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D(-)LACTATE METABOLISM IN BEEF CATTLE

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Introduction

Acidosis, the condition in ruminants resulting from an overconsumption of readily fermentable carbohydrate, has been reviewed extensively (MacKenzie, 1967; Dirkson, 1970; Dunlop, 1972; Brent, 1976; Elam, 1976; Huber, 1976; Cuonotte and Prins, 1979; Howard, 1981). Dunlop and Hammond (1965) defined the condition D(-)lactic acidosis based upon their data implicating D(-)lactate as the causative agent. They suggested that the systemic acidosis was the result of a buildup of D(-)lactate in the blood due to its apparent slow rate of metabolism.

Briefly, the condition is characterized by a rapid decline in rumen pH, following carbohydrate ingestion, with an accompanying rise in total rumen lactate (Dunlop and Hammond, 1965). The increased rumen lactate levels are apparently the result of lactate production exceeding rates of utilization, absorption and passage (Prins and Lankhorst, 1976). Increased rumen lactate levels are prevented on a practical basis by gradual increases in the intake of fermentable carbohydrate to enable lactate utilizing bacteria to become established. Several authors have reported on the microbial changes associated with high concentrate feeding (Allison et al., 1975; Huber et al., 1976; Mackie and Gilchrist, 1979; Mackie and Heath, 1979) and the subject has been reviewed (Schwartz and Gilchrist, 1975; Slyter, 1976).

A clinical sign of acidosis and one of major concern to the beef industry is anorexia. Tremere et al. (1968) reported that rumen acidity was not the sole influence in preventing animals from going off feed, whereas Fulton et al. (1979) reported that steers infused with hydroxides consumed significantly more feed, suggesting that rumen acidity plays a major role in intake regulation.

The role D(-)lactate plays in digestive disturbances has been depicted as detrimental, but the ability of the beef animal to metabolically eliminate D(-

)lactate has not been critically evaluated. Giesecke and Stangassinger (1979) demonstrated that in goats metabolism could account for 50 to 60% of the total elimination of D(-)lactate. The work reported herein was an attempt to better characterize the role of D(-)lactate in the beef animal as to dietary influences, to estimate the potential for its elimination metabolically, and to characterize its role in both acute and subacute digestive disturbances.

Review of Literature

Ruminal Lactate Production and Utilization

The proportion of D versus L lactate produced within the rumen appears to be under no defined regulatory mechanism, but rather is dependent upon time of sampling and diet fed. Giesecke et al. (1976) reported ratios of D:L ranging from 3:1 to 1:2 in sheep dosed intraruminally with various sugars. Results from in vitro incubations revealed that glucose, fructose and sucrose supported the greatest lactate production, with D production exceeding L (Prins and Lankhorst, 1976), whereas Lee and Matrone (1971) reported greater L production from diets supplemented with sodium or potassium bicarbonate. Studies with sheep (Giesecke and Geiges, 1974) demonstrated that as corn was added to a hay diet to reduce rumen pH from 6.5 to 5 the activity of bacterial amylase increased. This increased amylase activity results in an accumulation of glucose in the rumen (Ryan, 1964a,b). The glucose accumulation leads to a rapid fermentation and lactate accumulation. Prins and Lankhorst (1976) determined that an inhibition of methanogenesis and the resultant H_2 accumulation would enhance lactate production. Cuonotte et al. (1980) investigated factors controlling lactate production and found high NADH/NAD ratios to be the major influence favoring lactate formation. They postulated that the formation of NADH from NAD was an attempt to control rumen H^+ concentrations, thus as rumen pH declined it favored more lactate production. The major inhibitor of lactate production via the glycolytic scheme was found to be ATP (Cuonotte et al., 1980), so when a fermentation shifts from volatile fatty acid production to lactate, the decrease in ATP formation per mole glucose fermented would favor lactate production, and decreases in volatile fatty acid production do correspond with increased lactate production (Ryan, 1964a; Reid et al., 1957; Lee, 1977). The controlling factors in

rumen fermentation appear to favor lactate accumulation once a rapid fermentation is initiated. Lactate accumulation is also favored by catabolite repression of some species of lactate utilizing bacteria. The organism Selenomonas ruminantium has been shown to ferment glucose, sucrose and xylose preferentially over lactate (Hishinuma et al., 1968; Russel et al., 1979).

The role of lactate as an intermediate in volatile fatty acid production has been characterized. Heuter et al. (1956) demonstrated that lactate addition resulted in increases in rumen volatile fatty acids and Jayasuriya and Hungate (1959) reported that the total amount of substrate fermented via lactate was insignificant on roughage diets (.8%) but increased to 13% in the grain fed animal. Nakamura and Takahashi (1971) determined that 40% of acetate and 32% of propionate were formed via lactate when sheep were fed high concentrate diets. Baldwin et al. (1962) established the presence of two pathways of propionate formation from lactate, a randomizing route which proceeds via succinate and a nonrandomizing one which proceedes via acrylate. The contribution of the acrylate route appeared to increase as did carbohydrate availability in the diet. Satter and Esdale (1968) demonstrated that acetate was the major volatile fatty acid produced in the hay fed animal, but propionate production increased with lactate availability in the diet and that butyrate formation from acetate was obligatory with lactate fermentation in order that redox balance be maintained. The organism Megasphera elsdenii which ferments lactate via the acrylate pathway was studied to determine its contribution to total lactate utilization (Counotte et al., 1981). On roughage diets M. elsdenii fermented an average of 74% of the total lactate to propionate and butyrate. The contribution of M. elsdenii to total lactate utilization was increased as soluble sugars were increased in the diet due to catabolite repression of other

organisms. Increases in propionate production due to methane inhibitors resulted in a small increase in the contribution of the acrylate pathway but these increases were not totally responsible for the increase in propionate production (Prins and Van der Meer, 1976).

Attempts have been made to enhance lactate utilization in the rumen and thereby shorten the period of adaptation to high concentrate feeding. Lactic acid addition to the diet (Kunkle et al, 1976; Huntington and Britton, 1978, 1979) enhanced rates of in vitro lactate disappearance but these increases were not sufficient to prevent digestive disturbances or improve feedlot performance (Kunkle et al., 1976b). No differences were observed in the rate of in vitro lactate disappearance for either L or D-lactate (Ogimoto and Giesecke, 1974; Huntington and Britton, 1978, 1979; Byers and Goodall, 1979).

Alterations in rumen lactate accumulation have been achieved through the use of antibiotics. Dennis et al. (1981) reported that both monensin and lasalocid were effective in reducing lactate production and rate of fermentation from a variety of substrates, but both were more effective in the prevention of L production than D. Beede and Farlin (1979a) reported monensin to be ineffective in prohibiting lactate production but bacitracin, capreomycin disulfate, novobiocin and oxamycin were effective in decreasing lactate production. Confirming results were obtained in vivo with sheep for capreomycin disulfate and oxamycin (Beede and Farlin, 1977b), but lasalocid and monensin were effective in preventing digestive disturbances in cattle (Nagaraja et al., 1981). Muir and Baretto (1979) screened several of the sulfur containing peptide antibiotics, namely thiopeptin, sulfamycin and thiostrepton and found them to be selective for the organism Streptococcus bovis, one of the early, rapid lactate producers. Thiopeptin has proven useful in the prevention of acidosis in sheep (Muir et al.,

1980a,b) and cattle (Muir et al., 1981) and appears to prevent lactate production without depressing volatile fatty acid production. Nagaraja et al. (1982) concluded that monensin and lasalocid were equal to thiopeptin in their ability to prevent acidosis. Whether antibiotic addition as a prophylactic measure can be economically sound and withstand the pressures of society against antibiotics in animal feeds remains to be seen.

Lactate Absorption From the Rumen

Heuter et al. (1956) suggested that lactate was absorbed into the blood stream of sheep dosed intraruminally with lactate while Giesecke (1968) reported that elimination of lactate infused into the rumen of sheep could be accounted for as rumen absorption (57%), fermentation (34%) and passage (9%). Dunlop and Hammond (1965) inferred that there were no differences in the rate of D versus L absorption from the rumen. Factors influencing the rate of lactate absorption from the washed out sheep rumen were investigated (Williams and MacKenzie, 1965) and the rate of lactate absorption was proportional to concentration and inversely proportional to pH and tonicity. Rate of absorption was decreased by the addition of a mixture of volatile fatty acids at acid pH but not at alkaline. The rate of absorption of volatile fatty acids exceeded that of lactate in both acid and alkaline conditions. These authors concluded that the factors influencing lactate absorption were consistent with properties of diffusion through a lipid bilayer membrane.

The net portal absorption of D and L lactate were determined in sheep (Huntington et al., 1980) and cattle (Huntington et al., 1981) fed either all roughage or an 85% concentrate diet. Net absorption of D or L lactate did not increase in either set of animals when adapted to the 85% concentrate diet nor was there an increase in the A-V difference of D or L lactate across the gut in

sheep switched directly to an all concentrate diet. These authors suggested that perhaps total acid absorption (D and L-lactate and volatile fatty acids) was more beneficial in assessing the systemic acid insult to the animal than either D or L-lactate.

L-lactate Metabolism

The role of L-lactate to ruminant metabolism has been extensively studied in sheep and its significance appears very responsive to dietary influences. Turnover rates of L-lactate (mMole/hour per kg body weight) were .7 and 1.1 for fasted and fed sheep respectively (Annison et al., 1963) and rates were 78% greater in sheep fed ad libitum an 80% concentrate diet as compared to those fed at maintenance (Prior, 1978). Huntington et al. (1980) reported a 33% increase in L-lactate turnover for lambs fed an 85% concentrate diet versus lambs fed alfalfa hay. Turnover rates vary from .8 for fasted sheep (Annison et al., 1963) to 2.2 mM/hour per kg body weight in growing lambs (Prior and Christenson, 1977). A comparison of values based on metabolic body size ($kg^{.75}$) revealed similar turnover rates for sheep and cattle fed alfalfa hay (2.79 vs 2.79 mmol/h per $kg^{.75}$ for sheep and cattle respectively) but were higher for cattle when both were fed an 85% concentrate diet (3.79 vs 5.29 mmol/h per $kg^{.75}$ for sheep and cattle respectively). Reilly and Chandrasena (1978) determined that the production of lactate from glucose and glucose from lactate were both dependent upon the arterial concentrations of lactate and glucose respectively. Estimates of the contribution of lactate to glucose synthesis range from 5% in sheep (Annison et al., 1963) to 10% of the total glucose in lambs fed alfalfa hay (Huntington et al., 1980). The percentage of glucose derived from L-lactate decreased from 10 to 5.5 when lambs were switched from alfalfa hay to an 85% concentrate diet (Huntington et al., 1980). The decrease in the percent of

glucose derived from lactate did not occur when steers were subjected to the same diet switch (Huntington et al., 1981). Prior (1980) determined that nearly 50% of the glucose turnover was synthesized from lactate in the ovine fetus by 123 days of gestation.

Estimates on origins of the lactate pool include 50% produced from glucose (Annison et al., 1963), 12 to 13% from absorption and production by the gut (Weekes and Webster, 1975; Huntington et al., 1980) and the remainder produced endogenously. Baird et al. (1975) determined that in nonlactating dairy cows the gut would supply approximately 50% of the hepatic lactate utilization. Hepatic lactate uptake was stimulated by fasting and lactation in dairy cows (Baird et al., 1980) and decreased by propionate infusion in the lactating animal, while lactate infusion had no effect on propionate uptake.

The utilization of L-lactate for fatty acid synthesis in bovine adipose tissue has been demonstrated (Whitehurst et al., 1978). Rates of lactate utilization increase with age (Whitehurst et al., 1981) and are influenced by the level of feed intake (Prior, 1978). Prior and Jacobson (1979) reported that acetate incorporation into fatty acids was stimulated by L-lactate but not D, and that acetate inhibited lactate incorporation into fatty acids. Lactate utilization in ruminant adipose tissue appears to involve a functional ATP-Citrate lyase : NADP-Malate dehydrogenase pathway (Smith and Prior, 1981; Prior et al., 1981) and a functional alpha glycerol phosphate shuttle (Yang et al., 1982).

D(-)lactate metabolism

Differences in the utilization of D-lactate were first described in studies dealing with glycogen synthesis in rat liver (Cori and Cori, 1920). Studies using heart and liver slices from the rat and duck demonstrated that L-lactate was utilized at 3 to 5 times the rate of D-lactate (Brin et al., 1952). Tubbs and

Greville (1961), utilizing liver and kidney mitochondrial preparations from rabbits, reported that D was oxidized at a faster rate than L. These workers partially isolated an enzyme, D-2-hydroxyacid dehydrogenase (EC. 1.1.1.27) which was later purified and studied (Cammack 1969, 1970). The enzyme was shown to oxidize D-lactate to pyruvate, similar to L-lactate dehydrogenase, but differing in its cellular location. D-2-hydroxy acid dehydrogenase is mitochondrial whereas L-lactate dehydrogenase is located within the cytosol thus D-lactate must penetrate the mitochondria to be metabolized.

In a study comparing D-lactate utilization by the rat and rabbit (Giesecke et al., 1981) the rats oxidized a much greater percent (85%) of their dose of $[U\text{-}^{14}C]$ D-lactate than did rabbits (44%). The authors suggested that rabbits were similar to ruminants in their ability to oxidize D-lactate. In a previous report (Giesecke et al., 1980) D-lactate had been identified as a physiological isomer in the rat and that the major site of production was fermentation within the gut. Other workers (Brandt et al., 1982) feel that D-lactate is the product of methyl glyoxal metabolism via the glyoxylase system. Both of these sources may indeed contribute to the production of D-lactate in the rat, but the relative contribution of each has not been determined.

D-lactate produced from within the gut has been the only source identified in ruminants. While D-lactate metabolism in ruminants has not been extensively studied, the subject has been reviewed (Giesecke and Stangassinger, 1980). Experiments investigating the distribution of D and L-lactate in blood demonstrated that D produced a plasma level 2.5 times that of L when infused intravenously into sheep (Braide and Dunlop, 1969). Half-lives for D-lactate elimination increased from 25 to 160 minutes as D-lactate concentrations increased from .1 to 10 mM (Giesecke and Stangassinger, 1978). Estimates of the

amount of D-lactate metabolized by various tissues have been equivocal. Little or no utilization of D-lactate was reported in tissues from the digestive tract of cattle (Preston and Noller, 1973) or liver slices from sheep (Hinkson et al., 1967; Huber, 1969), but rates of CO_2 production from D-lactate varied from 117 to 51% of the rate of L-lactate in slices of goat rumen epithelia (Prins et al., 1974). Giesecke and Stangassinger (1976) observed 20 fold higher rates of oxidation of D-lactate for heart and kidney cortex as compared to rumen epithelium of sheep. Experiments with goats (Giesecke and Stangassinger, 1979) demonstrated that oxidation and gluconeogenesis could account for 50 to 60% of the total elimination of D-lactate at plasma levels of 3 mM while contributing 12 to 13% of the total glucose. No experiments have been conducted to ascertain the potential for metabolic elimination of D-lactate in cattle. Present data report only rates of utilization by tissues of the gut. The capacity of other tissues to metabolize D-lactate as well as the amount of D-lactate that is actually produced by the gut is information that is certainly lacking and is needed to evaluate the ability of cattle to metabolically eliminate D-lactate.

Net Portal Absorption

The ruminant animal provides a rather unique situation when considering nutrient availability to the animal. Rumen fermentation, albeit advantageous in the utilization of dietary fiber, produces a multitude of dietary end products for subsequent absorption and metabolism. Estimates of the composition of materials absorbed into the bloodstream is paramount in understanding and manipulating rumen fermentation. The net portal absorption technique invokes the Fick principle, which states that uptake or release by an organ of any substance is equal to the product of blood flow through that organ and the concentration difference of that substance as it passes through that organ (Webster,

1974). The term net absorption is applied since the material measured in venous blood may not have been absorbed from the gut, but rather a product of gut metabolism or the net remaining after gut metabolism. The determination of net absorption involves the sampling of arterial blood, the blood supplying the gut and a sample from the hepatic portal, the common vessel draining the gut. The concentration difference of these two samples times the blood flow in the portal vein gives an estimation of net absorption or utilization by the gut. Numerous procedures and approaches for the cannulation of the major vessels have been published for sheep (Katz and Bergman, 1969a; Yelverton et al., 1969) and cattle (Waldern et al., 1963; Olsen et al., 1967; McGilliard and Thorp, 1971; Symonds and Baird, 1973). McGilliard (1972) published an excellent summary of techniques and recommendations for catheter implantation and maintenance. Webster (1974) reviewed the theory and current practices for determining net portal absorption as did Bergman (1975) who included a summary of current knowledge on metabolite production and utilization.

The major difference in approaches to determining absorption from the gut lies in the means of estimating blood flow. Approaches have included thermo-dilution (Bensadoun and Reid, 1962; Bensadoun et al., 1962; Fegler and Hill, 1958; Webster and White, 1973), Doppler shift and telemetry (Carr and Jacobson, 1968; Sniffen and Jacobson, 1975) and isotope or dye dilution (McGilliard et al., 1971; Wangsness and McGilliard, 1972a,b; Katz and Bergman, 1969b). Wangsness and McGilliard (1972a) evaluated the dye dilution technique and measured the recovery of ^{14}C-glucose infused into the duodenum. Recoveries of ^{14}C for 7 experiments averaged 104% (range 89.1 to 114.4%). The authors concluded that blood flow measurements were most critical for accurate absorption results. Of the dyes chosen, p-aminohippuric acid has received the most attention. The

compound is cleared rapidly by the kidneys and therefore lends itself to continuous infusion. This technique has been used successfully in sheep (Bergman and Wolff, 1971; Tagari and Bergman, 1978; Heitman and Bergman, 1980a,b; Huntington et al., 1980), beef cattle (Prior et al., 1981; Huntington et al., 1981) and dairy cattle (Baird et al., 1975, 1979, 1980; Lenox et al., 1979; Huntington, 1982). The neglect in beef cattle is most evident and, as has been demonstrated in other species and classes of ruminants, the characterization of nutrients that are absorbed from the gut can provide invaluable information.

Glucose Metabolism

The topic of glucose metabolism has received much attention in ruminants and is a topic much too encompassing to cover in detail here. Excellent reviews are available for more detailed information (Leng, 1970; Bergman, 1973; Young, 1977). The purpose of this treatise is to review dietary and physiologic influences that have been shown to influence glucose metabolism. Much of the work on glucose metabolism has dealt with whole body metabolism of various radiolabelled forms of glucose. To begin one must first review the terminology that has evolved with these techniques of studying glucose metabolism. Bergman (1963) defined glucose (or other metabolite) turnover as the rate of movement of carbon atoms into and out of the glucose pool at a constant plasma glucose concentration. It is designed to represent both the production and utilization of glucose under steady state conditions. Perhaps a more frequently encountered term is irreversible loss. Judson and Leng (1972) defined irreversible loss as the rate at which a substance leaves the sampled pool never to return. Provided that they are determined by a common means (isotope chosen, molecule position and means of administration) and under steady state conditions, the terms turnover

and irreversible loss are synonymous and equivalent to another expression, entry rate. A final term, total entry rate, is defined as the rate of entry of all defined substance into a sampled pool (Judson and Leng, 1972). Total entry rate represents the sum of irreversible loss plus recycling. Recycling is the exit and reentry of a substance to the sampled pool without loss of its radioactive label, thereby providing an underestimation of the amount of actual utilization. For example, when studied using $[U\text{-}^{14}C]$-glucose, glucose is metabolized to lactate and resynthesized to glucose, thereby providing an underestimation of the actual glucose utilization. The situation can be resolved, however, through the use of combinations of isotope in various labelled positions; estimates of irreversible loss (turnover), total entry rate and recycling have been determined simultaneously (Muramatsu et al., 1974; Young et al., 1974; Herbein et al., 1978; Van Maanen et al., 1978; Buckley et al., 1982).

A summary of much of the early work in sheep (Bergman et al., 1974) reveals that glucose turnover is very responsive to changes in nutritional and physiologic state. Turnover rates increased from 3 to 4.6 g/hour for fed sheep as compared to fasted. Similar results were obtained in steers that were fed and fasted (Young et al., 1974). Turnover rates in sheep ranged from 13.3 for the lactating ewe to 7.5 for the twin pregnant ewe which declined to 4.6 g/hour when they were fasted. Wiltrout and Satter (1972) reported glucose turnover rates of 57 and 105 g/hour for dry and lactating cows respectively. Confirming responses to lactation have been reported in ewes (Gow et al., 1981) and goats (Buckley et al., 1982). Muramatsu et al. (1974) reported a rapid decline in glucose turnover for lambs from 5 to 21 days of age. Turnover rates adjusted for body weight remained 3 fold higher for 21 day old lambs as compared to adult sheep. Monensin, an antibiotic that increases ruminal propionate production, was shown