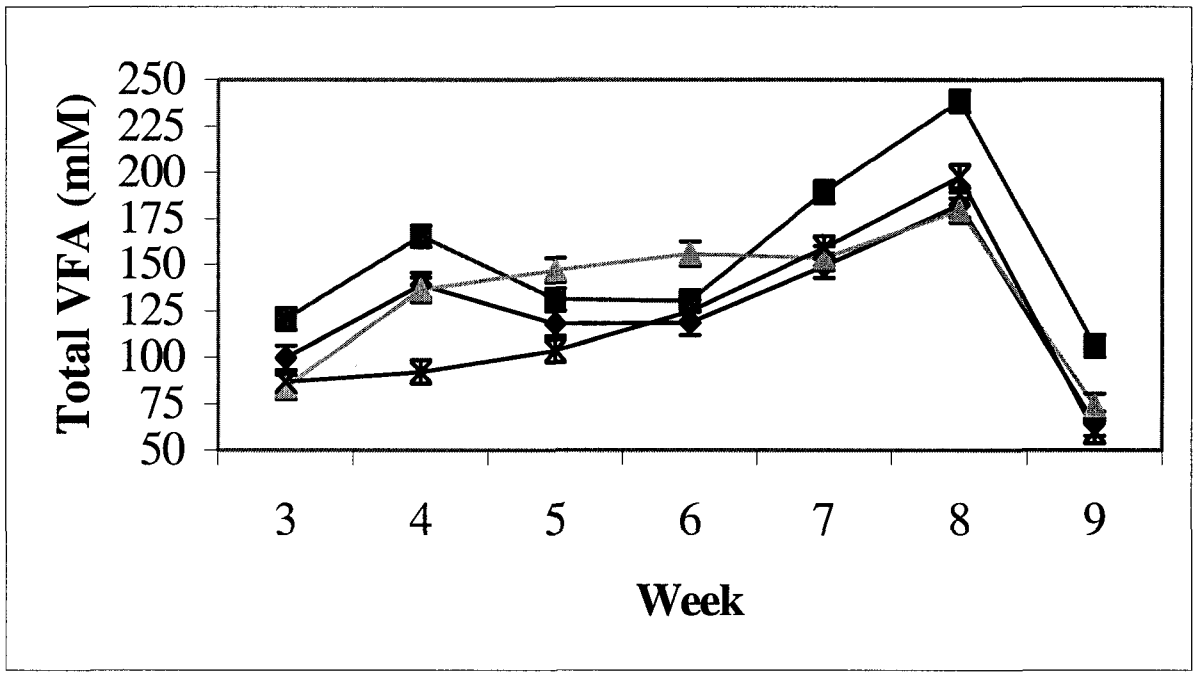

Figure 1. Total VFA concentrations in rumen fluid of calves fed various forms of diet (C ◆; G ■) and inclusions of forage (H1 ▲; H2 ×).

Molar concentrations of acetate also were greater in calves fed diet G than calves fed C ($P < 0.01$) (Table 4). Concentration of propionate also was greater in calves fed diet G versus C ($P < 0.01$) and diet H1 versus H2 ($P < 0.03$) (Table 4). Butyrate concentrations were greater in calves fed diet G than those fed C ($P < 0.01$) (Table 4). Concentrations of isovalerate were unaffected by treatment ($P = 0.50$). Calves fed diet G had higher concentration of valerate and isobutyrate than calves fed C ($P < 0.01$ and $P < 0.02$ respectively). Ratio of acetate to propionate tended to change with treatment ($P < 0.12$). Calves fed diet H2 had a higher ratio of acetate to propionate than calves fed H1 ($P < 0.03$). Beharka et al. (1998) also reported no difference in acetate to propionate ratio between calves fed different forms of diet (ground versus unground). Others have also observed an increase in acetate to propionate ratio in the rumen of calves fed diets with increasing levels of hay (Thomas and Hinks, 1982; Kerley et al., 1985).

Proportions of acetate, propionate, and valerate as a percent of total VFA tended to differ with treatment ($P < 0.09$, $P < 0.07$, and $P < 0.06$ respectively) (Table 4). Calves fed diet G tended to have a greater proportion of isobutyrate ($P < 0.09$), greater proportions of valerate ($P < 0.01$), and tended to have lower proportions of acetate ($P < 0.06$) than calves fed C. Calves fed diet C tended to have greater proportions of butyrate than calves fed H1 and H2 ($P < 0.10$). Quigley et al. (1992) reported similar results with the inclusion of hay in the diet. Calves fed H1 had greater proportions of propionate ($P < 0.01$) and lower proportions of isovalerate ($P < 0.05$) than calves fed H2.

Table 5. Effect of form of diet and inclusion of forage on ruminal VFA concentrations.

Item	Diet [1]				SE	Contrast *P* Values		
	C	G	H1	H2		C vs G	C vs H1 + H2	H1 vs H2
Acetate, m*M*	44.8	55.1	47.4	43.8	2.2	**	NS	NS
Propionate, m*M*	30.8	40.2	34.6	28.4	1.9	**	NS	*
Isobutyrate, m*M*	0.4	0.6	0.5	0.5	0.2	*	NS	NS
Butyrate, m*M*	11.2	12.6	10.3	9.3	0.8	**	NS	NS
Isovalerate, m*M*	0.9	0.9	0.7	1.0	0.1	NS	NS	NS
Valerate, m*M*	3.4	5.2	3.5	3.0	0.5	**	NS	NS
A:P[2]	1.6	1.5	1.4	1.7	0.1	NS	NS	*
Acetate, %	40.7	39.7	40.8	42.4	0.8	†	NS	NS
Propionate, %	28.0	28.6	29.5	27.1	0.7	NS	NS	**
Isobutyrate, %	0.4	0.5	0.4	0.5	0.1	†	NS	NS
Butyrate, %	9.4	9.5	8.5	8.6	0.4	NS	†	NS
Isovalerate, %	0.7	0.7	0.6	0.9	0.1	NS	NS	†
Valerate, %	3.0	3.6	2.8	2.7	0.3	**	NS	NS

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay.
[2]Ratio of acetate to propionate (m*M*).
†$P \leq 0.10$
*$P \leq 0.05$
**$P \leq 0.01$

β-Hydroxybutyrate Concentrations

Plasma concentrations of β-hydroxybutyrate (BHBA) did not differ with treatment prior to or after weaning, or during the entire trial (Table 6). This is in contrast to Quigley et al. (1992) who observed a decrease in plasma BHBA as hay intake increased. However this trial used restricted amounts of grain and ad libitum access to long hay postweaning. Therefore, calves did not consume consistent amounts of hay. Plasma BHBA concentrations did increase with age of the calf regardless of treatment ($P < 0.01$). Others have observed this increase in BHBA with age and increased consumption of fermentable carbohydrates

(Quigley et al., 1992; Greenwood et al., 1997). There were no significant differences throughout the entire trial ($P = 0.46$), as well as the preweaning ($P = 0.62$), or postweaning periods ($P = 0.36$). Others have also observed little influence of form of diet on plasma BHBA concentrations (Greenwood et al., 1997). Plasma BHBA concentrations were similar to those observed by Greenwood et al. (1997) who also utilized 15% bromegrass hay in the diets.

Table 6. Effect of form of diet and inclusion of forage on plasma BHBA.

	Diet[1]					Contrast *P* Values		
Item	C	G	H1	H2	SE	C vs G	C vs H1 + H2	H1 vs H2
BHBA, mmol/l								
Entire trial	0.16	0.17	0.16	0.15	0.01	NS	NS	NS
Preweaning	0.11	0.12	0.11	0.11	0.01	NS	NS	NS
Postweaning	0.35	0.37	0.33	0.32	0.03	NS	NS	NS

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay.
†$P \leq 0.10$
*$P \leq 0.05$
**$P \leq 0.01$

CONCLUSIONS

Addition of forage in a consistent particle size to the diet of young calves results in increased body weight after weaning. This increase in gain was due primarily to an improved feed efficiency. Addition of forage also altered rumen VFA concentrations. Starter supplemented with forage resulted in increased production of acetate in proportion to propionate. Increased feed efficiency and altered VFA production most likely resulted from an improved rumen environment. Addition of forage to the diet often stabilizes rumen pH due to the increased influx of saliva. This stable rumen pH allows populations of cellulolytic

bacteria to thrive. The addition of forage of a consistent particle size to the diet maintains intake and results in more efficient gain.

LITERATURE CITED

Anderson, K.L., T.G. Nagaraja, J.L. Morrill, T.B. Avery, S.J. Galitzer and J.E. Boyer. 1988. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215-1226.

Beharka, A.A., T.G. Nagaraja, J.L. Morrill, G.A. Kennedy and R.D. Klemm. 1998. Effect of form of diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946-1955.

Eadie, J.M. 1962. The development of rumen microbial populations in lambs and calves Under various conditions of management. J. Gen. Microbiol. 29:563-578.

Greenwood, R.H., J.L. Morrill, E.C. Titgemeyer and G.A. Kennedy. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. J. Dairy Sci. 80:2534-2541.

Hamada, T., S. Maeda and K. Kameoka. 1976. Factors influencing growth of rumen, liver, and other organs in kids weaned from milk replacers to solid foods. J. Dairy Sci. 59:1110-1118.

Hemken, R.W., G.W. Trimberger, and K.L. Turk. 1958. Growth rate of dairy calves fed on hay harvested at different stages of maturity and on two levels of grain. Bulletin #933 Cornell University Agricultural Experiment Station. Ithaca, N.Y.

Hibbs, J.W., H.R. Conrad, W.D. Pounden, and N. Frank. 1956. A high roughage system for raising calves based on early development of rumen function. VI. Influence of hay to grain ration on calf performance, rumen development, and certain blood changes. J. Dairy Sci. 39:171-179.

Hinders, R.G. and F.G. Owen. 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. J. Dairy Sci. 48:1069-1078.

Hodgson, J. 1971. The development of solid food intake in calves. 1. The effect of previous experience of solid food, and the physical form of the diet, on the development of food intake after weaning. Anim. Prod. 13:15-24.

Jahn, E., P.T. Chandler, and C.E. Polan. 1970. Effects of fiber and ratios of starch to sugar upon the performance of young ruminating calves. J. Dairy Sci. 53:466-474.

Kang, H.S. and J. Leibholz. 1973. The roughage requirement of the early-weaned calf. Anim. Prod. 16:195-203.

Kellaway, R.C., T. Grant, and J.W. Chudleigh. 1973. The roughage requirement of early weaned calves. Aust. J. Exp. Agric. Anim. Husb. 13:9-12.

Kellaway, R.C., D.J. Thomaon, D.E. Beever, and D.F. Osbourn. 1977. Effects of NaCl And $NaHCO_3$ on food intake, growth rate, and acid-base balance in calves. J. Agric. Sci. (Camb) 88:1-9.

Kerley, M.S., A.R. Kinser, J.L. Firkins, G.C. Fahey and L.L. Berger. 1985. Effects of roughage particle size on site of nutrient digestion and digesta flow through the gastrointestinal tract of sheep fed corncob-concentrate diets. J. Anim. Sci. 61:504-513.

Kincaid, R.L. 1980. Alternate methods of feeding alfalfa to calves. J. Dairy Sci. 63:91-94.

Klein, R.D., R.L. Kincaid, A.S. Hodgson, J.H. Harrison, J.K. Hillers, and J.D. Cronrath. 1987. Dietary fiber and early weaning on growth and rumen development in calves. J. Dairy Sci. 70:2095-2104.

Lammers, B.P., D.R. Buckmasters, and A.J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leibholz, J. 1975. Ground roughage in the diet of the early-weaned calf. Anim. Prod. 20:93-100.

Liang, Y.T., J.L. Morrill and J.L. Noordsy. 1967. Absorption and utilization of volatile fatty acids by the young calf. J. Dairy Sci. 50:1153-1157.

Littell, R.C., P.R. Henry, and C.B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

McCarthy, R.D. and E.M. Kesler. 1956. Relation between age of calf, blodd glucose, blood and rumen levels of volatile fatty acids, and in vitro cellulose digestion. J. Dairy Sci. 39:1280-1287.

McGavin, M.D. and J.L. Morrill. 1976. Dissection technique for examination of the bovine rumenoreticulum. J. Anim. Sci. 42:535-538.

Nocek, J.E., J.H. Herbein and C.E. Polan. 1980. Influence of ration physical form, ruminal degradable nitrogen and age on rumen epithelial propionate and acetate transport and some enzymatic activities. J. Nutr. 110:2355-2364.

Quigley, J.D., T.M. Steen and S.I. Boehms. 1992. Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed with or without hay. J. Dairy Sci. 75:228-235.

Sander, E.G., R.G. Warner, H.N. Harrison and J.K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42:1600-1605.

Santini, F.J., A.R. Hardie, N.A. Jorgensen, and M.F. Finner. 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66:811-820.

SAS User's Guide: Statistics, Version 6.04 Edition. 1989. SAS Inst., Inc., Cary, NC.

Stobo, I.J.F., J.H.B. Roy and H.J. Gaston. 1966. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. Br. J. Nutr. 20:171-188.

Stobo, I.J.F., C.S. Lucci, J.H.B. Roy, and M.W. Perfitt. 1985. Comparison of high-energy pellets containing processed fibre with a coarse concentrate mixture in relation to the development of solid food intake in the calf. Anim. Prod. 40:570.

Tamate, H., A.D. McGilliard, N.L. Jacobson and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Thomas, D.B. and C.E. Hinks. 1982. The effect of changing the physical form of roughage on the performance of the early-weaned calf. Anim. Prod. 35:375-384.

Thompson, G.B., L.D. Kintner and W.H. Pfander. 1958. Some effects of ration preparation on alterations of the rumen mucous membrane. J. Anim. Sci. 17:1220-1224.

Whitaker, R.T., W.J. Miller, J.L. Carmon and H.L. Dalton. 1957. Influence of level and source of crude fiber in calf starters on weight and feed consumption. J. Dairy Sci. 40:887-892.

Young, J.W., E.O. Otchere, A. Trenkle, and N.L. Jacobson. 1970. Effect of age on glucose, reducing sugars and plasma insulin in blood of milk-fed calves. J. Nutr. 100:1267-1274.

CHAPTER THREE

EFFECT OF VARIOUS LEVELS OF FORAGE AND FORM OF DIET ON RUMEN DEVELOPMENT AND GROWTH PERFORMANCE IN CALVES

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

J.A. Booth[1], H.D. Tyler[1], and J.A. Brumm[2]

ABSTRACT

Consumption of solid feed is essential for making the transition from a preruminant to ruminant animal. However, the optimal combination of feedstuffs required for optimal rumen development remains unclear. The amount of forage, if any, required by the young calf in unknown. The objective of this study was to determine the effect of form of diet (coarse versus fine) and inclusion of various levels of hay on rumen development in calves. Calves were offered ad libitum access to starter rations. Holstein, Jersey, Ayshire, and Brown Swiss calves (n = 56) were assigned to one of four treatments based on order of birth. Dietary treatments consisted of a commercial coarse starter (C), a ground starter (G), coarse starter with 7.5% grass hay of consistent particle size (H1), and coarse starter with 15% hay (H2). All diets were formulated to be isocaloric and isonitrogenous. Total ADF in diets were 7.43%, 10.54%, 10.74%, and 10.71% respectively. Weaning occurred when calves consumed 1.5% of initial body weight for 3 consecutive days. Calves were removed from the trial approximately 2 weeks after weaning. Jugular blood samples were obtained weekly and analyzed for β-hydroxybutyrate (BHBA). Body weight was also measured weekly. Intake of milk replacer did not differ among treatments. There was a tendency for starter intake to be greater for calves offered HI and H2 versus C. There was also a tendency for

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calves fed H1 and H2 to have a higher total dry matter intake than those offered C. Calves receiving diet C tended to consume more than calves fed G, and calves fed diets H1 and H2 tended to consume more total dry matter than those fed C. There were no differences in body weight gain with respect to treatment throughout the trial. Average daily gain and feed efficiency also did not differ among treatments. Age at weaning was also unaffected by treatment. Plasma concentrations of BHBA were unaffected by treatment. Daily scour scores, electrolyte and antibiotic administration also did not differ with treatment.

INTRODUCTION

Consumption of solid feed is essential to the physical development of the rumen and provides metabolites that direct the development of the ruminal mucosa. Solid feed is vital to the calf for making the transition from a preruminant animal to a functioning ruminant. However, there is still much controversy concerning the composition of starter that should be fed to preruminant calves especially regarding the level of forage those diets should contain.

The amount of roughage necessary in the diet of young calves is unclear (Klein et al., 1987). Trials investigating the use of forage in starter rations have yielded inconsistent results. Several investigators concluded that forage addition to the diet increases starter intake (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have seen a negative impact of forage addition on consumption of starter rations (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975). Inconsistent results may be influenced by forage composition and quality variation between the individual investigations. Forage quality is highly dependent on plant maturity. Forages that are more mature when harvested have reduced nutrient

content and lower palatability when compared to immature forages (Hemken et al., 1958). This results in variable consumption by the animal (Hemken et al., 1958).

Forage consumption promotes muscular development of the rumen (Tamate et al., 1962; Hamada et al., 1976). However, it does not promote production of sufficient concentrations of rumen VFA, especially butyrate, required for optimal papillae development. Fermentation of concentrates provides necessary butyrate to stimulate papillae development, but may promote keratinization of the papillae (Thompson et al., 1958; Hinders and Owen, 1965; Nocek et al., 1980). Forage is also important in the process of rumination and the flow of saliva into the rumen. This flow of saliva serves to buffer rumen contents, maintaining a stable pH (Hodgson, 1971). Therefore, the question remains unanswered as to how large of role forage should play in the diet of preruminant animals.

Particle size and the associated abrasive value of the forage also have significant effects on rumen development. Ration particle size influences the rumen environment, volatile fatty acid production, and papillae structure and function. Forage chopped or ground to fine particle sizes decrease rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). This decrease in pH is caused by a lack of rumination and saliva flow into the rumen (Kellaway et al., 1977; Santini et al., 1983). Rumen papillae are also influenced by the particle size of the ration. Papillae of animals receiving small forage particles have increased keratinization; increases in keratinization are theorized to decrease the percentage of metabolically active tissue (McGavin and Morrill, 1976). This decrease in active tissue results in decreased VFA absorption (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997a). The papillae begin to branch to compensate for the loss in metabolically active tissue (Sander et al., 1959; Tamate et al., 1962). Small forage particles

also do not increase the musculature of the rumen when compared to larger particle sizes (Greenwood et al., 1997a; Beharka et al., 1998).

Determining the proper level of forage, if any, to include in starter diets for optimal rumen development will benefit the producer greatly. Optimal rumen development will benefit the producer by shortening the length of time the calf requires milk replacer and allow early weaning of a mature ruminant calf. There are several advantages of early weaning when compared to the prolonged feeding of milk replacer. Labor is greatly reduced when calves are fed starter rations when compared to feeding milk replacer. Calves that are weaned earlier have fewer digestive problems (Klein et al., 1987). Determining the optimal level of forage necessary to develop the rumen properly will allow producers to wean younger calves that are more digestively mature. This should prevent the "slump" in intake and growth seen immediately after weaning.

Previous unpublished work in our laboratory has indicated that the inclusion of forage of consistent particle size in the starter diets of young calves is beneficial. When starter was offered in restricted amounts we observed increased body weight, average daily gain, and feed efficiency in the calves fed diets containing hay. Changes in VFA concentrations were also noted. The diets containing hay stimulated higher amounts of acetate production compared to propionate. This indicates a more stable rumen environment that supports the activity of cellulolytic bacteria (unpublished data). However, because of the restricted feeding regime, we were not able to measure the effects of forage addition on weaning, intake, and growth performance.

Therefore, the objective of this study was to determine the effect of form of diet and the inclusion of various levels of hay on rumen development in calves fed ad libitum.

MATERIALS AND METHODS

The institutional Animal Care and Use Committee approved all procedures used in this experiment.

Holstein (n = 48), Jersey (n = 3), Ayshire (n = 2), and Brown Swiss (n = 3) calves (total n = 56) were obtained from the Northeast Iowa Community College herd. At birth all calves received colostrum and injections of 5 cc Penicillin G Procaine and 4 cc Bo-Se vitamin E and selenium (Schering-Plough Animal Health, Union, NJ). Calves were alternately assigned to one of 4 treatment groups according to order of birth. Calves were housed in individual pens with wood shavings or straw for bedding. Water was offered on an ad libitum basis.

Diets consisted of commercial coarse starter (Land O' Lakes, Ft. Dodge, IA) (C), a starter with ground grain and intact pellets (G), coarse starter with 7.5% bromegrass hay of consistent particle size (H1), and coarse starter with 15% bromegrass hay of consistent particle size (H2). All diets were formulated to be isocaloric and isonitrogenous. The pellets in diets H1 and H2 were modified to account for the addition of hay (Table 1). Bromegrass hay used for H1 and H2 diets was chopped and sorted by particle size using a method described by Lammers et al. (1996). Hay particles used were approximately 8 to 19 mm in length. A commercial milk replacer (12.5% DM, 22% CP, 20% Fat, Land O' Lakes, Ft. Dodge, IA) was offered at 10% of initial body weight daily (Appendix A) via buckets until weaned. Weaning occurred when calves consumed 1.5% of initial body weight for 3 consecutive days (Greenwood et al., 1997b). All diets were offered from day one of age on an ad libitum basis. Samples of diets were collected weekly and stored (-20^{0}C) before analysis by a commercial laboratory (Iowa Testing Laboratory, Eagle Grove, IA) (Table 2).

Table 1. Ingredients of dietary treatments (as fed basis)

Ingredient	Concentration in Diet[1], %			
	C	G	H1	H2
Grass hay	------	------	7.50	15.00
Steam rolled corn	31.85	------	32.05	31.96
Fine cracked corn	------	31.85	------	------
Whole oats	10.00	------	9.81	9.86
Ground oats	------	10.00	------	------
Molasses	8.00	8.00	8.00	8.00
Preservative	0.15	0.15	0.14	0.13
Mixer Pellet[2]	50.00	50.00	42.50	35.05
Soybean meal	41.00	41.00	55.50	69.00
Wheat middlings	33.00	33.00	15.00	8.10
Soybean hulls	10.00	10.00	10.00	2.30
Distillers dried grains	5.00	5.00	5.00	5.00
Choice white grease	------	------	3.50	4.30
Molasses	1.50	1.50	1.50	1.50
Microingredients	9.50	9.50	9.50	9.80

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay

[2]Ingredients of mixer pellet listed as a percentage of the pellet mix (as fed basis)

Table 2. Composition of diets (DM basis).

Measurement	Diet[1]				
	C	G	H1	H2	Grass Hay[2]
DM (%)	90.50	90.50	90.30	89.60	86.23
CP (%)	22.76	22.60	23.51	21.73	15.56
EE (%)	3.51	3.12	4.74	5.50	------
NDF (%)	18.06	18.94	18.72	20.42	------
ADF (%)	7.43	10.54	10.74	10.71	37.57
Ca (%)	1.50	1.26	1.33	0.94	------
P (%)	0.66	0.59	0.58	0.49	------

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay

[2]Grass hay = bromegrass hay used in diets H1 and H2

Refusals of starter and milk replacer were recorded daily in addition to daily fecal scores (Appendix B). Daily administration of antibiotics and electrolytes were also recorded. Jugular blood was obtained and body weight was measured weekly. Calves were removed from the trial approximately 2 weeks after weaning.

Collection of Jugular Blood Samples and BHBA Analysis

Blood samples were obtained approximately 3 hours after the a.m. feeding once weekly. Blood was obtained by jugular venipuncture into evacuated tubes containing EDTA (K_3) as an anticoagulant. After collection, plasma was harvested by centrifugation (3,000 x g for 15 min) and placed in storage tubes and frozen (-20^0 C) until further analysis. Samples were transported to the Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory for analysis. Plasma samples were allowed to thaw and analyzed spectrophotometrically at 340 nm for concentrations of BHBA using a commercial reagent kit (Randox Laboratories Ltd., San Diego, CA).

Statistical Analysis

All data were analyzed using the mixed linear model (PROC MIXED) of SAS (1989). Covariance structure was modeled using an autoregression structure within animals and a random effect between animal (Littell et al., 1998). The model uses both RANDOM and REPEATED statements. This covariance structure specifies a random effect of differences between animals and creates a correlation structure within animals that decreases with increasing amount of time between measurements (Littell et al., 1998). Birth weight was used as a covariable to account for breed differences in birth weight. Least squares means and standard errors were obtained according to treatment and week/day by treatment. The data were analyzed for 4 treatments (coarse = C, ground = G, coarse with 7.5% hay = H1,

and coarse with 15% hay = H2). Main treatment effects used calf within treatment as the error term. Three orthogonal contrasts were made: G vs C, C vs H1 + H2, and H1 vs H2. Data were analyzed for the entire trial, preweaning, and postweaning time periods. Statistical significance was declared at probabilities < 0.05. Probability values between 0.05 and 0.15 were considered to be trends towards significance.

RESULTS AND DISCUSSION

Intake

Intake of milk replacer did not differ by treatment (P = 0.60). Intake of starter also did not differ by treatment over the entire trial (P = 0.31) or prior to weaning (P = 0.73). However, there was a tendency for starter intake to be greater for calves offered H1 and H2 versus C (P < 0.11) after weaning (Figure 1). Intake of starter and dry matter increased with age (P < 0.01). Total dry matter intake also did not differ by treatment over the entire trial (P = 0.25), but there was a tendency for calves receiving H1 and H2 to have a higher total dry matter intake than those offered C (P < 0.15) throughout the trial. This is similar to previous data from our lab utilizing similar treatments (unpublished data).

There was little variation in the intake of solid feed with respect to forage addition to the diet. This is in contrast to other investigations utilizing long roughage (Thomas and Hinks, 1982). The consistent particle size of the forage used in the dietary treatments results in less variation in intake. There was no difference in dry matter intake prior to weaning (P = 0.84). However, after weaning there was an effect of treatment on total intake (P < 0.15). Calves receiving diet C tended to consume more total dry matter than calves fed diet G (P < 0.15), indicating that form of diet impacts the intake of solid feed. Calves fed diets H1 and

H2 tended to consume more total dry matter than those fed C ($P < 0.11$) postweaning (Figure 2).

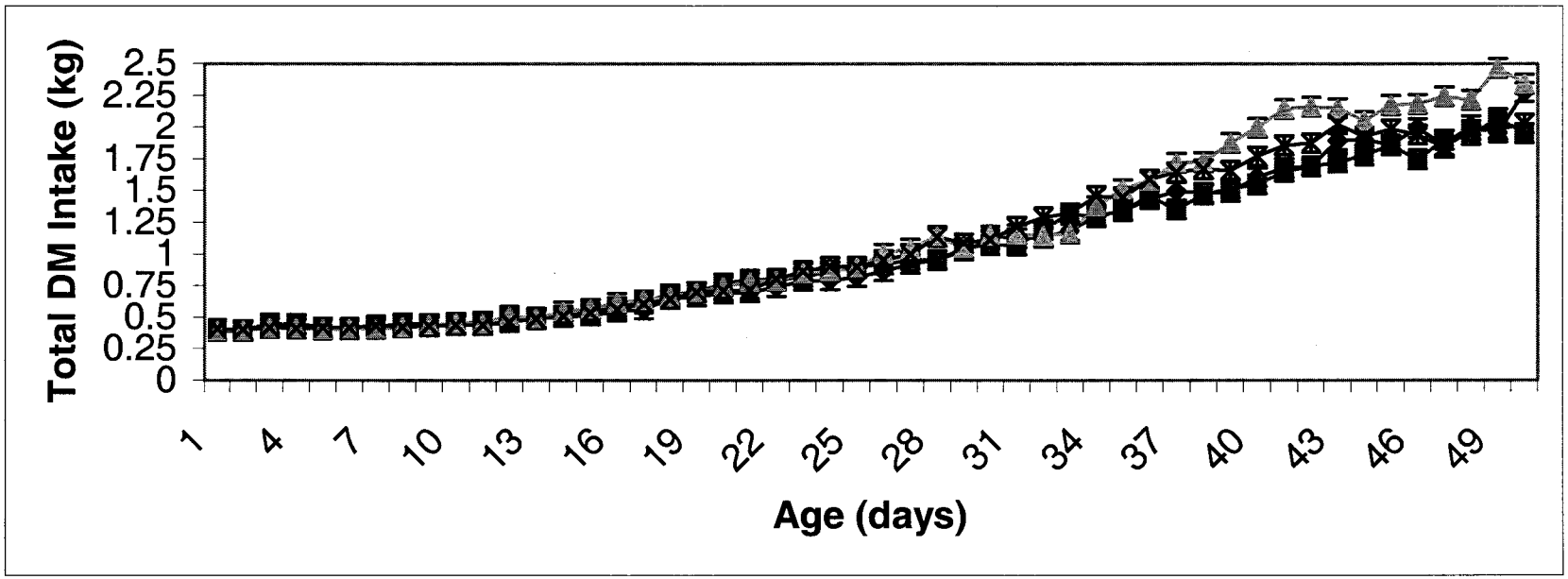

Figure 2. Total dry matter intake in calves fed various forms of diet (C ◆; G ■) and inclusions of forage (H1 ▲; H2 ×).

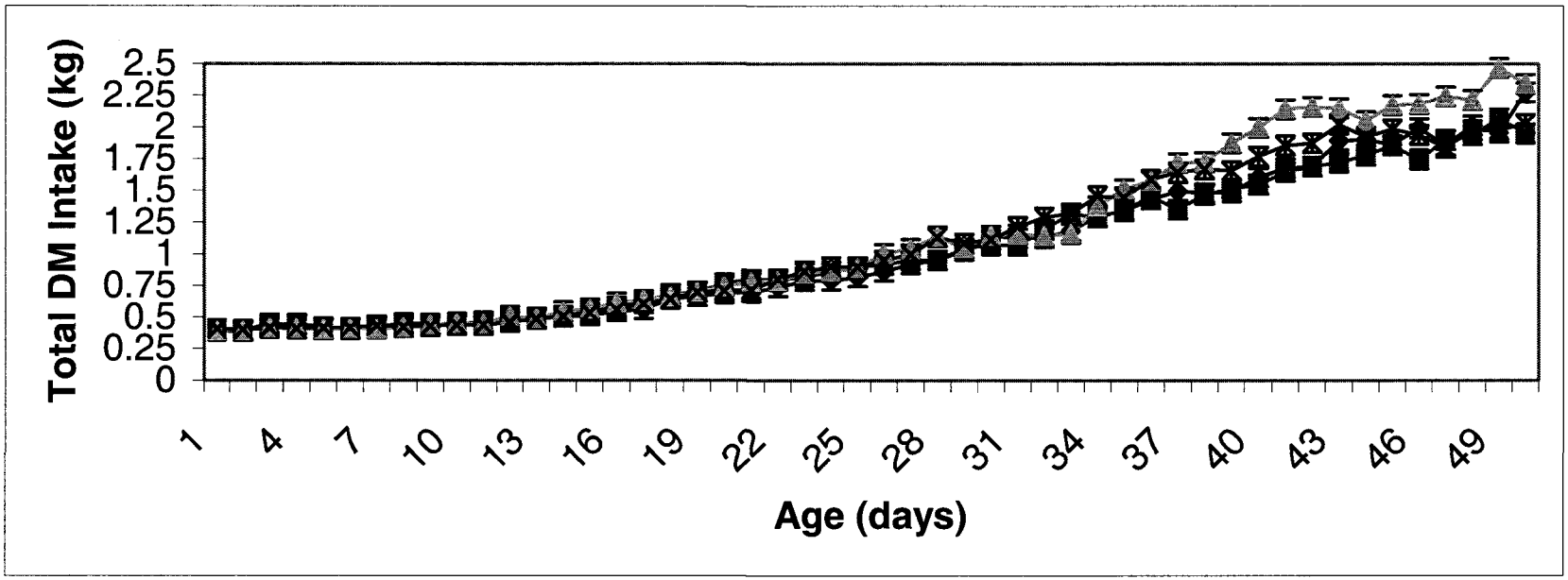

Figure 2. Total dry matter intake in calves fed various forms of diet (C ◆; G ■) and inclusions of forage (H1 ▲; H2 ×).

This is similar to other investigations that have shown an increase in starter consumption with the addition of forage to the diet (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have seen a negative impact of forage addition on the consumption of starter rations (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975). In a previous trial in our laboratory utilizing the same dietary treatments, there was no difference in starter or total dry matter consumption postweaning. However, the calves in that trial had restricted intake of solid feed prior to weaning. This perhaps altered the consumption of feed postweaning (unpublished data).

Body Weight Gain and Feed Efficiency

Body weight gain increased with age of the calves ($P < 0.01$). However, there were no differences in body weight gain with respect to treatment throughout the entire trial ($P = 0.72$), preweaning ($P = 0.75$), or postweaning ($P = 0.68$) (Table 3). This is similar to the results observed by Greenwood et al. (1997a). In contrast, Beharka et al. (1998) observed that calves fed ground diets had higher body weight than those fed unground rations. However, the ground diet did contain 25% alfalfa hay. In a previous trial from our lab, we observed that calves fed diet C were heavier than those fed G, and calves receiving diet H1 and H2 were heavier than those receiving diet C postweaning (unpublished data).

Average daily gain also did not differ by treatment over the entire trial ($P = 0.76$), preweaning ($P = 0.73$), or postweaning ($P = 0.53$) (Table 3). Feed efficiency, expressed as a ratio of kilograms of gain per kilogram of feed, also was unaffected by treatment. Gain to feed ratios were not different for the entire trial ($P = 0.85$), for the period prior to weaning ($P = 0.91$), or for the period after weaning ($P = 0.69$) (Table 3). Previous research from our lab

using similar treatments resulted in higher ADG and G/F in calves fed diet C versus diet G. In our previous trial, calves fed diets H1 and H2 had higher ADG and G/F than those consuming C when intake was restricted (unpublished data). Perhaps the restricted intake promoted increased feed utilization.

Table 3. Effect of form of diet and inclusion of forage on BW, ADG and G/F.

Item	Diet[1]				SE	Contrast *P* Values		
	C	G	H1	H2		C vs G	C vs H1 + H2	H1 vs H2
BW, kg								
Entire trial	49.6	50.5	51.0	50.9	1.0	NS	NS	NS
Preweaning	43.5	44.5	44.5	44.4	0.8	NS	NS	NS
Postweaning	57.8	58.6	60.0	59.8	1.5	NS	NS	NS
ADG, kg								
Entire trial	0.52	0.53	0.56	0.56	0.04	NS	NS	NS
Preweaning	0.32	0.38	0.35	0.35	0.04	NS	NS	NS
Postweaning	0.78	0.72	0.85	0.82	0.07	NS	NS	NS
G/F[2], kg								
Entire trial	0.55	0.60	0.56	0.62	0.06	NS	NS	NS
Preweaning	0.59	0.68	0.62	0.65	0.09	NS	NS	NS
Postweaning	0.50	0.50	0.51	0.57	0.04	NS	NS	NS

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay.
[2]Ratio of body weight gain (kg) to DM consumed (kg).
†$P \leq 0.10$
*$P \leq 0.05$
**$P \leq 0.01$

Calf Health

Daily scour scores, electrolyte and antibiotic administration did not differ with treatment. Scour scores were not different throughout the trial (P = 0.62). Mean fecal scores were 1.39, 1.50, 1.42 and 1.43 (SE = 0.06) for calves fed C, G, H1 and H2, respectively. The administration of antibiotics or electrolytes was also unaffected by treatment (P = 0.70).

Mean days given electrolytes or antibiotics were 0.06, 0.08, 0.06, 0.05 (SE = 0.02) for calves fed C, G, H1 and H2, respectively.

Age at Weaning

Weaning occurred according to starter intake by individual calves. The mean age at weaning (31 days) did not differ with treatment (P = 0.72). This indicates that there was little difference in dry matter consumption and minimal variation in intake among the treatments (Figure 3).

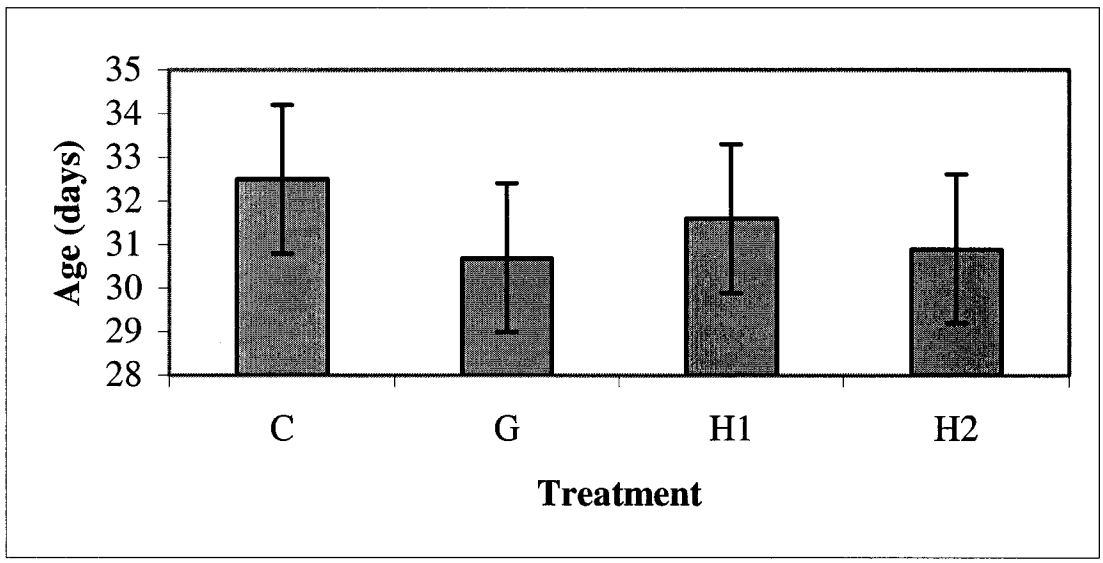

Figure 3. Weaning age of calves fed different forms of diet and various levels of hay ad libitum ([1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay).

β-Hydroxybutyrate Concentrations

Plasma concentrations of β-hydroxybutyrate (BHBA) did not differ with respect to treatment (Table 4). However, the plasma concentrations did increase with age of the calf regardless of treatment (P < 0.01). Others have observed this increase in BHBA with age (Quigley et al., 1992; Greenwood et al., 1997). There were no significant differences

throughout the entire trial (P = 0.91), as well as the preweaning (P = 0.83), or postweaning periods (P = 0.97). This indicates that there was little differences in the rumen metabolism of butyrate by diet.

Table 4. Effect of form of diet and inclusion of forage on plasma BHBA.

	Diet[1]					Contrast *P* Values		
Item	C	G	H1	H2	SE	C vs G	C vs H1 + H2	H1 vs H2
BHBA, mmol/l								
Entire trial	0.15	0.15	0.16	0.15	0.01	NS	NS	NS
Preweaning	0.07	0.07	0.08	0.08	0.01	NS	NS	NS
Postweaning	0.26	0.25	0.27	0.26	0.02	NS	NS	NS

[1]C = rolled corn, whole oats, and intact pellets; G = rolled corn and whole oats processed with a hammermill and intact pellets; H1 = rolled corn, whole oats, modified intact pellets and 7.5% grass hay; H2 = rolled corn, whole oats, modified intact pellets and 15% grass hay.
†$P \leq 0.10$
*$P \leq 0.05$
**$P \leq 0.01$

CONCLUSIONS

Prior to weaning there was little effect of treatment on intake and feed efficiency. However, after weaning the addition of forage in the diet promoted greater dry matter intake. The form of diet also affects intake of solid feed. This trial illustrates that forage of consistent particle size can be successfully utilized in starter rations of young calves. The maintenance of consistent particle size decreases the variation in intake commonly observed when long roughage is offered. The addition of forage may be an economical feedstuff for inclusion in starter rations for young calves. Further work is needed to determine the long-term effects of forage-containing diets.

LITERATURE CITED

Beharka, A.A., T.G. Nagaraja, J.L. Morrill, G.A. Kennedy and R.D. Klemm. 1998. Effect of form of diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946-1955.

Greenwood, R.H., J.L. Morrill, E.C. Titgemeyer and G.A. Kennedy. 1997a. A new method of measuring diet abrasion and its effect on the development of the forestomach. J. Dairy Sci. 80:2534-2541.

Greenwood, R.H., J.L. Morrill and E.C. Titgemeyer. 1997b. Using dry feed intake as a percentage of initial body weight as a weaning criterion. J. Dairy Sci. 80:2542-2546.

Hamada, T., S. Maeda and K. Kameoka. 1976. Factors influencing growth of rumen, liver, and other organs in kids weaned from milk replacers to solid foods. J. Dairy Sci. 59:1110-1118.

Hemken, R.W., G.W. Trimberger, and K.L. Turk. 1958. Growth rate of dairy calves fed on hay harvested at different stages of maturity and on two levels of grain. Bulletin #933 Cornell University Agricultural Experiment Station. Ithaca, N.Y.

Hibbs, J.W., H.R. Conrad, W.D. Pounden, and N. Frank. 1956. A high roughage system for raising calves based on early development of rumen function. VI. Influence of hay to grain ration on calf performance, rumen development, and certain blood changes. J. Dairy Sci. 39:171-179.

Hinders, R.G. and F.G. Owen. 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. J. Dairy Sci. 48:1069-1078.

Hodgson, J. 1971. The development of solid food intake in calves. 1. The effect of previous experience of solid food, and the physical form of the diet, on the development of food intake after weaning. Anim. Prod. 13:15-24.

Jahn, E., P.T. Chandler, and C.E. Polan. 1970. Effects of fiber and ratios of starch to sugar upon the performance of young ruminating calves. J. Dairy Sci. 53:466-474.

Kang, H.S. and J. Leibholz. 1973. The roughage requirement of the early-weaned calf. Anim. Prod. 16:195-203.

Kellaway, R.C., T. Grant, and J.W. Chudleigh. 1973. The roughage requirement of early weaned calves. Aust. J. Exp. Agric. Anim. Husb. 13:9-12.

Kellaway, R.C., D.J. Thomaon, D.E. Beever, and D.F. Osbourn. 1977. Effects of NaCl And $NaHCO_3$ on food intake, growth rate, and acid-base balance in calves. J. Agric. Sci. (Camb) 88:1-9.

Kincaid, R.L. 1980. Alternate methods of feeding alfalfa to calves. J. Dairy Sci. 63:91-94.

Klein, R.D., R.L. Kincaid, A.S. Hodgson, J.H. Harrison, J.K. Hillers, and J.D. Cronrath. 1987. Dietary fiber and early weaning on growth and rumen development in calves. J. Dairy Sci. 70:2095-2104.

Lammers, B.P., D.R. Buckmasters, and A.J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

Leibholz, J. 1975. Ground roughage in the diet of the early-weaned calf. Anim. Prod. 20:93-100.

Littell, R.C., P.R. Henry, and C.B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

McGavin, M.D. and J.L. Morrill. 1976. Dissection technique for examination of the bovine rumenoreticulum. J. Anim. Sci. 42:535-538.

Nocek, J.E., J.H. Herbein and C.E. Polan. 1980. Influence of ration physical form, ruminal degradable nitrogen and age on rumen epithelial propionate and acetate transport and some enzymatic activities. J. Nutr. 110:2355-2364.

Quigley, J.D., T.M. Steen and S.I. Boehms. 1992. Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed with or without hay. J. Dairy Sci. 75:228-235.

Sander, E.G., R.G. Warner, H.N. Harrison and J.K. Loosli. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42:1600-1605.

Santini, F.J., A.R. Hardie, N.A. Jorgensen, and M.F. Finner. 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66:811-820.

SAS User's Guide: Statistics, Version 6.04 Edition. 1989. SAS Inst., Inc., Cary, NC.

Stobo, I.J.F., C.S. Lucci, J.H.B. Roy, and M.W. Perfitt. 1985. Comparison of high-energy pellets containing processed fibre with a coarse concentrate mixture in relation to the development of solid food intake in the calf. Anim. Prod. 40:570.

Tamate, H., A.D. McGilliard, N.L. Jacobson and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Thomas, D.B. and C.E. Hinks. 1982. The effect of changing the physical form of roughage on the performance of the early-weaned calf. Anim. Prod. 35:375-384.

Thompson, G.B., L.D. Kintner and W.H. Pfander. 1958. Some effects of ration preparation on alterations of the rumen mucous membrane. J. Anim. Sci. 17:1220-1224.

Whitaker, R.T., W.J. Miller, J.L. Carmon and H.L. Dalton. 1957. Influence of level and source of crude fiber in calf starters on weight and feed consumption. J. Dairy Sci. 40:887-892.

Young, J.W., E.O. Otchere, A. Trenkle, and N.L. Jacobson. 1970. Effect of age on glucose, reducing sugars and plasma insulin in blood of milk-fed calves. J. Nutr. 100:1267-1274.

CHAPTER FOUR

EFFECT OF PLASMA PROTEIN AND FORM OF DIET IN MEAL FED CALVES

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

J.A. Booth[1], J.D. Quigley III[2], H.D. Tyler[1], and T.M. Wolfe[2]

ABSTRACT

Calves fed starter rations containing highly fermentable carbohydrates often experience dramatic changes in rumen and blood metabolites. It is not clear if these changes affect the animal's immune competence. Our objective was to compare the performance and immune response of calves fed two different forms of starter rations (coarse vs. finely ground) supplemented with either spray-dried animal plasma (SDAP) or an isonitrogenous combination of spray-dried red blood cells and whey protein concentrate (RBCW). Holstein bull calves (n = 28) were randomly assigned to one of four treatments in a replicated 4 x 4 Latin square design. Treatments consisted of coarse or finely ground commercial calf starter supplemented with either 5% SDAP or 5% RBCW. The trial consisted a 21- day pre-experimental period and 4 two-week treatment periods. During days 8-14 of each period, calves were limited to 90% of calculated average intake of the treatment diets from days 1-6 to induce meal feeding and rapid fermentation of carbohydrates. Body weight was measured on day 14 of each treatment period. At the same time, jugular blood samples were collected prior to (0 hour) and 8 hours post feeding and analyzed for urea N, NEFA, BHBA, WBC, RBC, hemoglobin, and hematocrit. Plasma urea N increased at 0 and 8 hours in calves fed SDAP and also in calves fed the finely ground diets. Concentrations of BHBA and NEFA

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[2] Committee member and research associate respectively. American Protein Company.

were not affected by treatment. Concentrations of RBC were higher at 0 and 8 hours in calves fed SDAP. Hemoglobin concentrations and hematocrit tended to be higher in calves fed SDAP at 0 hours. Concentrations of WBC were unaffected by treatment, however populations of specific white blood cells differed. This data suggests that the form of diet presented and the addition of spray dried animal plasma may influence blood metabolites associated with rumen function.

INTRODUCTION

Young dairy calves often consume starters containing sources of rapidly fermentable carbohydrates during the transition from liquid to solid feed, which leads to changes in rumen and blood metabolites. Consumption of concentrates containing rapidly fermentable carbohydrates results in increased rumen and blood volatile fatty acids (VFA) concentrations and increases blood β-hydroxybutyrate (BHBA) concentrations from butyrate oxidation in the rumen wall (Quigley et al., 1992). These types of feed can also increase lactic acid production and encourage keratinization of the rumen papillae. Changes in rumen and blood metabolites are greater when rations of small particle size are fed. Decreasing particle size of the diet accelerates rate of fermentation in the rumen environment resulting in even lower rumen pH and higher VFA production (Greenwood et al., 1997; Beharka et al., 1998). Diets containing small particle sizes also lack dietary abrasion required to maintain the integrity of the rumen epithelium (McGavin and Morrill, 1976).

Feeding starter in restricted amounts promotes rapid consumption of feed. Animals receiving restricted amounts of feed tend to consume the concentrates in the form of a meal. This meal feeding exacerbates changes in rumen metabolites. Quigley et al. (1992) observed rumen pH as low as 5.5 in calves receiving restricted access to concentrate diets. This

condition closely resembles acidosis. Due to the small stomach capacity of the young calf, a single meal may have a more profound impact on the rumen environment (Pounden and Hibbs, 1948). This condition is exaggerated when the diet lacks sufficient particle size. Small particle sizes decrease rumination and salivary flow to the rumen. This in turn lowers rumen pH even further.

It is not known whether these changes in rumen and blood metabolites impact the immune competence of the animal. If a large amount of fermentable carbohydrates are consumed in a single meal, metabolic acidosis may occur bacteria (Aiumlamai et al., 1992). When rumen acidosis is induced in young calves, endotoxins are produced from dying Gram-negative bacteria. These endotoxins promote the production of cytokines that initiate an immune response (Aiumlamai et al., 1992).

Spray-dried animal plasma (SDAP) has been successfully utilized in milk replacer fed to young calves (Drew, 1994; Morrill et al., 1995; Quigley and Bernard, 1996; Quigley et al., 2002). This product has also been successfully used in nursery diets for young pigs (Hansen et al., 1993; Kats et al., 1994). Spray-dried animal plasma has many advantages over milk products as a protein source. This product is highly digestible and has a desirable amino acid profile (Morrill et al., 1995). In addition, SDAP contains a source of immunoglobulins that may decrease the colonization of enteric pathogens (Drew, 1994). When added to nursery diets for young pigs, an increase in intake and average daily gain (ADG) are observed (Hansen et al., 1993; Kats et al., 1994). However, it is not known whether the addition of SDAP to the starter diets of young calves will yield similar results.

The objective of this study was to determine the effect of SDAP addition to the starter diets of young calves. In particular, we wanted to determine if SDAP would alleviate any

adverse metabolic changes that occur with the rapid consumption of fermentable carbohydrates in the diet.

MATERIALS AND METHODS

The institutional Animal Care and Use Committee approved all procedures used in this experiment.

Holstein bull calves (n = 28) obtained from sale barns were utilized in a replicated 4 x 4 Latin Square design. Calves were approximately 3 to 8 days of age at arrival. Upon arrival, calves were randomly assigned to one of 4 treatments. On the day of arrival, calves received one dose of a colostrum supplement (APC Lifeline Calf, Ames, IA). Calves were housed in individual hutches with wood shavings for bedding. Water was available at all times.

Calves were vaccinated based on the protocol of the research facility. On day 0 all calves received TSV-2 intranasal vaccine (Pfizer, New York, NY), 3 cc Naxcel IM (Pharmacia, Kalamazoo, MI), 1 cc Myosel (Vitamin E) IM (Schering Plough, Union, NJ), and Pasteurella vaccine SQ (Intervet, Millsboro, DE). Calves were vaccinated on days 10 and 30 with Bovishield (Pfizer, New York, NY) according to label directions.

The trial consisted of a 21-day pre-experimental period and 4 two-week treatment periods. During the pre-experimental period calves received a conventional commercial milk replacer (12.5% DM, 20% CP, 20% EE, Merricks Inc., Middleton, WI). Calves were offered approximately 227 grams of dry milk replacer in 1.8 liters of water twice per day. During this period calves were offered a commercial calf starter (DairyWay, Nutrena, Minneapolis, MN) for ad libitum consumption. This starter contained no additional molasses or animal

protein. All calves were weaned at the end of the 21-day pre-experimental period and placed onto the experimental treatments.

Dietary treatments consisted of a commercial coarse starter (Land O' Lakes, Ft. Dodge, IA) supplemented with either 5% spray dried animal plasma (AP-920, American Protein Company, Ames, IA) (C + SDAP) or with 5% of an isonitrogenous combination of spray-dried red blood cells and whey protein concentrate (Animix, Juneau, WI) (C + RBCW). A ground starter was also used in conjunction with SDAP (G + SDAP) and RBCW (G + RBCW). During days 1 to 7 of each treatment period calves received ad libitum access to diets. Average intake from days 1 through 6 was calculated. Calves were then offered 90% of the average intake from days 1-6 for days 7-14 to encourage meal feeding.

Refusals of starter and milk replacer, fecal scores (Appendix B), and daily administration of antibiotics and electrolytes were recorded daily. Jugular blood samples were obtained on arrival and at 0 and 8 hours after the morning meal on the last day of each treatment period (days 0, 35, 49, 63, and 77). Body weight was also measured on arrival and on the last day of each treatment period. Samples of dietary treatments were obtained weekly and stored (-20^{0}C) until analysis by an independent commercial laboratory (Silliker Laboratories of Iowa, Cedar Rapids, IA) (Table 1).

Table 1. Diet composition (dry matter basis).

Measure	Prewean Starter[1]	RBCW[2]	SDAP[3]	Diet[4] C + SDAP	F + SDAP	C + RBCW	F + RBCW
CP (%)	17.46	93.98	91.68	24.28	22.78	25.49	25.42
ADF (%)	6.17	------	------	5.13	5.25	5.25	5.41
Ash (%)	6.39	2.89	5.23	6.48	6.01	6.77	6.86
Ca (mg/100g)	1.07	0.83	0.54	1.15	0.94	1.13	1.20
Mg (mg/100g)	0.29	0.21	0.14	0.31	0.27	0.32	0.32
P (mg/100g)	0.83	0.24	0.79	0.63	0.56	0.60	0.61
K (mg/100g)	0.98	0.41	0.12	1.16	1.09	1.22	1.27

[1]Starter given during pre-experimental period prior to weaning
[2]Red blood cell whey protein concentrate
[3]Spray dried animal plasma
[4]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

Collection of Jugular Blood Samples and Analysis

Blood samples were obtained via jugular venipuncture at 0 and 8 hours after the a.m. meal. Blood was obtained in evacuated tubes containing EDTA (K_3) as an anticoagulant. After collection, plasma was harvested by centrifugation (3,000 x g for 15 min) and placed in storage tubes and frozen (-20^0 C) until further analysis.

Samples were then transported to the Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory for analysis. Plasma samples were allowed to thaw and then analyzed for β-hydroxybutyrate (BHBA) spectrophotometrically at 340 nm using a commercial reagent kit (Sigma Diagnostics, St. Louis, MO). Plasma samples were also analyzed for blood urea nitrogen (urea N) using a Roche/Hitachi 747 analyzer (Roche Diagnostics, Basel, Switzerland). Plasma concentrations of non-esterified fatty acids (NEFA) were also determined spectrophotometrically at 550 nm using a commercial reagent

kit (Wako Chemicals USA, Inc., Richmond, VA). Plasma concentrations of haptoglobin were measured via a single radial immunodiffusion plate test (Cardiotech Services, Inc., Louisville, KY).

Red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet concentrations, mean platelet volume (MPV), and red cell distribution width (RDW) were determined by a Cell-Dyn 3500 analyzer (Abbott Laboratories, Abbott Park, IL). The red blood cell count was directly measured. The MCV was mathematically derived from the RBC size distribution data. Hematocrit is calculated from the RBC count and MCV. The MCH was calculated from the RBC and HGB. The MCHC was calculated from the HGB and HCT. Red cell distribution width was derived from the RBC histogram. White blood cell counts were also directly measured, however, pathology personnel performed differential counts. Populations of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were measured. The cell cluster differentiation antigens (CD4 and CD8) positive cells and CD4 to CD8 ratios were determined by flow cytometry according to methods described by Wilson et al. (1996).

Statistical Analysis

All data were analyzed as a replicated Latin Square ANOVA using the general linear model (PROC GLM) of SAS (1989). Least square means and standard errors were obtained according to treatment. The data were analyzed for 4 treatments (coarse starter with 5% spray dried animal plasma = C + SDAP, ground starter with 5% spray dried animal plasma = F + SDAP, coarse starter with 5% red blood cell why protein concentrate = C + RBCW, and fine with 5% red blood cell why protein concentrate = F + RBCW). Three orthogonal

contrasts were made: C vs. F, SDAP vs. RBCW, and interaction between form of diet and plasma protein addition. Statistical significance was declared at probabilities < 0.05. Probability values between 0.05 and 0.15 were considered to be trends towards significance.

RESULTS AND DISCUSSION

Intake, Body Weight Gain, and Calf Health

Total dry matter intake did not differ with respect to treatment (P = 0.41). Intake of crude protein was higher for calves consuming diets containing RBCW compared to those consuming SDAP (P < 0.01). This reflects higher crude protein percentages of diets containing RBCW. Intake of fat was greater for calves consuming the coarse diets than for those consuming the fine form (P < 0.01). Body weight tended to be higher in calves consuming diets containing SDAP than those consuming RBCW (P < 0.11) (Table 2), in spite of higher intake of CP by calves receiving RBCW. When SDAP is added to nursery diets for young pigs, an increase in body weight is also observed (Hansen et al., 1993; Kats et al., 1994). There were also no differences in fecal scores with respect to treatment (P = 0.35), indicating that health of the animals was unaffected by plasma addition or form of diet.

Table 2. Intake of nutrients and body weight gain.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interaction
BW, kg	78.7	76.9	78.6	77.6	0.8	0.68	0.11	0.66
DMI, g/d	2250	2229	2287	2243	39	0.51	0.41	0.77
CP intake, g/d	471	495	451	493	8	0.17	0.01	0.26
EE intake, g/d	104	101	91	90	2	0.01	0.19	0.73

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

Plasma Urea N, NEFA, BHBA, and Haptoglobin

Plasma urea N concentrations were greater prior to the morning meal (0 hours) in calves fed the F diets ($P < 0.07$) and those receiving SDAP ($P < 0.01$). Calves receiving the F diets had higher urea N levels at 8 hours postfeeding ($P < 0.03$) (Table 3). Calves consuming SDAP also had higher urea N levels at 8 hours ($P < 0.04$) despite a lower level of crude protein in the diet. This may be due to differences in nitrogen availability as a result of differences in rumen protein degradability. Change from 0 to 8 hours with respect to urea N concentrations was unaffected by treatment ($P = 0.29$). Concentrations of NEFA did not differ with respect to treatment at 0 hours ($P = 0.77$), 8 hours ($P = 0.55$), and there was also no significant change from 0 to 8 hours ($P = 0.66$) (Table 3). This indicates that the treatments had little effect on lipid metabolism. Concentrations of plasma BHBA tended to be higher for calves receiving C at 0 hours post-feeding ($P<0.14$). However, there were no difference with treatment at 8 hours ($P = 0.27$) and the change from 0 to 8 hours was also not different with treatment ($P = 0.53$) (Table 3). There was little difference in rumen metabolism and utilization of butyrate with respect to treatment. All values were within normal ranges for calves this age (Staples et al., 1970; Knowles et al., 2000).

Table 3. Plasma urea N, NEFA, and BHBA.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interaction
Urea N (mg/dl)								
0	17.4	15.9	18.0	16.9	0.4	0.07	0.01	0.62
8	15.0	14.1	15.8	15.1	0.4	0.03	0.04	0.81
Change	-2.4	-1.8	-2.2	-1.8	0.5	0.82	0.29	0.82
NEFA (mg/dl)								
0	216	223	221	215	21	0.95	0.97	0.77
8	73	72	68	72	1	0.55	0.75	0.62
Change	-142	-152	-153	-144	21	0.96	0.99	0.66
BHBA (mg/dl)								
0	2.1	2.2	2.0	1.9	0.1	0.14	1.00	0.38
8	4.5	4.4	4.3	4.1	0.2	0.27	0.56	0.86
Change	2.5	2.3	2.3	2.3	0.2	0.70	0.53	0.77

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

Haptoglobin values are not reported here due to the failure of the commercial kit to provide a measureable low level standard. However, haptoglobin has been used as an indicator of the acute phase response in calves. Increasing levels of haptoglobin are indicative of both clinical and acute inflammation (Alsemgeest et al., 1995).

Blood Components

Red blood cell concentrations were greater in calves fed SDAP than those offered RBCW at 0 hours ($P < 0.05$) (Table 4). There was also an interaction between the form of diet offered and plasma addition at 0 hours ($P < 0.04$). At 8 hours post-feeding there was no effect of treatment on RBC populations ($P > 0.16$). There was an interaction between form of diet and plasma addition with respect to the change of RBC between 0 and 8 hours ($P < 0.15$). Hemoglobin concentrations tended to be higher for calves fed SDAP versus RBCW (Table 4). However, there were no differences with treatment at 8 hours post-feeding ($P >$

0.27). There were also no differences between 0 and 8 hours in hemoglobin concentration ($P > 0.27$). Hematocrit percentages were higher for calves receiving SDAP than RBCW at 0 hours post-feeding ($P < 0.07$) (Table 4). There were no differences at 8 hours ($P > 0.26$). Calves receiving the F diet had a greater decrease in hematocrit percentage than diet C post-feeding ($P < 0.07$). The differences in RBC, hemoglobin, and hematocrit may simply indicate differences in water intake and hydration of the calves. However, water intake was not measured in this trial. There were no treatment differences on MCV, MCH, MCHC, and MPV (Table 4). There was an interaction between form of diet and plasma addition on the change in concentration of platelets ($P < 0.06$) (Table 4). There was also an interaction at 0 and 8 hours on red blood cell width distribution ($P < 0.15$ and $P < 0.13$ respectively) (Table 4). All measurements were within normal ranges for calves of this age (Staples et al., 1970 and Knowles et al., 2000).

Table 4. Blood components including RBC, hemoglobin, hematocrit, and platelets.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interact.
RBC (10^6/μl)								
0	10.1	10.1	10.4	9.6	0.2	0.48	0.05	0.04
8	9.9	9.8	9.9	9.6	0.1	0.35	0.16	0.43
Change	-0.2	-0.4	-0.5	0	0.2	0.93	0.35	0.15
Hemoglobin (g/dl)								
0	12.3	11.6	12.0	11.5	0.4	0.57	0.14	0.72
8	11.3	11.3	11.4	11.1	0.1	0.75	0.27	0.37
Change	-1.0	-0.3	-0.6	-0.5	0.3	0.68	0.27	0.38
Hematocrit (%)								
0	33.0	33.1	34.0	32.8	0.3	0.39	0.13	0.07
8	32.0	31.9	32.1	31.3	0.4	0.46	0.26	0.31
Change	-1.0	-1.2	-1.9	-1.7	0.3	0.07	0.94	0.57
MCV (fl)								
0	32.6	32.7	32.7	32.9	0.1	0.38	0.3	0.64
8	32.4	32.6	32.5	32.6	0.2	0.63	0.29	0.85
Change	-0.2	-0.1	-0.2	-0.3	0.1	0.67	0.94	0.37
MCH (pg)								
0	11.5	11.5	11.5	11.5	0.1	0.43	0.67	0.43
8	11.4	11.5	11.1	11.6	0.2	0.63	0.22	0.38
Change	-0.1	0	-0.4	0	0.2	0.43	0.25	0.5
MCHC (g/dl)								
0	35.2	35.1	35.2	35.2	0.2	0.81	0.83	0.47
8	35.3	35.3	35.5	35.5	0.2	0.16	0.97	0.85
Change	0.1	0.2	0.4	0.3	0.2	0.32	0.80	0.62
Platelets (10^3/μl)								
0	826	836	861	817	28	0.77	0.53	0.33
8	868	835	839	847	19	0.66	0.57	0.29
Change	42	-1	-22	23	24	0.39	0.95	0.06
MPV (fl)								
0	4.59	4.42	4.66	4.69	0.41	0.67	0.86	0.8
8	3.98	4.34	4.75	4.42	0.36	0.23	0.98	0.33
Change	-0.44	0.06	0.04	-0.34	0.54	0.94	0.92	0.41
RDW (%)								
0	27.4	27.7	27.8	27.1	0.3	0.87	0.54	0.15
8	26.2	27.2	27.8	27.1	0.6	0.23	0.78	0.13
Change	-1.2	-0.3	0	-0.4	0.5	0.29	0.67	0.21

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

White Blood Cell Populations

Total blood white blood cell populations were unaffected by the form of diet of plasma addition alone ($P > 0.50$). However, a tendency towards an interaction was observed at 0 hours ($P < 0.13$). Individual populations of WBC were altered with treatment. Neutrophil concentrations tended to increase more in calves receiving C diets compared to those fed F ($P < 0.10$). Concentrations of lymphocytes tended to be greater in calves fed C at 0 hours than those fed F ($P < 0.09$). Lymphocyte concentrations decreased with time in calves fed C and increased in those fed F ($P < 0.05$). This decrease in lymphocyte concentrations may indicate an increase in the migration of the cells from the blood to the tissue. Monocyte concentrations tended to be higher in calves fed F diets than those fed C at 8 hours post-feeding ($P < 0.11$). There were no differences in eosinophil and basophil populations with treatment (Table 4), indicating there was no allergic reaction or response to parasites. Despite these changes, all values remained within normal reported values for calves of this age (Staples et al., 1970; Knowles et al., 2000), and are probably not indicative of a major stress response in these calves.

Table 5. White blood cell populations.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interact.
WBC (10^3/μl)								
0	8	8.2	8.3	7.7	0.3	0.69	0.50	0.13
8	8.5	8.8	8.6	8.3	0.4	0.61	0.96	0.37
Change	0.5	0.6	0.4	0.5	0.3	0.70	0.66	0.94
Neutrophils (10^3/μl)								
0	2.83	3.11	3.45	2.89	0.25	0.42	0.58	0.10
8	3.76	4.16	3.80	3.45	0.37	0.36	0.94	0.31
Change	0.93	1.07	0.34	0.49	0.35	0.10	0.69	0.99
Lymphocytes (10^3/μl)								
0	4.49	4.40	4.09	4.21	0.17	0.09	0.92	0.55
8	4.25	4.17	4.28	4.29	0.14	0.61	0.79	0.72
Change	-0.23	-0.27	0.19	0.05	0.19	0.05	0.66	0.78
Monocytes (10^3/μl)								
0	0.44	0.43	0.51	0.41	0.06	0.67	0.36	0.43
8	0.29	0.30	0.36	0.39	0.05	0.11	0.74	0.92
Change	-0.15	-0.12	-0.15	-0.02	0.08	0.53	0.32	0.53
Eosinophils (10^3/μl)								
0	0.12	0.17	0.13	0.11	0.03	0.44	0.70	0.33
8	0.11	0.11	0.10	0.11	0.03	0.82	0.92	0.99
Change	-0.01	-0.06	-0.03	-0.01	0.03	0.53	0.57	0.26
Basophils (10^3/μl)								
0	0.07	0.06	0.07	0.05	0.02	0.87	0.29	0.84
8	0.05	0.07	0.08	0.05	0.01	0.82	0.66	0.18
Change	-0.02	0.01	0.01	0.00	0.02	0.84	0.69	0.43

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

The proportion of individual WBC populations as a percentage of the total WBC also differed with treatment. The percentage of neutrophils as a portion of the total WBC was greater at 0 hours for calves fed C compared to F ($P < 0.05$). The increase in neutrophil

percentage also tended to increase more over the 8 hour period for calves fed C than F ($P < 0.08$). The proportion of lymphocytes was similar to the neutrophils. Calves fed C has higher proportions of lymphocytes at 0 hours ($P < 0.04$) and tended to have a greater increase over the 8 hours ($P < 0.08$). Once again, this may indicate a migration of the cells. Monocyte proportions were unaffected by treatment. Eosinophil and basophil proportions changed over time according to treatment. The form of diet offered altered eosinophil percentages. Calves fed C tended to have greater increases in eosinophils than those fed F ($P < 0.08$). Basophil proportions had a larger decrease in calves fed C than F ($P < 0.05$). Despite the changes associated with treatment, all proportions of individual WBC remained within normal ranges (Staples et al., 1970; Knowles et al., 2000), and therefore are probably not indicative of a major immunological response in these calves.

Table 6. White blood cell populations as a portion of total WBC.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interact.
Neutrophils (% WBC)								
0	34.0	34.9	39.0	36.7	1.8	0.05	0.70	0.36
8	40.6	43.2	42.8	40.6	2.0	0.92	0.92	0.23
Change	26.6	28.8	15.3	17.9	6.2	0.08	0.70	0.97
Lymphocytes (% WBC)								
0	58.1	57	52.4	55.6	1.7	0.04	0.52	0.21
8	53.9	51.2	51.1	53	2	0.77	0.84	0.24
Change	-6.0	-10.1	3.0	-1.8	4.8	0.08	0.36	0.94
Monocytes (% WBC)								
0	5.9	5.7	6.0	5.3	0.7	0.87	0.53	0.69
8	3.8	3.6	4.1	4.5	0.5	0.32	0.80	0.67
Change	23.8	-15.3	-7.2	23.7	24.9	0.88	0.87	0.18
Eosinophils (% WBC)								
0	1.3	1.7	1.5	1.6	0.3	0.77	0.43	0.56
8	1.1	1.2	1.3	1.4	0.2	0.43	0.7	0.96
Change	-62.9	-65.6	-36.4	-24.1	21.0	0.08	0.81	0.71
Basophils (% WBC)								
0	0.8	0.7	1.0	0.7	0.2	0.69	0.32	0.77
8	0.6	0.7	0.8	0.6	0.1	0.91	0.72	0.26
Change	-84.8	-27.4	-33.9	-69	20	0.82	0.57	0.05

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

CD4 and CD8 Cells

Cluster differentiation antigens (CD) are expressed in the membranes of lymphocytes and can serve as surface markers to identify cell populations. Lymphocytes that are CD4 positive bind to MHC II and are classified as helper T cells. These cells activate populations of other cells, such as macrophages, to destroy foreign molecules. Cells that are CD8

positive bind to MHC I and are classified as T cytotoxic cells. These cells kill targets directly.

CD4 positive cells were greater at 8 hours post-feeding in calves fed diets containing F compared to those fed C ($P < 0.03$). CD8 positive cells were unaltered with treatment ($P > 0.21$). The ratio of CD4 to CD8 positive cells was altered by plasma addition to the diet. Calves consuming SDAP tended to have a lower proportion of CD4 to CD8 at 8 hours post-feeding ($P < 0.15$). There was a tendency towards greater change in this ratio from pre-feeding to post-feeding in calves fed C than those receiving F ($P < 0.15$). There was also a tendency towards an interaction between form of diet and plasma addition with respect to the change in CD4 to CD8 over time ($P < 0.14$). (Table 5). However, the ratio of CD4 positive cells to CD8 positive cells did not differ greatly from the 2 to 1 ratio normally observed in calves. Despite the effects of treatment, all values remained within normal ranges for calves of this age (Wilson et al., 1996), and are probably not indicative of a major immunological response in these calves.

Table 7. CD4 and CD8 positive cells.

	Diet[1]					Contrast *P* Values		
	C+ SDAP	C+ RBCW	F+ SDAP	F+ RBCW	SE	Form	Plasma	Interact.
CD4								
0	21.77	22.19	23.59	22.85	1.16	0.29	0.89	0.62
8	22.79	22.75	24.40	26.71	1.25	0.03	0.37	0.36
Change	1.02	1.68	0.76	3.39	1.45	0.63	0.27	0.50
CD8								
0	10.97	13.38	12.58	12.86	1.06	0.61	0.21	0.31
8	12.12	11.86	11.81	11.98	1.04	0.93	0.97	0.84
Change	1.06	-0.83	-0.92	-0.88	1.21	0.41	0.45	0.43
CD4/CD8								
0	-13.74	2.33	1.04	2.53	7.84	0.34	0.27	0.36
8	2.48	2.69	-10.26	2.74	4.53	0.17	0.15	0.17
Change	16.68	0.21	-11.56	0.51	9.36	0.15	0.82	0.14

[1]C + SDAP = coarse starter with 5% spray dried animal plasma; F + SDAP = ground starter with 5% spray dried animal plasma; C + RBCW = coarse starter with 5% red blood cell whey protein concentrate; F + RBCW = ground starter with 5% red blood cell whey protein concentrate

CONCLUSIONS

The addition of SDAP to starter rations for young calves improves body weight gain and impacts specific WBC populations. However, the long-term effects of these changes are unknown. The form of diet offered also alters blood cell populations, and the impact of these changes is unknown. However, despite the changes in cell populations with treatment, all values remained well within normal ranges. Therefore, despite the significant statistical differences, these changes most likely do not represent significant physiological differences. These changes are probably not indicative of a major immunological shift in these animals. Further work is needed to determine the effect of such rations on animal health.

LITERATURE CITED

Aiumlamai, S., H. Kindahl, G. Fredriksson, L.E. Edqvist, L. Kulander, and O. Eriksson. 1992. The role of endotoxins in induced ruminal acidosis in calves. Acta Vet. Scand. 33:117-127.

Alsemgeest, S.P.M., I.E. Lambooy, H.K. Wierenga, S.J. Dieleman, B. Meerkerk, A.M. van Ederen, and Th. A. Niewold. 1995. Influence of physical stress on the plasma concentration of serum amyloid-A (SAA) and haptoglobin (HP) in calves. Vet. Q. 17:9-12.

Beharka, A.A., T.G. Nagaraja, J.L. Morrill, G.A. Kennedy and R.D. Klemm. 1998. Effect of form of diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946-1955.

Drew, M.D. 1994. Effects of immunoglobulin fortification of milk replacers on the performance of calves challenged with *Escherichia coli*. J. Dairy Sci. 77 (Suppl. 1):298. (Abstr.)

Greenwood, R.H., J.L. Morrill, E.C. Titgemeyer and G.A. Kennedy. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. J. Dairy Sci. 80:2534-2541.

Hansen, J.A., J.L. Nelssen, R.D. Goodband, and T.L. Weeden. 1993. Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71:1853-1862.

Kats, L.J., J.L. Nelssen, M.D. Tokach, R.D. Goodband, J.A. Hansen, and J.L. Laurin. 1994. The effect of spray-dried porcine plasma on growth performance in the early-weaned pig. J. Anim. Sci. 72:2075-2081.

Knowles, T.G., J.E. Edwards, K.J. Bazeley, S.N. Brown, A. Butterworth, and P.D. Warriss. 2000. Changes in the blood biochemical and hematological profile of neonatal calves with age. Vet. Rec. 147:593-598.

McGavin, M.D. and J.L. Morrill. 1976. Scanning electron microscopy of ruminal papillae in calves fed various amounts and forms of roughage. Am. J. Vet. Res. 37:497-508.

Morrill, J.L., J.M. Morrill, A.M. Feyerherm, and J.F. Laster. 1995. Plasma proteins and a probiotic as ingredients in milk replacer. J. Dairy Sci. 78:902-907.

Pounden, W.D. and J.W. Hibbs. 1948. The influence of the ration of grain to hay in the ration of dairy calves on certain rumen microorganisms. J. Dairy Sci. 31:1051-1054.

Quigley, J.D., III, T.M. Steen, and S.I. Boehms. 1992. Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed diets with or without hay. J. Dairy Sci. 75:228-235.

Quigley, J.D., III, and J.K. Bernard. 1996. Milk replacers with or without animal plasma for dairy calves. J. Dairy Sci. 79:1881-1884.

Quigley, J.D., III, C.J. Kost, and T.A. Wolfe. 2002. Effects of spray-dried animal plasma in milk replacers or additives containing serum and oligosaccharides on growth and health of calves. J. Dairy Sci. 85:413-421.

Staples, G.E., M.F. Andrews, R.M. Parsons, P.K. McIlwain, and C.N. Haugse. 1970. Young calves: relation of neonatal health status and sex to some blood components. J. Anim. Sci. 31:383-388.

Wilson, R.A., A. Zolnai, P. Ridas, and L.V. Frenyo. 1996. T-cell subsets in blood and lymphoid tissues obtained from fetal calves, maturing calves, and adult bovine. Vet. Immunol. Immunopathol. 53:49-60.

CHAPTER FIVE

GENERAL CONCLUSIONS

This research represents a continuing effort to determine the optimal ration formulation for preruminant calves. In contrast to older heifers and mature cows, the calf has unique dietary considerations. In order to formulate rations for the preruminant calf one must consider many factors. First, the combination of both liquid and solid feed in the diet markedly influences intake. It is relatively easy to meet the nutrient needs of a young calf with only liquid feed, however this diet does not promote rumen development. In addition, the consumption of large amounts liquid feed delays the consumption of solid feed. Furthermore, liquid feed does not provide the most economic rate of gain. The use of milk as a feedstuff requires additional labor and cost when compared to solid feed. The digestibility and utilization of solid feed also changes with the age of the calf and with the development of the rumen.

The consumption of solid feed is critical for the development of a functional rumen in the young calf. However, the optimal diet required for this transition has not yet been determined. Starter rations for young calves must contain sufficient amounts of fermentable carbohydrates to promote VFA production. The production of VFA, particularly butyrate, stimulate the development of the rumen epithelium. However, this cell growth is uncontrolled and requires sufficient particle size in the diet to discourage keratinization of the papillae. In previous investigations, forages provided this dietary abrasive value, and also promoted rumination and saliva flow into the rumen. The results from these investigations suggest that forage is a beneficial addition to the diet of the preruminant calf.

Results from previous investigations in this area have been inconsistent. Many investigators have found the addition of forage beneficial to starter intake (Jahn et al., 1970; Kang and Leibholz, 1973; Kellaway et al., 1973; Kincaid, 1980; Stobo et al., 1985; Thomas and Hinks, 1982). However, others have observed no effect or a negative impact on intake of solid feed (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975). The inconsistent findings of these studies may be a result of differences in the species of forage used, variations in forage quality, and differences in management strategies. The variation commonly seen in forage sources was decreased in these investigations by utilizing immature grass hay that was sorted for consistent particle size. This consistency of particle size decreased the variation in intake observed in other investigations when forages were offered.

The addition of forage of a consistent particle size to the diet of young calves was beneficial to the calves in these investigations. The addition of forage stimulated intake when diets were offered ad libitum, increased body weight gains, and promoted improved feed efficiency when intake was restricted. This increase in weight gain and feed efficiency is most likely due to a more stable rumen environment. Forage in the diet, in sufficient particle size, promotes rumination and increased saliva flow into the rumen. This maintains the rumen pH allowing cellulolytic populations of bacteria to thrive.

The rapid consumption of fermentable carbohydrates alters rumen and blood metabolites. At this time it is not clear if these changes affect the immune competence of the animals. The addition of SDAP to the diet improved body weight gain. This is similar to results observed in other species (Hansen et al., 1993; Kats et al., 1994). The form of diet offered (coarse versus fine) and the addition of plasma proteins also affected blood components and white blood cell populations. However, none of the changes observed were

beyond normal ranges for calves that age. This is probably not indicative of a major stress response in these meal-fed calves.

The addition of both forage of a consistent particle size and SDAP were beneficial to the growth of preruminant calves, and these feedstuffs should be considered as possible additions to starter rations. It appears that forage does play a beneficial role in the preruminant calf. This feedstuff would be an economically viable addition to the diet of the calf. However, the sorting of forage for consistent particle size is a time consuming process that would need to be mechanized. Further work needs to be done in order to determine the value of SDAP in the diet of young calves.

LITERATURE CITED

Hansen, J.A., J.L. Nelssen, R.D. Goodband, and T.L. Weeden. 1993. Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71:1853-1862.

Hibbs, J.W., H.R. Conrad, W.D. Pounden, and N. Frank. 1956. A high roughage system for raising calves based on early development of rumen function. VI. Influence of hay to grain ration on calf performance, rumen development, and certain blood changes. J. Dairy Sci. 39:171-179.

Jahn, E., P.T. Chandler, and C.E. Polan. 1970. Effects of fiber and ratios of starch to sugar upon the performance of young ruminating calves. J. Dairy Sci. 53:466-474.

Kang, H.S. and J. Leibholz. 1973. The roughage requirement of the early-weaned calf. Anim. Prod. 16:195-203.

Kats, L.J., J.L. Nelssen, M.D. Tokach, R.D. Goodband, J.A. Hansen, and J.L. Laurin. 1994. The effect of spray-dried porcine plasma on growth performance in the early-weaned pig. J. Anim. Sci. 72:2075-2081.

Kellaway, R.C., T. Grant, and J.W. Chudleigh. 1973. The roughage requirement of early weaned calves. Aust. J. Exp. Agric. Anim. Husb. 13:9-12.

Kincaid, R.L. 1980. Alternate methods of feeding alfalfa to calves. J. Dairy Sci. 63:91-94.

Leibholz, J. 1975. Ground roughage in the diet of the early-weaned calf. Anim. Prod. 20:93-100.

Stobo, I.J.F., C.S. Lucci, J.H.B. Roy, and M.W. Perfitt. 1985. Comparison of high-energy pellets containing processed fibre with a coarse concentrate mixture in relation to the development of solid food intake in the calf. Anim. Prod. 40:570.

Thomas, D.B. and C.E. Hinks. 1982. The effect of changing the physical form of roughage on the performance of the early-weaned calf. Anim. Prod. 35:375-384.

Whitaker, R.T., W.J. Miller, J.L. Carmon and H.L. Dalton. 1957. Influence of level and source of crude fiber in calf starters on weight and feed consumption. J. Dairy Sci. 40:887-892.

APPENDIX A

MILK REPLACER OFFERED BY INITIAL BODY WEIGHT

Initial Body Weight (lb)	Volume in Bottle/Feeding (pt)
20 - 30	1.0
31 - 40	1.5
41 - 51	2.0
52 - 61	2.5
62 - 72	3.0
73 - 82	3.5
83 - 92	4.0
93 - 103	4.5
104 - 113	5.0
114 -124	5.5
125 - 134	6.0

APPENDIX B

FECAL SCORE SYSTEM

Score	Description
1	Normal, firm
2	Soft, spreads easily
3	Runny, spreads easily
4	Watery, liquid consistency

APPENDIX C

GAS CHROMATOGRAPH VFA AND INTERNAL STANDARD SOLUTIONS

Rumen Fluid VFA Stock Standard:

Acid	mL acid/L water	Density (g/mL)	Molecular Weight (g/mol)	Final Conc. (mol/L)	Final Conc. (mM/L)
Acetic	6	1.0491	60.05	0.1048	104.8
Propionic	4	0.9920	74.08	0.0536	53.6
Isobutyric	1	0.9504	88.11	0.0108	10.8
Butyric	2	0.9640	88.10	0.0219	21.9
Isovalerate	1	0.9373	102.12	0.0092	9.2
Valerate	1	0.9391	102.12	0.0092	9.2

The stock standard listed above is then diluted 1:2, 1:4, and 1:6 with deionized water.

For the 1:2 dilution take 20 mls of stock solution + 20 mls water.
For the 1:4 dilution take 10 mls of stock solution + 30 mls water.
For the 1:6 dilution take 5 mls of stock solution + 25 mls water.

The dilutions are then treated with meta-phosphoric acid to mimic the sample solutions. All solution are diluted with a 5:1 ratio of solution to acid.

Once diluted with acid, the following standard solutions are produced:

	Level 1 (mM)	Level 2 (mM)	Level 3 (mM)	Level (mM)
Acetic	87.33	43.65	21.82	14.55
Propionic	44.66	22.32	11.16	7.44
Isobutyric	9.00	4.50	2.25	1.50
Butyric	18.23	9.11	4.56	3.04
Isovalerate	7.67	3.83	1.92	1.28
Valerate	7.66	3.83	1.92	1.28

Internal Standard Solution:

Solution is prepared by diluting 2 mls of 2-ethyl butyric acid in 15 mls of 90% ethanol and dilute to a final volume of 500 mls with deionized water.

APPENDIX D

VALUES AND STANDARD ERRORS FOR PARAMETERS MEASURED IN CHAPTER TWO

Milk replacer intake (kg DM).

Day	C	SE	G	SE	H1	SE	H2	SE
1	0.54	0.02	0.55	0.02	0.56	0.02	0.56	0.02
2	0.54	0.02	0.54	0.02	0.57	0.02	0.55	0.02
3	0.51	0.02	0.55	0.02	0.53	0.02	0.55	0.02
4	0.54	0.02	0.55	0.02	0.56	0.02	0.55	0.02
5	0.52	0.02	0.54	0.02	0.54	0.02	0.54	0.02
6	0.52	0.02	0.54	0.02	0.53	0.02	0.55	0.02
7	0.53	0.02	0.55	0.02	0.57	0.02	0.56	0.02
8	0.53	0.02	0.55	0.02	0.57	0.02	0.56	0.02
9	0.52	0.02	0.54	0.02	0.57	0.02	0.56	0.02
10	0.52	0.02	0.55	0.02	0.57	0.02	0.56	0.02
11	0.53	0.02	0.55	0.02	0.57	0.02	0.56	0.02
12	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
13	0.53	0.02	0.55	0.02	0.57	0.02	0.56	0.02
14	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
15	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
16	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
17	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
18	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
19	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
20	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
21	0.53	0.02	0.55	0.02	0.57	0.02	0.56	0.02
22	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
23	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
24	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
25	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
26	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
27	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
28	0.54	0.02	0.55	0.02	0.57	0.02	0.55	0.02
29	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
30	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
31	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
32	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
33	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
34	0.54	0.02	0.55	0.02	0.57	0.02	0.55	0.02
35	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
36	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
37	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
38	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
39	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
40	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
41	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
42	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02

Day	C	SE	G	SE	H1	SE	H2	SE
43	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
44	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
45	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
46	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
47	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
48	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
49	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
50	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
51	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02
52	0.54	0.02	0.55	0.02	0.57	0.02	0.56	0.02

P values:

Treatment ($P<0.72$)

Starter intake (kg DM).

Day	C	SE	G	SE	H1	SE	H2	SE
2	0.03	0.16	0.03	0.14	0.02	0.15	0.02	0.14
3	0.00	0.16	0.02	0.14	0.01	0.15	0.01	0.14
4	0.01	0.16	0.03	0.14	0.01	0.15	0.03	0.14
5	0.01	0.16	0.03	0.14	0.02	0.15	0.04	0.14
6	0.02	0.16	0.04	0.14	0.03	0.15	0.04	0.14
7	0.02	0.16	0.05	0.14	0.04	0.15	0.06	0.14
8	0.02	0.16	0.04	0.14	0.04	0.15	0.06	0.14
9	0.04	0.16	0.06	0.14	0.07	0.15	0.07	0.14
10	0.05	0.16	0.08	0.14	0.09	0.15	0.09	0.14
11	0.05	0.16	0.09	0.14	0.10	0.15	0.08	0.14
12	0.07	0.16	0.11	0.14	0.13	0.15	0.11	0.14
13	0.08	0.16	0.13	0.14	0.15	0.15	0.12	0.14
14	0.10	0.16	0.14	0.14	0.16	0.15	0.12	0.14
15	0.10	0.16	0.16	0.14	0.14	0.15	0.15	0.14
16	0.15	0.16	0.18	0.14	0.14	0.15	0.17	0.14
17	0.14	0.16	0.17	0.14	0.17	0.15	0.17	0.14
18	0.14	0.16	0.18	0.14	0.17	0.15	0.17	0.14
19	0.14	0.16	0.19	0.14	0.19	0.15	0.18	0.14
20	0.17	0.16	0.19	0.14	0.20	0.15	0.18	0.14
21	0.21	0.16	0.21	0.14	0.20	0.15	0.19	0.14
22	0.20	0.16	0.22	0.14	0.21	0.15	0.18	0.14
23	0.22	0.16	0.21	0.14	0.21	0.15	0.19	0.14
24	0.24	0.16	0.23	0.14	0.22	0.15	0.19	0.14
25	0.31	0.16	0.30	0.14	0.30	0.15	0.27	0.14
26	0.33	0.16	0.29	0.14	0.30	0.15	0.28	0.14
27	0.33	0.16	0.27	0.14	0.31	0.15	0.28	0.14
28	0.33	0.16	0.29	0.14	0.31	0.15	0.28	0.14
29	0.33	0.16	0.29	0.14	0.31	0.15	0.28	0.14
30	0.33	0.16	0.30	0.14	0.32	0.15	0.28	0.14
31	0.33	0.16	0.29	0.14	0.32	0.15	0.28	0.14
32	0.33	0.16	0.31	0.14	0.33	0.15	0.29	0.14
33	0.33	0.16	0.31	0.14	0.33	0.15	0.29	0.14
34	0.34	0.16	0.32	0.14	0.34	0.15	0.31	0.14
35	0.35	0.16	0.31	0.14	0.35	0.15	0.31	0.14
36	0.35	0.16	0.33	0.14	0.35	0.15	0.30	0.14
37	0.35	0.16	0.32	0.14	0.35	0.15	0.33	0.14
38	0.35	0.16	0.33	0.14	0.35	0.15	0.32	0.14
39	0.35	0.16	0.33	0.14	0.35	0.15	0.33	0.14
40	0.35	0.16	0.33	0.14	0.35	0.15	0.30	0.14
41	0.35	0.16	0.33	0.14	0.35	0.15	0.33	0.14
42	0.35	0.16	0.33	0.14	0.35	0.15	0.33	0.14
43	0.34	0.16	0.35	0.14	0.35	0.15	0.35	0.14
44	0.35	0.16	0.35	0.14	0.35	0.15	0.34	0.14
45	0.35	0.16	0.35	0.14	0.35	0.15	0.35	0.14
46	0.35	0.16	0.35	0.14	0.35	0.15	0.35	0.14
47	0.35	0.16	0.35	0.14	0.35	0.15	0.35	0.14
48	0.45	0.16	0.45	0.14	0.45	0.15	0.45	0.14

Day	C	SE	G	SE	H1	SE	H2	SE
49	0.45	0.16	0.45	0.14	0.45	0.15	0.45	0.14
50	0.45	0.16	0.44	0.14	0.45	0.15	0.45	0.14
51	0.45	0.16	0.44	0.14	0.45	0.15	0.45	0.14
52	0.45	0.16	0.45	0.14	0.45	0.15	0.45	0.14
53	1.62	0.16	1.29	0.14	1.45	0.15	1.48	0.14
54	0.98	0.16	1.15	0.14	1.24	0.15	1.25	0.14
55	1.33	0.16	1.35	0.14	1.64	0.15	1.51	0.14
56	1.30	0.16	1.37	0.14	1.64	0.15	1.66	0.14
57	1.51	0.16	1.57	0.14	1.81	0.15	1.86	0.14
58	1.65	0.16	1.59	0.14	2.02	0.15	1.96	0.14
59	1.82	0.16	1.77	0.14	2.21	0.15	2.02	0.14
60	2.06	0.16	2.07	0.14	2.09	0.15	2.00	0.14
61	2.03	0.16	2.09	0.14	2.46	0.15	2.15	0.14
62	2.21	0.16	2.13	0.14	2.52	0.15	2.28	0.14
63	2.45	0.16	4.13	0.14	2.65	0.15	2.44	0.14
64	2.21	0.16	1.85	0.14	2.53	0.15	2.32	0.14

P values:

Entire trial

Treatment ($P<0.63$)

C vs G ($P<0.84$)

C vs H1 + H2 ($P<0.29$)

H1 vs H2 ($P<0.44$)

Preweaning (days 2-52)

Treatment ($P<0.79$)

C vs G ($P<0.99$)

C vs H1 + H2 ($P<0.91$)

H1 vs H2 ($P<0.31$)

Postweaning (days 53-64)

Treatment ($P<0.59$)

C vs G ($P<0.81$)

C vs H1 + H2 ($P<0.22$)

H1 vs H2 ($P<0.55$)

Total dry matter intake (kg):

Day	C	SE	G	SE	H1	SE	H2	SE
1	0.54	0.03	0.55	0.02	0.56	0.02	0.56	0.02
2	0.57	0.03	0.57	0.02	0.59	0.02	0.58	0.02
3	0.52	0.03	0.57	0.02	0.54	0.02	0.55	0.02
4	0.54	0.03	0.58	0.02	0.57	0.02	0.58	0.02
5	0.53	0.03	0.58	0.02	0.56	0.02	0.58	0.02
6	0.54	0.03	0.58	0.02	0.57	0.02	0.58	0.02
7	0.54	0.03	0.60	0.02	0.61	0.02	0.61	0.02
8	0.55	0.03	0.59	0.02	0.61	0.02	0.61	0.02
9	0.57	0.03	0.60	0.02	0.64	0.02	0.63	0.02
10	0.57	0.03	0.63	0.02	0.66	0.02	0.65	0.02
11	0.58	0.03	0.64	0.02	0.67	0.02	0.64	0.02
12	0.60	0.03	0.66	0.02	0.70	0.02	0.67	0.02
13	0.61	0.03	0.68	0.02	0.72	0.02	0.67	0.02
14	0.64	0.03	0.69	0.02	0.73	0.02	0.68	0.02
15	0.64	0.03	0.71	0.02	0.71	0.02	0.70	0.02
16	0.68	0.03	0.73	0.02	0.71	0.02	0.73	0.02
17	0.67	0.03	0.72	0.02	0.74	0.02	0.73	0.02
18	0.68	0.03	0.73	0.02	0.74	0.02	0.73	0.02
19	0.67	0.03	0.74	0.02	0.76	0.02	0.73	0.02
20	0.71	0.03	0.74	0.02	0.76	0.02	0.74	0.02
21	0.75	0.03	0.76	0.02	0.77	0.02	0.75	0.02
22	0.74	0.03	0.77	0.02	0.78	0.02	0.74	0.02
23	0.76	0.03	0.76	0.02	0.78	0.02	0.75	0.02
24	0.78	0.03	0.78	0.02	0.79	0.02	0.75	0.02
25	0.85	0.03	0.85	0.02	0.86	0.02	0.83	0.02
26	0.87	0.03	0.84	0.02	0.86	0.02	0.84	0.02
27	0.87	0.03	0.82	0.02	0.88	0.02	0.84	0.02
28	0.86	0.03	0.84	0.02	0.88	0.02	0.84	0.02
29	0.86	0.03	0.84	0.02	0.87	0.02	0.84	0.02
30	0.87	0.03	0.85	0.02	0.89	0.02	0.84	0.02
31	0.87	0.03	0.84	0.02	0.89	0.02	0.84	0.02
32	0.87	0.03	0.86	0.02	0.90	0.02	0.85	0.02
33	0.87	0.03	0.86	0.02	0.90	0.02	0.85	0.02
34	0.88	0.03	0.87	0.02	0.91	0.02	0.86	0.02
35	0.89	0.03	0.86	0.02	0.92	0.02	0.86	0.02
36	0.89	0.03	0.88	0.02	0.92	0.02	0.86	0.02
37	0.89	0.03	0.87	0.02	0.92	0.02	0.89	0.02
38	0.89	0.03	0.88	0.02	0.92	0.02	0.88	0.02
39	0.89	0.03	0.88	0.02	0.92	0.02	0.89	0.02
40	0.89	0.03	0.88	0.02	0.92	0.02	0.86	0.02
41	0.89	0.03	0.88	0.02	0.92	0.02	0.89	0.02
42	0.89	0.03	0.88	0.02	0.92	0.02	0.88	0.02
43	0.87	0.03	0.90	0.02	0.92	0.02	0.90	0.02
44	0.89	0.03	0.90	0.02	0.92	0.02	0.90	0.02
45	0.89	0.03	0.90	0.02	0.92	0.02	0.90	0.02
46	0.89	0.03	0.90	0.02	0.92	0.02	0.91	0.02
47	0.89	0.03	0.90	0.02	0.92	0.02	0.90	0.02

Day	C	SE	G	SE	H1	SE	H2	SE
48	0.99	0.03	1.00	0.02	1.02	0.02	1.01	0.02
49	0.99	0.03	1.00	0.02	1.02	0.02	1.00	0.02
50	0.99	0.03	0.99	0.02	1.02	0.02	1.01	0.02
51	0.99	0.03	0.99	0.02	1.02	0.02	1.01	0.02
52	0.99	0.03	1.00	0.02	1.02	0.02	1.01	0.02
53	1.62	0.36	1.29	0.32	1.45	0.34	1.48	0.33
54	0.98	0.36	1.15	0.32	1.24	0.34	1.25	0.33
55	1.33	0.36	1.35	0.32	1.64	0.34	1.51	0.33
56	1.30	0.36	1.37	0.32	1.64	0.34	1.66	0.33
57	1.51	0.36	1.57	0.32	1.81	0.34	1.86	0.33
58	1.65	0.36	1.59	0.32	2.02	0.34	1.96	0.33
59	1.82	0.36	1.77	0.32	2.21	0.34	2.02	0.33
60	2.06	0.36	2.07	0.32	2.09	0.34	2.00	0.33
61	2.03	0.36	2.09	0.32	2.46	0.34	2.15	0.33
62	2.21	0.36	2.13	0.32	2.52	0.34	2.28	0.33
63	2.45	0.36	4.13	0.32	2.65	0.34	2.44	0.33
64	2.21	0.36	1.85	0.32	2.53	0.34	2.32	0.33

P values:

Entire trial

Treatment ($P<0.37$)

C vs G ($P<0.82$)

C vs H1 + H2 ($P<0.13$)

H1 vs H2 ($P<0.37$)

Preweaning (days 2-52)

Treatment ($P<0.58$)

C vs G ($P<0.94$)

C vs H1 + H2 ($P<0.26$)

H1 vs H2 ($P<0.40$)

Postweaning (days 53-64)

Treatment ($P<0.59$)

C vs G ($P<0.81$)

C vs H1 + H2 ($P<0.22$)

H1 vs H2 ($P<0.55$)

Body weight (kg).

Day	C	SE	G	SE	H1	SE	H2	SE
1	42.88	1.64	44.04	1.46	45.25	1.57	44.49	1.51
8	40.74	1.64	43.26	1.46	44.34	1.57	44.28	1.51
15	39.91	1.64	41.64	1.46	45.70	1.57	43.27	1.51
22	42.59	1.64	44.52	1.46	46.99	1.57	46.34	1.51
29	44.57	1.64	46.15	1.46	48.58	1.57	47.91	1.51
36	47.50	1.64	47.90	1.46	50.13	1.57	49.35	1.51
43	49.73	1.64	50.23	1.46	53.00	1.57	51.55	1.51
50	51.05	1.64	52.27	1.46	54.14	1.57	53.54	1.51
57	54.69	1.64	54.77	1.46	60.12	1.57	58.78	1.51
64	63.73	1.64	62.62	1.46	71.16	1.57	67.79	1.51

P values:

Entire trial

Treatment ($P<0.15$)

C vs G ($P<0.37$)

C vs H1 + H2 ($P<0.05$)

H1 vs H2 ($P<0.54$)

Preweaning (days 2-52)

Treatment ($P<0.28$)

C vs G ($P<0.62$)

C vs H1 + H2 ($P<0.07$)

H1 vs H2 ($P<0.63$)

Postweaning (days 53-64)

Treatment ($P<0.01$)

C vs G ($P<0.03$)

C vs H1 + H2 ($P<0.01$)

H1 vs H2 ($P<0.31$)

BHBA (mg/dl).

Day	C	SE	G	SE	H1	SE	H2	SE
1	0.66	0.18	0.92	0.16	0.77	0.17	1.06	0.17
8	0.79	0.18	0.70	0.16	0.74	0.18	0.76	0.17
15	0.63	0.19	0.76	0.18	0.70	0.17	0.73	0.18
22	0.88	0.18	0.96	0.16	0.90	0.18	0.91	0.17
29	1.44	0.18	1.46	0.16	1.38	0.18	1.35	0.17
36	1.54	0.18	1.58	0.16	1.41	0.17	1.31	0.17
43	1.77	0.18	1.69	0.16	1.73	0.17	1.44	0.17
50	1.83	0.18	1.88	0.16	1.64	0.17	1.62	0.17
57	3.34	0.18	3.29	0.16	3.27	0.17	3.16	0.17
64	3.91	0.18	4.40	0.16	3.69	0.17	3.47	0.17

P values:

Entire trial

Treatment ($P<0.46$)

C vs G ($P<0.17$)

C vs H1 + H2 ($P<0.50$)

H1 vs H2 ($P<0.75$)

Preweaning (days 2-52)

Treatment ($P<0.62$)

C vs G ($P<0.23$)

C vs H1 + H2 ($P<0.61$)

H1 vs H2 ($P<0.91$)

Postweaning (days 53-64)

Treatment ($P<0.49$)

C vs G ($P<0.20$)

C vs H1 + H2 ($P<0.49$)

H1 vs H2 ($P<0.65$)

Average daily gain (kg/d).

Day	C	SE	G	SE	H1	SE	H2	SE
8	-0.31	0.11	-0.11	0.10	-0.13	0.11	-0.03	0.10
15	-0.12	0.11	-0.23	0.10	0.19	0.11	-0.14	0.10
22	0.38	0.11	0.41	0.10	0.18	0.11	0.44	0.10
29	0.28	0.11	0.23	0.10	0.23	0.11	0.22	0.10
36	0.42	0.11	0.25	0.10	0.22	0.11	0.20	0.10
43	0.32	0.11	0.33	0.10	0.41	0.11	0.31	0.10
50	0.19	0.11	0.29	0.10	0.16	0.11	0.28	0.10
57	0.52	0.11	0.36	0.10	0.85	0.11	0.75	0.10
64	1.29	0.11	1.12	0.10	1.58	0.11	1.29	0.10

P values:

Entire trial

Treatment ($P<0.02$)

C vs G ($P<0.01$)

C vs H1 + H2 ($P<0.09$)

H1 vs H2 ($P<0.28$)

Preweaning (days 2-52)

Treatment ($P<0.96$)

C vs G ($P<0.78$)

C vs H1 + H2 ($P<0.68$)

H1 vs H2 ($P<0.94$)

Postweaning (days 53-64)

Treatment ($P<0.01$)

C vs G ($P<0.01$)

C vs H1 + H2 ($P<0.04$)

H1 vs H2 ($P<0.07$)

Feed efficiency (kg gain/kg feed).

Day	C	SE	G	SE	H1	SE	H2	SE
8	-0.56	0.15	-0.20	0.13	-0.23	0.14	-0.06	0.14
15	-0.19	0.15	-0.40	0.13	0.24	0.14	-0.20	0.14
22	0.55	0.15	0.57	0.13	0.25	0.14	0.55	0.14
29	0.35	0.15	0.29	0.13	0.27	0.14	0.25	0.14
36	0.47	0.15	0.29	0.13	0.25	0.14	0.23	0.14
43	0.36	0.15	0.38	0.13	0.45	0.14	0.34	0.14
50	0.21	0.15	0.32	0.13	0.17	0.14	0.31	0.14
57	0.43	0.15	0.30	0.13	0.65	0.14	0.58	0.14
64	0.62	0.15	0.53	0.13	0.67	0.14	0.59	0.14

P values:

Entire trial

Treatment ($P<0.41$)

C vs G ($P<0.20$)

C vs H1 + H2 ($P<0.30$)

H1 vs H2 ($P<0.74$)

Preweaning (days 2-52)

Treatment ($P<0.95$)

C vs G ($P<0.79$)

C vs H1 + H2 ($P<0.59$)

H1 vs H2 ($P<0.97$)

Postweaning (days 53-64)

Treatment ($P<0.01$)

C vs G ($P<0.01$)

C vs H1 + H2 ($P<0.03$)

H1 vs H2 ($P<0.12$)

Acetic acid concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	49.95	6.83	62.64	6.23	44.64	5.78	47.76	6.23
29	40.13	5.77	49.61	4.61	44.12	6.81	31.38	5.76
36	40.21	4.62	43.62	4.25	48.56	5.40	36.57	4.83
43	42.28	4.84	44.48	4.61	53.14	4.84	42.82	4.83
50	54.13	5.10	64.58	4.25	52.04	4.61	57.72	4.61
57	55.29	5.10	74.16	4.25	53.30	4.83	60.56	4.42
64	31.36	4.84	46.58	4.83	35.85	4.84	29.58	4.42

P values:

Treatment ($P<0.01$)

C vs G ($P<0.01$)

C vs H1 + H2 ($P<0.77$)

H1 vs H2 ($P<0.26$)

Propionic acid concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	35.16	5.71	40.66	5.20	29.09	4.84	26.74	5.21
29	32.79	4.83	44.36	3.86	31.14	5.68	21.01	4.82
36	27.07	3.87	31.48	3.56	36.94	4.52	22.69	4.04
43	25.12	4.06	28.27	3.85	34.83	4.05	26.29	4.04
50	26.34	4.27	39.14	3.56	33.99	3.86	28.79	3.86
57	44.54	4.27	59.97	3.56	46.29	4.04	49.67	3.70
64	24.61	4.06	37.36	4.05	29.57	4.05	23.15	3.70

P values:

Treatment ($P<0.01$)
C vs G ($P<0.01$)
C vs H1 + H2 ($P<0.79$)
H1 vs H2 ($P<0.03$)

Isobutyric acid concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	0.23	0.14	0.54	0.13	0.31	0.12	0.56	0.13
29	0.35	0.12	0.43	0.09	0.30	0.14	0.34	0.12
36	0.54	0.10	0.49	0.09	0.52	0.11	0.51	0.10
43	0.52	0.10	0.71	0.09	0.81	0.10	0.57	0.10
50	0.77	0.10	0.92	0.09	0.65	0.10	0.80	0.09
57	0.49	0.10	0.78	0.09	0.51	0.10	0.56	0.09
64	0.13	0.10	0.52	0.10	0.25	0.10	0.20	0.10

P values:

Treatment ($P<0.09$)
C vs G ($P<0.02$)
C vs H1 + H2 ($P<0.42$)
H1 vs H2 ($P<0.72$)

Butyric acid concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	10.03	2.44	11.82	2.22	6.55	2.07	8.39	2.23
29	12.96	2.06	11.57	1.65	11.68	2.43	6.87	2.06
36	9.29	1.65	10.17	1.52	9.00	1.93	7.10	1.73
43	8.44	1.73	9.79	1.64	11.11	1.73	9.93	1.72
50	13.03	1.82	16.20	1.52	11.79	1.65	14.00	1.65
57	17.81	1.82	15.22	1.52	15.96	1.72	13.94	1.58
64	6.77	1.73	13.38	1.73	6.11	1.73	5.09	1.58

P values:

Treatment ($P<0.02$)
C vs G ($P<0.01$)
C vs H1 + H2 ($P<0.17$)
H1 vs H2 ($P<0.38$)

Isovalerate concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	0.27	0.38	0.57	0.34	0.29	0.32	0.81	0.34
29	0.46	0.32	0.55	0.25	0.37	0.37	0.45	0.31
36	0.59	0.26	0.51	0.24	0.67	0.30	0.69	0.27
43	0.79	0.27	0.95	0.25	1.22	0.27	1.25	0.27
50	2.81	0.28	1.82	0.24	1.31	0.26	2.27	0.26
57	0.89	0.28	1.35	0.24	0.62	0.27	0.94	0.25
64	0.14	0.27	0.53	0.27	0.20	0.27	0.34	0.25

P values:

Treatment ($P<0.50$)
C vs G ($P<0.65$)
C vs H1 + H2 ($P<0.85$)
H1 vs H2 ($P<0.14$)

Valerate concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	4.42	1.60	5.24	1.46	2.31	1.36	2.97	1.46
29	3.82	1.35	5.09	1.08	4.03	1.59	2.23	1.35
36	3.16	1.09	3.28	1.00	3.49	1.27	2.36	1.13
43	2.90	1.14	3.65	1.08	4.20	1.14	3.49	1.13
50	3.77	1.20	5.46	1.00	3.98	1.08	3.94	1.08
57	4.20	1.20	5.40	1.00	4.57	1.13	4.40	1.04
64	1.48	1.14	8.20	1.14	1.97	1.14	1.64	1.04

P values:

Treatment ($P<0.01$)
C vs G ($P<0.01$)
C vs H1 + H2 ($P<0.83$)
H1 vs H2 ($P<0.49$)

Total VFA concentrations (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	99.72	19.64	120.63	17.90	83.90	16.63	86.81	17.93
29	138.72	16.60	165.56	13.26	136.22	19.57	92.21	16.57
36	118.14	13.29	131.29	12.22	146.98	15.54	103.54	13.90
43	118.41	13.92	130.14	13.24	155.83	13.91	124.61	13.89
50	149.36	14.67	189.47	12.21	153.65	13.27	159.21	13.26
57	182.58	14.66	238.57	12.22	179.36	13.90	197.75	12.71
64	64.19	13.92	106.03	13.90	73.96	13.91	59.76	12.71

P values:

Treatment ($P<0.01$)
C vs G ($P<0.01$)
C vs H1 + H2 ($P<0.92$)
H1 vs H2 ($P<0.10$)

Acetate to propionate ratio (mM).

Day	C	SE	G	SE	H1	SE	H2	SE
22	1.43	0.23	1.58	0.21	1.56	0.20	1.80	0.21
29	1.23	0.20	1.16	0.16	1.51	0.23	1.56	0.19
36	1.58	0.16	1.48	0.15	1.36	0.18	2.01	0.16
43	1.77	0.17	1.83	0.16	1.64	0.17	1.74	0.16
50	2.31	0.17	1.93	0.15	1.64	0.16	2.27	0.16
57	1.28	0.17	1.26	0.15	1.15	0.16	1.24	0.15
64	1.27	0.17	1.16	0.17	1.18	0.17	1.31	0.15

P values:

Treatment ($P<0.12$)
C vs G ($P<0.41$)
C vs H1 + H2 ($P<0.87$)
H1 vs H2 ($P<0.03$)

Acetic acid (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	49.91	2.12	52.11	1.92	54.18	1.81	54.62	1.93
29	31.99	1.78	34.03	1.44	36.94	2.07	38.33	1.77
36	38.52	1.45	37.76	1.34	37.53	1.68	40.91	1.50
43	40.48	1.52	39.23	1.43	38.34	1.51	38.50	1.50
50	40.92	1.60	38.45	1.33	38.30	1.44	40.81	1.44
57	34.68	1.59	34.67	1.34	33.22	1.50	34.16	1.39
64	48.25	1.52	41.56	1.50	47.40	1.51	49.49	1.39

P values:

Treatment ($P<0.09$)
C vs G ($P<0.06$)
C vs H1 + H2 ($P<0.34$)
H1 vs H2 ($P<0.16$)

Propionic acid (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	35.43	1.89	33.24	1.72	34.99	1.61	31.32	1.73
29	26.48	1.59	29.86	1.28	25.07	1.87	25.59	1.58
36	25.54	1.29	26.78	1.18	28.14	1.50	22.88	1.34
43	23.41	1.35	23.53	1.27	24.80	1.34	23.21	1.34
50	19.63	1.42	22.58	1.18	24.65	1.28	20.09	1.28
57	27.15	1.41	28.09	1.18	28.77	1.34	28.07	1.23
64	38.65	1.35	36.19	1.34	40.28	1.34	38.27	1.23

P values:

Treatment ($P<0.07$)
C vs G ($P<0.57$)
C vs H1 + H2 ($P<0.76$)
H1 vs H2 ($P<0.01$)

Isobutyrate (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	0.24	0.15	0.50	0.14	0.41	0.13	0.56	0.14
29	0.29	0.13	0.39	0.10	0.25	0.15	0.45	0.13
36	0.52	0.11	0.52	0.10	0.41	0.12	0.62	0.11
43	0.49	0.11	0.66	0.10	0.60	0.11	0.54	0.11
50	0.57	0.12	0.57	0.10	0.49	0.11	0.56	0.10
57	0.31	0.12	0.39	0.10	0.30	0.11	0.33	0.10
64	0.17	0.11	0.78	0.11	0.50	0.11	0.23	0.10

P values:

Treatment ($P<0.27$)
C vs G ($P<0.09$)
C vs H1 + H2 ($P<0.35$)
H1 vs H2 ($P<0.62$)

Butyrate (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	9.87	1.47	9.61	1.34	7.60	1.24	9.38	1.34
29	9.56	1.24	7.80	0.99	9.91	1.47	8.03	1.24
36	8.64	0.99	8.45	0.91	6.57	1.16	7.53	1.04
43	7.95	1.04	8.28	0.99	8.05	1.04	8.94	1.04
50	9.66	1.09	9.78	0.91	8.42	0.99	9.80	0.99
57	10.49	1.09	7.65	0.91	9.87	1.04	7.75	0.95
64	9.94	1.04	14.93	1.04	8.83	1.04	8.62	0.95

P values:

Treatment ($P<0.19$)
C vs G ($P<0.16$)
C vs H1 + H2 ($P<0.10$)
H1 vs H2 ($P<0.85$)

Isovalerate (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	0.29	0.31	0.71	0.28	0.36	0.27	0.80	0.28
29	0.37	0.26	0.61	0.22	0.33	0.30	0.58	0.26
36	0.59	0.22	0.66	0.20	0.55	0.25	0.82	0.22
43	0.77	0.23	0.86	0.21	0.93	0.23	1.22	0.22
50	2.03	0.24	1.10	0.20	0.95	0.22	1.55	0.22
57	0.55	0.24	0.66	0.20	0.37	0.22	0.51	0.21
64	0.45	0.23	0.60	0.23	0.34	0.23	1.01	0.21

P values:

Treatment ($P<0.30$)
C vs G ($P<0.94$)
C vs H1 + H2 ($P<0.93$)
H1 vs H2 ($P<0.06$)

Valerate (%).

Day	C	SE	G	SE	H1	SE	H2	SE
22	4.42	0.80	4.22	0.73	2.62	0.67	3.35	0.73
29	3.07	0.67	3.43	0.54	3.30	0.79	2.62	0.67
36	2.94	0.54	2.74	0.50	2.43	0.63	2.39	0.56
43	2.73	0.56	3.12	0.54	3.02	0.56	3.11	0.56
50	2.87	0.60	3.13	0.50	2.83	0.54	2.74	0.54
57	2.50	0.59	2.49	0.50	2.83	0.56	2.49	0.52
64	2.63	0.56	6.18	0.56	2.68	0.56	2.43	0.52

P values:

Treatment ($P<0.06$)

C vs G ($P<0.01$)

C vs H1 + H2 ($P<0.45$)

H1 vs H2 ($P<0.82$)

APPENDIX E

VALUES AND STANDARD ERRORS FOR PARAMETERS MEASURED IN CHAPTER THREE

Milk replacer intake (kg DM).

Age	C	SE	G	SE	H1	SE	H2	SE
1	0.39	0.03	0.39	0.03	0.40	0.03	0.40	0.03
2	0.39	0.03	0.39	0.03	0.40	0.03	0.40	0.03
3	0.40	0.03	0.40	0.03	0.41	0.03	0.41	0.03
4	0.40	0.03	0.40	0.03	0.41	0.03	0.41	0.03
5	0.40	0.03	0.40	0.03	0.41	0.03	0.41	0.03
6	0.40	0.03	0.40	0.03	0.41	0.03	0.41	0.03
7	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
8	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
9	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
10	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
11	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
12	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
13	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
14	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
15	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
16	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
17	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
18	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
19	0.40	0.03	0.41	0.03	0.41	0.03	0.41	0.03
20	0.37	0.03	0.41	0.03	0.41	0.03	0.41	0.03
21	0.37	0.03	0.41	0.03	0.41	0.03	0.39	0.03
22	0.37	0.03	0.38	0.03	0.38	0.03	0.39	0.03
23	0.37	0.03	0.38	0.03	0.38	0.03	0.39	0.03
24	0.36	0.03	0.38	0.03	0.38	0.03	0.39	0.03
25	0.33	0.03	0.38	0.03	0.35	0.03	0.33	0.03
26	0.30	0.03	0.36	0.03	0.35	0.03	0.29	0.03
27	0.30	0.03	0.31	0.03	0.35	0.03	0.26	0.03
28	0.27	0.03	0.28	0.03	0.35	0.03	0.26	0.03
29	0.27	0.03	0.26	0.03	0.26	0.03	0.17	0.03
30	0.27	0.03	0.26	0.03	0.22	0.03	0.17	0.03

P values:

Treatment ($P<0.96$)

C vs G ($P<0.75$)

C vs H1 + H2 ($P<0.80$)

H1 vs H2 ($P<0.75$)

Starter intake (DM kg).

Age	C	SE	G	SE	H1	SE	H2	SE
3	0.04	0.09	0.05	0.09	0.00	0.09	0.01	0.09
4	0.05	0.09	0.04	0.09	0.00	0.09	0.01	0.09
5	0.02	0.09	0.01	0.09	0.00	0.09	0.01	0.09
6	0.01	0.09	0.01	0.09	0.00	0.09	0.01	0.09
7	0.03	0.09	0.03	0.09	0.00	0.09	0.03	0.09
8	0.05	0.09	0.04	0.09	0.02	0.09	0.02	0.09
9	0.04	0.09	0.03	0.09	0.03	0.09	0.02	0.09
10	0.04	0.09	0.04	0.09	0.04	0.09	0.03	0.09
11	0.06	0.09	0.05	0.09	0.04	0.09	0.03	0.09
12	0.07	0.09	0.08	0.09	0.10	0.09	0.05	0.09
13	0.09	0.09	0.09	0.09	0.08	0.09	0.08	0.09
14	0.10	0.09	0.11	0.09	0.14	0.09	0.10	0.09
15	0.11	0.09	0.16	0.09	0.16	0.09	0.13	0.09
16	0.14	0.09	0.18	0.09	0.20	0.09	0.15	0.09
17	0.16	0.09	0.22	0.09	0.23	0.09	0.20	0.09
18	0.24	0.09	0.28	0.09	0.26	0.09	0.24	0.09
19	0.26	0.09	0.29	0.09	0.29	0.09	0.30	0.09
20	0.32	0.09	0.36	0.09	0.33	0.09	0.31	0.09
21	0.32	0.09	0.40	0.09	0.37	0.09	0.33	0.09
22	0.37	0.09	0.42	0.09	0.41	0.09	0.42	0.09
23	0.41	0.09	0.44	0.09	0.47	0.09	0.48	0.09
24	0.44	0.09	0.48	0.09	0.48	0.09	0.52	0.09
25	0.49	0.09	0.52	0.09	0.54	0.09	0.57	0.09
26	0.56	0.09	0.56	0.09	0.65	0.09	0.66	0.09
27	0.62	0.09	0.64	0.09	0.69	0.09	0.74	0.09
28	0.68	0.09	0.67	0.09	0.79	0.09	0.88	0.09
29	0.75	0.09	0.79	0.09	0.79	0.09	0.92	0.09
30	0.87	0.09	0.82	0.09	0.93	0.09	0.95	0.09
31	0.85	0.09	0.87	0.09	0.96	0.09	1.05	0.09
32	0.91	0.09	1.03	0.09	0.99	0.09	1.13	0.09
33	1.05	0.09	1.15	0.09	1.03	0.09	1.18	0.09
34	1.20	0.09	1.15	0.09	1.27	0.09	1.34	0.09
35	1.24	0.09	1.20	0.09	1.41	0.09	1.34	0.09
36	1.36	0.09	1.31	0.09	1.54	0.09	1.51	0.09
37	1.42	0.09	1.28	0.09	1.68	0.09	1.60	0.09
38	1.41	0.09	1.39	0.09	1.75	0.09	1.62	0.09
39	1.45	0.09	1.48	0.09	1.90	0.09	1.61	0.09
40	1.56	0.09	1.51	0.09	2.03	0.09	1.73	0.09
41	1.64	0.09	1.61	0.09	2.18	0.09	1.83	0.10
42	1.65	0.09	1.66	0.09	2.20	0.09	1.85	0.10
43	1.86	0.09	1.68	0.09	2.19	0.10	2.00	0.10
44	1.87	0.10	1.74	0.10	2.10	0.10	1.91	0.11
45	1.83	0.10	1.82	0.10	2.22	0.10	1.97	0.12
46	1.96	0.10	1.75	0.10	2.23	0.11	2.02	0.12
47	1.82	0.10	1.91	0.11	2.30	0.11	1.91	0.13
48	1.97	0.10	1.96	0.11	2.27	0.11	2.05	0.14
49	1.99	0.11	2.11	0.12	2.52	0.11	2.05	0.15

Age	C	SE	G	SE	H1	SE	H2	SE
50	2.33	0.12	2.00	0.13	2.40	0.12	2.10	0.16

P values:

Entire trial

Treatment (P<0.31)

C vs G (P<0.29)

C vs H1 + H2 (P<0.17)

H1 vs H2 (P<0.44)

Preweaning (age 3-30)

Treatment (P<0.97)

C vs G (P<0.90)

C vs H1 + H2 (P<0.65)

H1 vs H2 (P<0.87)

Postweaning (age 30-50)

Treatment (P<0.19)

C vs G (P<0.19)

C vs H1 + H2 (P<0.11)

H1 vs H2 (P<0.47)

Total dry matter intake (kg).

Age	C	SE	G	SE	H1	SE	H2	SE
1	0.39	0.07	0.39	0.07	0.39	0.07	0.41	0.07
2	0.39	0.07	0.39	0.07	0.40	0.07	0.40	0.07
3	0.44	0.07	0.45	0.07	0.42	0.07	0.42	0.07
4	0.45	0.07	0.44	0.07	0.41	0.07	0.41	0.07
5	0.42	0.07	0.41	0.07	0.41	0.07	0.42	0.07
6	0.41	0.07	0.41	0.07	0.41	0.07	0.42	0.07
7	0.42	0.07	0.43	0.07	0.41	0.07	0.43	0.07
8	0.45	0.07	0.44	0.07	0.42	0.07	0.42	0.07
9	0.45	0.07	0.44	0.07	0.44	0.07	0.43	0.07
10	0.44	0.07	0.45	0.07	0.46	0.07	0.44	0.07
11	0.47	0.07	0.46	0.07	0.45	0.07	0.44	0.07
12	0.47	0.07	0.49	0.07	0.51	0.07	0.46	0.07
13	0.49	0.07	0.50	0.07	0.49	0.07	0.49	0.07
14	0.50	0.07	0.51	0.07	0.55	0.07	0.51	0.07
15	0.52	0.07	0.57	0.07	0.57	0.07	0.54	0.07
16	0.55	0.07	0.58	0.07	0.62	0.07	0.56	0.07
17	0.56	0.07	0.62	0.07	0.64	0.07	0.61	0.07
18	0.65	0.07	0.68	0.07	0.66	0.07	0.65	0.07
19	0.67	0.07	0.70	0.07	0.70	0.07	0.70	0.07
20	0.69	0.07	0.77	0.07	0.75	0.07	0.71	0.07
21	0.70	0.07	0.80	0.07	0.78	0.07	0.72	0.07
22	0.74	0.07	0.80	0.07	0.79	0.07	0.80	0.07
23	0.79	0.07	0.82	0.07	0.85	0.07	0.86	0.07
24	0.79	0.07	0.86	0.07	0.86	0.07	0.90	0.07
25	0.82	0.07	0.90	0.07	0.89	0.07	0.90	0.07
26	0.86	0.07	0.92	0.07	1.00	0.07	0.95	0.07
27	0.92	0.07	0.95	0.07	1.04	0.07	1.00	0.07
28	0.96	0.07	0.95	0.07	1.14	0.07	1.13	0.07
29	1.03	0.07	1.04	0.07	1.05	0.07	1.09	0.07
30	1.14	0.07	1.07	0.07	1.15	0.07	1.12	0.07
31	1.13	0.07	1.07	0.07	1.16	0.07	1.22	0.07
32	1.13	0.07	1.19	0.07	1.14	0.07	1.29	0.07
33	1.18	0.07	1.32	0.07	1.16	0.07	1.32	0.07
34	1.30	0.07	1.29	0.07	1.38	0.07	1.46	0.07
35	1.34	0.07	1.35	0.07	1.51	0.07	1.45	0.07
36	1.43	0.07	1.45	0.07	1.58	0.07	1.59	0.07
37	1.49	0.07	1.35	0.07	1.72	0.07	1.64	0.08
38	1.48	0.07	1.47	0.08	1.73	0.07	1.67	0.08
39	1.49	0.07	1.52	0.08	1.88	0.07	1.66	0.08
40	1.61	0.07	1.55	0.08	2.00	0.07	1.77	0.08
41	1.68	0.08	1.65	0.08	2.14	0.08	1.86	0.08
42	1.69	0.08	1.69	0.08	2.16	0.08	1.88	0.08
43	1.89	0.08	1.72	0.08	2.15	0.08	2.02	0.09
44	1.90	0.08	1.78	0.08	2.05	0.08	1.93	0.09
45	1.86	0.08	1.86	0.08	2.18	0.09	1.98	0.10
46	1.99	0.08	1.75	0.08	2.18	0.09	1.94	0.11

Age	C	SE	G	SE	H1	SE	H2	SE
47	1.86	0.08	1.90	0.09	2.24	0.09	1.84	0.11
48	2.01	0.08	1.94	0.09	2.22	0.09	1.98	0.12
49	1.96	0.09	2.07	0.10	2.47	0.09	1.98	0.13
50	2.28	0.11	1.95	0.11	2.34	0.10	2.03	0.14

P values:

Entire trial

Treatment (P<0.25)

C vs G (P<0.28)

C vs H1 + H2 (P<0.15)

H1 vs H2 (P<0.34)

Preweaning (age 3-30)

Treatment (P<0.84)

C vs G (P<0.99)

C vs H1 + H2 (P<0.40)

H1 vs H2 (P<0.72)

Postweaning (age 30-50)

Treatment (P<0.15)

C vs G (P<0.15)

C vs H1 + H2 (P<0.11)

H1 vs H2 (P<0.41)

Body weight (kg).

Week	C	SE	G	SE	H1	SE	H2	SE
1	41.22	1.27	41.94	1.27	40.80	1.30	41.04	1.27
2	41.38	1.27	42.10	1.27	42.43	1.28	42.70	1.27
3	43.94	1.27	45.02	1.27	44.57	1.28	46.04	1.27
4	47.90	1.27	49.79	1.27	49.47	1.28	48.92	1.27
5	51.46	1.27	52.77	1.27	53.59	1.28	54.18	1.27
6	56.94	1.27	57.40	1.29	59.10	1.28	58.40	1.29
7	64.09	1.34	64.49	1.35	67.33	1.42	65.36	1.59

P values:

Entire trial

Treatment ($P<0.72$)

C vs G ($P<0.99$)

C vs H1 + H2 ($P<0.25$)

H1 vs H2 ($P<0.95$)

Preweaning (weeks 1-5)

Treatment ($P<0.75$)

C vs G ($P<0.68$)

C vs H1 + H2 ($P<0.31$)

H1 vs H2 ($P<0.93$)

Postweaning (weeks 6-7)

Treatment ($P<0.68$)

C vs G ($P<0.72$)

C vs H1 + H2 ($P<0.25$)

H1 vs H2 ($P<0.92$)

Average daily gain (kg/d).

Week	C	SE	G	SE	H1	SE	H2	SE
1	0.31	0.13	0.40	0.13	0.19	0.13	0.25	0.13
2	0.02	0.13	0.03	0.13	0.20	0.13	0.25	0.13
3	0.37	0.13	0.42	0.13	0.29	0.13	0.49	0.13
4	0.57	0.13	0.69	0.13	0.68	0.13	0.42	0.13
5	0.51	0.13	0.43	0.13	0.57	0.13	0.76	0.13
6	0.78	0.13	0.67	0.13	0.77	0.13	0.63	0.13
7	1.05	0.14	1.03	0.14	1.25	0.15	1.09	0.18

P values:

Entire trial

Treatment (P<0.76)

C vs G (P<0.64)

C vs H1 + H2 (P<0.34)

H1 vs H2 (P<0.87)

Preweaning (weeks 1-5)

Treatment (P<0.73)

C vs G (P<0.36)

C vs H1 + H2 (P<0.51)

H1 vs H2 (P<0.90)

Postweaning (weeks 6-7)

Treatment (P<0.53)

C vs G (P<0.20)

C vs H1 + H2 (P<0.47)

H1 vs H2 (P<0.81)

Feed efficiency (kg gain/kg feed).

Week	C	SE	G	SE	H1	SE	H2	SE
1	0.90	0.24	1.16	0.24	0.69	0.24	0.84	0.24
2	0.17	0.24	0.14	0.24	0.47	0.24	0.58	0.24
3	0.57	0.24	0.60	0.24	0.40	0.24	0.79	0.24
4	0.71	0.24	0.84	0.24	0.80	0.24	0.43	0.24
5	0.43	0.24	0.42	0.24	0.50	0.24	0.66	0.24
6	0.50	0.24	0.45	0.24	0.45	0.24	0.37	0.24
7	0.60	0.26	0.59	0.26	0.64	0.28	0.67	0.33

P values:

Entire trial

Treatment ($P<0.85$)

C vs G ($P<0.72$)

C vs H1 + H2 ($P<0.59$)

H1 vs H2 ($P<0.54$)

Preweaning (weeks 1-5)

Treatment ($P<0.91$)

C vs G ($P<0.57$)

C vs H1 + H2 ($P<0.69$)

H1 vs H2 ($P<0.83$)

Postweaning (weeks 6-7)

Treatment ($P<0.69$)

C vs G ($P<0.60$)

C vs H1 + H2 ($P<0.50$)

H1 vs H2 ($P<0.34$)

BHBA (mg/dl).

Week	C	SE	G	SE	H1	SE	H2	SE
1	0.5017	0.2088	0.3646	0.2089	0.5168	0.2165	0.3391	0.2031
2	0.5155	0.2025	0.5176	0.2028	0.5709	0.2037	0.4806	0.2031
3	0.7705	0.2025	0.7547	0.2028	0.9345	0.2037	0.7256	0.2031
4	1.0155	0.2025	1.139	0.2028	1.3188	0.2037	1.5306	0.2031
5	2.3252	0.2079	2.1004	0.2028	2.1852	0.2037	2.1413	0.2031
6	2.7662	0.2025	2.7228	0.209	3.2023	0.2037	2.8702	0.2091
7	3.1968	0.2241	3.1362	0.2251	2.8306	0.245	3.2053	0.2936

P values:

Entire trial

Treatment (P<0.91)

C vs G (P<0.53)

C vs H1 + H2 (P<0.73)

H1 vs H2 (P<0.82)

Preweaning (weeks 1-5)

Treatment (P<0.83)

C vs G (P<0.61)

C vs H1 + H2 (P<0.45)

H1 vs H2 (P<0.92)

Postweaning (weeks 6-7)

Treatment (P<0.97)

C vs G (P<0.67)

C vs H1 + H2 (P<0.96)

H1 vs H2 (P<0.87)