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EFFECTS OF AMINO ACID AND BRANCHED-CHAIN VOLATILE FATTY ACID ADDITIONS ON IN VITRO FERMENTATION OF DORMANT RANGE GRASSES

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Summary

Two-stage in vitro fermentation was used to screen five amino acids and three branched-chain volatile fatty acids as potential additions to a grain urea supplement for cows grazing dormant winter range. Urea addition alone increased dry matter and fiber digestibility of dormant cool season grasses. Methionine addition improved fiber digestibility and rate of fermentation of cool season grasses over urea alone. Compared to urea addition, the branched-chain volatile fatty acids did not increase dry matter or fiber disappearance or improve rate of fermentation of dormant range grasses. None of the buffer additions tested or urea increased digestibility of the dormant warm season grasses. This preliminary laboratory study indicates that methionine offers the greatest potential for addition to a grain urea supplement to increase utilization of dormant range grasses.

(Key Words: In Vitro Fermentation, Amino Acids, Branched-chain Volatile Fatty Acids, Dormant Range Grass.)

Introduction

In vitro fermentations are laboratory procedures designed to mimic rumen and/or lower gut digestion of ruminant animals. A single stage in vitro fermentation would indicate digestion of a feedstuff that occurs in the rumen. Rumen fluid from a rumen fistulated donor animal and buffers similar to those which are normally present in the rumen are mixed with a feed sample and maintained in a warm, oxygen-free environment for 48 hours. A two-stage in vitro fermentation would include the above first stage followed by an acid-pepsin digestion simulating feed breakdown that occurs in the abomasum and small intestine. These laboratory procedures allow for cost-effective preliminary screening of potential feed additives that may affect diet digestibility and animal performance.

Previous research has suggested that supplementation of amino acids, branched-chain volatile fatty acids or minerals may improve ruminal fermentation of low protein, dormant forages. Isolation of those beneficial components normally found in natural protein supplements could lead to enhanced utilization of lower cost nonprotein nitrogen sources. The objective of this study was to screen single amino acids and branched-chain volatile fatty acids as potential additions to a grain-urea supplement for cows grazing dormant winter range grasses.

Materials and Methods

Two-stage in vitro studies (replicated twice) were conducted separately for each of the predominant grass species inhabiting winter grazing areas located at the SDSU Range and Livestock Research Station near Cottonwood. Samples of cool season western wheatgrass and Japanese brome and a warm season shortgrass mixture of buffalograss and blue grama were hand-clipped from dormant standing vegetation in mid-March and oven dried at 60 °C prior to in vitro digestions. Rumen fluid was obtained from fistulated cows fed a mature grass hay composed of 63% (±10) western wheatgrass, 35% (±11) Japanese brome and 2% (±3) unidentified forage as determined by sorting 15 random subsamples. Analytical composition of grass samples and hay are listed in Table 1. Triplicate tubes consisting of additions of single amino acids (arginine, histidine, isoleucine, leucine and methionine), branched-chain volatile fatty acids (isobutyric, isovaleric and 2-methylbutyric acids), sodium sulfate, starch or urea alone were examined. All treatments except

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TABLE 1. CHEMICAL COMPOSITION OF DORMANT RANGE GRASSES AND GRASS HAY FED TO RUMEN FLUID DONORS[a]

Item	Western wheatgrass	Japanese brome	Shortgrass mixture	Grass hay
Dry matter, %	96.4	93.5	95.3	91.5
Crude protein, %	2.7	3.0	4.1	5.9
Ash, %	8.7	6.8	15.2	10.2
Calcium, %	.33	.24	.47	.40
Phosphorus, %	.05	.04	.06	.11
Sulfur, %	.07	.05	.07	.10
Neutral detergent fiber, %	69.9	68.7	65.1	63.9
Acid detergent fiber, %	43.6	50.6	35.9	33.7
Lignin, %	2.7	2.0	3.2	-

[a] Dry matter basis.

controls were balanced with urea to be isonitrogenous (50 mg of nitrogen/100 ml of buffer) and amino acids provided 25% of the total nitrogen/tube. Amino acid and branched-chain volatile fatty acid molar concentrations for each tube were the same. Sulfur levels were equal (7.14 mg/tube) for methionine and sodium sulfate treatments. Corn starch was provided at the same weight (33.3 mg/tube) as methionine and served as an energy control. Cumulative gas production as determined by a water displacement system was used to monitor in vitro fermentation rate of each tube over the first 48 hours. Neutral detergent fiber analysis was conducted on second stage in vitro residues to determine fiber digestibility.

Nonlinear iterative least squares regression procedures were used to generate estimated rate kinetics (lag time, fermentation rate and time of maximum rate, Figure 1) from cumulative gas production curves (Figure 2). Treatment effects were analyzed by least squares procedures using General Linear Model (GLM) of the Statistical Analysis System (SAS). Least squares means were separated by the Predicted Difference option.

Results and Discussion

The warm season shortgrass mix was slightly more digestible than western wheatgrass when no additional urea was added (Table 2). This difference can be explained by a greater disappearance of the fiber fraction for the shortgrass. When urea was provided, dry matter and neutral detergent fiber disappearance of western wheatgrass and Japanese

TABLE 2. EFFECTS OF UREA ADDITION ON IN VITRO DRY MATTER AND FIBER DISAPPEARANCE OF DORMANT RANGE GRASSES

Item	Western wheatgrass	Japanese brome	Shortgrass mixture
Dry matter disappearance, %			
Without urea	39.4[a]	42.1[b]	42.7[b]
With urea	49.6[d]	59.5[c]	43.1[b]
NDF disappearance, %			
Without urea	49.5[a]	50.1[a]	60.7[b]
With urea	62.3[b]	69.9[c]	61.6[b]

[a,b,c,d] Means within item without a common superscript differ ($P<.05$).

FIGURE 1
In vitro fermentation kinetics of western wheatgrass with methionine addition (example).

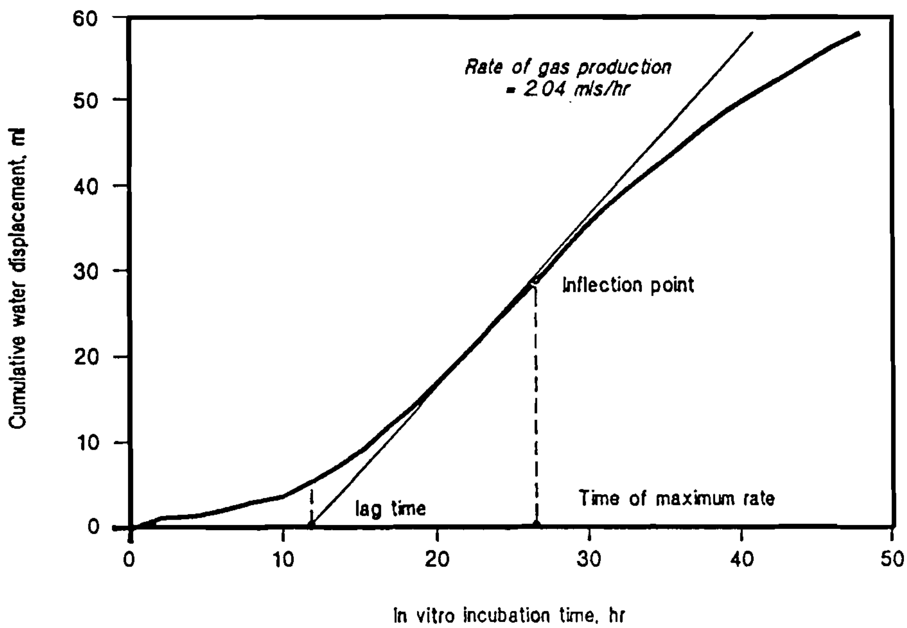
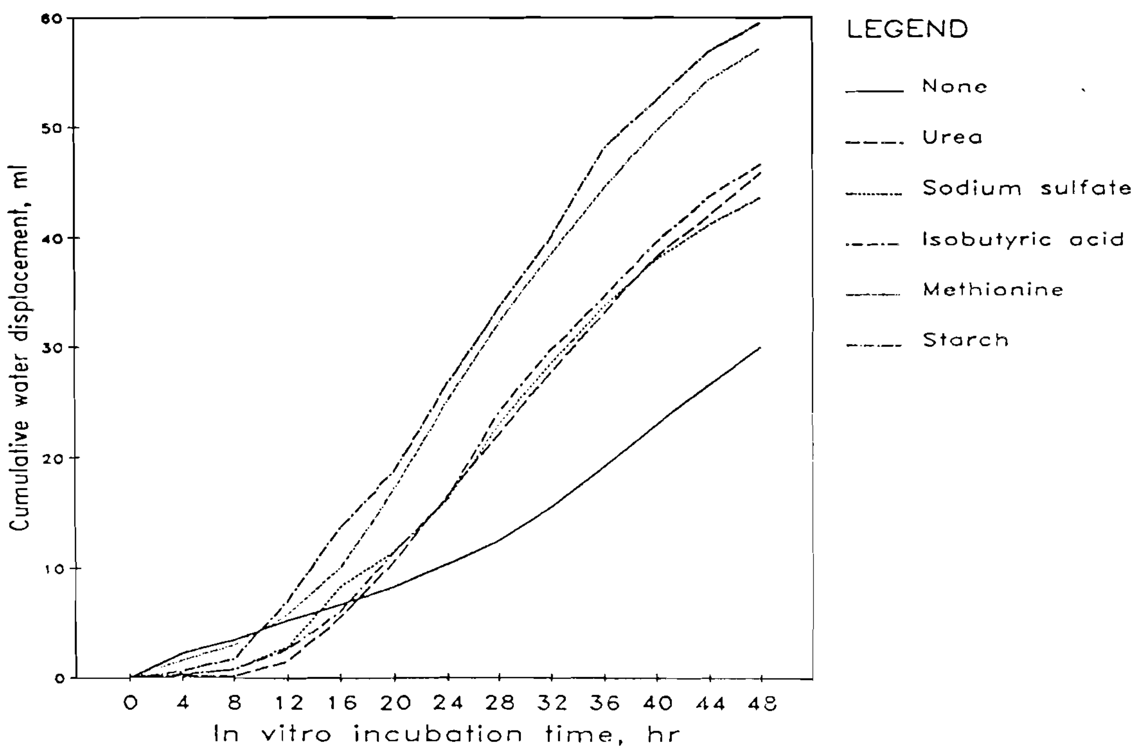


FIGURE 2
GAS PRODUCTION CURVES OF WESTERN WHEATGRASS WITH IN VITRO BUFFER ADDITIONS (example).



brome were increased ($P<.05$; 10 and 17%, respectively) suggesting that nitrogen was limiting for the cool season grasses. Urea addition did not improve digestibility of the warm season grasses which could be explained by its higher protein concentration (Table 1). Lignin and ash (primarily silica) content of the shortgrass mix were also higher than the cool season grasses which could limit digestibility.

Dry matter disappearance of western wheatgrass was not increased by in vitro buffer additions over urea alone and was lower ($P<.05$) when arginine was added (Table 3). Methionine improved ($P<.05$) neutral detergent fiber disappearance of western wheatgrass over urea alone and increased ($P<.05$) dry matter and fiber digestibility of Japanese brome (Table 4). Sodium sulfate increased ($P<.05$) neutral detergent fiber disappearance of Japanese brome over urea alone. Compared to grass alone none of the buffer amendments or urea affected digestibility of the warm season shortgrasses (Table 5).

Methionine addition increased ($P<.05$) rate of fermentation of western wheatgrass and methionine or isoleucine increased ($P<.05$) fermentation rate of Japanese brome (Tables 3 and 4). Fermentation rate was increased ($P<.05$) when histidine was added to shortgrasses. An increase ($P<.05$) in fermentation rate of dormant range forage when applied to the live animal grazing winter range could increase rate of passage and increase forage intake resulting in greater cow weight gains or reduced weight losses.

Lag time (the time prior to rapid microbial growth) was shortened ($P<.05$) by histidine addition and histidine, isoleucine, methionine or 2-methylbutyric acid decreased ($P<.05$) time of maximum fermentation rate for Japanese brome over urea alone. Amino acid or branched-chain volatile fatty acid buffer additions did not decrease lag phases or time of maximum fermentation rate for western wheatgrass or the shortgrasses. Sodium sulfate had no effect on in vitro rate kinetics of dormant range grasses. Corn starch increased ($P<.05$) in vitro fermentation rates of all grasses and shortened ($P<.05$) lag phases and the time at which maximal in vitro fermentation occurred (Tables 3, 4 and 5). Starch may have been used initially as a preferred energy source by some cellulolytic bacteria over the grass itself resulting in improved fermentation parameters as measured by gas production. This delay in forage digestion could explain why starch plus urea addition did not affect digestibility of dormant range grasses.

In vitro digestions for screening amino acid, branched-chain volatile fatty acid and sulfur buffer additions to dormant range grasses suggest that methionine supplementation offers the greatest potential for improving digestibility and rate of fermentation of dormant cool season grasses. None of the amendments tested or urea improved digestibility of the warm season shortgrasses. Currently grazing studies are being conducted to determine the effects of a methionine/grain-urea supplement on digestibility and intake of dormant winter range and cow performance.

TABLE 3. EFFECTS OF IN VITRO BUFFER ADDITIONS ON DIGESTIBILITY OF WESTERN WHEATGRASS

Addition	Dry matter disappear-ance, %	NDF disappear-ance, %	Lag time, hr	Fermenta-tion rate, ml H_2O/hr	Time of maximum rate, hr
Urea	49.0^{a}	60.4^{ab}	16.9^{a}	1.70^{bcde}	30.6^{ab}
Amino acids					
Arginine	45.2^{b}	62.9^{bc}	16.6^{ab}	1.54^{def}	31.2^{a}
Histidine	48.0^{ab}	61.8^{abc}	14.9^{abc}	1.88^{abc}	28.9^{abc}
Isoleucine	48.6^{a}	61.6^{abc}	15.3^{abc}	1.77^{abcd}	28.7^{bc}
Leucine	47.9^{ab}	63.2^{bc}	16.2^{ab}	1.77^{abcd}	30.1^{abc}
Methionine	49.8^{a}	64.6^{c}	15.3^{abc}	1.92^{a}	29.8^{abc}
Branched-chain volatile fatty acids					
Isobutyric acid	49.4^{a}	61.2^{abc}	16.6^{ab}	1.68^{cde}	29.8^{abc}
Isovaleric acid	47.1^{ab}	61.5^{abc}	15.5^{abc}	1.56^{def}	29.5^{abc}
2-methylbutyric acid	48.3^{ab}	62.7^{bc}	15.9^{ab}	1.43^{f}	29.3^{abc}
Na_2SO_4	48.6^{a}	60.5^{ab}	16.6^{ab}	1.50^{ef}	30.4^{abc}
Starch	47.6^{ab}	62.8^{bc}	13.4^{c}	1.94^{a}	28.1^{c}

^{a,b,c} Means within column without a common superscript differ ($P<.05$).

TABLE 4. EFFECTS OF IN VITRO BUFFER ADDITIONS ON DIGESTIBILITY OF JAPANESE BROME

Addition	Dry matter disappearance, %	NDF disappearance, %	Lag Lag time, hr	Fermentation rate, ml H_2O/hr	Time of maximum rate, hr
Urea	58.1^{bc}	68.8^{cde}	15.7^{ab}	1.81^{a}	31.4^{a}
Amino Acids					
Arginine	56.4^{cd}	68.2^{def}	13.5^{abc}	1.82^{a}	31.0^{ab}
Histidine	54.4^{de}	67.2^{ef}	12.1^{c}	1.93^{ab}	29.3^{bc}
Isoleucine	59.0^{bc}	71.4^{abc}	13.3^{bc}	2.05^{b}	29.5^{bc}
Leucine	52.7^{e}	65.6^{f}	14.4^{abc}	1.71^{a}	32.1^{a}
Methionine	63.0^{a}	74.0^{a}	14.2^{abc}	2.81^{d}	26.9^{d}
Branched-chain volatile fatty acids					
Isobutyric acid	58.9^{bc}	69.8^{bcde}	15.7^{ab}	1.84^{ab}	30.9^{ab}
Isovaleric acid	55.8^{cde}	69.2^{cde}	13.5^{abc}	1.81^{a}	30.4^{abc}
2-methylbutyric acid	57.2^{bcd}	70.0^{bcde}	13.5^{abc}	1.77^{a}	29.7^{bc}
Na_2SO_4	60.4^{ab}	72.55^{ab}	16.1^{a}	1.90^{ab}	30.6^{abc}
Starch	57.5^{bcd}	70.6^{bcd}	12.1^{c}	2.29^{c}	29.0^{c}

[a,b,c,d,e,f] Means within column without a common superscript differ (P<.05).

TABLE 5. EFFECTS OF IN VITRO BUFFER ADDITIONS ON DIGESTIBILITY OF SHORTGRASS MIXTURE

Addition	Dry matter disappearance, %	NDF disappearance, %	Lag time, hr	Fermentation rate ml H_2O/hr	Time of maximum rate, hr
Urea	43.8^{ab}	64.1^{ab}	9.3^{abc}	1.13^{ab}	22.4^{b}
Amino Acids					
Arginine	41.0^{bc}	63.6^{ab}	9.6^{abc}	1.10^{a}	25.3^{cde}
Histidine	43.6^{abc}	63.9^{ab}	8.9^{ab}	1.41^{cd}	22.4^{ab}
Isoleucine	44.0^{ab}	64.5^{ab}	10.4^{abcd}	1.22^{abc}	24.0^{bcd}
Leucine	44.1^{a}	65.2^{a}	11.5^{cd}	1.33^{bcd}	25.2^{cde}
Methionine	42.8^{abc}	63.8^{ab}	12.3^{d}	1.32^{bc}	26.5^{de}
Branched-chain volatile fatty acids					
Isobutyric acid	43.3^{abc}	64.7^{ab}	10.6^{bcd}	1.26^{abc}	23.1^{abc}
Isovaleric acid	42.9^{abc}	64.5^{ab}	10.2^{abcd}	1.16^{ab}	23.4^{abc}
2-methylbutyric acid	44.2^{a}	64.3^{a}	9.2^{abc}	1.11^{a}	22.3^{ab}
Na_2SO_4	41.7^{abc}	62.6^{ab}	10.5^{abcd}	1.05^{a}	22.5^{ab}
Starch	42.7^{abc}	64.1^{ab}	7.9^{a}	1.53^{d}	21.1^{a}

^{a,b,c} Means within column without a common superscript differ ($P<.05$).