Virginia Journal of Science
Volume 42, Number 1
Spring 1991

Influence of Flooded Soil on Chemical Composition of Annual Ryegrass and Digestibility by Meadow Voles*

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ABSTRACT

Flooding affects mineral composition of pasture grasses, but little is known concerning effects on fiber, organic-acid and amino-acid composition, dry matter digestibility, and mineral absorption by animals. 'Gulf' annual ryegrass (*Lolium multiflorum* Lam.) was grown on a Bucks loam (Typic Hapludult, fine-loamy, mixed, mesic) in a greenhouse to investigate the influence of flooding and 80% field capacity (FC) soil moisture on plant growth and chemical composition. Flooding increased soil pH, tended to increase ($P < 0.10$) soil exchangeable Al (modified aluminon method), and increased Al, Fe, Cu, neutral detergent fiber (NDF), acid detergent fiber ADF), hemicellulose, alanine ($P < 0.05$), valine and glutamate ($P < 0.07$) concentrations in ryegrass herbage. Magnesium, K, Zn, malate, fumarate ($P < 0.05$), and succinate ($P < 0.07$) concentrations were decreased by flooding. Meadow voles (*Microtus pennsylvanicus*) were fed the forages grown at two moisture levels over an 8-day period to evaluate mineral availability and forage digestibility. Apparent absorption of Mg and K was decreased ($P < 0.05$) in animals fed forage grown on flooded soil, but absorption of Al ($P < 0.12$), Fe ($P < 0.15$) and P ($P < 0.09$) tended to increase. Results suggest that forages grown under flooded conditions have altered amino acid, organic acid, mineral, and fiber concentrations, which could result in lowered performance of animals grazing these forages.

INTRODUCTION

Short-term flooding of pastures occurs frequently during periods of high rainfall, particularly on poorly drained soil. Reduced concentrations of Mg and Ca (Elkins and Hoveland, 1977) and increased Al (Muchovej, Allen, Martens, Zelazny and Notter, 1986) have been reported in forages in response to high soil moisture. Decreased Mg and Ca concentrations in forage grasses due to flooding (Elkins and Hoveland, 1977), high dietary Al (Dennis, 1971; Allen and Robinson, 1980), and increased concentrations of organic acids (Grunes, Stout and Brownell, 1970), have been suggested as contributing to the onset of grass tetany, a metabolic disorder of ruminants associated with deficiency or impaired utilization of Mg. Muchovej et al. (1986) suggested a relationship between organic acids and increased uptake of

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* The research was funded by a grant from the John Lee Pratt Animal Nutrition Program.

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Al by ryegrass under flooded conditions. They proposed a chelation mechanism which rendered Al available and non-toxic to the plant. Information on flooding effects on forage quality, including fiber, organic acid, and amino acid concentrations in grasses is scarce in the literature. Several authors have reported accumulation of various organic and amino acids in roots of plants under anaerobic soil conditions (Jackson and Drew, 1984), but effects of flooding on concentrations in forage plant tops are not well documented.

This experiment was designed to investigate the relative effects of flooding and 80% of field capacity (FC) soil moisture on fiber, organic acid, amino acid and mineral concentrations in annual ryegrass (*Lolium multiflorum* Lam.). The forages were fed to meadow voles (*Microtus pennsylvanicus*) in a digestion trial to further test effects of soil moisture on forage quality.

MATERIALS AND METHODS

A greenhouse experiment was conducted with the surface horizon of a Bucks loam (Typic Hapludult, fine-loamy, mixed, mesic) with a pH of 6.8 and 16, 91, 816, and 70 mg kg^{-1} dilute acid extractable [Mehlich I (Nelson, Hehlich and Winters, 1953)] P, K, Ca and Mg, respectively. Soil was air-dried, sieved through a 5-mm stainless steel screen, and weighed into plastic pots (12 kg of air-dry soil pot^{-1}). The soil was fertilized with reagent grade chemicals as follows: 150 mg kg^{-1} N as NH_4NO_3, 75 mg kg^{-1} P as $CaHPO_4\cdot 2H_2O$, 75 mg kg^{-1} K as KCl, 15 mg kg^{-1} Mg as $MgSO_4\cdot 7H_20$, 5 mg kg^{-1} Cu as $CuSO_4\cdot 5H_20$, 15 mg kg^{-1} Mn as $MnSO_4\cdot H_20$, 15 mg kg^{-1} Zn as $ZnSO_4\cdot 7H_20$, and 0.5 mg kg^{-1} B as $Na_2B_4O_{7}10\cdot H_2O$, according to soil test recommendations. Fertilizer was mixed with a 600 g subsample of soil from each pot. Amended subsamples were air-dried and returned to pots and total contents were mixed for 5 minutes in a V-shell blender.

Soil moisture treatments were 1) 80% FC and 2) flooding, beginning 21 days post seedling emergence and continuing for 30 days. For flooding, 1-2 cm of water were maintained above the soil surface until plants were harvested. Field capacity was determined by adding water to a compacted column of soil and measuring gravitational water content after drainage downward to dry soil (Sykes and Loomis, 1967). Following fertilizer application, all soils were brought to 80% FC and allowed to equilibrate for two weeks before planting. Watering to 80% FC was performed gravimetrically on a daily basis with distilled-deionized water, and flooding was maintained visually. Soil moisture treatments were replicated 12 times in a completely randomized design.

'Gulf' annual ryegrass was planted at 1.0 g per pot, and plants were thinned to 100 per pot when approximately 5 cm tall. Plants were harvested at soil level, at the end of the flooded period, rinsed with deionized water, freeze-dried, and ground in a stainless steel Wiley[*] mill to pass a 1-mm screen. Twelve replications per treatment were needed to produce sufficient dry matter for the feeding trial. Within each treatment, forage was randomly composited by weight among three replications for a final number of four replications per treatment for chemical analysis. Data are reported on a DM basis.

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Soil core samples were collected from each pot following plant harvest. Soil was analyzed without air-drying in order to maintain similar moisture conditions to those in the greenhouse. All analyses were adjusted to dry matter basis. Percentage soil moisture (gravimetrically), pH, exchangeable Al using a modified aluminon method (Jayman and Sivasubramaniam, 1974), P (Murphy and Riley, 1962), and Mg, Ca, and K (extraction with NH_4OAc and analysis by atomic absorption spectrophotometry) were determined on soil. Soil pH was determined with a glass electrode in a 1:1 mixture of soil and H_20 after 1 hr equilibration.

Forage was analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (NDF minus ADF), cellulose, lignin (Van Soest, 1963; Van Soest and Wine, 1968), minerals, organic acids and amino acids. Tissue samples were digested with 3:1 HNO_3:$HClO_4$ acid (Sandell, 1950) with modifications as described by Muchovej et al. (1986) and analyzed for Al, Fe, Zn, Cu, Ca, Mg, and K by atomic absorption spectrophotometry. Lanthanum chloride was included in dilutions for Mg and Ca analysis. Phosphorus was determined colorimetrically (Fiske and Subbarow, 1925).

For organic-acid analyses, plant material was extracted using a Soxhlet extractor with 80% (v/v) aqueous ethanol. Glutaric acid (500 ul of a 12 mM solution) was added as an internal standard. The extract was concentrated to dryness, resuspended in water, and solvent extracted with chloroform. The aqueous phase was fractionated using cation and anion exchange resins (Stumpf and Burris, 1979), and samples were analyzed by high-performance liquid chromatography (HPLC) using a 300 x 7.8 mm Bio-Rad Aminex HPX-87H* organic acids column with a mobile phase of 0.06 *N* H_3PO_4 and a flow rate of 0.6 ml/min. Organic acids were detected by absorbance at 214 nm.

For amino acid analysis, tissue was extracted using Soxhlet extractors and 65% (v/v) aqueous ethanol. Filtered extracts were passed through a C-18 Sep-Pak (Waters Associates). Fluorescent derivatives of primary amino acids were prepared using *o*-phthalaldehyde (OPA). The reaction solution was prepared by dissolving 50 mg of OPA in 1 ml of methanol, adding 50 μl β-mercaptoethanol, and bringing the solution to a final volume of 10 ml with 0.40 M sodium borate/KOH pH 9.5 containing 0.1% Brij 35. Samples (20 μl) were mixed with 100 μl of the reaction medium. After 1 minute, 20 μl of the mixture were analyzed by using a Beckman model 344 binary gradient HPLC system equipped with a 4.6 X 45 mm, 5 μm Altex Ultrasphere-ODS precolumn and a 4.6 X 250 mm, 5 μm Altex Ultrasphere-ODS analytical column maintained at 45°C following the protocol of Jones, Paabo and Stein (1981). OPA-amino acid derivatives were detected using a Gilson model 121 fluorescence detector equipped with a 9 μl flow cell and filters for excitation at 305 to 395 nm and emission at 430 to 470 nm. Detector range and time constant settings were 0.02 and 0.5, respectively.

Organic acids and amino acids in samples were identified by similarities in retention times to those of pure compounds (Sigma Chemical Company) and by increased peak areas observed upon spiking samples with the individual standards. Peak areas were determined using a Nelson Analytical model 4416X chromatog-

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raphy data system. Quantification was achieved using standard curves generated using the pure reagents.

Two digestion trials were conducted with four male meadow voles (avg. wt. 35.95 g) per treatment fed ryegrass forages from the greenhouse experiment to estimate in vivo dry matter and fiber digestibility and apparent mineral absorption. Meadow voles have been suggested to be suitable models for ruminants when evaluating forage of high digestibility (Keys and Van Soest, 1970).

Voles were trapped during December 1983 and January 1984 and placed individually in shoebox cages (12 by 18 by 27 cm) and fed Wayne Lab Chow and water ad libitum. Nine days prior to the trial, voles were switched to Wayne Rabbit Chow to increase fiber intake in preparation for the ground ryegrass diet. Seven days prior to each trial, voles were moved to false-bottom, stainless steel metabolism cages for total collection of feces. Trials consisted of a 3-day transition from rabbit chow to ryegrass, a 2-day preliminary period and a 3-day collection period during which total fecal excretions were collected. Animals were blocked by weight, location in the metabolism cage rack and randomly allotted to the two treatments. Voles were maintained in an environmental chamber at 17.2°C, 5% relative humidity and a light/dark schedule of 11:13 hours to minimize variation between trials. Trial variation was not significant, so data from the two trials were pooled. Feed for each treatment was a composite of all harvested, ground forage from each moisture treatment. Nitrogen was determined on the composited forage by micro Kjeldahl (Nelson and Sommers, 1973).

A blood sample was taken from each vole at the end of the trial, from the interorbital sinus, using 0.05 ml heparinized capillary tubes. Blood serum samples were diluted with 0.1% lanthanum chloride and were analyzed for Ca and Mg by atomic absorption spectrophotometry. Feed and feces samples were digested and analyzed for Al, Mg, Ca, K and P as described for the plant samples. Apparent absorption of minerals and apparent digestibility of fiber were calculated from total intake and excretion and analysis of feed and fecal samples.

Plant data were analyzed as a completely randomized design (Helwig and Council, 1979). Animal data were analyzed as a randomized block design.

RESULTS AND DISCUSSION

Soils. Bucks silt loam is a somewhat poorly drained series and commonly occurs in pasture land in Virginia. Soil samples analyzed at the beginning of the experiment indicated a pH of 6.8 and 70, 816, 91, and 16 mg $^{-1}$ extractable Mg, Ca, K, and P, respectively. Exchangeable Al was not detectable.

At the end of the treatment period, soil moisture was 19.9 and 36 g 100 g^{-1} soil for 80% FC and flooded soil, respectively. Soil pH was higher and exchangeable Al tended to be higher ($P < 0.10$) in flooded soil compared to soil maintained at 80% FC (Table 1). An increase in soil pH is commonly observed under flooded conditions. An increase in soil exchangeable Al in response to flooding was reported by Muchovej et al. (1986). The decreased pH in both flooded and 80% FC treated soils, compared to initial values, was probably due to acidity generated by fertilizer application.

Flooded soil contained higher concentrations of exchangeable Ca, Mg, and K than soil maintained at 80% FC. Moisture status had no significant effect on soil

TABLE 1. Soil pH, exchangeable Al and extractable minerals as influenced by 80% field capacity and flooding.

Item	Soil moisture		SE[1]
	80% FC	Flooded	
pH (H_2O)	5.25**	5.50	0.04
Exchangeable Al, cmol($1/3Al^{3+}$)kg	0.009[2]	0.021	0.004
Extractable minerals, mg kg^{-1}			
Ca	1171**	1283	20
Mg	117**	134	3
P	17	20	2
K	107**	149	5

** Indicates difference between values within a line ($P < 0.01$).
[1] Standard error of means.
[2] Values were different ($P < 0.10$).

TABLE 2. Yield, fiber and mineral concentrations[1] in annual ryegrass as influenced by 80% field capacity and flooded soil

Item	Soil moisture		SE[2]
	80% FC	Flooded	
Dry matter yield, g pot^{-1}	111.6**	88.8	4.3
NDF, mg $100mg-^1$	34.8**	36.7	0.3
ADF, mg $100mg^{-1}$	21.7*	22.7	0.2
Hemicellulose, mg $100mg^{-1}$	13.1*	14.0	0.2
Cellulose, mg $100mg^{-1}$	16.8	18.0	0.6
Lignin, mg $100mg^{-1}$	2.8	2.9	0.4
Mg, mg $100mg^{-1}$	0.37***	0.22	0.01
Ca, mg $100mg^{-1}$	1.24	1.27	0.02
K, mg $100mg^{-1}$	2.75***	2.07	0.08
P, mg $100mg^{-1}$	0.37	0.35	0.01
Cu, mg kg^{-1}	20***	27	1
Zn, mg kg^{-1}	118***	92	1
Al, mg kg^{-1}	213***	515	29
Fe, mg kg^{-1}	168***	318	16

*, **, *** indicate difference between values within a line ($P < 0.05$, 0.01, 0.001).
[1] Dry matter basis.
[2] Standard error of mean.

exchangeable P. Lower concentrations of Ca, Mg, and K in 80% FC soil probably reflected plant uptake of these nutrients.

Plants. Flooded soil decreased DM yield and increased plant concentrations of NDF, ADF and hemicellulose ($P<0.05$) in ryegrass, compared to soil at 80% FC (Table 2). Cellulose and lignin were not significantly affected by soil moisture. Aluminum, Fe, and Cu concentrations were higher ($P < 0.01$) in plants grown under flooded conditions, while concentrations of Mg, K and Zn were lower ($P<0.01$) than in plants grown at 80% FC. Calcium and P concentrations in ryegrass were not significantly affected by the two soil moisture treatments. Crude protein was 270 and 227 g kg^{-1} in forage grown at 80% FC and flooded soil moisture, respectively (data not shown). Based on NRC (1978) recommendations for laboratory animals, the protein levels should have been sufficient for meadow voles.

Reduction in DM yield due to flooding may have been due in part to loss of soil N by denitrification (Ponnamperuma, 1972). In the present study, N in ryegrass averaged 43.2 and 36.3 g kg^{-1} for 80% FC and flooded soil moisture treatments, respectively. Increased concentrations of Al and Fe, after 30 d flooding, are in agreement with results obtained by Muchovej et al. (1986) with ryegrass grown for 7 weeks in flooded soil in a greenhouse experiment. Cherney and Robinson (1985) found no relationship of soil moisture with Al accumulation by ryegrass grown in a growth cabinet with flooded periods of up to 21 days. Differences in results may be due to cultivar differences, difference in time plants were exposed to flooded soils or to an inherent difference among soils in Al availability under flooded conditions. The depressing effect of flooding on forage Mg concentration has been reported by Elkins and Hoveland, 1977. In the present study, flooding increased Cu and decreased Zn concentrations in ryegrass. Ponnamperuma (1972) reported similar results for Cu and Zn and suggested that for non-calcarous soils, long-term flooding increased Cu and decreased Zn availability.

Concentrations of succinate ($P<0.07$), malate, and fumarate ($P<0.05$) decreased while those of alanine ($P<0.01$), glutamate, and valine ($P<0.07$) increased in plants grown under flooded conditions (Table 3). Other amino acids identified and quantified in HPLC chromatograms, and citrate, were not significantly influenced by soil moisture.

Changes in permeability of cell membranes due to low soil O_2 may cause leakage of some substances, perhaps partially accounting for the decline in tissue concentrations of organic acids (Stolzy and Sojka, 1984). Organic acids are known constituents of root exudates, but the influence of soil moisture on these constituents is poorly understood. An increase in organic-acid concentration in the soil has been suggested to increase plant Al concentration and reduce Al toxicity (Muchovej et al., 1986). They found increased concentrations of Al in ryegrass with added increments of citric and nitrilotriacetic acid to soil.

The increase in certain amino acids during flooding agrees with results from Labanauskas, Stolzy and Handy (1974), who reported an increase in total free amino acids but a decreased sum of protein amino acids in citrus leaves of plants grown with low, as compared to normal, soil O_2. Free amino acids were measured in our experiment. Increased alanine in root and xylem sap was reported in several plants including pumpkins (*Cucurbita* sp. cv. Mozoleevskaya), tomatoes (*Lycopersicon* sp. cv. Bison) and some tree species grown under root anaerobiosis (Hook,

TABLE 3. Organic and amino acid composition[1] of annual ryegrass as influenced by 80% field capacity and flooded soil

Item	Soil moisture		
	80% FC	Flooded	SE[2]
Organic acids, mg g^{-1}			
Succinate[3]	77.4	44.6	10.4
Citrate	17.5	15.6	1.2
Malate	36.4*	24.6	3.2
Fumarate	0.07**	0.04	0.01
Amino acids,[4] $\mu g\ g^{-1}$			
Aspartate	21.9	23.4	5.6
Glutamate[5]	15.2	30.5	4.5
Asparagine	44.1	47.0	7.2
Serine	13.0	20.4	4.2
Alanine	39.2**	57.1	1.8
γ-aminobutyric acid	64.0	80.7	8.0
Valine	9.3*	14.0	1.5
Phenylalanine	48.9	47.6	6.5

*, **, indicate difference between values within a line ($P < 0.05, 0.01$).

[1] Dry basis.

[2] Standard error of mean.

[3] Values were different ($P < 0.07$).

[4] Data represent corrected peak areas 1 standard deviation chromatograms from HPLC for four replicates of each treatment except for asparagine under 80% field capacity soil moisture treatments when only three replicates are represented.

[5] Values were different ($P < 0.06$).

1984). Zemlianukhin and Ivanov (1978) suggested that increased CO_2 concentration, which accompanies ethanol fermentation, favored synthesis of γ-aminobutyric acid via α-ketoglutaric acid and glutamate. In our experiment, increases in alanine and glutamate occurred in response to flooding, but no effect was measured on γ-aminobutyric acid.

Animals. Feeding ryegrass grown with 80% FC or flooded soil moisture to meadow voles had no significant effect on body weight at the end of the trial. Dry matter intake and apparent digestibility of dry matter, NDF, ADF, hemicellulose and cellulose tended toward lower values with forage from flooded soil than in 80% FC soil, but the differences were not significant at the 0.05 level of probability (Table 4). The consistency of the lower numerical values for digestibility of DM and fiber components, coupled with the significant increase in NDF, ADF and hemicellulose concentrations in forage (Table 2), strongly suggest reduced digestibility of these components due to flooded soil.

Intake, fecal excretion, apparent absorption of Mg and serum Mg were decreased ($P < 0.05$) in voles fed ryegrass grown on flooded soil (Table 5). Serum Mg concentrations were 6.1 and 5.2 mg dl^{-1}. in voles fed forage grown with 80% and flooded soil moisture, respectively (data not shown). Intake and apparent absorption of K were decreased ($P < 0.05$) in voles fed forage grown on flooded soil. The bioavailability of P to meadow voles appeared to be very low, but was

TABLE 4. Dry matter intake and digestibility of dry matter and fiber components of ryegrass as influenced by 80% field capacity and flooded soil

Item	Soil moisture 80% FC	 Flooded	SE[1]
Dry matter intake, mg g^{-1} body weight	6.1	5.6	0.5
Apparent digestibility, g $100g^{-1}$			
Dry matter	67.4	64.0	3.2
NDF	54.7	45.1	5.2
ADF	52.6	42.0	5.9
Hemicellulose	58.3	50.0	4.2
Cellulose	51.6	37.8	7.2

[1] Standard error of mean.

enhanced in ryegrass grown on flooded soil although forage concentrations and P intake were not significantly affected by soil moisture. Animals fed ryegrass grown on flooded soils had increased intakes of Al and Fe ($P < 0.01$), and tended to show increased apparent absorption of Al ($P < 0.12$) and Fe ($P < 0.15$). Calcium, Cu and Zn intake and apparent absorption and serum Ca were not significantly influenced by soil moisture treatments (data not shown).

Lowered serum Mg may have been related to the decreased Mg intake although the Mg content of the diet (122 mg Mg 100 g^{-1} diet) was above a dietary level (40 mg Mg 100 g^{-1} diet) normally recommended for rodents (NRC, 1978). Lowered serum Mg could also have been related to dietary Al levels. Increased dietary Al, particularly when chelated with citric acid, has resulted in decreased serum Mg but had little effect on apparent absorption of Mg in sheep and cattle (Allen and Fontenot, 1984; Allen, Horn and Fontenot, 1986). Even small doses of chelated Al appear to have adverse affects on animals. As little as 5 mg Al kg^{-1} body weight day^{-1} as Al-nitrilotriacetic acid resulted in morphological damage to liver and kidney of rats (Ebina, Okada, Hamazaki and Midorikawa, 1984). Rats administered saline or Al as chloride or potassium sulfate were unaffected.

The decline in K concentration in forage could help to offset the adverse effects of decreased Mg in forage for grazing ruminants. Research has shown decreased Mg absorption in ruminants with increasing dietary K (Green, Fontenot and Webb, 1983).

Results of our experiments indicate that ryegrass grown under conditions of high soil moisture may have lower quality due to increased fiber and Al and decreased Mg and organic acid concentrations. The possibility of increased bioavailability of Al and its influence on grazing animals needs further investigation. Animals with elevated parathyroid hormone and/or impaired renal function may be particularly susceptible to adverse effects of ingested Al (Allen, 1987). The lowered K could improve bioavailability of Mg. Further research with animals fed forage grown under field conditions is needed to elucidate these effects.

TABLE 5. Intake, excretion and apparent absorption of minerals[1] of ryegrass as influenced by 80% field capacity and flooded soil moisture

Item	Soil moisture 80% FC	 Flooded	SE[2]
	- mg g-[1] body weight -		
Magnesium			
Intake	2.25**	1.22	0.14
Fecal excretion	0.37*	0.24	0.03
Apparent absorption	1.89**	0.98	0.13
Potassium			
Intake	17.3*	12.0	1.2
Fecal excretion	4.51	3.70	0.33
Apparent absorption	12.80*	8.30	1.07
Phosphorus			
Intake	2.28	1.96	0.17
Fecal excretion	2.49*	1.82	0.18
Apparent absorption	-0.20[3]	0.14	0.11
Aluminum			
Intake	0.12**	0.28	0.02
Fecal excretion	0.10**	0.23	0.02
Apparent absorption	0.02	0.05	0.01
Iron			
Intake	0.10**	0.17	0.01
Fecal excretion	0.09*	0.13	0.01
Apparent absorption	0.01	0.04	0.01

*, **, indicate difference between values within a line ($P < 0.05, 0.01$).

[1] Dry matter basis.

[2] Standard error of mean.

[3] Values are different ($P < 0.09$).

ACKNOWLEDGEMENTS

This research was funded by the John Lee Pratt Animal Nutrition Program and was conducted by T. H. Terrill as part of his Master of Science thesis research under the direction of Vivien G. Allen. J. P. Fontenot provided expertise on animal nutrition, J. A. Cranford directed management of meadow voles and J. A. Foster directed the analysis of amino and organic acids.

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