Influence of ruminal minerals on fiber utilization and supplementation on intake and nutrient balance of ewes

by Katherine Barnes Harris

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science

Montana State University

Abstract:
Four consecutive in vitro studies were conducted four weeks apart using two whiteface ewes as inocula donors to evaluate the effect of mineral concentration and inocula source on neutral detergent fiber (NDF) digestion. Diets consisted of grass-legume hay (trial 1), wheat straw (WS; trials 2 and 3) and anhydrous ammonia treated WS (WS + NH4; trial 4). Calcium, Mg, K or P were withheld from the buffer solution to reduce the absolute level of each mineral in the in vitro system. Inocula source affected (P<.05) rate and extent of NDF digestion. Inocula from trial 1 resulted in an increased (P<.05) rate for NDF digestion and greater (P<.05) potentially degraded NDF verses other inocula sources. Inocula from WS + NH4 resulted in a slower (PC.05) rate for NDF digestion but more (P<.05) potentially degraded NDF than WS. Potassium removal reduced (P<.05) the rate of digestion in trial 1 , but resulted in the highest (P,.05) rate in trial 2. In trial 4 removal of Mg and P reduced (P,.05) rate of NDF digestion. Potentially degraded NDF was lower (P<.05) with K removal in trials 2 and 3. These studies demonstrate that in vitro rate and extent of NDF digestion can be influenced by the mineral content of the rumen inocula. Magnesium, K and P appeared to influence NDF digestion more than Ca.

A supplementation trial evaluated the effects of supplementation on forage dry matter intake (DMI) and nutrient balance of gestating ewes grazing winter range. Sixteen ewes were randomly allotted to 1 of 2 daily treatments: control (no supplemental feed; C) and a pelleted supplement (.15 kg.hd-1.d-1; PS). Forage DMI was estimated as 1.8% of body weight with no difference (P> .05) between treatment groups. Supplemented ewes retained more (P<.05) N and gross energy daily than C ewes. Supplemented ewes were in positive N balance while C ewes were in negative balance. Mineral balances were similar (P> .05) between treatment groups with the exception of Mn which was greater (P<.05) in C ewes. Mineral balances were positive with the exception of Mg which was negative for both treatment groups (-.34 g/d; P>.05).

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

November, 1987

APPROVAL

of a thesis submitted by

Katherine Barnes Harris

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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AKNOWLEDGEMENTS

I would like to express my appreciation to the faculty and staff of the Department of Animal and Range Sciences, and the Montana Agriculture Experiment Station, Red Bluff Research Ranch and especially the following people:

Verl Thomas for his guidance, assistance, support and drive throughout my graduate program;

Mark Petersen, Rodney Kott and Mike McInerny for serving as members of my graduate committee;

Pat Jordan and Butch Anderson for their support and friendship during the winter collections;

Nancy Roth and Connie Clark for assistance with laboratory analyses;

Kathy Hanford and Steve Kachman for sharing their vast knowledge of statistical analyses and computers; and

I would especially like to thank my parents and family for their considerable support and faith in me.

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ABSTRACT

Four consecutive in vitro studies were conducted four weeks apart using two whiteface ewes as inocula donors to evaluate the effect of mineral concentration and inocula source on neutral detergent fiber (NDF) digestion. Diets consisted of grass-legume hay (trial 1), wheat straw (WS; trials 2 and 3) and anhydrous ammonia treated WS (WS + NH_4; trial 4). Calcium, Mg, K or P were withheld from the buffer solution to reduce the absolute level of each mineral in the in vitro system. Inocula source affected ($P<.05$) rate and extent of NDF digestion. Inocula from trial 1 resulted in an increased ($P<.05$) rate for NDF digestion and greater ($P<.05$) potentially degraded NDF verses other inocula sources. Inocula from WS + NH_4 resulted in a slower ($P<.05$) rate for NDF digestion but more ($P<.05$) potentially degraded NDF than WS. Potassium removal reduced ($P<.05$) the rate of digestion in trial 1, but resulted in the highest ($P<.05$) rate in trial 2. In trial 4 removal of Mg and P reduced ($P<.05$) rate of NDF digestion. Potentially degraded NDF was lower ($P<.05$) with K removal in trials 2 and 3. These studies demonstrate that in vitro rate and extent of NDF digestion can be influenced by the mineral content of the rumen inocula. Magnesium, K and P appeared to influence NDF digestion more than Ca.

A supplementation trial evaluated the effects of supplementation on forage dry matter intake (DMI) and nutrient balance of gestating ewes grazing winter range. Sixteen ewes were randomly allotted to 1 of 2 daily treatments: control (no supplemental feed; C) and a pelleted supplement (.15 $kg \cdot hd^{-1} \cdot d^{-1}$; PS). Forage DMI was estimated as 1.8% of body weight with no difference ($P>.05$) between treatment groups. Supplemented ewes retained more ($P<.05$) N and gross energy daily than C ewes. Supplemented ewes were in positive N balance while C ewes were in negative balance. Mineral balances were similar ($P>.05$) between treatment groups with the exception of Mn which was greater ($P<.05$) in C ewes. Mineral balances were positive with the exception of Mg which was negative for both treatment groups (-.34 g/d; $P>.05$).

INTRODUCTION

Throughout Montana native range provides much of the forage base for sheep production. In winter protein and energy concentration of range forage is low relative to the National Research Council (NRC, 1985) requirements. Van Dyne et al. (1964) and Thomas et al. (1986) reported an average crude protein (CP) content of 7.2% and 8.4%, respectively, in forage collected from rumen fistulated ewes grazing Montana winter range. These researchers did not determine nutritional adequacy of range forage because intake was not measured. The NRC (1985) recommends 9.3% CP for a 60 kg ewe during the first 15 weeks of gestation. Therefore, pregnant ewes grazing in the intermountain west are commonly supplemented with .15 to .23 kg of a grain based supplement containing 16 to 18% protein to offset the nutritional deficiencies of the forage (Van Dyne et al., 1964).

Recommendations for supplementation programs are determined by intake and(or) nutrient content of range forage and performance data of ewes. Measuring nutrient balance would assist in evaluating nutrients in the diet and allow for more precise formulation of supplements for ewes grazing winter range.

The microbial population of the rumen also has specific nutrient requirements. These requirements must be met in order to obtain optimum rumen fermentation of winter range forage. Durand and Kawashima (1980) reported that microbial growth and the various fermentation processes in the rumen can be depressed due to an inadequate supply of minerals.

The research reported herein consisted of two phases. A series of in vitro trials which investigated the affect of mineral concentration (Ca, Mg, K, and P) in an in vitro buffer solution and inocula source on rate and extent of fiber digestion of winter range forage. Phase II consisted of a winter feeding trial to determine the influence of supplementation on forage intake and nutrient balance of gestating ewes grazing winter range.

LITERATURE REVIEW

This review summarizes literature concerning: 1) the influence of minerals on ruminal microbial fiber utilization and 2) the effect of supplementation on ruminal rate of passage, total tract digestibility, intake, weight change and reproduction of sheep fed forage diets. A discussion of microbial mineral requirements and mineral functions for the following minerals: Ca, P, Mg, K, Mn, Cu and Zn is presented. Mineral requirements are related to fiber digestion. A second discussion describes fiber as it relates to rumen volume or fill, as well as rate and extent of digestion. In addition, the effect of supplementation and type of supplement on digestibility, intake and performance, as well as a brief overview of the effect of cold weather on intake is reviewed.

Influence of Minerals on Rumen Microbial Fiber Utilization

Importance of Minerals

Ruminant animals on roughage diets are dependent upon microorganisms for utilization of cellulose. If optimum cellulose degradation is to be attained the rumen environment must supply a balance of all nutrients required for optimal microbial activity (Martinez and Church, 1970). Nutrients are provided in ruminant rations to meet performance expectations and their presence in the rumen permits them to meet microbial requirements as well. There are two

classifications of inorganic requirements for rumen microorganisms: macrominerals and trace minerals. The macrominerals include sodium, potassium, calcium, magnesium, phosphorus, sulfur, and chloride. The trace minerals important to rumen microorganisms include iron, manganese, copper, molybdenum, zinc, cobalt, selenium and iodine (Hungate, 1966). Minerals such as zinc, copper, magnesium, molybdenum and cobalt are components of one or more enzymes. These minerals are concentrated by bacteria and their scarcity may limit microbial growth (Fenchel and Blackburn, 1979). Bacterial biosyntheses of secondary metabolites such as protease and riboflavin are affected by one or more metal ions which may be either required for or an inhibitor of a particular microbial product. These minerals include manganese, iron, copper, cobalt and zinc (Weinberg, 1983). Rumen microbial requirements for major minerals as shown by in vitro experiments are shown in Table 1. Major mineral content of rumen microorganisms is shown in Table 2 and trace mineral content of rumen microorganisms and of respective diet are shown in Table 3.

Saliva

Saliva is a lubricant that assists mastication and deglutition in all domestic animals. In ruminants, saliva is also important for regurgitation, remastication and transport of ingesta through the stomach to the small intestine. In addition, saliva forms a buffered medium in which the microorganisms of the rumen can florish (McDougall, 1948). Important nutrients contained in saliva include N, Na, K, Ca, Mg, P and Cl.

TABLE 1. RUMEN MICROBIAL REQUIREMENTS OF MAJOR MINERALS FOR CELLULOLYTIC ACTIVITY[a]

Sample[b]	P (mg/l)	Mg (mg/l)	Ca (mg/l)	K (g/l)	Na (g/l)
WCS[cd]	40[f] 40-90[e]	20-160[f]	50-300[e]	.5-2.0[e]	No effect
WCS[g]	10-100[f] 100-500[e]	5-10[e]	10-100[e]	.2-2.1[f]	.2-1.6[f]
SRF[d]	283-1033[e]	25[e]	20-40[e]	.05-.26[f]	.04-.2[f]

[a]adapted from Durand and Kawashima, 1980.
[b]WCS = washed cell suspension from rumen contents.
SRF = strained rumen fluid.
[c]WCS previously fermented in a P-deficient medium.
[d]Martinez, 1972.
[e]slight response to mineral addition.
[f]significant response to mineral addition.
[g]Uesaka et al., 1967.

TABLE 2. MAJOR MINERAL CONTENT OF RUMEN MICROORGANISMS (g/kg DRY MATTER) OBTAINED FROM SHEEP[a]

Diet	Nature of sample[b]	P	Mg	Ca
Semi-purified (post-feeding)[c]	WBS	14.0	.90	6.3
	AWBS	10.0	.13	2.0
	WMS	25.4	1.1	35.1
	AWMS	11.5	.4	3.2
Hay+concentrate (prefeeding)[d]	WBS	14.2		
	AWMS	7.2		
Mean of hay+ concentrate and hay alone (mean of pre and post feeding)[e]	WMS	16.7		
	SB	17.7		
	LB	12.4		
	P	12.5		

[a]adapted from Durand and Kawashima, 1980.
[b]WBS = washed bacterial sediment.
WMS = washed microbial sediment obtained after direct centrifugation of strained rumen fluid.
AWBS = acid-washed bacterial sediment (pH 2.8).
AWMS = acid-washed microbial sediment (pH 2.8).
SB = small bacteria.
LB = large bacteria.
P = protoza.
[c]Durand et al., unpublished.
[d]Van Newel and Demeyer, 1977.
[e]Durand and Kawashima, 1980.

TABLE 3. TRACE MINERAL CONTENT OF RUMEN MICROORGANISMS AND OF RESPECTIVE DIETS OF SHEEP[a]

Diet	Samples[b]	Fe	Mn	Zn	Cu
		mg/kg			
Hay[c]	diet	209	150	35	6.3
	MF	535-992	242-393	136-220	39-72
semi-purified[d]	diet	128	137	60	12
	WBS	445	133	242	35
semi-purified[d]	diet	128	137	1000	12
	WBS	850	90	3040	70
	AWBS	640	40	2140	60
semi-purified[d]	diet	128	137	115	12
	WMS	823	459	538	53
	AWMS	406	36	155	52

[a]adapted from Durand and Kawashima, 1980.
[b]MF = microbial fraction.
WBS = washed bacterial sediment.
AWBS = acid-washed bacterial sediment (pH 2.8).
WMS = washed microbial sediment.
AWMS = acid-washed microbial sediment (pH 2.8).
[c]Wetzel and Menke, 1978.
[d]Durand et al., unpublished.

Mayland and Lesperance (1977) compared the mineral composition of samples obtained from rumen fistulated cattle to that of the diet. These workers determined that fistula samples had relatively larger concentrations of ash, Si, Na, P, Zn, and Co than did diet samples due to salivary contamination. Small decreases were found in the Mg and Ca concentrations and small increases were found in N, K, Mn, Fe, and Mo values, but these were not generally different from diet concentrations. Therefore, dietary minerals influence salivary mineral concentration.

Rumen Fluid

The microbial population of rumen fluid is influenced by dietary factors (Giesecke, 1970). Elements must be released from food residues

in the rumen in order to be available to microorganisms (Playne et al., 1978). For example, feeding a poor quality diet results in rumen fluid with a low microbial count (Giesecke, 1970). Diet quality affects both microbial population and mineral content of rumen fluid (Table 4). Microbial growth and the various fermentation processes in the rumen can be depressed due to an inadequate mineral supply (Durand and Kawashima, 1980). However, some elements and insoluble complexes may be released by microbial enzymes such as P release from phytates (Weinberg, 1977). Dietary supplies of trace minerals have been shown to influence the efficiency of microbial fermentation and digestion (Durand and Kawashima 1980). Table 5 shows in vitro stimulatory effect of trace elements on different functions of rumen microorganisms.

Low concentrations of soluble trace elements are found in the supernate fraction of rumen fluid. This is due to formation of insoluble complexes and to uptake and accumulation of these minerals by microorganisms (Weinberg, 1977). Trace elements which are bound to bacterial cell walls may also be partially available for bacterial metabolism. In addition, protozoa mineral requirements can be partially met by engulfed bacteria. Therefore, it seems likely that trace mineral requirement of a microorganism is not necessarily met by the soluble form of that element (Durand and Kawashima, 1980). The addition of some elements can affect the solubility of others. For example, the solubility of Zn and Mn can be increased by addition of Cu, while Mn solubility can be increased by Zn (Durand and Kawashima, 1980). The concentration of trace elements in rumen fluid by dietary level and

TABLE 4. INFLUENCE OF DIETARY MINERAL LEVEL AND TYPE OF DIET ON MAJOR MINERAL CONTENT OF SHEEP RUMEN FLUID (RF)[a]

	P		Mg		Ca		K	
	diet	RF	diet	RF	diet	RF	diet	RF
Diet	g/kg	mg/l	g/kg	mg/l	g/kg	mg/l	g/kg	mg/l
Urea-maize concentrate[bc]	3.6	1300	1.5	80	9.2	450	5.7	.87
Lucerne hay[c]	2.8	370	2.1	110	12.8	180	23.9	2.53
semi-purified[bd]			<.17	11.4				
semi-purified[e]	.07	460			.7	27		
	3.4	781			7.3	82		
Lucerne + oat hulls[f]	1.7	300						
	9.4	950						
high in beet pulp[e]	1.3	690						
	1.6	930						
	3.2	1170						
semi-purified[bg]	0	80						
	7.0	760						

[a]adapted from Durand and Kawashima, 1980.
[b]strained rumen fluid.
[c]Durand et al., 1975.
[d]Tomas and Potter, 1976.
[e]Nel and Moir, 1974.
[f]Nel, 1974.
[g]Farries and Krasnodebska, 1972.

type of diet are shown in Table 6. Suggested trace element content of rumen fluid and the diet are shown in Table 7.

Calcium

Calcium requirements vary among rumen microorganisms. For example, protozoa appear to have higher calcium requirements than bacteria, and gram-positive bacteria have higher requirements than do gram-negative bacteria (Durand and Kawashima, 1980). Within microbial cells, the concentration of calcium is low in comparison to the external concentration. Internal enzymes requiring divalent cations can use Mg^{2+}

TABLE 5. IN VITRO STIMULATORY EFFECT OF TRACE ELEMENTS (mg/l) ON DIFFERENT FUNCTIONS OF RUMEN MICROORGANISMS[a]

Function	Nature of inoculum[b]	Fe	Mn	Zn	Co	Mo
Cellulolysis	WCS[f]	3-5[d]	5-30[b]	5-7[d]	3[d]	10-100[d]
	WCS + Chel[g]	.5-12.5[d]	7.5[c]	7.5[c]	e	
	SRF[g]	10[d]				10-1000[d]
Protein Synthesis	WCS[g]	5-40[c]				
	SRF[g]		e	e	.09-.18[c]	
	SRF + Chel[h]	1-2[a]				
	SRF[i]			20-80[c]		

[a]adapted from Durand and Kawashima, 1980.
[b]WCS = washed cell suspension.
SRF = strained rumen fluid.
Chel = addition of a chelating agent to the medium.
[c]slight response to mineral addition.
[d]significant response to mineral addition.
[e]no response to mineral addition.
[f]Church, 1976.
[g]Martinez, 1972.
[h]McNaught et al., 1950.
[i]Sonawane and Arora, 1976.

or Mn^{2+} in place of Ca^{2+}, therefore it has been concluded that there is no apparent intracellular role for calcium (Silver, 1977). Extracellular enzymes requiring calcium include proteases, nucleases, lipases, α -amylases and cellulases. In in vitro systems, many enzymes can utilize calcium in place of Mg^{2+} as substrate for numerous ATP-kinases such as nucleotide kinases and sugar kinases. However, other kinases cannot utilize Ca^{2+} because the Ca^{2+} ATP complex does not fit into the active site of the enzyme (Silver, 1977).

While calcium is necessary for nitrogen fixation by many bacteria such as Azotobacter vinelandii, it is not known whether all bacteria have a calcium requirement for growth. Deficiency of this mineral however, can cause growth defects and may alter metabolic processes requiring a calcium activated enzyme such as α -amylase. It has not

TABLE 6. CONCENTRATION OF TRACE ELEMENTS IN SHEEP RUMEN FLUID (RF) ACCORDING TO DIETARY LEVELS AND TYPE OF DIET[a]

	Fe		Mn		Zn		Cu		Mo	
Diet	diet	RF	diet	RF	diet	RF	diet	RF	diet	RF
	mg/kg									
dried grass[bd]			60	.39	40	.25	6	.12		
semi-purified[be]	128	2.14	137	5.0	60	2.0	12	.38		
semi-purified[ce]	128	14.3	137	14.5	60	12.3	12	1.3		
semi-purified[be]	128	4.0	137	11.0	1000	5.1	12	.32		
semi-purified[bf]									.53	.02
semi-purified[bf]									.48	.046

[a]adapted from Durand and Kawashima, 1980.
[b]supernate fraction after centrifugation at 20,000-36,000 g to eliminate bacteria.
[c]strained rumen fluid.
[d]Wetzel and Menke, 1978.
[e]Durand et al., unpublished.
[f]Grace and Suttle, 1979.

been established whether a calcium deficiency can actually occur in vivo, (Durand and Kawashima, 1980). Rumen microbial requirements for Ca appear to be met at levels of 10-40 mg/l (Table 1).

TABLE 7. SUGGESTED TRACE ELEMENT CONTENT OF RUMEN FLUID AND DIET[a]

	Fe	Mn	Zn	Co	Cu	Mo
In rumen fluid (mg/l)	1-10	1-10	.2-1	.1-.5	.01-.25	1-10
In diet (mg/kg)	120	120	50	.5-1	5-10?	?

[a]Durand and Kawashima, 1980.

Phosphorus

Phosphorus is necessary for carbohydrate fermentation and is a constituent of primary cell metabolites such as nucleotides and of coenzymes such as flavin phosphate, pyridoxal phosphate and thiamine pyrophosphate. Eighty percent of the phosphorus in rumen bacteria is found in nucleic acids while 10% is in phospholipids (Durand and Kawashima, 1980). Phosphorus occurs nearly exclusively as phosphates. Organic phosphates may be taken up directly or they are first hydrolyzed by extracellular alkaline phosphatases, but organic P may be very resistant to hydrolysis and not readily available to microorganisms. In the cell, orthophosphate is coupled to ADP to form ATP. Phosphate is essential for the transfer of energy and phosphorylations, and for the synthesis of nucleic acids in all living cells. Bacteria may store phosphorus in volutin granules and this pool may take up a considerable fraction of the total cell phosphorus (Fenchel and Blackburn, 1979).

Milton and Ternouth (1984) demonstrated an 8 to 40% increase in NDF digestion with the addition of P to buffered ruminal or caeco-colic

digesta using rumen fluid obtained from sheep fed a high calcium-low phosphorus diet, thus concluding that microorganisms in the large intestine have a P requirement similar to rumen microorganisms for the digestion of NDF. A mean level of about 100 mg/l of available P in the rumen is adequate for cellulolytic activity (Durand and Kawashima, 1980; Table 1).

Magnesium

Magnesium is necessary for many bacterial cell processes such as cell growth and normal cell division and this requirement is higher for cell division than for cell growth. This has been determined because cells grow without dividing when there is insufficient magnesium present, resulting in the formation of filamentous cells (Jasper and Silver, 1977). While magnesium requirements for growth are considered absolute, in some bacteria magnesium requirements can be reduced by addition of manganese (Jasper and Silver, 1977). Gram-positive bacteria have been found to have higher magnesium requirements than gram-negative bacteria, which has been attributed to the differences in intracellular permeability for magnesium (Lichstein, 1983). Intracellular magnesium is associated with the ribosomes and the synthesis of nucleic acids. Ribosomes are sensitive to Mg^{2+} deprivation and proteosynthesis is disturbed in many bacteria lacking magnesium. Magnesium is also involved in preserving the intregity of cell membranes and a deficiency often induces morphological changes that may affect cell function (Durand and Kawashima, 1980). Many bacterial enzymes such as phosphohydrolases and phosphotransferases are activated by magnesium. Rumen microbes may become magnesium deficient when the

animal is fed poor quality grass hay with a low magnesium content or when fed a diet of young grasses with a high soluble nitrogen content which can result in high NH_3 concentrations and the formation of insoluble magnesium ammonium phosphate.

Durand and Kawashima (1980) reported that in some studies, the addition of magnesium to in vitro systems improved cellulose digestion, while in other studies, the omission of magnesium and manganese resulted in severe reduction of cellulolysis, though the omission of magnesium alone had no effect. Other studies (Martin et al., 1964 and Ammerman et al., 1971), reported no improvement in in vitro cellulose digestion with supplemented magnesium when rumen inocula donors were fed diets adequate in magnesium, unless excess absorbed magnesium was removed through dilutions and fermentations in a magnesium-free medium. However, when sheep and cattle were fed purified diets devoid of magnesium, results showed reduced in vitro and in vivo cellulose digestion and changes in volatile fatty acid concentrations. These results support the importance of adequate magnesium in the ruminant's diet for microbial growth and function. Magnesium levels of 5-25 mg/l in rumen fluid appear to be adequate (Table 1).

Potassium

Potassium is required by microorganisms, though it can be replaced partially or totally by rubidium. Most rumen bacteria contain enzymes whose activity requires potassium. Potassium is essential for protein synthesis as well as for glycolysis. It is associated with the ribosomes in Aerobacter aerogenes and is bound to anionic polymers of cell walls in Bacillus subtilis (Caldwell et al., 1973).

Potassium and sodium are required for optimum fermentation in the rumen, but the required levels for microorganisms have not been established. High levels of potassium are necessary for Bacteroides for maximum growth when levels of sodium are low. While potassium content in rumen fluid of 800 mg/l may satisfy microbial requirements, adequate buffering capacity will depend on the supply of other cations (Durand and Kawashima, 1980).

Manganese

Manganese is required in trace quantities for growth of most cells, is a cofactor for a number of enzymatic reactions (Hungate, 1966) and is required for synthesis of many secondary metabolites such as antibodies. Enzymes may have a specific requirement for manganese in one microbial strain, while in another the requirement may be replaced by Mg^{2+} or another divalent cation such as Zn^{2+} or Co^{2+} (Weinberg, 1977). Manganese operates during glycolysis in the decarboxylation reactions of the citric acid cycle (Weinberg, 1977) and stimulates CO_2 fixation in the production of succinic acid by Ruminococcus flavefairens (Durand and Kawashima, 1980). Manganese can also serve as an alternate cofactor to Mg^{2+} in a large number of enzymes. Martinez and Church (1970), using washed suspensions of rumen microorganisms, reported up to 19% increased cellulose digestion with the addition of 5-30 ppm manganese and 24% depression of cellulose digestion with the addition of 100 ppm manganese. A Mn level of 1-10 mg/l in the rumen fluid is suggested (Table 7).

Copper

A small amount of copper may stimulate microbial protein synthesis (Durand and Kawashima, 1980). Copper can be either absorbed from or secreted into the stomach (Purser et al., 1984). Saxena and Ranjhan (1978) found increased cellulose digestion by 17% with supplementation of cobalt and copper to calves fed straw diets. Martinez and Church (1970) reported a 33% depression in cellulose digestion with the addition of 1 ppm copper to washed suspensions of rumen microorganisms. Copper levels in rumen fluid should be from .01-.25 mg/l (Table 7).

Zinc

Zinc is essential to all living systems and plays an important role in stabilizing various cell components such as ribosomes and membranes. Microbial zinc metalloenzymes include intracellular (DNA polymerase and RNA polymerase), extracellular (amylase and neutral proteases), and wall-associated (alkaline phosphatase) enzymes. The relatively high concentrations of zinc present in cell membranes are possibly associated with phospholipids and may also interact with various membrane-bound enzymes. The presence of zinc in bacterial cell walls contributes to stabilizing the interactions between various components of the wall, possibly binding the cells either to particles or other cells. Thus zinc may play an important role in the adherence of cellulolytic rumen bacteria to feed fiber (Durand and Kawashima, 1980). A deficiency of zinc adversely affects numerous cell characteristics including DNA and RNA levels, protein synthesis, as well as carbohydrate and phosphate metabolism (Failla, 1977). Martinez and Church (1970) reported the addition of 5-7 ppm zinc resulted in a

stimulatory effect on cellulose digestion while the addition of 20 ppm zinc depressed cellulose digestion. Rumen fluid should contain from .2-1 mg/l Zn (Table 7).

In summary, microorganisms are necessary for fiber digestion in ruminant animals. Nutrients, including minerals must be provided for microorganisms to meet their requirements in order to obtain maximum growth and cellulose utilization. Important minerals to consider are Ca, P, Mg, K, Mn, Cu, and Zn.

Affect of Supplementation on Rate of Passage, Digestibility, Intake, and Performance of Sheep Fed Forage Diets

Passage Rate and Digestibility

Intake and digestibility of feeds by ruminants are dependent upon the interaction of the diet, animal and feeding environment (Mertens, 1987b). Diets selected by grazing animals are the result of the interaction between animal preference, limitations of selection opportunity (distribution of plant species) and animal-based limitations (the extent to which dietary preferences are modified by the size of the mouth parts and mode of biting) (Grant et al., 1985). The digestibility of the diet of free-grazing animals is a product of a range of influences including diet selection, fermentation rate and passage rate factors such as ruminal retention and turnover within the gastrointestinal system of that particular animal. Therefore digestibility is not solely a characteristic of the diet consumed (Huston et al., 1986). The extent of digestion of fiber is the ultimate determination of digestibility and, along with intake,

determines the amount of digestible energy consumed (Huston et al., 1986).

Neutral detergent fiber (NDF) has been shown to be highly correlated with the volume or bulk density of feeds (Mertens, 1987b). Neutral detergent fiber should be most highly related to the space-occupying, or fill effect, of the diet (Mertens, 1987b). A high content of structural carbohydrates which are slowly fermented (Van Soest, 1975) will lead to a higher degree of rumen fill while soluble constituents in feeds dissolve and contribute very little to the fill effect (Mertens, 1987b). Non-fiber organic materials may contribute substantially to the whole tract digestion of energy. Such materials tend to be highly digestible either within or past the rumino-reticulum in foraging animals (Goering and Van Soest, 1970). Ulyatt and Egan (1979) determined that the water-soluble carbohydrates, organic acids and pectin of all diets studied were almost completely digested and that approximately 79 to 94% of potentially digestible hemicellulose and 87 to 97% of the cellulose were digested in the rumen.

The rate and extent of NDF digestion is the result of the interactions between the microbial population and the substrate. Digestion continues until passage of the digesta particles from the rumen (Huston et al., 1986). Rate of digestion appears minimally related or unrelated to animal species as such but may become characteristic of the host animal in response to the dietary behavior and(or) fluid dynamics in the gastrointestinal tract. Mertens (1987b) described intraruminal digestion as a competition between the rate of digestion and the rate of passage for potentially digestible substrate.

Upon introduction of forage into the rumen, a lag period occurs before digestion is initiated then increases to a maximum as the fast-digesting pool is fermented, followed by a decline in rate associated with the slow digesting pool. Increasing dietary concentration of slowly degraded or indigestible material reduces the rate of digestion and physical fill becomes limiting (Mertens and Ely, 1982; Van Soest, 1982; Mertens, 1983). Aitchison et al. (1986) support the hypothesis that degree of rumen fill is involved in the control of voluntary intake, particularly with grass hays. The extent of rumen fill is governed by factors which affect digestion in and passage from the rumen. However, as digestible energy content is increased metabolic controls become the dominant factors limiting intake (Aitchison et al, 1986).

Intake

In sheep, feed intake is controlled by energy demand for maintenance and production up to the limits of gastrointestinal capacity (Mertens, 1987a). Gut capacity is adequate for dry ewes to meet their energy requirements from roughages, until 30 d before lambing (NRC, 1987). Less than one month before lambing, depression in feed consumption may be the result of increasing competition for space within the abdomen between the rapidly growing fetus and the rumen (Lewis and Shelton, 1983). From conception to approximately the 120th d of pregnancy the abdominal wall expands to accommodate the increase in uterine volume, but after this rumen volume decreases (Forbes, 1969). While level of hay intake has been shown to be related to ruminal volume in late pregnancy or in very fat ewes, these same factors do not

seem to affect the volume of digesta post ruminally. Forbes (1969) found no significant relationship between voluntary intake and the volume of intestinal contents.

Huston and Engdahl (1983) reported that ewes had the greatest fill, longest retention time, and slowest rate of flow during winter when forage was dormant, fiberous, and low in digestibility. Supplemental feeding of concentrates and increasing levels of supplemental feed from 0 to 500 g/d tended to decrease fill, shorten rumen retention time and increase flow rate. Huston (1983) reported fecal output was highest during winter and not affected by supplemental feed level. Huston (1983) suggested that intake was limited during winter by maximum passage of undigested residues. Huston (1983) also reported that forage intake (2.1% of body weight) was not significantly different for non-lactating nonpregnant ewes or pregnant ewes carrying either single or twin fetuses.

Early researchers noted grazing behavior differences between supplemented and unsupplemented animals and found that animals may substitute supplement for grazing and therefore reduce forage intake and weight gain. McClymont (1956) reported that sheep grazing pasture gained more than those allowed 3 h a day of supplemental grazing on oats. This was due to anticipation of supplementation which resulted in decreased grazing time.

Holder (1962) reported that with Merino sheep grazing unimproved native pasture, feeding a oat grain supplement significantly depressed grazing time (28%) and pasture intake (36%) in comparison to unsupplemented sheep. Mixing supplemented and unsupplemented sheep

resulted in a decrease (12 to 16%) in grazing time of the unsupplemented sheep due to a "social inhibition" caused by the decreased grazing time of the supplemented sheep.

In a review, Allison (1985) noted that evidence is accumulating for the importance of supplemental protein and energy in relation to voluntary intake of forages. Generally, it has been found that addition of readily available carbohydrates to a roughage diet decreases voluntary intake, while addition of protein supplements to low quality roughage diets (below 8-10% crude protein) increases rate of digestion and voluntary intake. The increase in intake is generally attributed to increasing rumen microbial activity and consequently rate of passage.

Judkins et al. (1985) indicated that protein supplementation of wintering steers did not influence botanical or chemical composition of their diets or the amount of forage consumed. Cook and Harris (1967) reported that cattle receiving .22 kg mixed supplement daily ate .20 kg less range forage than nonsupplemented animals. However, total intake of digestible protein and ME in the combined ration of supplement and forage was greater than the nonsupplemented group in spite of the decreased forage intake resulting from supplementation. Clanton and Zimmerman (1965) found that protein supplementation is effective in stimulating voluntary intake of cattle only when the protein content of the forage is low (below 8.4%).

Kartchner (1981) found that providing a soybean meal protein supplement to range cattle grazing a mixed shrub and grass vegetation during the fall and winter improved forage dry matter intake and digestibility under severe winter conditions when grass was limited and

shrubs constituted a majority of the diet. However, under the same conditions, feeding a low level (.7 kg/d) of barley provided no apparent benefit when compared to unsupplemented animals.

Supplementation and Performance

Poor quality roughages are often unable to support rumen conditions that are conducive to optimal microbial activity because of their deficiency in total nitrogen, true protein, readily fermentable carbohydrates and minerals (Ndlovu and Buchanan-Smith, 1985). Ndlovu and Buchanan-Smith (1985) found that alfalfa supplementation improved rumen environment (increased ruminal ammonia-N levels) and rate of in sacco fiber digestion. Other workers found higher digestion of dry matter (DM), organic matter (OM), crude protein (CP) and gross energy (GE) with roughages supplemented with concentrate than with roughages alone (Antoniou and Hadjipanayiotou, 1985), and improved fiber digestion and microbial protein synthesis with urea treated straw (Merry et al., 1984). Using pregnant ewes fed diets of hay and concentrates providing varying levels of protein (from 2.0 to 7.2 g/kg $W^{\cdot 73}$) and high (150 kcal ME/kg $W^{\cdot 73}$) or low (125 kcal ME/kg $W^{\cdot 73}$) energy, Robinson and Forbes (1967) determined protein utilization at different stages of gestation. These workers reported apparent digestibility of both DM and CP decreased with decreasing protein intake and with the high energy diet apparent dry-matter digestibility increased and the apparent digestibility of CP decreased. With the high energy diet and high protein diet at all stages of gestation, absolute retention of N increased. In addition, nitrogen retention also increased with advancing pregnancy.

Kartchner (1981) found that supplemented animals on winter range with relatively mild climatic conditions and non-limiting forage showed no change in body condition in comparison to unsupplemented animals. However, others found supplemented cows on winter range gain more or maintain weight better and wean heavier calves than unsupplemented animals (Thomas, 1982; Thomas et al., 1960). This implies that the supplemented cows were in better body condition than the control cows. Harris et al. (1956) reported that supplements of protein, energy and phosphorus increased weight gains of pregnant ewes and supplemented ewes produced more lambs than those that were not supplemented. Harris et al. (1956) also reported that ewes in good condition could lose some weight during the winter grazing season and still produce efficiently.

Van Horn et al. (1959a) reported that increasing supplemental feeding levels during winter reduced the percentage of dry ewes and lambs lost from birth to weaning. Increasing feeding levels increased body weight gains of ewes during winter, birth weight of lambs, pounds of lambs weaned per ewe and grease and clean fleece weights. Van Horn et al. (1959b) determined that it was profitable to feed a moderate amount of supplement (.15 to .23 kg) during gestation, but it was not more profitable to feed a high protein supplement (36%)than a low protein supplement (16 to 18% protein).

High protein supplements are better for ruminants on winter range than high energy supplements even when energy is low in the diet because energy supplements such as corn and barley tend to reduce the digestibility of cellulose and other carbohydrates and do not substantially increase the overall energy intake (Cook and Harris,

1968). This is probably due to the microbial shift from cellulose fermentation to the more easily fermented starch. However, protein supplements such as soybean meal and cottonseed meal increase the digestibility of most nutritional constituents of the diet (Cook and Harris, 1968). This is probably due to the increased N supply to the microorganisms which results in a positive influence on fiber digestion. Weight gain resulting from protein supplementation is probably more closely associated with meeting the animal's requirements at the tissue level, by increasing the supply of microbial protein reaching the small intestines in contrast to an increased release of energy from the carbohydrate fraction of the forage (Rittenhouse et al., 1970). Therefore, protein supplementation is actually done to provide a positive influence on fiber digestion and forage intake (Clanton, 1981).

Cold Stress

Probably the one single weather condition that has most influence on grazing behavior is cold temperature (Young, 1983). Although cattle generally increase their intake as temperatures fall, they are less efficient and gain and produce less (Malechek and Smith, 1976; Young, 1981). As temperatures drop below homeostasis, more heat is required for the animal to maintain homeothermy or the same level of daily activity. The thermoneutral zone (TNZ) is the range of effective ambient temperatures in which the heat from normal maintenance and production functions of the animal in nonstressful situations offsets the heat loss to the environment without requiring an increase in rate of metabolic heat production (NRC, 1981). The lower border of the TNZ

can be defined as the lower critical temperature, which is the point below which an animal must increase its rate of heat production in order to maintain body temperature. The lower critical temperature may also be described as the point at which animal performance begins to decline as temperatures become colder (Kott, 1985). The lower critical temperature for a mature ewe at maintenance with 5cm fleece is 9 C (Blaxter, 1967).

Grazing accounts for the greatest amount of energy expenditure, and temperature and wind velocity have effects on time spent grazing (Malechek and Smith, 1976; Adams, 1984). Voluntary intake increases during exposure to cold relative to the animals TNZ and reaches a maximum before animals are severely cold stressed. When temperatures drop below the lower critical temperature, grazing animals reduce activity to conserve energy and start shivering (Malechek and Smith, 1976). However, daily temperature fluctuations around the TNZ do not influence intake (NRC, 1981). Reductions in grazing time can be expected with both falling temperatures and increasing wind speeds (Adams, 1984).

In summary, the digestibility of the diet of free-grazing ruminants is influenced by diet selection as well as fermentation rate and passage rate. Forage intake is influenced by several factors such as digestibility of the diet, supplementation, and the environment.

EXPERIMENTAL PROCEDURE

In Vitro Trials

Four consecutive in vitro trials were conducted four weeks apart using two mature western whiteface ewes as innocula donors. The same animals were used in all trials. An attempt was made to decrease the concentration of Ca, P, K, and Mg in the rumen fluid. Table 8 gives the description of the in vitro trials. In the first trial the animals were fed ad libitum chopped grass-legume hay. After completing the first in vitro trial the diets were changed to chopped wheat straw (WS) and fed ad libitum through the end of the third in vitro trial at which time the animals were fed ad libitum anhydrous ammonia treated wheat straw (WS + NH_4). All diets were fed for four weeks prior to collection of rumen fluid. Iodized salt was available free choice and no other supplemental minerals were provided during the trial. The winter range forage was a composite of rumen extrusa collections from ewes grazing winter range at the Red Bluff Research Ranch near Norris, Montana from a previous study (Thomas et al., 1986). Nutrient composition of forage fed and extrusa is shown in Table 9. The extrusa samples were freeze-dried, ground through a 1 mm screen and .25 g weighed into 50 ml in vitro fermentation tubes. Mineral composition of diets and extrusa is shown in Table 10. In each trial the Ca, P, Mg, or K were withheld from the control buffer solution (McDougall, 1948; Table 11). The control buffer was modified as follows: Ca removed; NaCl replaced $CaCl_2$, 2) P

TABLE 8. DESCRIPTION OF IN VITRO TRIALS

Trial	number animals[a]	diet fed	length of feeding prior to in vitro, d
1	2	grass-legume	28
2	2	wheat straw	28
3	2	wheat straw	28
4	2	ammoniated wheat straw	28

[a]The same animals were used in all in vitro trials.

TABLE 9. NUTRIENT COMPOSITION OF EXTRUSA AND FORAGE FED INOCULA DONORS (% dry matter)

Item	Trial 1[a]	Trial 2[b] and 3[b]	Trial 4[c]	extrusa
NDF, %	59.5	69.5	63.0	64.5
ADF, %	39.6	48.1	36.9	42.7
CP, %	7.4	4.5	10.5	8.4

[a]grass legume.
[b]wheat straw.
[c]ammoniated wheat straw.

TABLE 10. MINERAL COMPOSITION OF FORAGES FED AND RUMEN EXTRUSA (% dry matter)

Mineral	Trial 1[a]	Trial 2[b] and 3[b]	Trial 4[c]	extrusa
Ca	.4350	.3138	.1785	.6703
Mg	.1610	.0873	.1783	.0763
K	2.0486	1.8696	1.2533	.4762
P	.1765	.1187	.0783	.3614

[a]grass legume.
[b]wheat straw.
[c]ammoniated wheat straw.

TABLE 11. CHEMICAL SUBSTITUTIONS IN BUFFER SOLUTION

Ingredient	Substitution
$NaHCO_3$	----
Na_2HPO_4	$NaHCO_3$
KCl	NaCl
NaCl	----
$MgSO_4$	$(NH_4)_2SO_4$
$CaCl_2$	NaCl

TABLE 12. MINERAL COMPOSITION OF RUMEN FLUID

Mineral	Trial 1[a]	2[b]	3[b]	4[c]
Ca, % DM[d]	.9252	.6910	.7773	.4249
Mg, % DM	.3858	.2563	.2853	.1630
K, % DM	9.3783	6.6694	7.4667	3.8013
P, % DM	2.3694	2.0504	2.0342	3.5362
Ca, mg/l[e]	185.0	138.2	155.4	84.9
Mg, mg/l[e]	77.1	51.2	57.0	32.6
K, g/l[e]	1.87	1.33	1.49	.76
P, mg/l[e]	473.8	410.0	406.8	707.2

[a]grass legume.
[b]wheat straw.
[c]ammoniated wheat straw.
[d]DM = dry matter.
[e]Assumes a DM content of 2% based on laboratory observations.

removed; $NaHCO_3$ replaced Na_2HPO_4, 3) Mg removed; $(NH_4)_2SO_4$ replaced $MgSO_4$ and 4) K removed; NaCl replaced KCl. Following adjustment of pH to 6.9, 20 ml of buffer were added to the forage sample. Rumen fluid used as innocula was collected, composited and strained through sixteen layers of cheesecloth to remove large digesta particles. Samples of the rumen fluid were frozen for later freeze-drying and analysis of mineral content (Ca, P, Mg and K) by inductive plasma coupling (OSAES, 1986; Table 12). Rumen ammonia levels at the time of the innocula collection were analyzed from frozen samples (AOAC, 1980). The rumen fluid was continuously agitated at 39 C while 5 ml were added into each in vitro tube. Fermentation tubes were flushed with CO_2, capped, stirred and incubated at 39 C for 6, 12, 18, 24, 36 and 48 hours. Fermentation was halted using .5 ml mercuric chloride. After incubation, tubes were centrifuged at 2000 rpm for 15 minutes, decanted, dried at 60 C for 48 h, and weighed to determine DM content. The concentration of NDF was determined for each residue (Van Soest and Wine, 1967).

h, and weighed to determine DM content. The concentration of NDF was determined for each residue (Van Soest and Wine, 1967).

Rate and potentially degradable NDF were determined using the equation $y = ae^{-kt} + u$ where y = predicted amount remaining at time t; a = potentially degradable portion; e = 2.718; k = relative rate of potentially degradable portion; t = time in hours; and u = potentially undegradable portion (Mertens, 1977). Data were analyzed using a split plot analyses of variance by the General Linear Model procedure of SAS (1985). Dependent variables were rate and extent of potentially degraded NDF. Independent variables were trial (inocula source), buffer and trial by buffer interactions. There were three replications within each trial by buffer subcell, as such error is an estimate of replication within trial by buffer interaction.

Supplementation Study

A winter feeding trial was initiated in December 1985 at the Montana State Agricultural Experiment Station, Red Bluff Research Ranch near Norris, Montana. Elevation and annual precipitation ranged from 1,402 to 1,889 m, and 35.5 to 43.1 cm, respectively. Snow cover is uncommon and rarely persists for more than a few days. The upland vegetation is a typical foothill bunchgrass type. Bluebunch wheatgrass (Agropyron spicatum) and Idaho fescue (Festuca idahoensis) are the major grasses. Rubber rabbitbrush (Chrysothamnus nauseous), fringed sagewort (Artemisia frigida), lupine (Lupinus spp.), milkvetch (Astragalus spp.) and western yarrow (Achillea millefolium) are commonly occurring shrubs and forbs (Thomas et al., 1986).

for approximately 1 h and fed supplement. All animals were turned out onto the range and allowed to graze for approximately 8 h before returning to the bedground. From the 600 western whiteface ewes, sixteen ewes ages 3 to 4 were selected for random allocation to 1 of 2 treatments: control (no supplemental feed; C) and a pelleted barley-soybean meal supplement (.15 $kg \cdot hd^{-1} \cdot d^{-1}$; PS; Table 13). Treatments began approximately 120 d prior to and continued until 30 d prior to the first expected lambing date (December 11, 1985 to March 11, 1986). All ewes were weighed and assigned a body condition score at the beginning of the trial and at 28 d intervals thereafter. Body condition score was based on a scale of 1 to 5 with a score of 1 designating an emaciated ewe and 5 designating an obese ewe (Russel et al., 1969).

Ewes were fitted with fecal bags and four ninety-six hour total fecal and urine collection periods were conducted during January 6 to 10 (P1), January 20 to 24 (P2), February 3 to 7 (P3), and February 17 to 20 (P4). Fecal bags were changed every 24 h and excreta weighed, mixed and subsampled. Excreta samples were frozen and later freeze-dried. Daily samples from each ewe were ground in a Wiley mill through a 1 mm screen and composited by collection period. Composited samples consisted of 1% of each daily total excreta DM during each collection period. The composited excreta samples were analyzed for DM, ash, CP (AOAC, 1980), neutral detergent fiber (Van Soest and Wine, 1967), and Ca, P, Mg, K, Mn, Cu and Zn content by inductive plasma coupling (OSAES, 1986).

Forage samples were obtained using total rumen evacuation (Lesperance et al., 1960) and were collected three times during each

Forage samples were obtained using total rumen evacuation (Lesperance et al., 1960) and were collected three times during each fecal collection period. Rumen extrusa was collected from 6 rumen fistulated ewes and used for nutrient analysis as fistulated and non-fistulated animals of similar history and nutritional background do not differ in grazing behavior or diet composition (Forbes and Beattie, 1987). Rumen fistulated animals were used rather than esophageal fistulated animals due to the stressful conditions on winter range. Extrusa samples were hand squeezed to decrease salivary contamination, frozen and later freeze-dried. Samples were ground through a 1 mm screen and analyzed for DM, ash, CP (AOAC, 1980), acid detergent insoluble nitrogen (ADIN, Goering and Van Soest, 1970), NDF, acid detergent fiber (ADF) (Van Soest and Wine, 1967), in vitro indigestible NDF (IVNDF) (Barnes, 1969; Van Soest and Wine, 1967) and mineral content by inductive plasma coupling (OSAES, 1986). Mineral analyses were similar to those previously described for excreta samples. Means for each sample analyses were calculated (SAS, 1985) within each collection period and these means were used to estimate nutrient quality of the forage.

Indigestible NDF has been found to be a useful internal marker (Lippke et al., 1986), therefore forage and excreta NDF were used to estimate forage dry matter intake (DMI). This corrected for urinary contribution to the sample. In addition, supplement NDF was determined to correct for supplement contribution in excreta. The formulas (Ansotegui, 1986) used to estimate forage intake were:

$$\text{kg NDF intake/d} = \frac{\text{kg fecal NDF/d - supplement IVNDF g}}{\text{\% forage IVNDF}} \times 100$$

$$\text{kg forage intake} = \frac{\text{kg NDF intake}}{\text{\% forage NDF}} \times 100$$

Forage intake, fecal output, nitrogen, energy and mineral balance data were analyzed using a split plot analyses of variance by the General Linear Model procedure of SAS (1985). Dependent variables were DMI and nutrient intake, output, and balance (N, ME, Ca, P, Mg, K, Mn, Cu and Zn). Independent variables were supplementation (C or PS), period, supplement by period interaction and ewe within supplement. Ewe within supplement was used as an error term to test significance level for supplement and supplement by period. Preliminary analyses were conducted on breed and age however, there were no differences (P>.10).

TABLE 13. INGREDIENT AND CHEMICAL COMPOSITION OF FEED SUPPLEMENT

Item	% dry matter
Ingredient composition[a], %	
Barley	79.5
Soybean meal	15.0
Molasses, cane	5.0
Dicalcium phosphate	.25
Trace mineral salt	.25
	100.00
Chemical composition	
Crude protein, %	20.2
Energy, Mcal/kg	4.03
NDF, %	15.7
ADF, %	5.9
ADIN, %	.7
In vitro NDF digestibility, %	92.1
Minerals, %	
Ca	.21
P	.48
Mg	.18
K	.96
Minerals, ppm	
Mn	35.2
Cu	15.2
Zn	50.7

[a]Supplement contained 2,200 IU/kg vit. A and 272 IU/kg vit. D.

RESULTS

In Vitro Trials

Inocula from ewes fed different diets affected (P<.05) rate and extent of potentially degraded NDF (Table 14). Inocula from ewes fed grass-legume hay resulted in increased (P<.05) rate for NDF digestion and greater (P<.05) potentially degraded NDF verses other inocula sources (Table 14). Rate per h ranged from a high of .188 %/h (trial 1) to a low of .071 %/h (trial 4). Inocula from anhydrous ammonia treated WS resulted in a slower (P<.05) rate for NDF digestion (.071 vs .106 and .091 %/h) but more (P<.05) potentially degraded NDF (55.8 vs 48.9 and 50.6 %) than WS not treated with ammonia.

Calcium removal from the buffer had no effect on rate or extent of potentially degraded NDF in any trial in comparison to the control (Table 15). Removal of potassium reduced (P<.05) the rate of digestion in trial 1 (grass-legume; .146 vs .199 %/h) and tended (P>.05) to reduce rate in trial 4 (WS + NH_4) in comparison to the control (.066 vs .104 %/h; Table 15). However, in trial 2 (WS) the highest (P<.05) rate constant for NDF digestion in comparison to the control was observed with K removal (.154 vs .089 %/h). In trial 4 (WS + NH_4) removal of Mg and P reduced (P<.05) rate of NDF digestion versus the control (.062 and .056 vs .104 %/h).

Potentially degradable NDF was lower (P<.05) when K was removed from the buffer in comparison to the control in trial 2 (WS; 42.5 vs

48.7 %) and 3 (WS; 48.0 vs 54.7 %). Phosphorus removal reduced extent of NDF digestion in trial 3 (WS) in comparison to the control (48.7 vs 54.7 %). In trial 4 ($WS+NH_4$), Mg removal increased ($P<.05$) extent of NDF digestion in comparison to the control (60.2 vs 53.6 %).

TABLE 14. EFFECT OF RUMEN INOCULA SOURCE ON RUMINAL AMMONIA LEVEL AND RATE AND EXTENT OF IN VITRO NEUTRAL DETERGENT FIBER DIGESTION

Item	Trial 1[a]	2[b]	3[b]	4[c]	SE[d]
Ruminal ammonia, mg/dl	12.4	7.3	5.1	11.2	
Rate, %/h	.188[e]	.106[f]	.091[f]	.071[g]	.006
Potentially degraded NDF, %	73.1[e]	48.9[f]	50.6[f]	55.8[g]	.88

[a]Grass legume.
[b]Wheat straw.
[c]Ammoniated wheat straw.
[d]Standard error of least square mean.
[e,f,g]Means within the same row with different superscripts differ ($P<.05$).

TABLE 15. THE EFFECT OF RUMINAL INOCULA SOURCE AND MINERALS ON RATE AND EXTENT OF IN VITRO NEUTRAL DETERGENT FIBER DIGESTION

Treatment	Trial 1[a]	2[b]	3[b]	4[c]
	% per h			
Control	.199[d]	.089[ef]	.074[d]	.104[d]
Ca removed	.197[d]	.124[de]	.109[d]	.066[de]
Mg removed	.205[d]	.073[f]	.088[d]	.062[e]
K removed	.146[e]	.154[d]	.088[d]	.066[de]
P removed	.191[d]	.089[e]	.095[d]	.056[e]
SE[g]	.01	.01	.01	.01
	Potentially degraded NDF, %			
Control	71.9[de]	48.7[d]	54.7[d]	53.6[e]
Ca removed	72.4[de]	47.3[df]	50.9[de]	55.5[de]
Mg removed	69.2[e]	53.0[d]	50.5[de]	60.2[d]
K removed	77.4[d]	42.5[f]	48.0[e]	52.5[e]
P removed	74.4[de]	53.0[d]	48.7[e]	57.0[e]
SE[g]	1.9	1.9	1.9	1.9

[a]Grass legume.
[b]Wheat straw.
[c]Ammoniated wheat straw.
[d,e,f]Means within same column with different superscripts differ ($P<.05$).
[g]Standard error of least square means.

Supplementation Study

Extrusa samples contained an average of 7.7% CP with a low of 6.9% CP during P1 and a high of 8.5% CP during P2 (Table 16). These results were similar to those reported by Van Dyne et al. (1964) and Thomas et al. (1986) on Montana winter range. Approximately 10% of the protein was bound to the fiber fraction of the forage (ADIN .81% / CP 7.7%). Forage samples contained an average of 4.1 Mcal/kg gross energy. In-vitro NDF digestibility averaged 47.1 percent. Mineral concentrations reported in Table 8 were used to calculate mineral intake.

Mean forage DMI during the wintering period was 1.8 and 1.7% of body weight respectively for nonsupplemented and supplemented ewes (Table 17). There were no differences ($P>.05$) in DMI when expressed on a g/d basis (1117 vs 1070g) or when expressed as a percent of body weight (BW; Table 17). Forage DMI (g/d and as %BW) was highest ($P<.05$) during P2, and higher during P4 than P1 and P3 (Table 17).

There were no differences ($P>.05$) in N intake (15.5g vs 18.7g) between treatments, however N intake was below the NRC (1985) requirement (19.8g N/d, Table 18). Nitrogen intake and excretion were greatest ($P<.05$) during P2 and higher ($P<.05$) during P4 than P1 and P3 (Table 19). The NRC (1985) N intake requirement (19.8 g N/d) was met (23.2 g N/d) during P2 when ewes had the greatest ($P<.05$) forage DMI, but N intake was below NRC (1985) requirement level throughout the rest of the winter period. Supplemented ewes retained more ($P<.05$) g N/d and were in positive N balance while C ewes were in negative N balance

TABLE 16. CHEMICAL COMPOSITION AND DIGESTIBILITY OF RUMEN EXTRUSA COLLECTED FROM EWESa

Item	Period 1	2	3	4	Mean	SE[a]
Crude protein, %	6.9	8.5	7.0	7.1	7.7	.2
Gross energy, Mcal/kg	4.2	4.2	4.1	4.1	4.1	.01
NDF, %	70.9	64.2	70.6	70.1	67.9	.8
ADF, %	44.7	41.5	43.4	45.2	43.2	.6
ADIN, %	.51	.91	.64	.93	.81	.05
In-vitro NDF digestibility, %	45.3	49.5	43.7	46.7	47.1	.7
Ash, %	10.8	10.5	11.3	10.5	10.8	.2
Ca, %	.45	.74	.47	.57	.60	.20
P, %	.24	.37	.34	.35	.35	.09
Mg, %	.05	.08	.044	.051	.06	.03
K, %	.27	.47	.31	.42	.40	.16
Mn, ppm	38.3	55.2	42.3	56.8	50.7	18.6
Cu, ppm	2.8	6.2	2.3	5.2	4.7	2.7
Zn, ppm	10.2	21.6	8.8	17.6	16.2	8.2

[a]Standard error of means.

TABLE 17. EFFECT OF SUPPLEMENT AND PERIOD ON FORAGE DRY MATTER INTAKE (DMI) AND ORGANIC MATTER INTAKE (OMI)

	Supplement None	.15	SE[a]	Period 1	2	3	4	SE[a]
DMI, g/d	1117	1070	50.9	1015[c]	1221[e]	1008[c]	1113[d]	15.5
DMI, % BW[b]	1.8	1.7	.07	1.6[c]	2.0[e]	1.6[c]	1.8[d]	.002
DMI, % $BW^{.75}$	5.1	4.8	.2	4.6[c]	5.5[e]	4.5[c]	5.0[d]	.07
OMI, g/d	1252	1199	57.0	1137	1368	1130	1247	17.3
OMI, % BW	2.0	1.9	.08	1.8	2.2	1.8	2.0	.002
OMI, % $BW^{.75}$	5.7	5.3	.2	5.1	6.1	5.0	5.6	.08

[a]Standard error of least square mean.
[b]Body weight.
[c,d,e]Means within same row with different superscripts differ (P<.05)

during the wintering period (.0004 vs -.003; Table 19). Despite dietary intake levels above NRC (1985) requirement during P2, ewes were in negative N balance.

Gross energy (GE) intake was higher (P<.05) for PS ewes than for C ewes (5.0 vs 4.6 Mcal) and followed forage DMI resulting in the highest (P<.05) GE intake during P2 and higher (P<.05) GE intake during P4 than

TABLE 18. DAILY NITROGEN (N), METABOLIZABLE ENERGY (ME) AND MINERAL INTAKE OF GESTATING EWES GRAZING WINTER RANGE AND 1985 NRC REQUIREMENT

	Supplement			
Item	None	.15 kg/d	SE[a]	NRC[b]
N intake, g	15.5	18.7	1.3	19.8
ME, Mcal	.91[e]	1.46[f]	.06	2.6
Calcium, g	5.68	6.02	.3	3.2
Phosphorus, g	3.69[c]	4.18[d]	.1	3.4
Magnesium, g	55.0	61.0	2.0	160.0
Potassium, g	4.18[e]	5.29[f]	.1	6.5
Manganese, mg	54.7	57.2	.2	26.0
Copper, mg	4.8[e]	6.6[f]	.2	9.1
Zinc, mg	16.1[e]	22.8[f]	.7	26.0

[a]Standard error of means.
[b]NRC (1985) requirements for 60 kg ewe during the first 15 weeks of gestation.
[c,d]Means within column with different superscripts differ (P<.05).
[e,f]Means within column with different superscripts differ (P<.01).

P3 or P1 (Table 20). Excreta energy output (3.7 and 3.6 Mcal/d; Table 20) was similar (P>.05) between treatments. Therefore PS ewes had a higher (P<.05) energy balance or metabolizable energy (ME) intake which resulted in a higher (P<.05) percentage of dietary energy retained and therefore greater energy efficiency than C ewes (28.5 vs 18.8 %; Table 20). Metabolizable energy intake was highest (P<.05) during P2 followed by P4, P3 then P1. Fecal and urinary energy output decreased (P<.05) from P1 to P2 (3.8 vs 3.6 Mcal/d; Table 20) and tended to decrease from P2 to P3 (3.6 vs 3.5 Mcal/d) and from P3 to P4 (3.5 vs 3.4 Mcal/d). Energy efficiency followed the same order as energy balance (P2>P4>P3>P1; P<.01; Table 20).

Intakes of Ca, P and Mn (Table 18) were above and Mg, K, Cu and Zn were below the NRC (1985) requirements while. Supplementation had no affect (P>.05) on dietary intake of Ca, Mg and Mn (Table 21). The PS ewes had greater intakes (P<.05) of K, P, Cu and Zn than C ewes, but

TABLE 19. EFFECT OF SUPPLEMENTATION AND PERIOD ON NITROGEN (N) INTAKE, OUTPUT AND BALANCE OF GESTATING EWES GRAZING WINTER RANGE

Item	Supplement			Period				
	None	.15 kg/d	SE[a]	1	2	3	4	SE[a]
N intake, g/d	15.54	18.73	1.3	13.76[b]	23.21[b]	13.70[b]	16.73[c]	.48
N output, g/d	15.54	18.73	1.3	13.76[b]	23.21[d]	13.70[b]	16.73[c]	.48
N balance, g/d	- .003[d]	.0004[e]	.0007	- .003[b]	- .003[b]	.001[c]	.001[c]	.0005

[a]Standard error of least square means.
[b,c,d]Means within rows with different superscripts differ (P<.01).

TABLE 20. EFFECT OF SUPPLEMENTATION AND PERIOD ON ENERGY INTAKE, OUTPUT, BALANCE AND EFFICIENCY OF GESTATING EWES GRAZING WINTER RANGE

	Supplement			Period				
Item	None	.15 kg	SE[a]	1	2	3	4	SE[a]
Intake, Mcal/d	4.6[b]	5.0[c]	.124	4.5[d]	5.4[f]	4.4[d]	4.8[e]	.06
Output, Mcal/d	3.7	3.6	.07	3.8[f]	3.6[e]	3.5[de]	3.4[d]	.04
Balance, Mcal/d	.9[d]	1.4[e]	.08	.69[d]	1.7[g]	.87[e]	1.4[f]	.03
Efficiency, %	18.8[d]	28.5[e]	1.3	14.9[d]	31.6[g]	19.6[e]	28.9[f]	.54

[a]Standard error of least square mean.
[b,c]Means within rows with different superscripts differ (P<.05).
[d,e,f,g]Means within rows with different superscripts differ (P<.01).

intake levels were below NRC (1985) requirements with the exception of P (Table 18). All ewes also had similar (P>.05) daily mineral outputs with the exceptions of K, Cu and Zn which were greater (P<.05) in PS ewes (Table 21). All mineral balances were positive with the exception of Mg (Table 21). As mineral intake increased, concentration of minerals in the excreta increased, therefore balances were not different (P>.05) between treatments with the exception of Mn which was higher (P<.05) in C than PS ewes (32.2 vs 26.8 mg/d). The C ewes retained a greater (P<.05) percentage of their dietary Cu (81.3 vs 37.0 %) and Zn (91.2 vs 50.7 %) and tended (P>.05) to retain more of the dietary K (44.5 vs 35.9%) and Mn (71.2 vs 55.1%) than PS ewes which suggests C ewes might have been stressed for these minerals. Effect of period on mineral intake, output, balance and efficiency (Table 22) as expected generally followed DMI.

TABLE 21 MINERAL INTAKE, OUTPUT AND BALANCE OF GESTATING EWES GRAZING WINTER RANGE

	Intake			Output			Balance			Efficiency[a]		
Mineral	None	.15 kg	SE[b]	None	.15 kg	SE[b]	None	.15 kg	SE[b]	None	.15 kg	SE[b]
Ca, g	5.6	6.0	.3	3.6	3.8	.3	2.0	2.1	.1	51.3	57.9	12.6
P, g	3.6[c]	4.1[d]	.1	2.0	2.2	.1	1.6	1.8	.09	41.6	43.7	2.2
Mg, g	.54	.61	.02	.91	.94	.1	- .36	- .33	.09	---	---	---
K, g	4.1[e]	5.2[f]	.1	2.7[c]	3.5[d]	.2	1.4	1.7	.1	44.5	35.9	5.3
Mn, mg	54.7	57.1	2.3	2.3	3.0	.3	32.3[c]	26.8[d]	1.7	71.2	55.1	6.0
Cu, mg	4.7[e]	6.5[f]	.2	2.3[e]	4.2[f]	.1	2.5	2.4	.1	81.3[e]	37.0[f]	2.1
Zn, mg	16.5[e]	22.6[f]	.7	6.0[e]	12.2[f]	.8	10.5	10.4	.7	91.2[e]	50.7[f]	7.5

[a]Percent dietary mineral retained.
[b]Standard error of least square mean.
[c,d]Means within an intake, output or balance column with different superscript differ (P<.05).
[e,f]Means within an intake, output or balance column with different superscript differ (P<.01).