Malate Content of Forage Varieties Commonly Fed to Cattle

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ABSTRACT

The objective of this study was to determine the concentration of malate in forage varieties at different stages of maturity. Five alfalfa varieties (Alfagraze, Apollo Supreme, Cimarron, Crockett, and Magnum III) and three bermudagrass varieties (Coastal, Tifton-78, and Tifton-85) were collected at different stages of maturity. Samples were collected from replicate plots (n = 3) of each alfalfa variety at 9, 18, 28, 35, and 42 d of maturity; bermudagrass hay samples were composited from six bales of each variety from two cuttings staged to be harvested at 27 and 41 d of maturity. Malate was extracted from the samples and quantitated by high performance liquid chromatography using an organic acid column. As maturity increased, the concentration of malate declined in both plant species. Concentrations of malate were numerically higher in two alfalfa varieties (Crockett and Magnum III) at 35 and 42 d of maturity than in all other alfalfa varieties. Concentrations of malate in bermudagrass at 41 d of maturity were lower than concentrations of malate in all alfalfa varieties at 42 d of maturity. Malate declined as maturity increased in the Coastal and Tifton-78 varieties. Because malate stimulates the utilization of lactate by the predominant ruminal bacterium *Selenomonas ruminantium*, some of the benefits associated with alfalfa in the diets of dairy cattle may be due to the malate in this forage.
(**Key words**: malate, forage, rumen, microorganisms)

INTRODUCTION

The dicarboxylic acid, malate, stimulates the uptake of lactate as much as 10-fold by the predominant ruminal bacterium *Selenomonas ruminantium* (13, 14, 15, 16, 24). Concentrations of malate between 0.03 and 10 m*M* increased the uptake of lactate in a dose-response manner (14). Based on our observation, the low concentrations of organic acids (including malate) other than VFA in ruminal fluid may potentially limit the growth of *S. ruminantium*. When mixed ruminal microorganisms were incubated in a medium that contained cracked corn or soluble starch, malate treatment decreased concentrations of lactate and increased final pH (2, 12). Those results suggested that increased dietary concentrations of malate might help reduce problems associated with ruminal acidosis by stimulating lactate utilization by *S. ruminantium*.

Plants are a rich source of nutrients that can be utilized by both the ruminant animal and the mixed ruminal microbial population. Intermediates of the citric acid cycle accumulate in plant tissue and may represent as much as 10% of the DM of grasses (22). Malate can constitute up to 1.5% of the DM of mature grasses (22). Inclusion of malate as a feed additive in the diets of ruminants is currently not economically feasible; however, forages that are high in organic acids might provide a vehicle for the inclusion of malate in the diets of ruminants. Therefore, the objective of this study was to determine the concentrations of malate in five alfalfa varieties and three bermudagrass hay varieties at different stages of maturity.

MATERIALS AND METHODS

Samples of alfalfa (*Medicago sativa*) were collected from a test plot at the University of Georgia Plant Sciences Farm (Watkinsville). Samples were randomly taken from within a 1.5-m × 4.6-m plot of alfalfa by clipping the plants 2 cm above the soil surface on d 9, 18, 28, 35, and 42 from triplicate plots (n = 3) of each variety [Alfagraze (WA-89), Apollo

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Supreme, Cimarron, Crockett (84634), and Magnum III] from late June to August 1994. The alfalfa stand was 4 yr old, and the soil type was a Cecil sandy clay loam (clayish, kaolinitic, thermic, Typic Kanhapludult; pH 6.2). A soil test taken in February 1994 indicated that available P and K measured 88 and 194 kg/ha, respectively. No additional fertilizer was applied. The mean temperature and daylength from June 20 to August 1 ranged between 25.0 and 25.6°C and 14 h 15 min and 13 h 24 min, respectively. Bermudagrass (*Cynodon dactylon*) hay samples were obtained from well-established 0.81-ha pastures of each variety (Coastal, Tifton-78, and Tifton-85) at the University of Georgia Coastal Plain Station (Tifton). Pastures were mowed and fertilized (24:6: 12 of N-P-K; 336 kg/ha) on May 11, 1994. Each pasture was mowed, and the residue was removed on June 7. Half of each pasture was mowed, and the residue was removed on June 21. On July 18, hays at 4 to 6 wk of maturity were harvested; hays were baled on July 19. Hay samples were composited from six randomly selected 25-kg bales of each variety that had been harvested at 27 and 41 d of growth. Samples were obtained using a Penn State Forage Auger (Nasco, fort Atkinson, WI).

All samples were taken to the laboratory, frozen, freeze-dried, and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA). Organic acids were extracted from the samples by a variation of the hot water extraction method of Jones and Barnes (8). Ground samples (0.5 g) were mixed with 12.5 ml of deionized water and boiled in a 100°C water bath for 30 min. The samples were then mixed using a glass stirring rod to release organic acids from the cellular membranes. This slurry was filtered using a Buchner funnel (Whatman no. 1 filter; Whatman, Clifton, NJ), and the flask and residue were rinsed with 12.5 ml of deionized water to remove excess organic acid. The filtrate was centrifuged ($10{,}000 \times g$ for 10 min at 25°C) and filtered through a 0.45-μm membrane filter prior to HPLC analysis. Malate was quantitated by HPLC (Shimadzu LC-10AS liquid chromatograph, RID-6A refractive index detector, SCL-10A system controller, SIL-10A autosampler, C-R5A integrator, 50-μl loop; Shimadzu Scientific Instruments, Columbia, MD) at 50°C using a Bio-Rad HPX-87H organic acid column (Bio-Rad Laboratories, Hercules, CA) (11, 19). Samples were eluted from the column with 0.013*N* H_2SO_4 at a flow rate of 0.5 ml/min.

Data were analyzed using the GLM procedure of SAS (20) for a completely randomized design with five varieties and five maturities of alfalfa and three varieties and two maturities of bermudagrass hay. Means for concentrations of malate are reported, and significance was determined at $P < 0.05$ and $P < 0.10$. The least significant difference method was used to determine significance between treatment means. Regression lines (SigmaPlot®; Jandel Corp., San Rafael, CA) of mean values are presented in Figure 1 to compare the rate of decline of malate in all five alfalfa varieties.

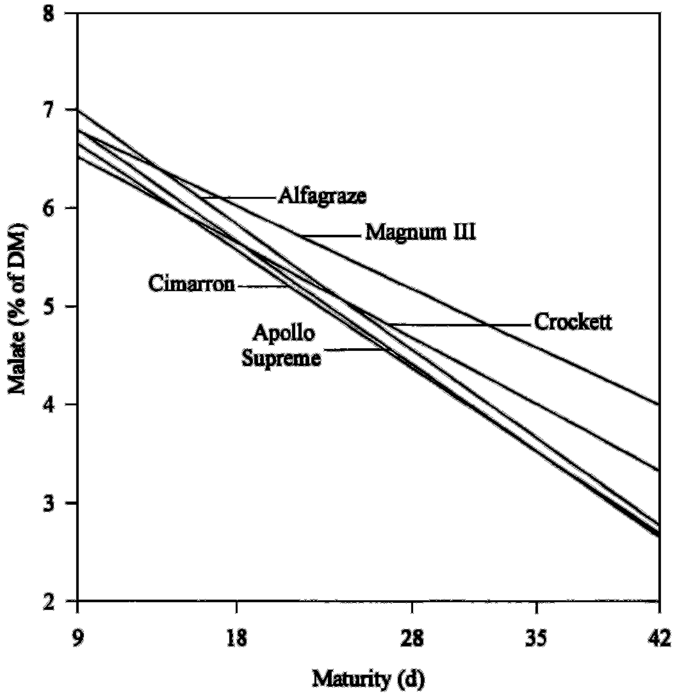

Figure 1. Regression analysis of concentrations of malate (percentage of DM) in five alfalfa varieties. Regression equations and r^2 values for each variety are Alfagraze: $y = -0.120x + 7.744$, $r^2 = 0.84$; Apollo Supreme: $y = -0.126x + 7.333$, $r^2 = 0.94$; Cimarron: $y = -0.128x + 8.143$, $r^2 = 0.96$; Crockett: $y = -0.097x + 7.397$, $r^2 = 0.92$; and Magnum III: $y = -0.0846x + 7.55$, $r^2 = 0.80$.

RESULTS AND DISCUSSION

Photosynthesis utilizes the activity of the citric acid cycle in plants to fix carbon dioxide into biomass. Growing plants accumulate intermediate metabolites of the citric acid cycle, and tissues associated with photosynthesis, such as leaves, tend to accumulate the highest concentrations of malate (25). Alfalfa is included in many dairy cattle diets in the US because it is a high quality forage. Alfalfa contains high concentrations of trace minerals and macrominerals, which may improve ruminant performance by providing limiting nutrients to the ruminant and to its gastrointestinal microbial population (5). To our knowledge, concentrations of malate in modern cultivars of alfalfa have not been examined, but, because of the positive effect that malate has on the utiliza-

TABLE 1. Concentration of malate in alfalfa varieties at different maturities.

	Concentrations of malate in alfalfa					
Maturity	Alfagraze	Apollo Supreme	Cimarron	Crockett	Magnum III	SEM
	(% of DM)					
9 d	7.5[a]	7.2[a]	6.8[a]	6.5[a]	6.9[a]	0.99
18 d	4.5[b,e]	5.6[ab,de]	6.4[a,d]	5.9[a,de]	6.4[ab,d]	0.55
28 d	4.2[bc]	3.9[b]	4.2[b]	4.1[ab]	4.3[c]	0.23
35 d	3.8[bc,de]	3.3[b,e]	3.5[bc,de]	4.5[ab,d]	4.5[bc,d]	0.38
42 d	2.9[c,e]	3.2[b,de]	2.9[c,e]	3.3[b,de]	4.5[bc,d]	0.44
SEM	0.50	0.68	0.40	0.71	0.55	

[a,b,c]Means within a column with no common superscripts differ ($P < 0.05$).

[d,e]Means within a row with no common superscripts differ ($P < 0.10$).

tion of lactate by the predominant ruminal bacterium *S. ruminantium* (13, 14, 15, 16), perhaps malate content influences the quality of this important forage in high producing dairy cows.

Concentrations of malate declined ($P < 0.05$) as maturity of all alfalfa varieties advanced (Table 1). Compared with the concentration of malate at 9 d, concentrations in Alfagraze declined ($P < 0.05$) as maturity increased. Concentrations of malate continued to decrease after 18 d of maturity ($P < 0.10$), and concentrations were always below those observed at 9 d ($P < 0.05$) for all stages of maturity. Concentrations of malate in Apollo Supreme declined in a manner similar to that described for Alfagraze. Compared with concentrations at 9 d, concentrations of malate in Apollo Supreme were lower ($P < 0.05$) at 28 d. After 28 d of maturity, concentrations of malate continued to decline ($P < 0.10$) and were always below ($P < 0.05$) those found in the samples at 9 d of maturity. Concentrations of malate in Cimarron were lower ($P < 0.05$) at 28 d of maturity than at 9 and 18 d. Concentrations of malate in Crockett declined similarly. Concentrations of malate in Crockett at 9 d were numerically lower than those in all varieties examined; however, the decline in concentrations of malate over time with Crockett was less than that of the previously described varieties. Concentrations of malate were not reduced ($P < 0.05$) until 42 d of maturity. Concentration of malate in Magnum III at 9 d was intermediate to those of the other alfalfa varieties. Concentrations of malate declined as maturity increased as in all other varieties; however, Magnum III tended to have the highest concentrations of malate from 18 to 42 d. Both Magnum III and Crockett had similar concentrations of malate at 35 d. Concentrations of malate in Magnum III were lower ($P < 0.05$) at 28 d of maturity than at 9 d and then remained constant up to 42 d.

Regression analysis indicated that concentrations of malate in the varieties of alfalfa examined in this study decreased at different rates as maturity increased (Figure 1). Cimarron had the highest rate of decline, followed by Apollo Supreme, Alfagraze, Crockett, and Magnum III, respectively. No differences in concentrations of malate were observed at 9 d among the five alfalfa varieties (Table 1). Alfagraze had the numerically highest malate content initially, but, the decline in malate was so great after 18 d that concentrations of malate were numerically lower than three other varieties by 42 d (Table 1). At 28 d of maturity, concentrations of malate were not different among varieties. At 35 d of maturity, concentrations of malate were lower for Apollo Supreme than for Crockett and Magnum III. After 42 d of maturity, concentrations of malate in Alfagraze and Cimarron were lower than those in Magnum III. No differences in the concentration of malate were detected among the other alfalfa varieties.

Because alfalfa production is more difficult in the southeastern US, there is interest in developing improved varieties of bermudagrass for use in the diets of dairy cattle (26). Because low quality is often associated with this forage, many dairies in the Southeast feed high concentrations of cereal grains to high producing cows. High amounts of grain in the diet are associated with subclinical ruminal acidosis, which causes reduced feed efficiency in dairy cows (21). Therefore, a reduction in the accumulation of lactate in the rumen of these high producing cows through enhanced utilization of lactate by *S. ruminantium*, may possibly improve production efficiency.

Because soluble nutrient losses are associated with hay production, concentrations of malate were expected to be lower in hay samples than in freshly harvested alfalfa. Coastal bermudagrass hay tended to have the lowest concentration of malate of all bermudagrass varieties examined at 27 and 41 d

TABLE 2. Concentration of malate in bermudagrass hay varieties at different maturities.

Maturity	Concentrations of malate in bermudagrass hay			SEM
	Coastal	Tifton-85	Tifton-78	
	(% of DM)			
27 d	$2.7^{a,d}$	2.8^{d}	$4.5^{a,c}$	0.21
41 d	1.9^{b}	2.3	2.3^{b}	0.26
SEM	0.15	0.22	0.30	

[a,b]Means within a column with no common superscripts differ (P < 0.05).

[c,d]Means within a row with no common superscripts differ (P < 0.05).

(Table 2). Concentrations of malate in Coastal and Tifton-78 were lower (P < 0.05) at 41 d than concentrations at 27 d, but concentrations of malate in Tifton-85 showed little change. At 27 d of maturity, malate in Tifton-78 was higher (P < 0.05) than that in the other bermudagrass hay samples. Concentrations of malate were similar in all three bermudagrass varieties at 41 d.

Recognizing that the bermudagrass samples were grown and preserved under different conditions than were the alfalfa varieties, we compared concentrations of malate of the two forages. Concentrations of malate in bermudagrass were lower (P < 0.05) than those in alfalfa varieties at an equivalent maturity, except for Tifton-78 at 27 d of maturity (Table 1 vs. Table 2). At 42 d, concentrations of malate in freshly cut samples of alfalfa were up to twofold higher than those observed in the bermudagrass hay samples at 41 d.

In early studies (18), tobacco leaves and rhubarb were found to contain high concentrations of malate. Malate in those leaves was almost exclusively in the L-isomeric form (17). When tobacco plants were grown in darkness, concentrations of malate in leaves declined rapidly, but concentrations in the stalk did not vary (25), probably because of different respiration rates between cells in the photosynthetic leaf and structural stalk (25). Concentrations of malate were constant throughout the leaf, but concentrations of other organic acids varied along a concentration gradient (25).

Organic acids constitute as much as 10% of the dry weight of grasses (4, 5, 22). Jones and Barnes (8) surveyed several varieties of grasses for organic acid content. Malate was found to decline as plants matured and made up approximately 1.5% of the DM of mature grasses (8, 9). Concentrations of malate especially were noted to decline over time in wheatgrass pasture (1). Our results agree with the trends found for other forage grasses.

Lactate is produced in the rumen by fermentation of starch or sugars by saccharolytic bacteria. Accumulation of lactate causes ruminal acidosis, which decreases feed efficiency because of reduced intake and other physiological problems (21). Acidosis is a problem in the dairy industry, especially in high producing dairy cows fed high grain diets (7). Ruminal lactate can be fermented by *S. ruminantium* to form propionate (6), and the uptake of lactate by this predominant ruminal bacterium is stimulated in the presence of malate (13, 14, 15, 16). Furthermore, recent research (12) showed that, when mixed ruminal microorganisms fermented cracked corn or soluble starch, malate treatment decreased concentrations of lactate and CH_4 as well as the ratio of acetate to propionate in a manner analogous to ionophore treatment. Addition of malate to the diet of crossbred feedlot steers has shown potential for improving performance by reducing days on feed and increasing feed efficiency of these steers (23).

Concentrations of malate in this study (Table 1) were approximately twofold higher than those reported in the literature for other forage species (8, 9, 22). Concentrations of malate were higher (P < 0.05) in mature (42 d) alfalfa varieties than in bermudagrass hays (41 d) (Table 1 vs. Table 2). Seed producers have selected alfalfa varieties based on digestibility and soluble nutrient content, and by doing so, have increased the ratio of leaf to stem of many varieties. Malate is found primarily in leaf tissue of plants because of the activity of the citric acid cycle (25); thus, plant breeders have indirectly selected for increased malate in these plants.

Given that concentrations of malate ranged between 2.9 and 4.5% of alfalfa DM at 42 d of maturity (Table 1), we can estimate how much malate might be available in the rumen of a dairy cow fed alfalfa at 42 d of maturity. If the amount of alfalfa consumed per day is 6.0 kg in a TMR (26) and the concentration of malate in Alfagraze is 2.9%, the animal would consume 174 g of malate. If the ruminal volume is approximately 70 L, then the intraruminal concentration of malate would be 2.5 g/L or 18.6 m*M* (molecular mass of malate is 134.1 g/L; Sigma Chemical Co., St. Louis, MO). If similar calculations are made for 4.5% of DM malate, Magnum III would provide 29 m*M* intraruminal malate. Concentrations of malate between 0.03 and 10 m*M* increased the uptake of lactate by *S. ruminantium* in a dose-response fashion, and the 10 m*M* concentration was the most stimulatory (14). Therefore, all alfalfa varieties examined in this study appeared to provide adequate concentrations of malate to stimulate lactate utilization by *S.*

ruminantium maximally. However, because of ruminal dilution rate as well as the utilization of malate by ruminal microorganisms (3, 19), it is unlikely that all malate would be readily available. In addition, release of this organic acid may be dependent on at least some digestion of the plant cell wall. In vitro studies (3, 19) have shown that 7.5 m*M* malate is completely fermented within 10 to 24 h by mixed ruminal microorganisms.

Malate can be purchased in bulk quantities, and, at current prices, the use of malate as a feed additive (80 g/d per head) is estimated to cost $0.15 to $0.19/d per head under feedlot conditions, which, at present, might prohibit the inclusion of malate in the diets of feedlot or dairy cattle. However, selection for and incorporation of forage varieties that are high in malate into the ruminant diet might improve ruminal fermentation. Perhaps the high concentrations of malate observed in this study account for some of the high quality associated with alfalfa. More research is needed to evaluate whether or not ensiling has any effect on the concentrations of malate in forage.

CONCLUSIONS

Monetary losses attributed to ruminal acidosis have been conservatively estimated at $60 to $100 million/yr for US feedlots (10). Economic losses associated with acidosis in the dairy industry are not available, but, because of the energy demands on high producing dairy cows, these losses are probably significant (7). Therefore, by decreasing the accumulation of lactate in the rumen, producers may possibly reduce losses associated with ruminal acidosis. Concentrations of lactate are reduced by the addition of organic acids to the in vitro fermentation by mixed ruminal microorganisms (2, 12). Organic acids are commonly found in plants and can constitute a significant percentage of the DM. Consequently, incorporation of forage varieties that are high in malate may provide a vehicle for economically including malate in the diet.

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