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EFFECTS OF LASALOCID ON RUMINAL AND BLOOD METABOLITES IN YOUNG DAIRY CALVES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

SUZETTE INLOW BOEHMS

December 1991

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DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. John Benton Inlow who have given me the opportunity to continue my education with love and support

and

to my husband John Philip Boehms to whom I will always be grateful for his love, support and tolerance.

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ABSTRACT

Effects of lasalocid on peripheral concentration of blood ketones, plasma nonesterified fatty acids, glucose, insulin, urea-nitrogen, and volatile fatty acids and ruminal pH, ammonia-nitrogen and volatile fatty acids were examined using 16 Holstein bull calves. Ruminal cannulas were installed surgically at 5 ± 2 d of age. Calves were fed 1.8 kg milk replacer twice daily to weaning at 8 wk and a commercial pelleted calf starter once daily for ad libitum consumption to a maximum of 4.5 kg. Blood was collected once weekly for 12 wk and analyzed for ß-hydroxybutyrate and acetoacetate. Blood ß-hydroxybutyrate increased with increasing dry matter intake and was affected significantly by treatment post-weaning. Blood acetoacetate response was similar to ß-hydroxybutyrate but without treatment effect. Plasma nonesterified fatty acids decreased with age suggesting that calves were under little stress. Plasma glucose increased to wk 5 then decreased to concentrations expected for mature ruminants. Plasma insulin responded to plasma glucose by increasing to weaning then decreased across all treatments. Plasma insulin correlated .41 to plasma glucose suggesting that insulin responds to other metabolites in addition to glucose. Plasma urea-nitrogen increased with age due to

an increase in crude protein intake. Plasma total VFA increased with increasing dry matter intake and correlated .62. Plasma acetate, which composed 93 percent of plasma total VFA, correlated to dry matter intake (r = .62). Plasma propionate (r = .53) and butyrate (r = .02) were less highly correlated with DMI. Ruminal pH decreased with increased dry matter intake (r = .68). Calves fed lasalocid tended to maintain a higher ruminal pH than control calves. Ruminal ammonia-nitrogen increased across all treatments throughout the study. Ruminal VFA increased as a function of dry matter intake (r = .74). Molar percent ruminal acetate decreased and percent propionate increased throughout the study. Percent butyrate tended to be lower for calves that received lasalocid ruminally. Data indicate that ketone body concentration resulted from alimentary ketogenesis and lasalocid decreased blood ketone levels. No protein-sparing effect was observed in this study. Both ruminal and plasma volatile fatty acids responded to dry matter intake. Lasalocid shifted molar percentages of ruminal VFA away from acetate and butyrate in favor of propionate.

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CHAPTER I

INTRODUCTION

During the periweaning period, digestive and metabolic processes undergo a transition from those typical of pre-ruminants to those of ruminants. The transitional phase is characterized by anatomical and metabolic changes that occur. Development of mature ruminal function has been shown repeatedly to be dependent upon dry feed intake (Roy, 1980; Anderson et al., 1987a; Godfrey, 1961). Volatile fatty acid production as a result of carbohydrate fermentation by microbes initiates ruminal mucosal development (Roy, 1980; Pounden and Hibbs, 1948; Leibholtz, 1975; Dziuk, 1984; Sakata and Tamate, 1978; 1979).

Increased in butyrate influenced metabolic activity of ruminal epithelium (Manns and Boda, 1966). Feeding rations to promote elevated concentrations of ruminal butyrate as suggested by Murdock and Wallenius (1980) would hypothetically increase rate of ruminal maturation. Butyrate is metabolized primarily by ruminal epithelium to ß-hydroxybutyrate in ruminants reaching a maximum rate by 8 wk (Bergman, 1971). ß-hydroxybutyrate is released into bloodstream and used for energy by peripheral tissues.

Quigley et al. (1991a) reported that at weaning there was a dramatic increase in ketones to concentrations exceeding 2000 μM while plasma non-esterified fatty acids and glucose concentrations remaining within normal ranges. The authors proposed that hyperketonemia was alimentary and not hepatic. It has not been established whether elevated blood ketones are characterized by over-production of ketones by splanchnic tissues or under-utilization of ketones by peripheral tissues. Regardless, excess ketone bodies are excreted via urine which potentially represent a loss of energy to the calf.

Increasing maturation rate of rumen epithelium may be an ideal method for increasing calf productivity. In addition, decreasing excess ketone production might increase energetic efficiency of the calf. Orally administered lasalocid, a carboxylic polyether ionophore antibiotic, alters ruminal fermentation (Anderson et al., 1988) and affects metabolism independent of the rumen (Bergen and Bates, 1984). Reported post-ruminal responses of lasalocid may influence animal growth rates (Anderson et al., 1988). Feeding lasalocid may alter rumen microbes by selective inhibition to produce a higher concentration of propionate, decrease acetate:propionate ratio and decrease concentration of butyrate (Schelling, 1984;

Bergen and Bates, 1984; Anderson et al., 1988). With decreased ruminal butyrate, blood ß-hydroxybutyrate may be decreased, thereby reducing urinary ketone excretion.

The objective of this study was to evaluate effects of lasalocid on blood and ruminal metabolites and routes of administration in young calves during the periweaning period.

CHAPTER II

REVIEW OF LITERATURE

Ruminal Development

Introduction

During the early post-natal period, digestive and metabolic processes undergo a transition from those typical of pre-ruminants to those of mature ruminants. The transitional phase is characterized by anatomical and metabolic changes, including a rapid increase in size and capacity of the forestomach (rumen, reticulum and omasum) in relation to other organs of the digestive tract (Roy, 1980). Physiologically, concentrations of blood metabolites such as glucose, non-esterified fatty acids, ketone bodies [β-hydroxybutyrate (βHOB) and acetoacetate (ACAC)], VFA and urea-nitrogen (PUN), change in response to ruminal development. Concentrations of ruminal VFA and ammonia N (NH_3-N) change in response to the introduction of feed into the rumen and subsequent fermentation and pH. Microbial populations are established in the rumen at an early age. Certain dietary factors have been associated with controlling digestive capacity and function and enzymatic and metabolic activities. Prior to ruminal development, milk, a primary source of nutrients, flows directly to abomasum via esophageal groove closure. This

closure is stimulated by suckling stimulus as well as certain compounds (Roy, 1980).

Establishment of Rumen Microbial Population

To understand metabolic changes taking place during transition to the ruminant state, a knowledge of ruminal microbial population establishment is imperative. At birth, the neonate's rumen is without any characteristic bacteria of the mature ruminant (Lengemann and Allen, 1955). Although several theories of rumen inoculation have been proposed, none have been complete. Hungate (1966) suggested that newborn ruminants were microbially inoculated by swallowing saliva and digesta from the dam. Others speculated that sources of microbial inoculation were the dam's birth canal, manure, bedding and environmental flora, animal to animal contact, udder and milk (colostrum), and other food sources (Yokoyama and Johnson, 1988). Milk backflow from abomasum contains various microbes (Lactobacilli, Escherichia coli, and streptococci) that may aid in inoculating the rumen (Quigley, 1983; Roy, 1980; Mann and Oxford, 1954; 1955). All mentioned speculations concur that establishment of bacterial populations begins shortly after birth which was shown by Ziolecki and Briggs, (1961) when they found small

numbers of bacteria from rumen samples of calves only a few hours old.

With inclusion of dry feed into the diet, microbial populations increase and begin to shift from aerobic to facultative and obligate anaerobes (Lengemann and Allen, 1959; Williams and Dinusson, 1972). Along with the increasing microbial population, there is a subsequent increase in ruminal development. When milk is the sole source of nutrition, the rumen develops very slowly and is delayed in acquisition of mature characteristics (Smith, 1959; Lengemann and Allen, 1959). Yokoyama and Johnson (1988) conducted a study with four groups of calves fed either a milk diet for 9 weeks, normally-fed milk plus hay and concentrate, or limit-fed milk plus hay and concentrate, with and without antibiotics. Milk-fed calves maintained high numbers of aerobic bacteria (2.4×10^8/g of rumen contents) until solid food was provided at 9 weeks of age. Cellulolytic activity increased until four weeks. Ruminal activity of calves fed milk plateaued after four weeks. In other groups, cellulolytic activity increased gradually and by 6 weeks of age adult concentrations were reached. Predominant ruminal bacteria varied with age and diet received by the calf. In the study by Yokoyama and Johnson (1988), predominant bacteria

were identified as the rumen population became established. In week 1, Butyrivibrio-like strains were isolated, followed by Ruminococcus (wk 3), and Bacteriodes succinogenes, B. ruminicola, and Selenomonas-like (wk 6). Up to 3 weeks of age, major bacteria present in the rumen were different than those in mature cattle. At 6 weeks, predominant strains normally present in adults were found, in addition to species not found in adults. By 9 to 13 weeks of age in calves normally fed, ruminal bacterial populations were indicative of the adult ruminant. It should be noted that calves fed rations with different grain to roughage ratios will likely develop different microbial population (Pounden and Hibbs, 1948).

Establishment of Ruminal Protozoa

Much research has been done concerning establishment of protozoal populations in the rumen. Bryant et al., (1958) observed that calves require intimate contact with protozoal-infected animals for establishment of their own protozoal populations. The authors also suggested a sequence in establishment of different protozoal groups, i.e., Entodinium, Diplodinium, and holotrichs, respectively. Establishment of ciliate protozoa can occur at an early age (Pounden and Hibbs, 1948) and the rumen can be maintained devoid of ciliated protozoa by isolation

from mature ruminants (Pounden and Hibbs, 1950). A significant factor affecting establishment of ruminal protozoa is ruminal pH and diet. Diets higher in forage resulting in a ruminal pH of approximately six or greater favor establishment of ciliates early in life (Eadie and Hobson, 1962). High concentrate feeding and associated pH below six will slow establishment or cause disappearance of ciliates already present (Bryant et al., 1958; Conrad et al., 1950; Eadie et al., 1959; Eadie and Mann, 1970).

Ruminal flagellate protozoa are generally found in great numbers in very young calves and in much lower numbers in adult ruminants. Species most commonly found are callimastic, chilomastix, monocercomonas, tetratrichomonas and pentatrichomonas (Church, 1969). Eadie (1962) suggested that flagellates are sensitive to pH in a manner similar to ciliates, although flagellates do not appear to require direct contact for transmission.

Anatomical Changes

At birth, the abomasum is the only functional compartment of the stomach of the calf (Roy, 1980). Ingestion of dry feed provides the stimulus needed for the reticulorumen to undergo anatomical changes to more suitably accomodate ruminal fermentation and absorption of fermentative end-products (Leibholtz, 1975). When calves

were fed a combination of milk, grain and hay at 4 weeks of age, the reticulorumen comprised 64 percent of total stomach volume (Huber, 1969). This value increased to approximately 75 percent at 12 weeks of age (Tamate et al., 1962; Warner et al., 1956). This trend continued until the reticulorumen comprised about 87 percent of total stomach volume (Warner and Flatt, 1964). At 12 weeks of age the capacity of the reticulorumen and total stomach was about twice as large for calves fed a combination of milk, grain and hay in comparison to calves fed milk only (Tamate et al., 1962). Others have shown that dry feed stimulated reticuloruminal and omasal growth with abomasal size remaining similar between grain and hay fed calves and milk-fed calves (Warner et al., 1956). Warner and Flatt (1964) concluded that reticuloruminal volume of calves on normal hay-grain rations reached adult proportions of 23 to 36 l/100 kg of ingesta-free body weight by 12 to 16 weeks; however, the omasum continued to grow in proportion to body size until approximately one year of age.

Papillary Development

Growth and elongation of rumen papillae are associated with functional development of the rumen (Huber, 1969). Normal development of rumen papillae by

calves receiving solid feed has been attributed to presence of VFA produced as microbial end-products of fermentation (Brownlee, 1956; Flatt et al., 1958; Sander et al., 1959; Tamate et al., 1962; Sakata and Tamate, 1978). Order of effectiveness in stimulating rumen mucosal growth and development is butyrate, propionate, and acetate (Sander et al., 1959; Sakata and Tamate, 1978; 1979).

Reticulorumen papillae at birth are less than 1 mm in length but grow rapidly with the proper stimuli to reach their maximum length of 5 to 7 mm by 8 weeks of age (Tamate et al., 1962; 1964; Warner et al., 1956). Tamate et al., (1964) showed that calves with high ruminal VFA concentrations and calves fed grain and hay had a much greater degree of papillary development than did only milk-fed calves. Other work has shown much shorter papillary length in calves fed all grain diets as opposed to rations of hay and grain; apparently, some hay is necessary for optimum papillary growth (Tamate et al., 1962).

Physiological Activity of Rumen Mucosa

Ruminal mucosa is the absorptive surface of the rumen. Metabolic activity increases with structural development. Ruminal mucosa takes up VFA (in order of

preference) butyrate, propionate, and acetate, respectively (Sutton et al., 1963a), as well as other ruminal metabolites and fermentation products such as ammonia (Quigley, 1983). Mucosal layer of the rumen is capable of converting ruminal butyrate to ßHOB and ACAC (Annison et al.,1957; Brown et al., 1960; Hodson et al., 1952; Johnston et al., 1961; Joyner et al., 1963; Knox, 1960; McGilliard et al., 1965; Pennington, 1952; Pennington and Pfander, 1957; Sutton et al., 1963b and Huber,1969). It is evident that growth, absorptive capacity, and metabolic activity of forestomach mucosa are related closely and depend upon one another (Huber, 1969).

End-products of Fermentation

As rumen development occurs, there is an increase in production of primary end-products of microbial fermentation, primarily VFA and microbial cells, serving as energy and protein sources to the host, respectively (Roy, 1980). Also produced are minor quantities of methane, carbon dioxide, heat, and NH_3-N which indicates a loss of energy and N from the rumen (Russell and Hespell, 1981; Quigley, 1983). Volatile fatty acids are produced in the rumen by rumen microbes metabolizing carbohydrates and proteins. Carbohydrates are degraded from complex or simple forms which are commonly found in plant material,

either complex or soluble fractions (Russell and Hespell, 1981). Ruminal microbes are responsible for degrading the fractions, releasing soluble mono- and disaccharides via microbial cellulases (Russell and Hespell, 1981). Generally, microbes degrade complex sugars via glycolysis to produce pyruvate which may then follow different pathways depending upon bacterial species and environmental conditions and substrate concentration all producing VFA (Fahey and Berger, 1988).

Ammonia is the chief end-product of ruminal protein hydrolysis (el Shazy, 1958; Abou Akkada and H. el sayed Osman, 1967) and because ammonia is not found in peripheral blood, it has been shown that it is absorbed via the portal system and is converted to urea in the liver (Lewis, 1960; Owens and Zinn, 1988). Urea may be returned to the rumen via salivary passage (Abou Akkada and H el Sayed Osman, 1967) or lost in the urine.

Dietary proteins entering the rumen may be degraded by extracellular microbial proteases forming short-chain peptides as end-products (Herdt, 1991). These peptides are formed and absorbed into microbial cell bodies. Within the microbial cells, peptides are utilized either for formation of microbial protein or further degraded for

production of energy through the formation of VFA and ammonia (Herdt, 1991).

Nitrogen nutrition of the ruminant depends largely on availability of protein and energy to microbes for fermentation (Herdt, 1991), as well as relative proportions of ruminal ammonia present. Ammonia may be produced from the action of bacterial urease, dietary and recycled urea, and/or microbial catabolism of amino acids and peptides (Allison, 1970; Pittman and Bryant, 1964; Pittman et al., 1967; Russell and Hespell, 1981).

Ammonia exists in a dynamic ruminal pool which may be used as the nitrogen source for amino acid biosynthesis by rumen microbes (Russell and Hespell, 1981). That portion not taken up by microbes may diffuse passively across rumen wall. The weak base behavior of ammonia is reflective of the dependency of the rate of ammonia proportions increase at higher pH and ammonia diffuses much more rapidly than do ammonium ions. Therefore, increased rates of absorption that occurred at a higher pH was due to increased concentration of free ammonia (Merchen, 1988). If the ruminant consumes excessive amounts of rapidly hydrolyzed nonprotein nitrogen, there will be an accumulation of ammonia in the peripheral circulation causing ammonia toxicity.

As the rumen develops, ammonia levels decrease with age from approximately 20 to 23 mg/dl to 5 to 8 mg/dl at 12 to 17 weeks of age (Anderson et al., 1987a). Conversely, Godfrey (1961) found extremely low levels of ruminal ammonia at birth which increased and peaked at about 3 weeks of age. Observed decrease in ruminal ammonia concentration is indicative of an increase in microbial ammonia utilization and an increase in the ability of rumen mucosa to absorb free ammonia due to microbial hydrolysis of dietary nitrogen (Anderson et al., 1987a).

Ruminal pH was mentioned briefly in previous sections. According to Anderson and coworkers (1987b), ruminal pH decreases with age from 6.5 to 7.0 to as low as 5.5 with initiation of dry feed intake. All ruminal activity is either directly or indirectly dependent upon ruminal pH levels. Savage et al., (1942) indicated that dry feed intake was a prerequsite for early development of rumen microbial population and the amount of grain be less than that of roughage to insure rumen pH was high enough to allow establishment of cellulolytic bacteria and protozoa. Also, other microbes must first be established in the rumen to provide proper conditions necessary for initial establishment (VFA, redox potential, pH and

ammonia levels). Diets differing in grain to hay ratios resulted in different ruminal pH levels due to the shift of microbial population to accomodate dietary composition (Roy, 1980). Diets of high concentrate and low roughage content show decreased rumen pH due to greater numbers of streptococci (S. bovis and S. faecium) which produce more lactate and less acetate. High grain diets also increase the rate of fermentation as a result of available carbohydrates (Roy, 1980; Ziolecki and Briggs, 1961). Escherichia coli and Aerobacter aerogenes are present and produce acids and gases (Yokoyama and Johnson, 1988). If these microbes are allowed to continue, ruminal pH will drop even further to approximately 5.7 where facultative anaerobes are inhibited. When these conditions prevail, lactate is not utilized, causing pH levels to be depressed further. Lactobacilli will then begin to predominate (Yokoyama and Johnson, 1988).

When diets high in roughage are consumed, microbial populations develop to accomodate diet. Streptococci do not increase in numbers as with the high concentrate diet; therefore, pH levels remain at or near neutrality (7.0) and cellulolytic strains predominate (Eadie and Mann, 1970).

Since rumen flora and fauna are affected by pH, logically, fermentation products are affected. Slyter and colleagues (1966) observed, as pH dropped from 6.0 to 5.5 and 5.0, total fermentation products measured decreased markedly. Most significantly, a decrease in acetate and methane production was related directly to decreased pH and to most celluloytic activity being inhibited at pH < 6.0. Molar percentages of propionate increased with decreasing pH due to a greater decrease in production of other VFA, although propionate also decreased with depressed ruminal pH (Slyter et al., 1966).

Along with ruminal metabolite changes with rumen development, circulating metabolites are altered also. In response to ruminal VFA concentration increasing with age, peripheral blood VFA concentrations also increase with age (Roy, 1980; Quigley et al., 1991b). Roy reported also VFA levels higher in calves consuming a high roughage diet than receiving a high-concentrate or all-concentrate diet.

It has been documented that concentration of blood glucose is higher in calves prior to ruminal development. As the calf ages and rumen development is initiated, there is a decline in glucose concentrations (Conrad et al., 1950; Craine and Hansen, 1952; Hibbs et al., 1952; McCandles and Dye, 1950; Warner et al., 1953; McCarthy and

Kesler, 1956). Roy (1980) reported blood glucose levels declined from 100 mg/dl to about 60 mg/dl at approximately 6 weeks of age.

The observed decline in blood glucose concentrations is due to either a decrease in fetal erythrocytes which are high in glucose and are replaced by erythrocytes that are low in glucose content, or an increase in production of VFA in large intestine (Reid, 1950). McCarthy and Kesler (1956) stated that there is a change in energy metabolism from glucose being the major source of energy to short-chain fatty acids serving to meet a large portion of energetic needs.

Ruminal butyrate and, to some extent acetate, can be converted to ACAC and ßHOB during absorption across ruminal mucosa (Brockman, 1976; Seto et al., 1955; Stevens, 1970; Leighton et al., 1983; Heitmann et al., 1987) to serve as an energy source for ruminants. Since ruminal VFA concentrations increase with age, conversion of VFA to ketones by epithelium and liver increase upon initiation of dry feed consumption. Quigley et al., (1991a) observed a dramatic increase in peripheral concentrations of ketones immediately post-weaning to levels typically seen in cases of subclinical ketosis with no obvious effects associated with ketotic cattle such as

hypoglycemia or elevated NEFA. Hepatic ketogenesis follows concentrations of NEFA which decrease with age in normal calves.

As reported previously, ruminal ammonia-nitrogen levels decrease with age and rumen development (Anderson et al., 1987a). Blood PUN fluctuations are a direct result of ruminal ammonia production from protein catabolism (Lewis, 1957). Considering the positive relationship between ruminal ammonia and blood PUN, it can be deduced that blood PUN levels decrease with age and rumen development of calves (Lewis, 1957).

Plasma non-esterified fatty acid (NEFA) concentrations generally decrease with ruminal development (Quigley et al., 1991a). When calves undergo stressful conditions, an increase in NEFA and a resultant increase in hepatic ketogenesis is observed due to an immediate need for extra energy.

Ionophores and Rumen Development

Introduction

Increasing maturation rate of rumen epithelium may be an ideal method for increasing calf productivity. Earlier ruminal development and DMI allows for early weaning, which has several benefits including reduced expense of

liquid feeding, reduced labor costs, and reduced incidence of disease. One method to achieve early ruminal development is to offer dry feed at a young age and wean calves as soon as they consume .5 to 1 kg/d. Morrill et al. (1984) suggested feeding a prestarter supplement to stimulate ruminal development and compensate for reduced milk intake. Early weaning and early dry feed consumption may be beneficial to the calf (Anderson et al., 1987a). Benefits of early dry feed consumption include acceleration of rumen motility (McGilliard et al., 1965), VFA production (Russell and Hespell, 1981) and absorption (Sutton et al., 1963a). However, there are some disadvantages to early weaning. Lower ruminal pH observed in early weaned calves is unfavorable for establishment of cellulolytic bacteria and ruminal protozoa. Anderson et al. (1987b) observed high numbers of methanogens in early weaned calves, increasing methanogenesis which could contribute to reduced growth efficiency (Thornton and Owens, 1981).

In reviewing effects of ionophores observed in mature ruminants (Bergen and Bates, 1984; Schelling, 1984), it may be beneficial to feed ionophore antibiotics to neonatal calves to promote productive efficiency. According to Anderson et al. (1988), calves fed lasalocid

showed increased productive efficiency after week 5; presumably, ruminal physiological and microbial activities were greater than when calves received no lasalocid. Supplemental feeding of lasalocid after dry feed intake increased - approximately 4 to 5 weeks of age - stimulated an increase in ruminal VFA concentration with a lower acetate to propionate ratio. No significant differences in ruminal NH_3-N were observed between calves fed lasalocid and those that did not receive lasalocid. Also reported by Anderson et al. (1988), total viable anaerobic and amylolytic bacterial counts were higher in lasalocid-fed calves. Lasalocid-resistant, lactate-producing, lactate-utilizing, or methanogenic bacterial species in the rumen were not affected significantly in the mentioned study (Anderson et al., 1988).

Potential Benefits

Feeding lasalocid to growing ruminants may offer valuable benefits both ruminal and post-ruminal. A reduction in methogenesis by reducing precursors such as hydrogen and formate (Chen and Wolin, 1979; Bartley and Nagaraja, 1982) would result in a decreased energy loss to the animal. As well, an increase in propionate production at the expense of acetate production would be energetically beneficial to the growing ruminant. Another

hypothetical ruminal effect in the presence of lasalocid is a decrease in butyrate metabolism via rumen epithelium which would decrease excessive alimintary ketogenesis relative to utilization by peripheral tissues. It was suggested by Quigley et al. (1991a) that enzymatic mechanisms responsible for ketone body metabolism in growing ruminants may not utilize increased ketone concentration produced by ruminal fermentation.

When feeding concentrates high in fermentable carbohydrate to ruminants, a condition develops commonly known as lactic acidosis (Elam, 1976). This condition is resultant of production and accumulation of abnormal amounts of lactic acid in the rumen (Dunlop, 1976). Ionophores have exhibited characteristics required for preventing lactic acidosis by inhibiting the majority of lactate-producing bacteria and are insensitive to lactate-fermenting bacteria. As a result, ionophores depress lactate production and magnify lactate utilization (Bartley and Nagaraja, 1982).

Although lasalocid is not approved as a prevention of bloat, studies have shown a surprising effectiveness of lasalocid in controlling and preventing grain bloat (Bartley et al., 1975; Bartley and Nagaraja, 1982). Lasalocid's unique ability to inhibit growth of all

nutritionally important strains of S. bovis, the causative organism of grain (feedlot) bloat (Van Soest, 1982; Bartley et al., 1975), may reduce the incidence of grain bloat.

Other benefits have been shown by Sinks (1990) concerning lasalocid's post-ruminal mode of action. When calves exposed to a coccidial challenge of Eimeria bovis were fed lasalocid, DMI and BW gain were increased in calves fed lasalocid. Further, lasalocid ameliorated observed reductions in DMI, ADG, and feed efficiency caused by coccidial challenge. This study resulted in a similar manner as did Fitzgerald and Mansfield (1973) confirming the efficacy of lasalocid in controlling coccidiosis.

Although ruminal and post-ruminal influences of lasalocid have been identified in mature ruminants, the nature of lasalocid's effects have not been fully delineated in immature ruminants. Some effects have been described previously but more information is needed to fully understand effects of lasalocid on metabolism in the young calf.

CHAPTER III

MATERIALS AND METHODS

Animal Assignment and Feed Management

Sixteen Holstein bull calves were assigned randomly at birth to one of four treatments in a modified split-plot experimental design. Treatments were: no lasalocid (C); lasalocid (1 mg/kg BW/d) added to milk replacer (M), or administered via ruminal cannula (R), and 0.5 mg lasalocid/kg BW/d added to milk replacer and via ruminal cannula (M + R). Lasalocid was allocated based on previous week's BW. Lasalocid in a calcium carbonate carrier was administered to M and M + R treatments by placing measured dosage into nipple bottles that contained milk replacer. Lasalocid was administered to the rumen in R and M + R treatments by mixing with distilled water and adding to the rumen via ruminal cannula. Lasalocid was provided by Hoffmann-LaRoche, Nutley, New Jersey.

Calves remained with dam for approximately 3 d of age and were moved to individual pens (1.8 m^2) bedded with non-cedar shavings at ambient temperature. Ruminal cannulas were installed surgically at an average of 5 d of age (SD = 2). A multispectrum antibiotic (Combiotic by Pfizer) was used post-cannulation for 4 days to minimize

incidence of surgical infections. The 12 wk study was initiated immediately following cannulation.

Calves were fed 1.8 kg milk replacer twice daily (approximately 0800 and 1630 h) from nipple bottles until weaning at wk 8. Calves were offered commercially prepared calf starter[1] once daily (0800 h) for ad libitum consumption to a maximum of 4.5 kg (Table 1). Calf starter contained high fiber by-products to stimulate development of ruminal function (Murdock and Wallenius, 1980). Refused calf starter was weighed daily and reported weekly. Fresh water was available at all times.

Body weights were measured at birth, on d 0 (first d of study) and every 7 d thereafter.

Calf starter was sampled once per shipment and analyzed for dry matter (DM) and crude protein (CP) (AOAC, 1980), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Goering and Van Soest, 1970), Ca, Mg, and K (atomic absorption spectrophotometry), and P (Hawk et al., 1948).

Blood Sampling and Analysis

Blood was collected weekly (on Wednesday) approximately 2 h after morning feeding by one jugular venipuncture into evacuated tubes (approximately 10 ml)

[1]Total Calf Ration One, Tennessee Farmer's Cooperative, LaVegne, TN.

with no anticoagulant, and 2 ml were added to 2 ml 1 M perchloric acid and placed on ice prior to transport to laboratory. Deproteinated blood was centrifuged and supernatant was analyzed for ßHOB and ACAC (Heitmann et al., 1987). Remaining blood was added to 400 µl of 6% EDTA; plasma was harvested and stored at 20° C prior to analysis of nonesterified fatty acids NEFA.[2]

A second set of tubes containing 143 USP units sodium heparin as anti-coagulant were placed on ice prior to transport to laboratory. Plasma was harvested and frozen (-20° C) prior to analysis of glucose[3], VFA by gas chromatography[4] (Quigley et al., 1991b), insulin (Yalow, 1976), and PUN.[5]

A third sample of jugular blood (approximately 5 ml) was collected and placed on ice prior to transport to the laboratory. Serum was harvested and frozen (-20° C) prior to analysis of serum total protein[6] and Ca, Mg, K, and Na

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[2]NEFA-C-Kit, Wako Pure Chemical Industries, Osaka, Japan.

[3]Glucose Kit No. 510. Sigma Chemical Co., St. Louis, MO.

[4]HP 5890 splitless injection with a 38:1 ratio cross-linked FFAP 10 m x 0.53 mm capillary column.

[5]Urea-nitrogen kit no. 640. Sigma Chemical Co., St. Louis, MO.

[6]Total Protein Kit No. 540. Sigma Chemical Co., St. Louis, MO.

(atomic absorption spectrophotometry) (Emmel et al., 1977).

Rumen Sampling and Analysis

Rumen samples were taken weekly (on Wednesday) approximately 2 h after morning feeding after blood was sampled. Approximately 40 ml of ruminal fluid were collected via vacuum pump from the ruminal cannula and pH was measured immediately using a portable pH electrode. Samples were frozen quickly in liquid nitrogen and placed on ice prior to transport to laboratory. Samples remained frozen (-20° C) until analyzed for VFA by gas chromatography[7] and NH_3-N.[8]

Statistical Analysis

Dependent variables were analyzed as a modified split-plot design (SAS, 1985) using the model:

$$Y_{ijk} = \mu + T_i + C_{(i)j} + P_k + (TP)_{ik} + e_{ijk}, \text{ where}$$

Y_{ijk} = dependent variable

μ = overall mean

T_i = effect of ith treatment

$C_{(i)j}$ = effect of jth calf nested within i^{th} treatment

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[7]HP 5890 Split injection with 38:1 ratio cross-linked FFAP 10 m x 0.53 mm capillary column.

[8]Modified Urea Nitrogen Kit No. 640. Sigma Chemical Co., St. Louis, MO.

P_k = effect of kth period

$(TP)_{ik}$ = effect of treatment * period interaction

e_{ijk} = residual.

Term $C_{(i)j}$ was used as error term to test differences due to treatment. Standard errors of treatment means were calculated as described by Gill (1978). Single degree of freedom contrasts were used to test differences between C treatment and lasalocid treatments, combined effect of R and M versus M + R, and R versus M treatments. Period 3 was analyzed for treatment differences using a single degree of freedom contrast testing C and M versus R and M + R treatments. Level of significance $P < .05$ was used unless noted otherwise.

Initial BW was analyzed as a completely randomized experimental design. Final BW was analyzed by analysis of covariance using initial BW as covariable.

CHAPTER IV

RESULTS AND DISCUSSION

Experimental starter (Table 1) contained alfalfa meal, cottonseed and soybean hulls as sources of fiber to stimulate ruminal development. Chemical composition of experimental starter (Table 2) averaged 19.2% crude protein (CP) which was 2.2% higher than formulated. Due to the inclusion of soybean and cottonseed hulls at 20.0 and 7.5% respectively, starter averaged 35.7% NDF.

All calves remained relatively healthy throughout the study. Health related problems were minor and unrelated to treatment. Three cases of bloat (C calves) and one case of bloody scours of non-coccidial origin (during last week of the study) were observed.

Least squares means of BW and ADG (Table 3) were somewhat greater than other studies reported from Tennessee Experiment Station (Quigley and Bearden, 1990). As expected, ADG was affected by week of study, and was lower during period 1 (0-3 wk) which could be attributed to low levels of starter consumption and stresses from cannulation and introduction to a new environment. As calves began consuming calf starter, ADG increased due to changes in ruminal fermentation (Foreyt et al., 1986) and increasing gut fill (Owens and Goetsch, 1988) which

TABLE 1. Formulation of commercial[1] starter.

Item	%
Corn, ground	20.0
Soybean meal, 48%	7.5
Soybean hulls	20.0
Wheat midds	25.0
Cottonseed hulls	7.5
Cottonseed meal	5.8
Alfalfa meal	5.0
Molasses	3.0
Bentonite/limestone	3.1
Vitamins/minerals/additives	3.1
Crude protein[2]	17.0
TDN	74.0
ADF	7.4

[1]Ingredients expressed on an as-fed basis.

[2]Nutrients expressed as a percent of dry matter.

TABLE 2. Chemical composition of commercial calf starter[1].

Item Calf starter	
DM	89.4
	% DM
CP	19.2
NDF	35.7
Ether extract	.38
Ca	.89
P	.64
Mg	.32
K	1.21
Na	.41

[1]Mean of 5 observations.

TABLE 3. Least squares means of body weight (BW), average daily gain (ADG), dry matter intake (DMI), and gain to feed ratio (G/F).

Item	Treatments[1]					Contrasts[2]		
	C	M	R	M+R	SE	1	2	3
BW, kg								
0- 3 wk	45.2	42.3	41.6	47.1	.6	NS	NS	NS
4- 8 wk	63.6	61.8	54.8	64.9	.7	NS	NS	NS
9-12 wk	96.8	87.2	82.4	92.3	.9	NS	NS	NS
0-12 wk	68.3	63.7	59.2	67.7	.8	NS	NS	NS
ADG, kg								
0- 3 wk	.36	.25	.16	.23	.12	NS	NS	NS
4- 8 wk	.77	.76	.67	.77	.10	NS	NS	NS
9-12 wk	1.06	.84	.92	.87	.13	NS	NS	NS
0-12 wk	.76	.66	.62	.67	.06	NS	NS	NS
DMI, kg/d								
0- 3 wk	.54	.52	.42	.58	.04	NS	NS	NS
4- 8 wk	1.74	1.70	1.34	1.58	.06	NS	NS	NS
9-12 wk	3.12	2.68	2.68	2.73	.05	NS	NS	NS
0-12 wk	1.81	1.65	1.47	1.63	.04	NS	NS	NS
G/F								
0- 3 wk	.67	.48	.38	.40	.04	NS	NS	NS
4- 8 wk	.44	.45	.50	.49	.04	NS	NS	NS
9-12 wk	.34	.31	.34	.32	.05	NS	NS	NS
0-12 wk	.42	.40	.42	.41	.03	NS	NS	NS

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrasts: 1 = C vs lasalocid treatments; 2 = average of R and M vs M + R; 3 = rumen treatment vs milk treatment; NS = P > 0.10.

appeared to increase proportionally to BW. No significant treatment differences were observed which may have been a function of strict management and controlled environment. Although calves were altered surgically, ADG was greater than intact animals at Tennessee Experiment Station (Sinks et al., 1990). Van Soest (1982) reported a considerable list of dietary factors that would mimic lasalocid's effects on animal response consisting of increased intake and ground, pelleted feed. This study involved relatively high intake of a ground, pelleted ration constituting a possible masking of effects of lasalocid.

Dry matter intake (DMI) was not affected significantly by treatment (Table 3). Starter was fed to a maximum of 4.5 kg/d (as-fed basis); only two calves reached maximum starter intake by 12 wk. Intake of minerals (Ca, Mg, K and Na) was unaffected by treatment, and increased as a function of DMI. Inclusion of lasalocid did not affect feed efficiency (G/F) of calves (Table 3).

Least squares means of ruminal pH are in Table 4. Ruminal pH declined ($P < .001$) as the study progressed and calves began consuming dry feed. A decrease in ruminal pH with development of ruminal function has been reported

TABLE 4. Least squares means of ruminal pH and ammonia-nitrogen (NH_3-N).

Item	Treatments[1]					Contrasts[2]		
	C	M	R	M+R	SE	1	2	3
pH								
0- 3 wk	5.91	6.15	6.53	6.15	.16	*	NS	NS
4- 8 wk	5.49	5.51	5.64	5.56	.01	NS	NS	NS
9-12 wk	5.11	5.15	5.40	5.28	.05	NS	NS	NS
0-12 wk	5.52	5.61	5.84	5.66	.05	*	NS	*
NH_3-N(mg/dl)								
0- 3 wk	13.86	9.27	12.30	8.53	1.65	NS	NS	NS
4- 8 wk	16.43	15.36	14.83	17.16	1.60	NS	NS	NS
9-12 wk	17.96	16.01	21.53	25.40	1.60	NS	NS	NS
0-12 wK	16.09	13.60	16.25	17.17	1.03	NS	NS	NS

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrasts: 1 = C vs lasalocid treatments; 2 = R and M vs M + R; 3 = R vs M; NS = P > .10; * = P < .10.

(Anderson et al., 1987a; Owens and Goetsch, 1988). Over the 12 wk study, ruminal pH of calves receiving lasalocid appeared to be higher than pH of those receiving no lasalocid ($P < .10$), and those on treatment R tended to be higher than calves on treatment M ($P < .10$). During period 3 (9-12 wk), calves receiving lasalocid had significantly higher ruminal pH than those on treatments C and M ($P < .05$; Table 5). Lasalocid decreases molar proportion of acetate and butyrate thereby reducing hydrogen gas production in the rumen and increasing ruminal pH (Bergen and Bates, 1984).

Least squares means of ruminal NH_3-N are in Table 4. Although others (Dinius et al., 1976; Van Nevel and Demeyer, 1977; Chalupa, 1980) have reported a decrease in ruminal NH_3-N in lasalocid-fed calves which suggests a "protein-sparing" effect, no significant differences among treatments were observed. An increase in ruminal NH_3-N with week of study was observed ($P < .001$). Means of this study are somewhat higher than those reported previously (Quigley and Bearden, 1990). Increased ruminal NH_3-N concentrations could be attributed to increased crude protein intake (CPI) which was correlated to DMI ($r = .998$). Calves receiving lasalocid had significantly

TABLE 5. Least squares means of body weight (BW), average daily gain (ADG), dry matter intake (DMI), ruminal pH (pH), ammonia-nitrogen (NH_3-N), blood ß-hydroxybutyrate (ßHOB), acetoacetate (ACAC), nonesterified fatty acids (NEFA), glucose (GLU), urea-nitrogen (PUN), insulin, ruminal total FVA (TVFA), % ruminal acetate (% ACET) % ruminal propionate (% PROP), % ruminal butyrate (% BUTY), Acetate to Propionate (A:P), Plasma total VFA (PTVFA), % plasma Acetate (% PACE), % plasma propionate (% PROP), % plasma butyrate (%PBUTY), plasma acetate to propionate (PA:PP).

	Treatments[1]					Contrasts[2]
Item	C	M	R	M+R	SE	1
BW	96.8	86.6	82.4	92.3	4.3	NS
ADG	1.1	.8	.9	.9	.1	NS
DMI	3.1	2.7	2.7	2.7	.1	NS
pH	5.2	5.2	5.4	5.3	.1	**
NH_3N	17.8	15.7	22.0	25.5	2.2	***
ßHOB	881.7	996.7	646.5	639.6	64.1	***
ACAC	21.2	20.8	15.6	15.7	2.6	**
NEFA	122.0	89.3	105.1	99.4	7.8	NS
GLU	4.9	4.5	4.9	4.3	.1	NS
PUN	4.4	3.4	3.9	4.6	.2	NS
INSULIN	127.8	138.3	111.7	98.3	16.0	*
TVFA	175.5	170.1	176.9	188.5	10.1	NS
% ACET	51.7	54.2	50.9	51.92	1.2	NS
% PROP	35.3	32.9	38.6	36.7	1.4	**
% BUTY	12.9	12.8	10.3	11.4	1.0	**
A:P	1.5	1.7	1.3	1.5	.1	**
PTVFA	1.0	1.0	.9	.8	.1	*
% PACET	94.3	93.1	93.9	94.7	.6	NS
% PPROP	4.0	4.0	4.8	4.0	.3	NS
% PBUTY	1.6	2.9	1.2	1.3	.5	*
PA:PP	25.2	26.9	20.0	25.7	2.1	NS

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrast: 1 = C and M versus R and M + R; NS = P > .10; * = P < .10; ** = P < .05; *** = P < .01.

higher NH_3-N than those on C and M treatments during period 3 ($P < .01$; Table 5).

Least squares means of PUN are in Table 6. As expected, PUN increased with week of study ($P < .001$) due to an increase in CPI as well as a decrease in ruminal NH_3-N absorption due to a low ruminal pH. Calves on M + R treatment had significantly higher concentration of PUN ($P < .05$) than the average of M and R treatments. At lower pH, less NH_3-N is absorbed across rumen wall to liver which results in less urea being produced (Van Soest, 1982). Since there is a high concentration of NH_3-N in rumen, less urea is recycled to rumen and the urea pool is in excess (Van Soest, 1982). However, the urea pool size is regulated by renal threshold and excess urea is excreted via urine. Calves on M + R treatment had significantly higher concentrations of PUN ($P < .05$) than the average of M and R treatments. This higher concentration of PUN follows a tendency of lower ruminal pH and higher ruminal NH_3-N also exhibited by M + R calves. Many authors suggested that ionophores produce a protein sparing effect (Dartt et al., 1978; Rust et al., 1978; Beede et al., 1980; 1978; Joyner et al., 1979) primarily when a low protein diet is offered. There are inconsistent reports from others (Dinius et al., 1976;

Table 6. Least squares means of blood ß-hydroxybutyrate (ßHOB), acetoacetate (ACAC), plasma non-esterified fatty acids (NEFA), glucose (GLU), urea-nitrogen (PUN) and insulin.

	Treatments[1]					Contrasts[2]		
Item	C	M	R	M+R	SE	1	2	3
ßHOB (μM/L)								
0- 3 wk	140.12	121.52	99.93	128.16	20.89	NS	NS	NS
4- 8 wk	487.99	429.75	410.76	435.79	42.81	NS	NS	NS
9-12 wk	877.18	1000.59	646.54	639.61	63.59	NS	**	**
0-12 wk	503.88	512.46	386.13	403.85	25.40	NS	NS	***
ACAC (μM/L)								
0- 3 wk	6.28	6.50	5.13	4.16	1.69	NS	NS	NS
4- 8 wk	10.97	11.04	12.72	11.61	2.12	NS	NS	NS
9-12 wk	21.26	20.84	15.59	15.71	2.70	NS	NS	NS
0-12 wk	12.69	12.66	11.27	10.58	1.30	NS	NS	NS
NEFA (μM/L)								
0- 3 wk	177.97	190.95	210.23	218.89	30.02	NS	NS	NS
4- 8 wk	150.79	143.29	146.55	143.63	13.25	NS	NS	NS
9-12 wk	123.01	89.09	105.06	99.36	7.42	**	NS	NS
0-12 wk	150.61	140.86	153.38	153.17	11.02	NS	NS	NS
GLU (mM/L)								
0- 3 wk	4.81	4.78	4.71	4.47	.31	NS	NS	NS
4- 8 wk	5.27	4.93	5.16	4.61	.21	NS	*	NS
9-12 wk	4.61	4.49	4.59	4.44	.11	NS	*	NS
0-12 wk	5.04	4.76	4.96	4.46	.12	NS	*	NS
PUN (mm/L)								
0- 3 wk	3.21	.32	3.10	3.71	.16	NS	**	NS
4- 8 wk	3.36	3.23	3.48	4.18	.14	NS	NS	NS
9-12 wk	4.21	3.27	3.88	4.63	.16	NS	*	NS
0-12 wk	3.60	3.23	3.49	4.18	.10	NS	**	NS
INSULIN (μU/ml)								
0- 3 wk	80.99	74.92	68.06	76.46	15.60	NS	NS	NS
4- 8 wk	60.94	87.13	67.97	78.85	12.21	NS	NS	NS
9-12 wk	62.70	70.94	55.83	49.15	6.57	NS	NS	NS
0-12 wk	68.80	79.43	64.26	68.98	7.14	NS	NS	*

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrasts: 1 = C vs lasalocid treatments; 2 = average of R and M vs M + R; 3 = R vs M; NS = P > 0.10; * = P < .10; ** = P < .05; *** = P < .01.

Poos et al., 1979) concerning a protein sparing effect. These data indicated no protein sparing effect, possibly due to the high CP formulation of the ration.

Plasma glucose least squares means are in Table 6. Glucose levels increased from 4.47 mM in period 1 (wk 0-3) to 5.27 mM in period 2 (wk 4-8). Throughout the remainder of the study, concentrations decreased in a manner typical of developing ruminants (Roy, 1980). Plasma glucose concentrations for M + R calves were lower than the average of M and R treatments ($P < .05$) throughout the study. Overall, plasma glucose concentrations were affected significantly by week of study ($P < .001$) until weaning. During period 3 (postweaning), means did not differ between treatments. When adding ionophores to diets, which are reported to provide more gluconeogenic substrates, peripheral concentrations could be altered (Bergen and Bates, 1984; Schelling, 1984; Judson and Leng, 1973). As ruminal function matures, source and length of time for glucose to reach the peripheral circulation may change due to a change in the source of glucose. Initially, blood glucose is derived from milk; with increasing DMI, fermentation of carbohydrates to propionate and subsequent hepatic gluconeogenesis would contribute to blood glucose (Martin et al., 1983).

Plasma insulin (Table 6), responded to plasma glucose concentrations by increasing until weaning at 8 wk ($P < .05$). Postweaning treatments did not differ significantly, but all decreased significantly from periweaning period ($P < .05$). Overall, plasma insulin of calves on treatment R were significantly lower when compared to calves on treatment M ($P < .10$) supporting a similar observation with plasma glucose. Insulin concentrations in calves receiving lasalocid in period 3 were significantly lower than calvs on C and M treatments ($P < .10$; Table 5). Plasma insulin correlated with plasma glucose ($r = .41$; $P < .01$), (Table 7), which suggests that plasma insulin responds to other metabolites besides glucose.

Concentration of plasma NEFA (Table 6) was elevated at the initiation of the study, possibly due to stresses of birth and cannulation. Means across all treatments decreased significantly ($P < .001$) by week until the postweaning period (9-12 wk) when concentrations were relatively unchanged. Low concentrations of NEFA after weaning suggests the calves were under little stress. Concentration of plasma NEFA was not affected significantly by treatment within preweaning and periweaning periods, but calves that received lasalocid

Table 7. Coefficients of Correlation[1] of dry matter intake (DMI); ruminal ammonia-nitrogen (NH_3-N), acetate (ACET), propionate (PROP), butyrate (BUTY), total VFA (TVFA), and pH; blood -hydroxybutyrate (BOHB) and acetoacetate (ACAC); plasma nonesterified fatty acids (NEFA), glucose (GLU), urea-nitrogen (PUN), insulin, acetate (PACET), propionate (PPROP), butyrate (PBUTY), and total plasma VFA (PTVFA).

	NH_3-N	ACET	PROP	BUTY	TVFA	pH	BHOB	ACAC	NEFA	GLU	UREA-N	INS	PACET	PPROP	PBUTY	PTVFA
DMI	.36	.71	.73	.47	.74	.68	.78	.51	-.46	0^c	.35	$-.12^a$	.62	.53	$.02^c$	.62
NH_3-N		.41	.54	$.15^b$	.45	-.24	$.17^b$	$.05^c$	-.20	0^c	$.17^b$	$-.09^c$	$.13^a$	$.18^b$	$-.15^b$	$.13^a$
ACET			.86	.70	.98	-.73	.52	.31	-.39	$.13^a$	$.18^b$	$-.04^c$	.34	.41	$.07^c$	.35
PROP				.55	.93	-.59	.47	.28	-.35	$.09^c$	$.17^b$	$-.07^c$	.35	.49	$-.04^c$	.36
BUTY					.74	-.65	.54	.41	-.30	$.09^c$	.32	-.05	.21	.24	.36	.23
TVFA						-.74	.55	.34	-.39	$.12^c$	.22	-.05	.35	.45	.08	.36
pH							-.57	-.38	.48	$-.08^c$	-.20	.06	-.35	-.47	.15	-.37
BHOB								.80	-.38	$-.12^a$	.30	-.13	.67	.48	.23	.68
ACAC									-.27	-.22	.23	-.11	.46	.29	.25	.47
NEFA.										$-.05^c$	$-.18^b$	$.12^c$	-.28	-.38	$-.04^c$	-.30
GLU											$-.14^b$	.41	-.11	.08	$-.02^c$	$-.10^c$
PUN												$-.10^c$	.24	.12	.13	.24
INSULIN													$-.06^c$	0^c	$.06^c$	$-.06^c$
PACET														.67	$.10^c$	.99
PPROP															$.18^b$	.71
PBUTY																$.16^b$

[1]All coefficients were significant ($P < .01$), except [a]($P < .10$) and [b]($P < .05$) and [c]($P > .10$).

had significantly lower NEFA levels in postweaning period than calves on C treatment ($P < .05$).

Serum total protein and Ca, Mg, K and Na were also analyzed also but no significant differences were observed; data are not presented.

Blood ßOHB concentrations (Table 6) tended to be affected by lasalocid treatment ($P < .10$) overall but were affected significantly in postweaning period (9-12 wk) ($P < .01$). When analyzed by period, ßHOB showed no significant differences to weaning. In the postweaning period, calves that received lasalocid had lower ßHOB concentrations than calves receiving no lasalocid ($P < .01$; Table 5).

Upon initiation of the study, ßHOB levels were very low and increased with advancing age and DMI. Correlation of ßHOB and DMI ($r = .80$) (Table 7) suggests that as DMI increased, ßHOB increased as well. At weaning, concentrations of treatments began distinguishing themselves. Concentrations of ßHOB in calves no longer receiving lasalocid (C and M treatments) continued to increase postweaning but R and M + R concentrations began to decrease.

Concentrations of ßHOB indicated the presence of ruminal acetate and butyrate which are ketogenic in young

ruminants (Sutton et al., 1963a). Wardrop and Coombe (1961) stated that development of ruminal function after first few weeks after dry feed consumption begins was followed by increased concentrations of VFA in the rumen. Metabolism of butyrate by ruminal epithelium as well as by liver in the adult ruminant is known to result in the production of ketone bodies (Pennington, 1952). The ability of the rumen epithelium to make ketone bodies in the presence of butyrate is extremely low at birth but increases with age to a maximum concentration at 8 wks of age (Hird and Weidemann, 1966).

Recently, Quigley et al. (1991a) reported that ketone body production increased dramatically at weaning to levels typical of sub-clinical ketosis. However, calves remained healthy and free of any visible sign of ketosis. This study indicated while calves were still consuming milk, levels of ßHOB were not statistically different and were relatively low but across all treatments continued to increase with age and DMI. It has been documented that ketone production can occur also in the cecum of young calves in the presence of butyrate (Walker and Simmonds, 1962).

Concentrations of ßHOB postweaning increased substantially; in calves no longer receiving lasalocid,

ßHOB concentrations continued to increase and reached sub-clinical ketotic levels as high as 1663 μM which is similar to data of Quigley et al. (1991a). However, plasma glucose did not fall to levels observed often with ketosis. As well, plasma NEFA levels were consistently within normal ranges.

Concentrations of blood ACAC (Table 5) responded similarly to ßHOB throughout the study beginning at low levels initially and increasing with age and DMI. Blood ACAC correlated positively with DMI ($r = .51$) (Table 7) which also suggests that increases in ketogenesis were the result of increased DMI. Concentrations of blood ACAC were similar across all treatments throughout the study.

Levels of ßOHB reflected ruminal VFA production, especially butyrate. Ruminal VFA (acetate, propionate, and butyrate) least squares means are in Table 7. Individual VFA are reported in molar percentages. Total ruminal VFA (TVFA) concentrations increased with week of study ($P < .001$) as a function of increasing DMI ($r = .74$) (Table 8). In period 1, calves on treatment C tended to have a higher TVFA concentration ($P < .09$) than calves receiving lasalocid, especially when lasalocid was given directly into the ruminal cannula. Because TVFA concentrations indicate an increase in ruminal metabolic

TABLE 8. Least squares means of total ruminal VFA (TVFA) and molar percentages of ruminal acetate propionate and butyrate.

Item	Treatments[1]					Contrasts[2]		
	C	M	R	M+R	SE	1	2	3
TVFA (mM/L)								
0- 3 wk	86.1	68.6	43.1	62.0	11.6	*	NS	NS
4- 8 wk	156.5	152.5	141.5	158.0	7.3	NS	NS	NS
9-12 wk	173.4	165.8	175.8	189.6	6.8	NS	NS	NS
0-12 wk	140.3	129.8	121.2	139.9	5.7	NS	NS	NS
% Acetate								
0- 3 wk	58.9	61.4	63.5	59.6	2.9	NS	NS	NS
4- 8 wk	53.7	56.2	53.6	54.2	1.4	NS	NS	NS
9-12 wk	51.0	54.0	50.8	52.0	1.1	NS	NS	NS
0-12 wk	54.6	57.6	55.9	55.1	1.1	NS	NS	NS
% Propionate								
0- 3 wk	29.1	28.9	29.1	29.2	2.3	NS	NS	NS
4- 8 wk	32.4	31.4	32.9	28.9	1.4	NS	NS	NS
9-12 wk	35.9	33.6	38.7	36.2	1.3	NS	NS	NS
0-12 wk	32.5	30.8	33.4	31.3	1.0	NS	NS	NS
% Butyrate								
0- 3 wk	12.0	9.7	7.5	11.3	1.9	NS	NS	NS
4- 8 wk	13.9	12.4	13.5	16.9	1.4	NS	*	NS
9-12 wk	13.0	12.4	10.5	11.8	1.0	NS	NS	NS
0-12 wk	12.9	11.6	10.7	13.6	1.0	NS	NS	NS

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrasts: 1 = C vs lasalocid treatments; 2 = average of R and M vs M + R; 3 = rumen treatment vs milk treatment; NS = P > .10; * = P < .10.

activity, reduced ruminal VFA suggests that lasalocid may have inhibited proliferation of ruminal bacteria during the first few weeks. Generally, establishment of ruminal bacteria in the first few weeks is characterized by large numbers of aerobes (Lengemann and Allen, 1959) and facultative anaerobes (Anderson et al., 1987b).

Molar proportions of acetate, propionate and butyrate were unaffected by treatment, although there was a decrease in percent acetate ($P < .001$) and an increase in percent propionate ($P < .001$) as the study progressed. During period 3 (9-12 wk), calves receiving lasalocid had significantly greater percent propionate than calves on C and M treatments ($P < .05$; Table 5). Molar percent butyrate increased from period 1 (0-3 wk) to period 2 (4-8 wk) ($P < .02$). Calves receiving lasalocid had lower molar percent butyrate than other calves from 9-12 wk ($P < .05$; Table 5).

Plasma VFA (acetate, propionate and butyrate) least squares means are in Table 9. Individual VFA are reported in molar percentages. Total plasma VFA (PTVFA) concentrations increased with increasing DMI from period 2 to period 3 ($P < .01$), and correlated 0.62 with DMI (Table 7). This correlation suggests that a majority of the

Table 9. Least squares means of plasma total volatile fatty acids (PTVFA), and molar percentages of plasma acetate (PACET), propionate (PPROP) and butyrate (PBUTY).

	Treatments[1]					Contrasts[2]		
Item	C	M	R	M+R	SE	1	2	3
PTVFA (mM)								
0- 3 wk	.50	.54	.46	.54	.05	NS	NS	NS
4- 8 wk	.56	.70	.57	.55	.04	NS	NS	**
9-12 wk	1.01	.95	.91	.79	.06	NS	NS	NS
0-12 wk	.68	.73	.64	.62	.03	NS	NS	*
% PACET								
0- 3 wk	90.7	94.5	95.2	92.8	1.6	NS	NS	NS
4- 8 wk	92.4	94.4	93.2	93.3	1.0	NS	NS	NS
9-12 wk	94.0	93.3	93.9	94.7	.6	NS	NS	NS
0-12 wk	92.6	93.9	94.1	93.6	.7	NS	NS	NS
% PPROP								
0- 3 wk	2.63	3.23	3.14	3.52	.59	NS	NS	NS
4- 8 wk	3.93	4.03	4.39	3.44	.38	NS	NS	NS
9-12 wk	4.35	4.27	4.85	4.03	.22	NS	NS	NS
0-12 wk	3.61	3.90	4.14	3.64	.25	NS	NS	NS
% PBUTY								
0- 3 wk	6.62	2.28	1.63	3.63	1.60	NS	NS	NS
4- 8 wk	3.70	1.59	2.41	3.25	.95	NS	NS	NS
9-12 wk	1.63	2.42	1.23	1.25	.55	NS	NS	NS
0-12 wk	3.81	2.13	1.80	2.75	.71	NS	NS	NS

[1]Treatments: negative control (C); lasalocid in milk (M); lasalocid injected into rumen via cannula (R); and lasalocid administered in milk and rumen (M + R).

[2]Contrasts: 1 = C vs lasalocid treatments; 2 = average of R and M vs M + R; 3 = R vs M; NS = P · .10; * = P < .10; ** = P < .05; *** = P < .01.

increase in PTVFA is due to an increase in DMI which results in more substrate fermentation in the rumen.

Over the entire 12 wk study, calves receiving lasalocid ruminally had significantly lower PTVFA levels than calves on M treatment ($P < .05$). When analyzed by period, VFA in plasma of calves on treatment R was significantly lower than treatment M ($P < .05$) during periweaning period. During period 3, calves on R and M + R treatments has significantly lower PTVFA concentrations than did calves on C and M treatments ($P < .10$; Table 5).

Plasma acetate comprised more than 90% of PTVFA in this study. The high coefficient of correlation of plasma acetate and PTVFA ($r = .99$) suggests that PTVFA concentrations are dependent on effects of plasma acetate. No significant treatment differences were observed ($P > .05$) in plasma acetate percent.

Molar percent of plasma propionate increased significantly by week throughout the study ($P < .001$) without effect of treatment. Initially percent propionate increased slowly, then more rapidly following weaning.

Changes in percent plasma butyrate were affected significantly by week of study ($P < .05$) in period 2 (4-8 wk). Percent plasma butyrate increased from period 1 to period 2, then decreased to period 3 in the R calves.

This trend was not statistically significant but did follow the trend of ruminal butyrate percentage. Percent plasma butyrate was affected significantly by treatment in period 3 ($P > .10$; Table 5) in that calves receiving lasalocid had a lower percent butyrate than those no longer on lasalocid treatment.

Coefficients of correlation among selected variables in this study (Table 7) generally indicate consistent metabolic changes with an increase in DMI as well as ruminal development. Blood ßHOB and ACAC correlated .80 with each other indicating a similar source of ketogenesis throughout the study (Quigley et al., 1991a). As mentioned previously, ßHOB and ACAC correlated .78 and .51 respectively, with DMI also indicating that blood ßHOB concentration was influenced by alimentary ketogenesis.

Plasma NEFA correlated -.38 and -.27, respectively, with ßHOB and ACAC. Plasma glucose correlated -.12 and -0.22 respectively, with ßHOB and ACAC. Both NEFA and glucose correlations with ßHOB and ACAC further support the hypothesis that the source of ketones observed in this study was of alimentary instead of hepatic origin which is the source of sub-clinical hyperketonemia ketogenesis (Bergman, 1971).

CHAPTER V

CONCLUSIONS

Blood ketones increased substantially with dry matter intake. In calves not receiving lasalocid, ketone levels reached concentrations indicative of hyperketonemia post-weaning. However, calves remained healthy throughout the study. Plasma NEFA and glucose remained within normal ranges suggesting that increased blood ketones resulted from alimentary and not hepatic ketogenesis. It is not known if this excessive concentration of blood ketones was the result of over production or under utilization of ketones by peripheral tissues. Excessive blood ketones are excreted via urine which suggests a loss of energy to the calf. This study showed that lasalocid decreased post-weaning blood ketone levels via lasalocid's ruminal mode of action which would decrease the suggested loss of energy.

Lasalocid increases ruminal efficiency by decreasing molar percent acetate and butyrate which also decreases production of methane. As well, lasalocid may allow for the calf to be more efficient by decreasing alimentary ketogenesis which results in less ketones being excreted - which is less energy lost to the growing calf.

Additional research is required to investigate the potential decrease of metabolic efficiency due to ketones being lost to urinary excretion and further use of ionophores to minimize metabolic inefficiencies, primarily hyperketonemia.

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APPENDIXES

APPENDIX A

PLASMA UREA-NITROGEN ANALYSIS

Urea-nitrogen kit No. 640. Sigma Chemical Co., St. Louis, MO is based on the following reactions:

$$\begin{matrix} H_2N \\ C = O \\ H_2N \end{matrix} + H_2O \xrightarrow{\text{Urease}} 2\ NH_3 + CO_2$$

Reagents

1) Phenol Nitroprusside Solution
Phenol, 50 g/L, sodium nitroprusside and stablizier. Store in refrigerator [2-6°C].

2) Alkaline Hypochlorite Solution
Sodium hypochlorite, 0.2%, in alkali.
Store in refrigerator [2-6°C].

3) Urease Buffer Reagent
Buffered urease from jack beans, 100 units, when prepared. Sodium azide added as preservative.
Store in refrigerator [2-6°C].

4) Urease Solution
Prepared by reconstituting vial of Urease Buffer Reagent with 30 ml H_2O. Stable at least 1 month when refrigerated [2-6°C].

5) Urea Nitrogen Standard Solution
Urea at a urea-nitrogen level of 30 mg/dl [10.7 mmol/L] and benzoic acid as preservative. Store in refrigerator [2-6°C].

Sample Collection and Storage

Plasma: Add whole blood directly to a tube containing anticoagulant. Most common anticoagnlauts may be used. However, fluoride must not be used since it inhibits urease action. Ammonium salts of anticoagulants must be avoided due to sensitivity of the reaction. Centrifuge tubes for 15 to 20 minutes and harvest plasma.

Procedure

1) Label test tubes for standards, samples and blanks; in duplicate.

2) To appropriate tubes, add 900 µl saline and 100 µl standard, plasma and blank.

3) Vortex thoroughly.

4) To second set of tubes, add 100 µl of diluted standard, plasma and blank; in duplicate.

5) Add 250 µl urease solution to each tube.

6) Vortex thoroughly.

7) Incubate at 37°C for 5-10 minutes.

8) Add to each tube in the following order:

   a) 0.5 ml phenyl nitroprusside; vortex
   b) 0.5 ml alkaline hypochlorite; vortex
   c) 2.0 ml dH_2O; vortex

9) Allow color to develop at room temperature for 20-30 minutes.

10) Read absorbance at 570 nm.

Calculation

$$\text{Urea-nitrogen (mg/dl)} = \frac{[A]\ \text{unknown}}{[A]\ \text{standard}} \times 30$$

APPENDIX B

PLASMA GLUCOSE ANALYSIS

Plasma glucose kit No. 510. Sigma Chemical Co., St. Louis, MO. Quantitative enzymatic colorimetric in vitro determination in whole blood, serum, or plasma is based on the following coupled enzymatic reactions:

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{Glucose}} \text{Gluconic Acid} + 2H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{O-Dianisidine}} \longrightarrow \underset{\text{(brown)}}{\text{Oxidized O-Dianisidine}}$$

The intensity of the brown color measured at 425-475 nm is proportional to the original glucose concentration.

Reagents

1) PGO Enzyme, Catalog No. 510-6 (prepared capsule). Contains 500 units of glucose oxidase, 100 purpurogallin units of peroxidase and buffer salts. Store in refrigerator [2-6°C].Combined enzyme-color reagent solution is prepared by combining 100 ml enzyme solution and 1.6 ml color reagent solution. Solution must be stored in amber bottle and refrigerated at 2-6°C.

2) O-Dianisidine Dihydrochloride, catalog No. 510-50. Preweighed vial containing 50 mg O-dianisidine dihydrochloride must be reconstituted in the vial with 20 ml dd H_2O and refirgerated at 2-6°C.

3) Glucose Standard Solution, catalog No. 635-100. Solution contains 100 ml/dl ß-glucose in a 0.1% benzoic acid solution and stored at 2-6°C.

4) 0.3N Barium Hydroxide Solution. Catalog No. 14-3.

5) 0.3N Zinc Sulfate Solution. Catalog No. 14-4.

Sample Collection and Preparation

Blood samples should be collected into a container containing appropriate anticoagulant. Centrifuge blood for 10-15 minutes and harvest plasma.

Procedure

1) Label test tubes for blank, standard and samples; in duplicate.

2) Add 0.25 ml plasma to sample tubes. Add 0.25 ml d H_2O to blank tubes. Add 0.25 ml glucose standard to standard tubes.

3) Add 1.75 ml dH_2O to all tubes.

4) Add 1.0 ml 0.3N BaOH to all tubes.

5) Vortex thoroughly.

6) Add 1.0 ml 0.3N $ZnSO_4$ to all tubes.

7) Vortex thoroughly.

8) Centrifuge in high speed refrigerated centrifuge for 20 minutes.

9) Label second set of tubes for supernatant; in duplicate.

10) Pipette 0.2 ml supernatant into second set of tubes.

11) Add 2 ml PGO enzyme solution to all tubes.

12) Incubate at 37°C in hot water bath for 30 minutes.

13) Read absorbance at 450 nm.

Caution: Avoid direct exposure to sunlight or bright light. Following incubation, remove tubes from water bath prior to reading.

Calculation

$$\text{Plasma glucose (mg/dl)} = \frac{[A]\ \text{unknown}}{[A]\ \text{standard}} \times 100$$

APPENDIX C

PLASMA NONESTERIFIED FATTY ACID ANALYSIS

NEFA-C kit from Wako utilizes an in vitro enzymatic colorimetric method for the quantitation of nonesterified fatty acids. This kit is based on the following reactions:

NEFA are activiated to CoA ester by acetyl CoA synthetase (ACS).

$$RCOOH + ATP + CoA \longrightarrow Acyl\text{-}CoA + AMP + 2\ Pi$$

Acyl CoA is oxidized by acyl-CoA oxidase (ACOD) to produce H_2O_2.

$$Acyl\ CoA + O_2 \xrightarrow{ACOD} 2,3\text{-trans-Enoyl-CoA} + H_2O_2$$

H_2O_2 is acted on by peroxidase (POD) in the presence of 3-methyl-N-ethyl-N-beta-hydroxyethyl-aniline (MEHA) with 4-amino antipyrine (AAP) for form a product with a purple color which is measured colorimetrically at 550 nm.

$$2H_2O_2 + MAHE + AAP \longrightarrow Purple\ Quinone\ product + H_2O$$

Reagents

1) Color Reagent A

Each vial contains:

a)	ACS (acyl coenzyme A synthetase)	3U
b)	AOD (ascorbic oxidase)	15U
c)	CoA (coenzyme A)	7 mg
d)	ATP (adenosine triphosphate)	30 mg
e)	4-amino antipyrine	3 mg

2) Diluent for color reagent A

Contains 65 ml of an aqueous solution of the following:

a)	Phosphate buffer (pH = 6.9)	0.05M
b)	Magnesium chloride	3mM
c)	Surfactant	
d)	Stabilizers	

3) Color Reagent B

Each vial contains:

a)	ACOD (acyl, coenzyme A oxidase)	132U
b)	POD (peroxidase)	150U
c)	MEHA (3-methyl-N-ethyl-N-ß-hydroxyethyl-aniline)	4 mg

4) Diluent for color reagent B

Contains 130 ml of an aqueous solution of the following:

a)	Phenoxyethanol	0.3% (v/v)
b)	Surfactant	

5) NEFA Standard Solution

Contains 10 ml of an aqueous solution with a known concentration of a non-esterified fatty acid:

a)	Oleic acid	1.0 mM
b)	Surfactant	
c)	Stabilizers	

Sample Collection and Storage

Plasma: Blood samples should be collected into a container with anticoagulant. Heparin should not be used since it stimulates the activity of lipoprotein lipase which ultimately releases non-esterified fatty acids. Centrifuge tubes for 15 to 20 minutes and collect plasma.

Procedure

1) Color Reagent A Solution

a) add 10 ml diluent for color reagent A
b) mix well
c) pour contents into appropriate amber bottle
d) add to vial, 13.3 ml dH_2O
e) pour dH_2O into bottle
f) refrigerate
(stable for 5 days only)

2) Color Reagent A Solution

a) add 10 ml diluent for color reagent B
b) mix well

c) pour vial contents into appropriate amber bottle
d) add to vial, 33.3 ml dH_2O
e) pour dH_2O into bottle
f) refrigerate
(stable for 5 days only)

3) Standards

a) Label duplicate tubes for 0, 125, 250, 500 and 1000
b) To 1000 standard:
add 25 µl of NEFA standard
c) To 500 standard:
add 25 µl of NEFA standard and 25 µl of dH_2O; vortex
d) To 250 standard:
add 25 µl of 500 standard and 25 µl of dH_2O; vortex
e) To 125 standard:
add 25 µl of 250 standard and 25 µl of dH_2O; vortex
f) Remove 25 µl of 125 standard solution and discard
g) Add 25 µl of dH_2O to 0 tubes

4) Label duplicate tubes for samples.

5) Add 25 µl vortex plasma.

6) Add 350 µl Reagent A to all tubes.

7) Vortex

8) Place all tubes in water bath at 37°C for exactly 20 minutes.

9) Add 800 µl Reagent B to all tubes.

10) Vortex

11) Place all tubes in water bath at 37°C for exactly 20 minutes.

12) Add 400 µl of dH_2O to all tubes.

13) Vortex

14) Allow to set 5 minutes at room temperature.

15) Read absorbance at 550 nm.

Calculations

$$\text{NEFA} = \frac{[\text{A}]\ \text{sample}}{[\text{A}]\ \text{standard}} \times \text{NEFA concentration}$$

$$\text{where NEFA concentration} = \frac{[\text{A}]\ \text{sample}}{[\text{A}]\ \text{NEFA standard solution}}$$

APPENDIX D

SERUM TOTAL PROTEIN ANALYSIS

Total Protein Kit No. 540. Sigma Chemical Co., St. Louis, MO is based on the following reactions:

$$\text{Copper} + \text{Serum Proteins} \xrightarrow{\text{alkaline pH}} \text{Copper-Protein Complex (purple)}$$

The intensity of the color is proportional to the total protein concentration.

Reagents:

1) Biuret Reagent, Catalog No. 540-2 contains copper sulfate 0.15 [w/v], sodium hydroxide [w/v], with tartrate and iodide added. Store at room temperature [18-20°C]. Suitable for use in the absence of precipitate.

2) Protein Standard Solution, Catalog No. 540-10. Contains Albumin [human] 5 g/dl, and globulin [human] 3 g/dl. Sodium azide, 0.05, added as preservative. Store tightly capped in refrigerator [2-6°C]. Discard if turbidity develops.

Sample Collection and Storage

Blood samples should be collected into a serum separator tube. Centrifuge blood for 10-15 minutes and harvest serum.

Procedure

1) Label duplicate tubes for standard, sample and blank.

2) For Blank, add 0.5 ml dH_2O.

3) For Standard, add 0.5 ml protein standard solution.

4) For Samples, add 0.5 ml serum.

5) Add 2.5 ml Biuret Reagent to all tubes.

6) Vortex

7) Allow to stand 15 minutes at room temperature [18-26°C].

8) Read absorbance at 540 nm.

Calculation

$$\text{Serum Total Protein} = \frac{[A]\ \text{sample}}{[A]\ \text{standard}} \times 8^{*}$$

*Concentration [g/dl] of protein standard solution.

APPENDIX E

SERUM POTASSIUM ANALYSIS

Potassium Analysis, as in Atomic Absorption Methods Manual, vol. 1, Standard Conditions for Flame Operation, by Robert H. Emmel, John J. Sotera and Ronald L. Stux. 1977.

Reagents

1) 6 N HCl

2) 0.5% Cesium Chloride

Procedure

1) Ash sample in muffle furnace at 600°C 8 hours or overnight.

2) Redissolve ash with 3 ml 6 N HCl.

3) Wash sample from crucible with glass dH_2O to a known volume (usually 25 ml).

4) Shake sample and allow to settle.

5) Dilute sample as necessary to read on AA, adding CsCl (0.5%) in a 1:5 ratio to the final dilution; that is, 2 ml CsCl in 10 ml final volume.

6) Read on AA.

Potassium is highly ionized in an air acetylene flame. By adding 0.1% solution Cesium chloride in final dilution, this ionization is greatly reduced.

APPENDIX F

SERUM SODIUM ANALYSIS

Sodium analysis, as in Atomic Absorption Methods Manual, vol. 1, Standard Conditions for Flame Operation, by Robert H. Emmel, John J. Sotera and Ronald L. Stux. 1977.

Reagents

1) 6 N HCl

Procedure

1) Ash sample in muffle furnace at 600°C 8 hours or overnight.

2) Redissolve ash with 3 ml 6 N HCl.

3) Wash sample from crucible with glass dH_2O to a known volume (usually 25 ml).

4) Shake sample and allow to settle.

5) Dilute sample as necessary to read on AA.

6) Read on AA.

APPENDIX G

SERUM CALCIUM AND MAGNESIUM ANALYSIS

Calcium and Magnesium analysis, as ain Atomic Absorption Methods Manual, vol. 1, Standard Conditions for Flame Operation by Robert H. Emmel, John J. Sotera and Ronald L. Stux. 1977.

Reagents

1) 6 N HCl

2) Lanthium oxide

Procedure

1) Ash sample in muffle furnace at 600°C 8 hours or overnight.

2) Redissolve ash with 3 ml 6 N HCl.

3) Wash sample from crucible with glass dH_2O to a known volume (usually 25 ml).

4) Shake sample and allow to settle.

5) Dilute sample as necessary to read on AA, adding La_3O_3 (0.5%) in a 1:5 ratio to the final dilution.

6) Read on AA.

APPENDIX H

BLOOD ACETOACETATE ANALYSIS

Acetoacetate analysis must be done on same day of sampling.

Reference: Williams, D. H. and J. Mellanby. 1965. Acetoacetate and D-(-)-beta-hydroxybutyrate. In: Methods of Enzymatic Analysis, 2nd ed. (Bergmeyer, H. V., ed.), p. 454. Academic Press, New York.

Reagents

1) Phosphate buffer (pH = 6.8, 0.1 M)

   a) 1.36 g KH_2PO_4 ——> 100 ml
   b) 1.74 g K_2HPO_4 ——> 100 ml

   Mix equal volumes of (a) and (b).
   Check pH, add (a) or (b) if necessary.

2) NADH (~ 1mM)
   Boehringer. Grade II, disodium salt, 97% pure.
   Catalog No. 128023.
   0.005 g ——> 6 ml
   Make up on day of use.

3) 3-hydroxybutyrate dehydrogenase
   Boehringer, catalog No. 127841.
   5 mg/ml.

4) Acetoacetate standards
   Sigma. Lithium salt. 90-95% pure.
   Stock solution 1.5mM
   0.01620 g ——> 100 ml
   Make up on day of use.
   Dilute to the following concentrations for the working standards:

   a) 0.05 mM ——> 2 ml 1.5 mM + 18 ml dH_2O
   b) 0.10 mM ——> 12 ml (a) + 8 ml dH_2O
   c) 0.075 mM ——> 4 ml (a) + 4 ml dH_2O
   d) 0.05 mM ——> 10 ml (b) + 10 ml dH_2O
   e) 0.025 mM ——> 10 ml (d) + 10 ml dH_2O

   Also use 0 mM as blank.

Procedure

1) Treatment of whole blood (carry out at 0-4°C)

   a) Mix 2 ml whole blood and 2 ml $HClO_4$ immediately after sampling.
   b) Centrifuge at approximately 5,000 g for 15 minutes.
   c) Neutralize excess $HClO_4$ with KOH so that final pH = 6-8.
   d) Centrifuge off the potassium perchlorate formed at 100 g for 15 minutes.
   e) Pour off supernatant.

   Final dilution of blood is approximately 2x.

2) Treatment of standards

   Standards must be taken through the entire procedure as small amounts of perchlorate do affect enzyme activity.

   a) Pipette into a graduated conical centrifuge tube:

      1. 1 ml standard + 1 ml 1 M $HClO_4$.
      2. Neutralize to pH = 6-8.
      3. Make volume up to 2.5 ml with dH_2O.
      4. Centrifuge 1000 g for 10-15 minutes.

3) Pipette into tubes:

   a) 0.5 ml buffer.
   b) 1.0 ml sample (for fed animal; 0.5 ml for fasted animal).
   c) 0.05 ml NADH

4) Read E_1 at 340 nm.

5) Add 0.005 ml ß-hydroxybutyrate dehydrogenase.

6) Incubate at room temperature for approximately 20 minutes.

7) Read E_2 at 340 nm.

Calculation

1) Calculate $E_1 - E_2$ for all standards, samples and blanks.

2) Calculate ΔE std = ΔE blk
ΔE sample - ΔE blk

3) Plot a standard curve of ΔE std versus concentration.

4) Read concentration of samples from curve.

5) Multiply by appropriate dilution factors.

APPENDIX I

BLOOD ß-HYDROXYBUTYRATE ANALYSIS

ß-hydroxybutyrate analysis samples may be stored at -20°C for at least one week.

Reference: Williams, D. H. and J. Mellanby. 1965. Acetoacetate and D-(-)-beta-hydroxybutyrate. In: Methods of Enzymatic Analysis, 2nd ed. (Bergmeyer, H. V., ed.), p. 454. Academic Press, New York.

Reagents

1) tris-HCl buffer (pH = 8.5, 0.1M)
   2.42 g tris ——> approximately 50 ml
   pH ——> 8.5 with 1 M HCl
   volume ——> 200 ml

2) Hydrazine-tris buffer
   Make up on day of use.
   2.5 ml hydrazine hydrate
   0.05 g EDTA
   12.5 ml 1 M HCl
   volume ——> 50 ml with tris-HCl buffer
   check pH = 8.5

3) NAD^+ (approximately 14 mM)
   Boeringer. Grade II. Free acid.
   98% pure. Catalog No. 127990.
   0.03 g ——> 3 ml

3) ß-hydroxybutyrate standards
   Calbiochem. Sodium salt. Enzymatic purity approximately 96%.
   Stock 2nM 0.0656 g ——> 250 ml
   Working standards - dilute to the following concentrations:

   a) 2.0 mM ——> 20 ml stock std. + 0 ml dH_2O
   b) 1.6 mM ——> 16 ml stock std. + 4 ml dH_2O
   c) 1.2 mM ——> 12 ml stock std. + 8 ml dH_2O
   d) 0.8 mM ——> 8 ml stock std. + 12 ml dH_2O
   e) 0.4 mM ——> 4 ml stock std. + 16 ml dH_2O

   Also use 0 mM as blank.

Procedure

1) Treatment of whole blood (carry out at 0-4°C)

   a) Mix 2 ml whole blood and 2 ml $HClO_4$ immediately after sampling.
   b) Centrifuge at approximately 5,000 g for 15 minutes.
   c) Neutralize excess $HClO_4$ with KOH so that final pH = 6-8.
   d) Centrifuge off the potassium perchlorate formed at 1000 g for 15 minutes.
   e) Pour off supernatant.

   Final dilution of blood is approximately 2x.

2) Treatment of standards

   Standards must be taken through the entire procedure as small amounts of perchlorate do affect enzyme activity.

   a) Pipette into a graduated conical centrifuge tube:
      1. 1 ml standard + 1 ml 1 M $HClO_4$
      2. Neutralize to pH = 6-8.
      3. Make volume up to 2.5 ml with d H_2O.
      4. Centrifuge 1000 g for 10-15 minutes.

3) Pipette into tubes:

   a) 0.25 ml sample (for fed animal; 0.1 ml for fasted animal)
   b) 0.75 ml dH_2O (for fed animal; 0.9 ml for fasted animal)
   c) 0.5 ml buffer
   d) 0.05 ml NAD^+

4) Read E_1 at 340 nm.

5) Add 0.005 ml ß-hydroxybutyrate dehydrogenase.

6) Incubate at room temperature for about 45 minutes.

7) Read E_2 at 340 nm.

Calculation

1) Calculate $E_2 - E_1$ for all standards, samples and blanks.

2) Calculate ΔE std - ΔE blk
ΔE sample - ΔE blk

3) Plot a standard curve of ΔE std versus concentration.

4) Multiply by appropriate dilution factors.

Note:

NAD^+ and hydrozine form a complex which absorbs at 340 nm. Therefore, a slow constant increase in absorbance occurs. This is similar to the acetoacetate assay but in reverse. So, pipette NAD^+ and enzyme and take to absorbance readings at timed intervals as described to the acetoacetate assay.

APPENDIX J

PLASMA INSULIN ANALYSIS

Insulin analysis - points to remember:

1) Keep all reagents and tubes on ice and cold as much as possible.

2) Calculate volumes of reagents needed before assay and mix accordingly to avoid waste.

3) Keep frozen reagents in a non-frost-free freezer.

4) BSA-PBS-EDTA should not be kept more than 4 weeks at 2-4°C.

5) GPA-BSA-PBS-EDTA should not be saved and used again after more than 2 or 3 days.

6) Use "scrupulously" cleaned glassware.

7) "Precision" pipetting is essential.

8) Pipette solutions directly to the bottom of culture tubes as much as possible.

9) Centrifuge using at least 1.5 g in a refrigerated centrifuge (2-4°C).

Reagents

1) NaH_2PO_4 - H_2O
Fisher Scientific Company

2) NaCl
Fisher Scientific Company

3) Disodium ethylene diamine tetraacetate (EDTA)
Fisher Scientific Company

4) Merthiolate (thimerosal)
Sigma Chemical Company

5) 5 M NaOH
100 g ——> 500 ml dH_2O

6) Bovine serum albumin (BSA)
RIA grade
Sigma Chemical Company

7) Guinea pig serum (GPS)
Research Products International (RPI)

8) Bovine pancreatic crystallin insulin
Sigma Chemical Company

9) Guinea pig anti-bovine insulin (1st antibody)
Miles Laboratory, Inc.

10) ^{125}I-insulin
Amersham Corporation

11) Goat anti-guinea pig IgG (2nd antibody)
Research Products International (RPI)

12) Borosilicate culture tubes
12 x 75 mm
Fisher Scientific Company

Reagent Preparation
(for 3 assays of 123 tubes each)

1) Phosphate buffered saline (PBS-EDTA)
Dissolve in 800 ml dd H_2O:
1.38 g NaH_2PO_4-H_2O
8.77 h NaCl
9.30 g EDTA - 2 H_2O disodium salt
Adjust pH to 7.6 with 5 M NaOH.
Add 100 mg thimerosal and bring to 1 L in a volumetric flask and store at 2-4°C.

2) 1% BSA-PBS
Add 2.5 g 1% BSA to cold PBS in a beaker and allow to dissolve (approximately 2-3 hours) while kept at 2-4°C.
After BSA is dissolved, readjust pH to 7.6 using 5 M NaOH.
Bring to desired amount in volumetric flask and store at 2-4°C.

3) 0.25% GPS - 1% BSA-PBS-EDTA
Add 250μl GPS to 1% BSA-PBS and bring to 100 ml in a volumetric flask.

4) 1st antibody

a) Reconstitute lyophilized guinea pig anti-bovine insulin according to manufacturers instructions.
b) Use 1 ml cold ddH_2O and then add 9 ml 1% BSA-PBS-EDTA to give a 1/10 dilution. This solution is then frozen in 0.15 ml aliquots (use polystyrene tubes).
c) 1/8000 dilution is used for the assay. To obtain this dilution, 0.05 ml of the 1/10 dilution is diluted to 40 ml using 1% BSA-0.25% GPS-PBS-EDTA.

5) Insulin standards

a) 81.63 mg of bovine insulin (Sigma #15500) was diluted in 500 ml 1% BSA-PBS-EDTA (at room temperature with 1-2 hr stirring) to yield a 4 U/ml solution. Our insulin lot contained 25.5 U/mg, therefore, 81.63 mg = 2000 U.
b) 1 ml of this 4 U/ml solution was then diluted to 100 ml in 1% BSA-PBS-EDTA to give a 40 mU/ml solution. The 40 mU/ml solution was frozen in 0.5 ml aliquots using liquid nitrogen and stored at -20°C for future use.
c) To prepare standard solution, 0.2 ml of the 40 mU/ml solution was diluted to 4 ml using 1% BSA-PBS-EDTA to give a 2 mU/ml solution. The following dilutions of the 2 mU/ml solution are then used to give solutions utilized for a standard curve:

Standard	2mU/ml solution	BSA-PBS-EDTA
200 μU/ml	1.0 ml	9.0 ml
120	0.6	9.4
80	0.4	9.6
40	0.2	9.8
20	0.1	9.9
12	0.06	9.94
8	0.04	9.96
4	0.02	9.98
1	0.005	9.995

200 μl of these solutions is then used in each standard tube.

6) Labelled insulin
Labelled insulin is diluted with 1% BSA-PBS-EDTA to give a solution containing enough ^{125}I-insulin so that approximately 10,000 cpm (counts per minute) are in a 100 μl sample.

7) 2nd antibody
RPI antibody is thawed and diluted 1/80 using 1% BSA-PBS. Currently, RPI antibody is frozen in 1 ml aliquots which can be diluted to 80 ml.

8) Guinea pig serum (GPS)
Guinea pig serum should be frozen in 400 μl aliquots.

Procedure

1) Day 1

a) Label culture tubes.
b) Mix 1% BSA with PBS-EDTA before use (usually the night before) and have its pH at 7.6
c) Thaw an aliquot each of 1st antibody, insulin standard and GPS. Prepare insulin standard solutions, GPS-BSA-PBS-EDTA (100 ml is sufficient for 3 assays) and 1st antibody as previously described.
d) Dispense BSA-PBS-EDTA and GPS-BSA-PBS into tubes as needed, standard solutions and plasma samples. Add 1st antibody to all but total and nonspecific binding tubes. Vortex tubes and incubate 24 hours at 2-4°C.

2) Day 2

a) Prepare labelled insulin.
b) Add 100 μl to all tubes.
c) Vortex.
d) Incubate 24 hours at 2-4°C.

3) Day 3

a) Prepare 2nd antibody.
b) Add 200 μl to all but total tubes.
c) Vortex.
d) Incubate 48 hours at 2-4°C.

4) Day 5

a) Centrifuge at 3 g for 30-35 minutes.
b) Pour off supernatant into waste bottle (quickly and gently) and place upside down in Kimwipes in a foil lined box for at least 15 minutes.
c) Wipe out the top half of each tube with a Kimwipe on a wooden applicator stick and count for one minute.

APPENDIX K

BLOOD VOLATILE FATTY ACID ANALYSIS

Blood volatile fatty acid analysis was reported by Quigley, III, 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. 47:258.

Reagents

1) H_2O-Triton
   20 μl Triton X-100 per 250 ml dH_2O

2) 1 N NaOH
   40.0 g NaOH per liter

3) 10 mM NaOH
   Make stock 1.0 N NaOH (40 g/l) and dilute 5 ml to 500 ml each day of use.

4) 1 N HCl
   83 ml per liter.

5) 16% H_3PO_4

6) $ZnSO_4$, 0.175 (5%)
   100g $ZnSO_4 \cdot 7\ H_2O$ (reagent grade, unefforvesced) dissolve in dH_2O to make 2 liters.

7) $Ba(OH)_2$, 0.15 M
   95g $Ba(OH)_2 \cdot 8\ H_2O$; dissolve in recently boiled and cooled dH_2O to make 2 liters.

   Always use highest purity water.
   $ZnSO_4$ and $Ba(OH)_2$ must exactly neutralize each other. To check:

   a) Pipette exactly 10 ml of $ZnSO_4$ solution to a flask containing about 40 ml dH_2O; add a few drops of phenophthalein indicator (0.5 g/dl in 95% ETOH).

   b) Titrate with $Ba(OH)_2$ solution, slowly and with constant agitation.

c) Titrate until 1 drop of $Ba(OH)_2$ solution turns the titrated solution a faint pink which lasts for 1 minute.
The 10 ml $ZnSO_4$ should require 10.0 ± 0.05 ml $Ba(OH)_2$.
Keep solutions tightly stoppered - $Ba(OH)_2$ will have a white precipitate of $BaCO_3$ with exposure to air.

8) Standards

a) Stock Blood Standard

Stock	mw	g/l	final concentration (mM)
Acetate	60.0	3.0	2.0
Propionate	74.0	0.9	0.5
Butyrate	88.1	0.9	0.4

b) Stock Internal Standard
0.762 g 2-ethylbutyric acid m2 = 116.16, qs to 100 ml dH_2O.

c) Working Internal Standard (ISTD)
Add 4 ml of each stock 2-ethylbutyric to 200 ml volumetric prior to use, qs to volume. Use 2 ml of ISTD per sample.

d) Working Blood Standard
Add 4 ml stock blood standard, 4 ml each. Stock ISTD to 100 ml volumetric. Add 16% H_3PO_4 and qs to volume. Make a working standard with each set of samples, as working ISTD is prepared.

Sample Preparation

1) Add 2 ml whole blood or plasma to 6 ml H_2O/Triton X.

2) Vortex; incubate at room temperature for 5 minutes.

3) Add 4 ml 0.3 N $Ba(OH)_2$.

4) Vortex; incubate at room temperature for 5 minutes.

5) Add 4 ml 5% $ZnSO_4$.

6) Vortex; incubate at room temperature for 5 minutes.

7) Centrifuge for 15 minuts at 15,000 x g.

8) Pour supernatant fluid into 2nd tube containing 2 ml ISTD.

9) Vortex.

Ion Exchange Columns

1) Materials

   a) AG 50 W-X 8 - 100 - 200 mesch (cation; top)
   b) BIO-REX 5 - 100 - 200 mesh (anion; bottom)
   c) Polypropylene Econo-Column catalog number 731-1550 (Bio-Rad Laboratories, Richmond, CA 94804).

2) Pack columns by preparing a slurry of resin in dH_2O. Allow resin to settle and pour off fines 1 to 3 x.

3) Agitate slurry by swirling and pipette into columns, CATION - 1.8 ml resin; ANION - 0.6 ml resin.

4) Prepare columns in following manner:

   Cation:
   a) Fill reservoir (10 m) with 1 M NaOH once (stir resin).
   b) Rinse twice with dH_2O.
   c) Fill reservoir with a M HCl twice.
   d) Rinse twice with dH_2O (fill reservoir).

   Anion:
   a) Fill reservoir twice with 1 N NaOH (stir resin with first NaOH).
   b) Rinse twice with dH_2O.

5) Always leave columns in H_2O overnight. Never allow to dry out.

Procedure

1) Using pasteur pipette, stir up each column immediately after unplytting the outlet prior to use. This will prevent air bubbles and promote an even flow through columns. After dH_2O has dripped through, place cation column over anion column.

2) Pour supernatant + ISTD into top of cation column, allow to drip through both columns, simultaneously and completely. NOTE: All supernatant will not go on column at once.

3) Rinse tubes twice with 2 ml dH_2O and pour onto columns. Let each rinse drop through completely before adding the next.

4) Rinse cation (top) column once with 2 ml dH_2O.

5) Remove cation column.

6) Rinse anion (bottom) column with w ml dH_2O.

7) Add 10 ml 10 mM NaOH to anion column and collect in 20 x 150 mm screw top tubes or 50 ml beakers.

8) Place beakers in forced-air oven (50°C) and allow to dry.

9) Reconstitute in 1.0 ml o-phosphoric acid (109 ml/l).

10) Transfer to GC vials.

Regeneration of ion exchange columns.

Must regenerate prior to and following each run.

1) Cation (top) column

   a) Fill column with 1 M NaOH once - stir up resin.
   b) Rinse twice with dH_2O.
   c) Fill column with 1 M HCl twice.
   d) Rinse twice with reservoir dH_2O.

2) Anion (bottom) column

a) Fill column twice with 1 N NaOH - stir up resin with first NaOH,
b) Rinse twice with dH_2O.

Chromatography

1) Column and Packing:

2 m x 2 mm glass column placked with 80/120 Carbopak B-DA 4% Carbowax 20 M (Supelco, Inc., Bellefonte, PA).

2) Carrier gas:

N_2, 24 ml/min.
Column is maintained at 175°C, inlet and detector are 200°C.

3) Run time is 30 minutes.

APPENDIX L

RUMINAL VOLATILE FATTY ACID ANALYSIS

Reagents

1) Working Internal Standard (ISTD)
353 ml 85% H_3PO_4
5.5106 g 2-3thylbutyric acid
1641 ml dH_2O

Sample Preparation

1) Freeze raw ruminal fluid immediately in liquid nitrogen to slow NH_3 escape.

2) Frozen samples stable for at least one week.

Procedure

1) Thaw ruminal fluid.

2) Centrifuge in refrigerated centrifuge at 10,000 x g for 10 minutes.

3) Remove 2.5 ml aliquots from supernatant and place in labelled tube.

4) Add 1 ml ISTD to each sample.

5) Vortex

6) Incubate in cooler for 30 minutes.

7) Centrifuge at 13,000 x g for 15 minutes.

8) Pour supernatant off into a labelled snap-top vial. Do not transfer any precipitated protein. Fill vial approximately 3/4 full.

9) Freeze sample until future use.

10) Thaw sample.

11) Recentrifuge sample if protein is present.

12) Transfer sample to G.C. vials and cap.

13) Inject sample into G.C. (gas chromatograph)

Column Packing

1) Use a 6" glass-coiled or 2 mm U-shaped column.

2) Packing material = SP12000.

3) Attach the column to a vacuum line with tygon tubing (use a safety flash between the column and vacuum outlet).

4) Unused columns should be washed and dried with acetone under vacuum. Used columns should be washed with an acid cleaning solution, dH_2O, and acetone, repectively.

5) After drying, put a glasswool plug in one end; attach this end to vacuum line.

6) Fit opposite end of column with a small funnel using a short piece of tygon tubing.

7) Apply vacuum and pour packing material into funnel until no more is taken up by vacuum. Gently tap column with a rubber stopper along its entire length to ensure uniform packing. Continue tapping column and add more packing material until the desired amount is reached, i.e., within 5-6 inches of the top of injection end of the column.

8) Remove column and place a glass wool plug in the injection side of the column.

9) Place a column tag with type of packing and an arrow pointing the direction of the carrier gas flow on it (injection ——> detector).

10) Cure column in GC for 72 hours at a carrier gas flow equal to or greater than the expected determination use (30-60 cc/minute).

Standard Preparation

Molecular weight	Acid	g/l
60 g	Acetic acid	3.5211
74	Propionic	3.5308
88	Isobutyric	0.2706
88	Butyric	1.5023
102	Isovaleric	0.3583
102	Valeric	0.5474
116	Caproic	0.5192
116	2-ethylbutyric	1.8309

Calculation

1) For each acid take:

$$\frac{\text{actual g/l wt}}{\text{molecular wt.}} \times 1000$$

which gives mm/l or μm/ml.

2) For calculations:

$$\frac{\text{Std 2-EB}}{\text{Sample 2-EB}} = \text{2-EB correction factor}$$

3) For each acid:

$$\frac{\text{Sample x 2 EB correction factor}}{\text{std acid}} \times \text{calculated acid value}$$

Calculated acid values utilized are as follows:

A = 58.7941
P = 47.3918
IB = 3.1431
B = 11.6517
IV = 3.5205
V = 5.0764

APPENDIX M

RUMINAL AMMONIA ANALYSIS

Ruminal ammonia analysis follows outline defined in Urea-nitrogen kit No. 640. Sigma Chemical Co., St. Louis, MO.

Reagents

1) Phenol nitroprusside solution
phenol, 60 g/L, sodium nitroprissued and stablizier. Store in refrigerator [2-6°C].

2) Alkaline hypochlorite solution
Sodium hypochlorite, 0.2%, in alkali.
Store in refrigerator [2-6°C].

3) Ammonium chloride
Fisher standards, 1000 ppm.

Standard Preparation

1) Assume 1000 ppm as NH_3
1,000 mg NH_3 per liter; which equals 100 mg NH_3 per 100 ml.

2) To prepare standards:

	NH_3 Standards	dH_2O	Concentration
Add:	5 ml	5 ml	50 mg/100 ml
	2.5 ml	7.5 ml	25 mg/100 ml
	1.25 ml	8.75 ml	12.5 mg/100 ml
	.625 ml	9.375 ml	6.25 mg/100 ml

Procedure

1) Label duplicate tubes for blank, standard, rumen samples.

2) Add 900 µl saline + 100 µl dH_2O for blank, 100 µl for standards and 100 µl ruminal fluid in appropriate tubes.

3) Vortex.

4) Add 100 µl above dilution to second set of labeled tubes.

5) Add (in following order):

   a) 0.5 ml phenyl nitroprusside; vortex
   b) 0.5 ml alkaline hypochlorite; vortex
   c) 2.0 ml dH_2O; vortex

6) Allow color to develop at room temperature for 20-30 minutes.

7) Read absorbance at 570 nm.

Calculation:

$$NH_3\text{-}N = \frac{[A]\ \text{sample}}{[A]\ \text{standard}} \times 30 - \frac{5}{6}$$

APPENDIX N

SURGICAL PROCEDURE

PRE-OPERATIVE CARE AND ANESTHESIA

Calves were fasted 10 to 12 hours prior to surgery to prevent bloat, emesis, and other gastrointestinal distrubances. Water was available at all times.

Prior to surgery, calves were equipped with a face mask and initially anesthetized with halothane-oxygen mixture (5% halothane @ 2.0 l/min O_2). Immediately following loss of mandible tension and swallowing reflex, calves were intubated with an endotracheal tube through which surgical level anesthesia was introduced and maintained (2% halothane @ 2.0 l/min O_2). Determination of surgical level anesthesia was made by lack of pedal reflex, pupil dilation, eye position, and, finally, lack of response to scapel.

ABDOMINAL SURGERY: PLACEMENT OF CANNULA

Calves were placed in a left lateral recumbency and prepared for asceptic surgery. The area between the last rib and the sixth lumbar vertebra midway to the linea alba was clipped, shaved, scrubbed with iodine, and rinsed with alcohol. An incision approximately 5 cm in length was made starting caudal to last rib. Indision continued through skin, muscle layers, and into the peritoneal cavity. Dorsal sac of the rumen was located, grasped with clamps, and exteriorized through the incusion. An incision approximately 2 cm in length was made into the dorsal sac of the rumen and cannula inserted. A purse-string sugure of #0 degradable catgut will close ruminal incision. Cannula was held in place in the surgical opening, and layers were closed with #0 non-degradable silk in inverse order. External washer was placed over cannula barrel and secured in place with hose clamp. Cannula opening was sealed with screw cap.

POST-OPERATIVE CARE

Following the surgical procedure, calves were placed on sternum and closely supervised until able to stand without assistance. Combiotic (5 ml) was administered immediately following surgery and at 24 hour intervals for 4 days thereafter. Furacin past with a milk analgesic was applied along the suture line once daily until the sutures

were removed beginning at the time of surgery. Incision area was cleaned daily. Sutures were removed 10 days after surgery. Calves were moved to metabolism facility following surgery to begin experiment.

DISPOSITION OF CALVES

At the termination of this experimental protocal, all cannulas were removed and calves were returned to Knoxville Dairy where they were used for follow-up studies and disposed by farm manger.

VITA

Suzette Inlow Boehms was born on November 2, 1965 in Dyersburg, Tennessee. Her parents are John Benton Inlow and Sue Kennedy Inlow. She attended elementary school at Lara Kendall in Ridgely, Tennessee and Margaret Newton in Tiptonville, Tennessee and graduated from Lake County High School in Tiptonville in 1984. She then attended Middle Tennessee State University in Murfreesboro for two semesters. She transfered to the College of Agriculture, Department of Animal Science at The University of Tennessee at Martin where she graduated with a Bachelor of Science degree in 1988. In Fall of 1988, she began her graduate studies at The University of Tennessee, Knoxville. While attending The University of Tennessee, Knoxville, she worked as a graduate teaching assistant and gained valuable experience in the classroom. She received the Outstanding Teaching Award for the College of Agriculture in 1991. On May 26, 1990, she married John Philip Boehms, son of James H. and Lyla Boehms of Knoxville, Tennessee.

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