

Influence of genotype on in vitro dry matter disappearance rate, estimated microbial yield and in vivo digestive physiology of barley
by Ruth Ellen Kemalyan

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
Effects of genotypic variation among barley cultivars were evaluated under in vitro and in vivo. In vitro dry matter disappearance rate and estimated microbial yield (as measured by purine accumulation) was measured using one normal barley (Cl 15478), 5 proanthocyanidin-free mutant cultivars (ANT 534, ANT 563, ANT 230, ANT 231, ANT 246) and 2 cross-line barleys (CA 602914, CA 57163). Four cultivars were selected for use in a sheep metabolism study. Cultivars were analyzed for starch, protein, neutral detergent fiber (NDF), fiavonoid and total phenolic compounds and $ -glucan content. Correlation coefficients were calculated between the chemical constituents, DMDR and purine accumulation. Cultivars differed (P<.05) with respect to DMDR and purine accumulation. Negative relationships (P<.05) were detected between DMDRand starch (r=-.44), fiavonoid compounds (r=-.30) and phenolic compounds (r=-.31). Purine accumulation was positively associated with protein content (r=.30) and NDF (r=.46) and negatively with starch (r=-.38), fiavonoid compounds (r=-.32), phenolics (r=-.37) and $ glucan (r=-.29). The metabolism study investigated one normal barley, (Cl 15478), 3 proanthocyanidin-free mutant cultivars (ANT 534, ANT 246, ANT 231) and com. Diets were formulated to provide equal starch, protein and NDF. The diets were fed to mature ruminally and duodenally cannulated Rambouillet ewes in a 5 X 5 Latin square design. Ruminal diy matter and organic matter digestibility in ewes was affected by diet (P<.05). Viscosity and pH of ruminal fluid were similar in all ewes. Ruminal viscosity appeared to be inversely related to $ -glucan content of the grain. Ruminal and duodenal NH3 concentrations were similar in ewes fed all diets. The ruminal digestion of starch (P<.01) and duodenal starch flow (P<.05) in ewes was influenced by diet. Effects of diet fed to ewes were found in duodenal CP flow (P<.05), dietary escape protein flow (P<.01) with dietary escape protein flow greater (P<.01) in ewes fed com. Microbial nitrogen concentration in the duodenum were similar in ewes fed each diet. Microbial efficiencies differed (P<.05) in ewes fed the various diets with values ranging from 13.3 to 21.4 g microbial protein produced/100 g OM digested. Results from this study demonstrate variation among lines of barley for in vitro DMDR and estimated microbial yield. These results also demonstrate genotypic variation among barley cultivars site and extent of digestion.

INFLUENCE OF GENOTYPE ON IN VITRO DRY MATTER DISAPPEARANCE RATE, ESTIMATED MICROBIAL YIELD AND IN VIVO DIGESTIVE PHYSIOLOGY OF BARLEY

by

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ABSTRACT

Effects of genotypic variation among barley cultivars were evaluated under in vitro and in vivo. In vitro dry matter disappearance rate and estimated microbial yield (as measured by purine accumulation) was measured using one normal barley (CI 15478), 5 proanthocyanidin-free mutant cultivars (ANT 534, ANT 563, ANT 230, ANT 231, ANT 246) and 2 cross-line barleys (CA 602914, CA 57163). Four cultivars were selected for use in a sheep metabolism study. Cultivars were analyzed for starch, protein, neutral detergent fiber (NDF), flavonoid and total phenolic compounds and $ -glucan content. Correlation coefficients were calculated between the chemical constituents, DMDR and purine accumulation. Cultivars differed ($P<.05$) with respect to DMDR and purine accumulation. Negative relationships ($P<.05$) were detected between DMDR and starch ($r=-.44$), flavonoid compounds ($r=-.30$) and phenolic compounds ($r=-.31$). Purine accumulation was positively associated with protein content ($r=.30$) and NDF ($r=.46$) and negatively with starch ($r=-.38$), flavonoid compounds ($r=-.32$), phenolics ($r=-.37$) and $ -glucan ($r=-.29$). The metabolism study investigated one normal barley, (CI 15478), 3 proanthocyanidin-free mutant cultivars (ANT 534, ANT 246, ANT 231) and corn. Diets were formulated to provide equal starch, protein and NDF. The diets were fed to mature ruminally and duodenally cannulated Rambouillet ewes in a 5 X 5 Latin square design. Ruminal dry matter and organic matter digestibility in ewes was affected by diet ($P<.05$). Viscosity and pH of ruminal fluid were similar in all ewes. Ruminal viscosity appeared to be inversely related to $ -glucan content of the grain. Ruminal and duodenal NH_3 concentrations were similar in ewes fed all diets. The ruminal digestion of starch ($P<.01$) and duodenal starch flow ($P<.05$) in ewes was influenced by diet. Effects of diet fed to ewes were found in duodenal CP flow ($P<.05$), dietary escape protein flow ($P<.01$) with dietary escape protein flow greater ($P<.01$) in ewes fed corn. Microbial nitrogen concentration in the duodenum were similar in ewes fed each diet. Microbial efficiencies differed ($P<.05$) in ewes fed the various diets with values ranging from 13.3 to 21.4 g microbial protein produced/100 g OM digested. Results from this study demonstrate variation among lines of barley for in vitro DMDR and estimated microbial yield. These results also demonstrate genotypic variation among barley cultivars site and extent of digestion.

CHAPTER 1

INTRODUCTION

Barley is a widely cultivated grain used for malted beverages, human and livestock feeds. In Montana, barley is an important commodity. In 1987, Montana ranked second in the nation in the production of barley. Returns from barley account for eight percent of agricultural revenues (Montana Agricultural Statistics, 1987).

As a feed grain, barley is commonly used for both ruminants and non-ruminants. However, as a concentrate feed for ruminants, barley has limitations. Digestive disorders such as ruminal acidosis and bloat have been associated with feeding high-concentrate barley diets to ruminants. Although barley is a concentrated energy source and contains more crude protein than corn (NRC, 1984), corn has remained the preferred feed grain for cattle and sheep finishing diets. Several reasons exist for this situation; digestive disorders are not as likely to occur when feeding corn-based concentrate diets and animals receiving corn are believed to make more efficient use of the starch in the diet (Waldo, 1973).

Differences among barley cultivars have been identified with respect to the nutritional value of cultivars for nonruminants. Protein quality and biological value has been shown to vary (Hofer et al, 1983; Øverland, 1988) for nonruminants.

Information regarding cultivar differences as they pertain to ruminant usage of barley is limited. Investigation of barley cultivars for ruminants began at

Montana State University in 1985. Clark and Petersen (1987) found differences among cultivars with respect to in vitro dry matter disappearance rates and estimated in vitro microbial yield. These measures reflect functions which are of direct importance to the ruminant. If such differences are also present in vivo, it is possible that some barley cultivars are more suitable than others for ruminant feed. Identification and/or development of cultivars which may be best utilized by sheep or cattle may provide Montana barley producers an expanded market for their product.

The objectives of this study were:

1. To determine the influence of barley genotype on in vitro dry matter disappearance rate and estimated microbial yield.

2. To determine the relationship between in vitro dry matter disappearance rate and microbial yield to in vivo duodenal flow of nutrients in mature ewes fed barley diets differing in genotype.

CHAPTER 2

LITERATURE REVIEW

Barley as a Feed Grain for Ruminants

Feeding grain to ruminants is generally implemented when production and economic demands require rapid weight gain. The starch in cereal grains provides a concentrated source of readily fermentable substrate for microorganisms in the rumen. Corn is often the grain of choice due to its higher concentrations of starch compared to barley. Sorghum also has a higher starch content than barley, while wheat and rye are usually comparable to that of barley. While crude protein of wheat may be greater, barley contains more crude protein than corn, oats and sorghum. Of the more common feed grains, oats contain the highest percentage of dry matter as crude fiber (12.2%). Barley contains approximately 6% fiber with corn, wheat, rye and sorghum containing 2.5-3.2%, 2.3-2.1%, 2.6% and 2.7% respectively. In a study comparing the feeding value of sprouted wheat to sound wheat and barley, Preston et al.(1980) found no differences due to grain type in feedlot performance traits (average daily gain, intake, feed/gain) or in carcass characteristics (dressing percentage, quality grade or yield grade). Malcolm and Moss (1984) found similar production of milk and butterfat in Holstein cows fed corn, barley or 50:50 corn-barley diets.

Cultivar Effects on Performance

Limited information is available regarding the effect of cultivars upon animal performance by ruminants. In a comparison of malting and feed barleys, Hinman (1979) reported improved feed efficiency when feeding Klages, a malting barley, vs. Steptoe, a feed barley, though no difference in daily gain was seen. Newman and McGuire (1985) suggested the difference in feed efficiency might be attributed to the higher test weight of the Klages which suggests a higher percentage of starch and therefore a greater energy density. In a similar study, Preston and Herlugson (1980) fed the cultivars Boyer and Steptoe to yearling steers. No apparent difference in performance was seen when barleys were rolled. However, when fed whole, cattle fed Boyer gained 10 - 12% faster and more efficiently than those fed Steptoe. The carcasses of cattle fed Boyer were leaner than those fed Steptoe. The barleys were similar in nutrient composition, though Steptoe had slightly higher crude protein, crude fiber, acid detergent fiber contents and a slightly higher test weight.

In another study comparing the Andre, Steptoe and Klages cultivars in a steer finishing trial found no differences between cultivars for average daily gain. The cultivar Andre required protein supplementation to maintain comparable feed efficiency (Muirhead, 1987).

Physical and Chemical Characteristics of Barley

Physical Characteristics.

The physical form of the barley plant is highly variable within and between varieties, however the general morphology of the kernel is similar among genotypes. Kernel shape ranges from elongated with tapered ends to nearly round. The outer most layer of the kernel is the husk, which may be adhered to the kernel or may easily separate from it. Below the husk lies the pericarp layer and the testa, or seed coat. The pericarp, the remains of the ovary wall, is fused to the testa to provide a protective covering for the tissues within the kernel. Most of the fiber in the barley grain is found in the husk, pericarp and testa (Briggs, 1978). The tissues found within the testa are the starchy endosperm and kernel embryo. The endosperm, the largest tissue within the testa, is the storage place for the energy reserves of the grain as well as up to 80% of the total protein in the grain (Chung and Pomeranz, 1985). The outer layer of the endosperm is known as the aleurone layer. The kernel embryo lies toward the base of the kernel.

Chemical Characteristics.

Starch Starch is the major chemical component of the barley grain. The starch granules are laid down within the amyloplasts of the starchy endosperm as the seed matures. In the central region of the starchy endosperm, the starch granules are packed into a protein matrix. The starch granules tend to be larger toward the center of the endosperm and decrease in size and quantity of starch in

the subaleurone region (Briggs, 1978). The starch is deposited first in large granules. As the kernel matures, small granules are deposited (Karlsson et al., 1983). Small granules account for up to 90% of the number of granules but only about 10% of the starch weight (Kang et al., 1985).

Two major groups of starches may be identified based upon the X-ray diffraction patterns cast by their crystalline structures (French, 1973). Cereal starches are generally represented as A - type, while root and tuber starches are B - type. Chain length may be a factor in determining starch type. French and Youngquist (1960) determined that short chain amyloses crystallize in the A form. Longer chains tended to prefer the B form. Karlsson et al. (1983) suggested that variations in the synthesis of starch would result in variations in the final structure of the starch and upon the total amount of carbohydrates produced. These factors may cause variation in the swelling of the granules during processing or feeding which does affect the degradation activity of enzymes.

The starch polysaccharide is composed of " -glucan and consists of amylose (straight chain, " (1-4) glucosidic linkages) and amylopectin (" (1-4) chains are branched with " (1-6) linkages) (Briggs, 1978). In barley, the ratio of amylose to amylopectin is primarily genetically determined (Morrison, et al. 1986), though minor variations may be attributed to environmental conditions during early phases of the development of the grain.

Briggs (1978) states that most British barleys contain starch comprised of 22-26% amylose and 74-78% amylopectin. Amylose content in barley can vary from 0-50%.

Over 90% of the starch in barley is fermented in the rumen, as compared to 84% of the starch in corn (Waldo, 1973). This extensive fermentation is believed to be a factor in the digestive disorders associated with feeding high concentrate barley diets. Starch which bypasses rumen fermentation is hydrolyzed directly to glucose in the small intestine thereby avoiding energy losses through fermentation. Because more corn starch than barley starch bypasses the rumen to the small intestine, Waldo (1973) estimated the efficiency of use of metabolizable energy to be 47% for barley and 53% for corn (Waldo, 1973).

Fiber Most of the fiber in barley is located in the husk, pericarp and testa (Briggs, 1979). The husk consists of thickened cell walls which contain most of the lignin found in the grain. In addition, cellulose, hemicellulose, pentosans, mannan, uronic acids as well as substantial amount of silica make up the tissue of the husk. In fully ripened grain, the pericarp is dead tissue and the testa is constructed of "crude cellulose" (Briggs, 1978). Aastrup et al. (1984) identified the testa as the location in barley of proanthocyanidins, a type of phenolic compound.

Bacic and Stone (1981) evaluated the chemical components of the aleurone cell wall and found it contained 44% xylose, 29% glucose, 23% arabinose, 2% mannose and 2% galactose. Arabinoxylan and 1,3:1,4-$ -glucan constituted 60%

and 20% of the polymers in the aleurone cell walls. The susceptibility of xylan to acid hydrolysis is similar to that of starch (van Soest, 1987). Wohlbier et al. (1969) as cited by Czerkawski (1986) indicated that the digestibility of xylan in the rumen may reach 96% provided the structure is accessible to rumen microorganisms. Ballance and Manners (1978) evaluated the components of the cell walls in the endosperm. Their study indicated 70-72% of the carbohydrate polymer fraction consisted of mixed linked $ -glucan. Most of the cell wall was found to consist of carbohydrate and was analyzed as 74% glucose, 13% D-xylose, 10% L-arabinose and 2.5% D-mannose.

Beta-glucan A class of soluble non-starch carbohydrate, $ -glucan, makes up 1.5 - 8.0% of barley. Beta-glucan content varies among cultivars and is dependent upon genetic and environmental growing conditions. Thomke et al. (1980) concluded that mixed-linked 1-3:1-4 $-glucans caused decreased growth and interfered with digestibility of organic matter, crude protein and energy in growing pigs. However, other studies have reported digestion of $-glucan by swine (Weltzien and Aherne, 1987; Graham et al.,1986b). Beta-glucan is responsible for restricting protein and starch absorption in poultry resulting in reduction of intake and feed efficiency (Hesselman and @man, 1986). The effects seen in non-ruminants and the viscous nature of $ -glucan has lead to the postulation that this soluble carbohydrate may be a causative factor associated with bloat in ruminants fed high-barley diets (Engstrom and Mathison, 1988). Although $ -linkages are

readily attacked by rumen microorganisms, $-glucans may remain intact long enough to increase the viscosity of the rumen fluid and thereby lead to bloat problems. However, Engstrom and Mathison (1988) suggest that this may not be the case. In their study using feedlot steers, $ -glucan levels ranging from 3.5% to 4.8 % of dry matter appeared to have no effect on the performance of steers fed high-concentrate barley diets. The authors suggested the range of $ -glucan may have been too narrow to detect differences.

Protein The range in protein content of barley is more variable than other components. Protein content of barley is known to vary inversely with the starch content (Briggs, 1978). Proteins found in barley as in other cereals are: the albumins (water soluble) and globulins (salt soluble), prolamine (soluble in aqueous alcohol) and glutelins (soluble in acid or alkaline solutions). The water and salt soluble proteins (albumins and globulins) make up 15 to 30% of the total grain nitrogen (Shewry et al., 1984). These proteins are the main components of the aleurone layer and the kernel embryo, and contain relatively high levels of lysine and threonine. The prolamines, known in barley as hordein, are the major form of storage protein in barley. Hordein is considered a low quality protein for nonruminants due to its low lysine content. The relative quantity of hordein to albumin and globulin is a determining factor in barley protein quality. Glutelins also serve as storage proteins. Starch cells in barley are surrounded by a wall which includes a protein - possibly a glutelin (Foster and Prentice, 1987). Ten to twelve

percent of the total nitrogen in the kernel is non-protein nitrogen (peptides, free amino acids, nitrates) (Newman and McGuire, 1985).

Protein supplied to ruminants must meet the needs of both the ruminal microorganisms and the host animal. Proteins entering the rumen are subject to hydrolysis to amino acids by the microorganisms. The amino acids are further broken down to ammonia, carbon dioxide and organic acids. Ammonia is the chief source of nitrogen used by ruminal microorganisms for growth and reproduction. Microorganisms reorganize the products of proteolysis into different amino acids and peptides which may be incorporated into microbial protein. Thus, the protein presented to the animal in the feed is not necessarily the protein presented to the small intestine. Protein solubility (or insolubility) is generally considered more important than protein quality when evaluating protein for ruminant use (Church, 1986). Proteins which are more soluble may be expected to be more readily available sources of nitrogen for microbes in the rumen (Van Soest,1982). Proteins which are more insoluble and escape ruminal degradation (bypass protein) may be expected to deliver more intact dietary protein and amino acids to the small intestine. Bypass protein, however, may not be satisfactorily digested in the small intestine. In addition, partial ruminal degradation of protein may result in a poor balance of amino acids delivered to the duodenum (NRC, 1984). Mahadevan et al. (1980) concluded that solubility alone may not be an indication of the degree of susceptibility of proteins to proteases of rumen bacteria. Structural characteristics of the proteins are probably important determining factors.

In a study comparing sorghum, corn and barley, Spicer et al.(1986) found no differences in total nitrogen in the abomasum of steers fed barley- and corn-based diets. However, partitioning the nitrogenous components showed less barley protein escaping rumen degradation than corn or sorghum.

Phenolic compounds Plants contain a wide array of phenolic and polyphenolic compounds. Many colors seen in flowers, fruits and vegetables may be attributed to the phenolic compounds in the plant tissues. Phenolic compounds are derivatives of the aromatic amino acids; tyrosine, phenylalanine and tryptophan. Barley contains a wide number of phenolic substances. These compounds are found in numerous forms such as tyrosine, phenolic acids, esters, glycosides, lignans and related substances.

The four major biochemical classifications of phenolic compounds include flavonoids, terpenoids, benzoic acids and cinnamic acids. The flavonoid compounds are numerous, widely distributed in plants and often have physiologic activities in addition to lending color to plants (Harbourne et al., 1975). Included in this group of compounds are the proanthocyanidins. Flavonoid compounds which yield anthocyanidins when treated with acid are collectively referred to as proanthocyanidins (Weinges and Nader, 1982). In barley, the aleurone and the pericarp layers contain proanthocyanidins (Aastrup et al., 1984). Phenolic anthocyanidin pigments are responsible for the red and blue colors seen in some barley tissues (Briggs, 1978). Research by the brewing industry demonstrated that

proanthocyanidins form complexes with proteins in beer creating a haze of insoluble precipitates (von Wettstein et al., 1980). Development of proanthocyanidin-free mutant barley varieties alleviated the precipitation problem in beer (von Wettstein et al, 1980). Munck (1981) suggested that similar precipitates may form in the digestive tract of animals thereby reducing protein availability. In rat and chick trials, Newman et al. (1984) demonstrated more highly digestible protein in proanthocyanidin-free mutant cultivars compared to normal barley. Øverland (1988) demonstrated numerically small though statistically significant differences in protein digestibility between proanthocyanidin-free mutant cultivars and their normal parent barleys.

Phenolic compounds are known to have an inhibitory effect on a wide variety of microorganisms in many environments (Akin and Rigsby, 1985; Theodorou et al., 1987). Microbial growth and cellulolytic activity of rumen microorganisms may be inhibited by phenolic compounds (Akin, 1982, Chesson, et al.,1982; Jung et al., 1983). Nordkvist et al.(1984), evaluated eight abraded fractions of barley grain for analysis of bound phenolic acids. Vanillic, p-coumaric, ferulic and di-ferulic acids were identified in all eight fractions isolated. Jung and Fahey, (1983) demonstrated that vanillin is an effective inhibitor of in vitro starch digestion by rumen microorganisms. Phenolic compounds are also known to interact with proteins reducing their nutritional value for animals. Sorghum cultivars with high levels of tannins have poorly digestible protein compared to cultivars with low levels (Cousins et al., 1981).

CHAPTER 3

MATERIALS AND METHODS

In Vitro Evaluation

From the barleys evaluated by Clark and Petersen (1987), eight cultivars were selected for reevaluation. The selection of these barleys was based upon in vitro dry matter disappearance rate (DMDR) and purine accumulation. The cultivars selected included CI 15478, a normal barley; ANT 534, ANT 563, ANT 230, ANT 231, ANT 246, proanthocyanidin-free mutant cultivars; CA 602914, and CA 57163, cross-line barleys. The barleys were analyzed for crude protein content (AOAC,1980), neutral detergent fiber (NDF) fraction (Van Soest and Robertson, 1980), starch content (@man and Hesselman, 1984), total flavonoid content (Truelson, 1984), total polyphenol content (Folin and Denis, 1912), and total $ - glucan content (McCleary et al.,1985)(Table 1).

Limitations of laboratory space and equipment precluded simultaneous analysis of the cultivars. Multiple runs were conducted evaluating four cultivars in each run. Cultivars were assigned to runs such that each cultivar was included in at least three runs. Appendix Table 14 illustrates the arrangement of cultivars and fermentation runs. The inclusion of cultivars in multiple runs allowed comparisons across runs.

TABLE 1. CHEMICAL COMPONENTS OF NORMAL, PROANTHOCYANIDIN-FREE AND CROSS-LINE BARLEYS

Barley	Starch %	Protein %	NDF[a] %	$-glucan[b] %	Flavonoids[c] mg/g	Phenols[d] mg/g
CI 15478	53.4	12.9	12.3	4.51	0.78	3.58
ANT 534	49.1	13.0	15.5	4.99	0.29	1.92
AN5 536	48.9	11.0	14.6	4.97	0.02	1.51
ANT 230	52.9	11.9	12.8	5.04	0.01	1.41
ANT 231	49.6	13.9	11.8	4.22	0.16	1.64
ANT 246	52.3	13.1	13.9	3.85	0.12	1.65
CA 602914	52.2	12.2	13.4	4.74	0.02	1.34
CA 57163	54.5	12.0	14.6	4.79	0.02	1.35

[a]Neutral Detergent Fiber
[b]Total $-glucan
[c]Total flavonoids compounds
[d]Total polyphenolic compounds

The barleys were evaluated for DMDR using a modified Tilley and Terry in vitro fermentation procedure as described by Harris (1970). Barleys were ground in a Udy mill to pass a 1 mm screen. Half gram samples were soaked with 30 ml McDougal's buffer (McDougal, 1948), inoculated with 7 ml of ruminal fluid, and then incubated at 39A C in tubes capped with Bunsen valves. The ruminal inoculum was a composite of fluid collected from two ruminally cannulated cows fed 4.5 kg barley daily. Fermentation was ceased after 0, 3, 6, 9, 12, 15, 18, 21, 24, 36, and 48 hours of incubation using .5 ml 5% mercuric chloride solution. Three tubes containing samples of each cultivar were treated at each time interval. The entire contents of each tube was dried at 60AC in a forced air oven for 48 hours.

Disappearance rates for dry matter were determined using the equation:

$$\text{DM residue remaining} = D\, e^{(-kd)(t)} + U$$

where D = the potentially degradable fraction, -kd = rate constant for DM disappearance, t = time (hrs) and U = undegradable fraction (Robinson et al., 1986).

Dried residues were analyzed for purine content using the technique described by Zinn and Owens (1986). A curve representing purine concentration over time was developed and the area under the curve was utilized to estimate microbial yield as described by Burden et al. (1978).

Relationships between chemical components and dry matter disappearance rate and purine accumulation were characterized by simple regression techniques using the Correlation Procedures of the General Linear Model of the Statistical Analysis System (SAS), (1984).

Metabolism Study

From the eight barleys evaluated in the in vitro procedure, four were chosen for in vivo evaluation. The cultivars chosen included the normal barley Klages, the proanthocyanidin-free mutant cultivars Advance ANT 534, Gunhild ANT 246, and Tron ANT 231. Barley fed in the in vivo study was grown under irrigation three miles west of Bozeman, MT by Western Plant Breeders Association. These barleys were characterized using the in vitro procedure described above.

Experimental diets were formulated using the four selected barleys (Table 2). A fifth diet was formulated using corn as the primary ingredient. Diets were formulated to supply equal amounts of starch (350 g/d), crude protein (170 g/d) and NDF (235 g/d).

Diets were fed to five mature (3 and 4 yrs) Rambouillet ewes fitted with ruminal and duodenal cannulae (Aquilar and Depeters, 1988). Animals were fed twice daily at 6 a.m. and 6 p.m. and were dosed at feeding with 2.5 g Cr_2O_3 which was used as an external digesta marker. Diets were randomly assigned to ewes during five periods in a 5 X 5 Latin square arrangement of treatments. Periods consisted of two weeks for diet adaptation and one week following adaptation for sample collections.

Approximately 250 ml digesta were collected from the duodenum 0, 3, 6, and 9 h post feeding. Ten ml samples were immediately weighed and dried for analysis of dry matter and ash contents (AOAC, 1980). A 50 ml sample of digesta was acidified with 3 ml of 6 N HCl and frozen for ammonia analysis by magnesium oxide distillation (AOAC, 1980). The remaining digesta was frozen upon collection and subsequently freeze-dried. The freeze-dried samples were finely ground in a Braun hand held coffee/spice mill (2 min). Quarter gram samples of dry digesta were analyzed for chromic oxide (Fenton and Fenton, 1979) for determination of duodenal digesta flow. Crude protein was determined using Kjeldahl procedures (AOAC, 1980) and starch content was determined according to @man and Hesselman (1984).

One day following the duodenal sampling, ruminal digesta was collected at 0, 1, 2, 3, 4, 5, 6, 7, 8.5, 10 and 12 h post-feeding. Ruminal pH was measured immediately upon collection using a combination glass electrode pH meter. Ruminal fluid was extracted by squeezing digesta through 4 layers of cheese cloth. Fifty ml of ruminal fluid were acidified with 3 ml of 6 N Hcl and frozen for later analysis of ammonia using the magnesium oxide distillation procedure (AOAC, 1980). Twenty ml ruminal fluid were frozen and later composited for viscosity measurements using a Haake falling ball viscometer set at 30AC. In order to have adequate fluid to fill the viscometer, samples collected during the first 6 collections were composited for viscosity measurements as were samples from the last 5 collections. Pellets containing microbial cells harvested from experimental ewes were utilized for determination of nitrogen:purine ratio as described by Smith and McAllan (1974). This ratio serves to allow calculation of microbial nitrogen flow in the duodenum.

Two days following ruminal collections, in situ dry matter disappearance was evaluated using polyester bags with a surface area of 50 cm^2 and pore size of 53' 10μ (Nocek, 1988). Twenty-eight bags containing 1 g of grain (ground to pass a 2 mm screen) representative of the diet received by each ewe were suspended in the rumen of the animal. Two ewes received an additional 14 empty bags to be used as blanks. Two bags containing grain were removed from each ewe at each collection. At each collection one blank bag was removed from each of the two ewes with blanks. Bags were treated with 5 ml 5% $HgCl_2$ and rinsed in cold water until the water ran clear. Bags were dried at 60C for 48 h and weighed. Dry

matter disappearance was determined by weight difference. Weight changes in the blank bags were utilized as correction values which account for the influx of ruminal debris into grain-filled bags removed at the same time intervals. Collection times were .75, 1.5, 2.25, 3.0, 3.75, 4.5, 5.25, 6.0, 7.5, 8.25, 9.0, 10.5, 12 and 24 hours post feeding. The 6 p.m. feeding was eliminated in order to maintain uninterrupted incubation conditions.

Statistical Analysis

In Vitro.

Data from the in vitro study were analyzed using analysis of variance. Where a significant F-statistic was detected, means were compared using the Least Significant difference method on the General Linear Model procedure of Statistical Analysis Systems (SAS),(1986). The main effects in the statistical model were barley and run. Linear regression using the procedures of SAS (1986) was used to generate correlation coefficients for the chemical components of barley and DMDR and purine accumulation.

Metabolism Study.

Analysis of the data from the metabolism study was conducted using analysis of variance procedures on the General Linear Model of SAS. The main effects in the model were period, ewe and treatment. Least Squares Means were compared where

significant F-statistics were found using the Least Significant Difference mean test. Correlation coefficients between in vitro DMDR and ruminal starch digestibility and between in vitro purine accumulation and duodenal microbial nitrogen were generated by linear regression using the procedures of SAS (1986).

CHAPTER 4

RESULTS AND DISCUSSION

In Vitro Study

The DMDR and purine yield differed ($P<.05$) among barley cultivars (Table 2.) Disappearance rates ranged from 8.47%/hr (ANT 230) to 10.14%/hr (ANT 231). Purine yield in 48 hours ranged from 7.60 mg (CI 15478) to 8.72 mg (ANT 246) which were different ($P<.05$).

TABLE 2. THE INFLUENCE OF BARLEY CULTIVAR ON DRY MATTER DISAPPEARANCE RATE (DMDR) AND PURINE YIELD IN BARLEY DURING IN VITRO FERMENTATION.

Item	DMDR (%/Hr)	S.E.	Purine (mg)	S.E.
ANT 231	10.14^{b}	0.41	8.00^{abc}	0.22
CA 602914	9.25^{ab}	0.42	7.62^{bc}	0.28
ANT 563	9.23^{ab}	0.42	7.91^{abc}	0.23
CA 57163	8.8^{a}	0.41	7.81^{bc}	0.22
ANT 246	8.67^{a}	0.41	8.72^{ad}	0.21
CI 15478	8.66^{a}	0.33	7.60^{bc}	0.17
ANT 534	8.55^{a}	0.46	8.53^{acd}	0.24
ANT 230	8.47^{a}	0.41	8.09^{abc}	0.22

Means in columns with different superscripts differ, ($P<.05$).

The correlation between DMDR and purine yield, NDF or CP content of barley was not significant (P>.1) (Table 3.) A positive association was seen between CP and NDF and purine accumulation. While the CP effect might be expected, the correlation (r=.46) between NDF and purine was unexpected. This correlation may be due to the cation-exchange capacity of the fiber matrix. Cation exchange capacity is an important factor in microbial attachment (Allen et al., 1985) and can be used as a measure of the binding ability of the fiber matrix (McBurney et al.,1986).

The association between DMDR and purine yield may be described as quadratic (r=.89). As might be expected, purine yield initially increased with increasing DMDR. As DMDR continued to accelerate however, purine yield peaked and began to decline. The results indicate less purine yield for several of the most rapidly fermenting cultivars and greatest accumulation for barleys with more moderate DMDR. The cultivar with the highest DMDR (ANT 231) showed less purine yield than the cultivar with the lowest DMDR. This relationship is apparently contrary to the long standing assumption that increased fermentation leads to increased microbial yield (nrskov, 1971; Waldo, 1973). The response reported in this study is supported by the work of Hespell and Bryant (1979) who reported decreased microbial yield in a ruminal environment characterized by adequate levels of readily available energy but limiting concentrations of free nitrogen.

TABLE 3. CORRELATIONS (r) OF CHEMICAL CONSTITUENTS OF BARLEY WITH IN VITRO DRY MATTER DISAPPEARANCE RATE AND PURINE ACCUMULATION

		Rate	Purine	Protein	NDF	Starch	Flav[a]	Phenol[b]	β-Glu
Rate									
	r	1.0	0.03	0.06	-0.07	-0.44	-0.30	-0.31	-0.02
	P	0.0	0.77	0.62	0.53	<0.01	0.01	<0.01	0.83
Purine									
	r			0.30	0.46	-0.38	-0.32	-0.37	-0.29
	P			<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Protein									
	r				-0.42	-0.08	0.38	0.29	-0.67
	P				<0.01	0.49	<0.01	0.01	<0.01
NDF									
	r					-0.28	-0.41	-0.43	0.41
	P					0.01	<0.01	<0.01	<0.01
Starch									
	r						0.23	0.27	-0.13
	P						0.04	0.02	0.26
Flav[a]									
	r							0.99	-0.20
	P							<0.01	0.07
Phenol[b]									
	r								-0.19
	P								0.10

[a]Total flavonoid compounds
[b]Total polyphenolic compounds

An unexpected negative association was seen between starch and DMDR ($P<.01$). This surprising result may be explained by the positive correlation of polyphenolic compounds with starch ($r=.27$). Flavonoid and total polyphenolic contents were negatively associated with DMDR ($P<.01$). In vitro studies by Jung and Fahey (1983) demonstrated a linear decrease in cellulose and starch digestion with increasing levels of phenolic compounds. Thus, while the expected response to an increase in starch concentration would be more rapid DMDR, the corresponding increase in phenolic compound levels may inhibit microbial function thereby reducing DMDR. Flavonoid compounds were also found to be negatively associated with purine accumulation ($r=-.32$). This correlation may be an indication of restriction of free nitrogen by flavonoids. This contention is supported by Munck (1981) who suggested that proteins may be precipitated in the digestive tract by proanthocyanidins. The negative correlation between content of polyphenolic compounds and purine accumulation ($r=-.37$) may indicate restricted activity of rumen microorganisms as suggested by Theodorou et al. (1987) and Jung and Fahey (1983). Though no association ($P>.1$) was seen between DMDR and $ -glucan content, $-glucan did demonstrate a negative association ($P<.05$) with purine yield ($r=-.29$). This result may be an indication of inhibition of the attachment of microorganisms. Attachment inhibition of this type has been reported in the presence of methylcellulose, a soluble carbohydrate (Kudo, et al., 1986).

The results of this study demonstrate that rates of in vitro DMDR and estimated microbial yield can be attributed to genotype. In addition, chemical

constituents of barley were shown to influence DMDR and estimated microbial yield and may serve as predictive tools to assess the feeding value of various barleys.

Metabolism Study

The barleys used in the in vitro study (harvested in 1986) ranked in order of increasing rate: Advance ANT 534 < Klages < Gunhild ANT 246 < Tron ANT 231 with DMDR of 8.55, 8.66, 8.67 and 10.14 %/h, respectively. Barleys harvested in 1988 for use in the metabolism trial ranked Advance ANT 534 < Gunhild ANT 246 < Tron ANT 231 < Klages with DMDR values of 5.69 < 6.88 < 7.42 < 8.52 %/h, respectively. The proanthocyanidin-free mutant cultivars did not change ranking relative to one another. However, the normal barley Klages ranked as the fastest fermenting barley in the second evaluation was the third fastest fermenting barley in the first experiment. Variation between in vitro runs is to be expected, in particular when evaluating cultivars from different production years. Therefore, the change in ranking reported here should not be considered a discredit to the experiment. It is important to note that only one cultivar changed rank.

The composition of the treatment diets is listed in Table 4. The chemical analysis of the diets is listed in Table 5. By design, dry matter and organic matter intake were the same among ewes fed each diet (Table 6). Flow of DM to the small intestine was different ($P<.05$) (Table 7). Rate of DM flow ranged from 357.4 g/d for the Tron ANT 231 diet to 442.0 g/d for the corn diet. Organic matter flow to

the duodenum also differed (P<.01) with values ranging from 343.5 g/d (Tron ANT 231) to 438.5 g/d (corn).

TABLE 4. COMPOSITION OF TREATMENT DIETS (% AS FED)

	Diets[a]				
Ingredients	KLA	ADV	GUN	TRON	CORN
Barley	60.5	67.9	62.1	64.6	-
Corn	-	-	-	-	63.5
Beet Pulp	36.5	29.0	34.9	32.9	33.7
Urea	1.8	1.9	1.8	1.2	1.6
Dical Phos[b]	.8	1.1	.9	1.0	.6
TMS[c]	.2	.2	.2	.2	.2
Vitamin A,D,E[d]	.1	.1	.1	.1	.1

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246 TRON = Tron ANT 231
b dicalcium phosphate
c TMS = Trace Mineralized Salt
d Vitamin A and D premix containing 20,000,000 IU Vit.A and 4,000,000 IU Vit. D. per pound of premix.

TABLE 5. CRUDE PROTEIN (CP), STARCH AND NEUTRAL DETERGENT FIBER (NDF) CONTENT OF TREATMENT DIETS (%)

	Diets[a]				
Item	KLA	ADV	GUN	TRON	CORN
CP	16.4	16.5	14.4	15.5	13.5
STARCH	33.0	33.5	29.7	42.3	30.2
NDF	30.6	31.3	29.8	35.1	23.8

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246 TRON = Tron ANT 231

TABLE 6. DAILY INTAKE (G) OF DRY MATTER (DM), ORGANIC MATTER (OM), STARCH, CRUDE PROTEIN (CP) AND NEUTRAL DETERGENT FIBER (NDF)

	Diets[a]				
Item	KLA	ADV	GUN	TRON	CORN
DM	1010.0	995.0	1010.0	1022.0	1207.0
OM	1008.0	994.0	1008.0	1020.0	1197.0
CP	174.8	171.6	151.2	171.7	165.2
STARCH	351.0	349.0	313.0	384.0	450.0
NDF	326.4	325.8	313.0	446.6	253.5

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231

TABLE 7. INFLUENCE OF TREATMENT DIET ON PERCENT RUMINAL DIGESTION AND DUODENAL FLOW OF DRY MATTER (DM) AND ORGANIC MATTER (OM)

Item[a]	DM Digestion	DM Flow	OM Digestion	OM Flow
KLA	62.3^{ab}	380.6^{ab}	62.7^{ab}	371.8^{a}
ADV	57.7^{a}	426.4^{ab}	57.6^{a}	421.5^{b}
GUN	58.0^{ab}	424.4^{ab}	58.4^{ab}	421.2^{b}
TRON	69.2^{b}	357.4^{a}	63.5^{ab}	343.5^{a}
CORN	56.2^{a}	442.0^{b}	65.3^{b}	438.5^{b}
S.E.	2.45	24.3	2.17	16.3

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231
Means in rows with different superscripts differ, $P<.05$.

The percentage of DM digested in the rumen expressed as a percentage of DM fed ranged from 56.2% for corn to 69.2 % for Tron ANT 231 (Table 7). Hynd and Alden (1985) reported 75-83% DM digestibility of barley fed to sheep. Rumen

digestible OM differed (P<.05) with values ranging from 57.6% in ewes fed Advance ANT 534 to 65.3% in ewes fed corn. These values are similar to those of Morgan et al. (1989), who reported OM digestibility of 69% for a high concentrate barley diet and 66% for a similar corn diet.

Bloat and ruminal acidosis are digestive disorders often associated with feeding high-concentrate barley diets. In this study, barley genotype or corn had no effect (P>.1) on ruminal pH. Ruminal pH in ewes ranged from 5.6 to 5.8. Hynd and Alden (1985) reported a mean pH of 5.6 in sheep fed high barley diets with no apparent symptoms of acidosis. Thick, viscous ruminal contents traps gases produced during fermentation. If the animal is unable to expel these gases, bloat may result. While no differences were seen in ruminal fluid viscosity across treatments, viscosity appeared to be inversely related to $ -glucan content of the grain. Although bloat in animals fed concentrates is more commonly associated with barley, it is interesting to note that ruminal fluid viscosity was greatest in animals fed the corn diet. Viscosities, expressed in centipoise units (cp) ranged from 2.8 cp (Advance ANT 534) to 5.1 cp (corn) (Table 8). Engstrom and Mathison (1988) found no negative effects on performance of feedlot steers due to $ -glucan in barley based finishing diets. The same study estimated the ruminal digestibility of $-glucan to be approximately 98.1%.

Interesting results were derived from the in situ digestibility measurements (Table 9). No differences among barley cultivars were seen and all barleys were more extensively digested (P<.01) in 24 hours than corn. Digestion values for all

grains were well below accepted values (nrskov, 1986; Waldo, 1973) of over 90% for barley and 82-86% for corn. Values for the barley cultivars ranged from 48.3 to 59.9 % which were different (P<.05) than corn (9.8%).

TABLE 8. INFLUENCE OF TREATMENT DIET ON RUMINAL FLUID VISCOSITY AND TOTAL BETA-GLUCAN CONTENT

Diets[a]	$-glucan total %	Viscosity (cp[b])	S.E.[c]
KLA	4.26	3.9	.77
ADV	4.63	2.8	.77
GUN	3.45	4.5	.77
TRON	4.12	4.2	.77
CORN	.13	5.1	.77

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246 TRON = Tron ANT 231
b centipoise units
c Standard error of the mean viscosities

Barleys ranked differently than when evaluated for DMDR. While the Tron ANT 231 had a significantly higher IVDMDR than the other cultivars, no difference in in situ DMDR was found. The Advance ANT 246 appeared to have the most rapid in situ DMDR relative to the other cultivars, yet had one of the slowest IVDMDR values.

The in situ digestibility values are questionable due to the extremely low values. It is possible that the thick, viscous nature of the ruminal fluid interfered with normal fermentation of grain samples inside the bags. Odle and Schaefer (1987) suggested the viscous ruminal fluid of concentrate fed animals tended to

plug the pores in nylon bags preventing the escape of gases. Ballooning of the bags was seen in this study in all periods and all treatments, which made rinsing the bags difficult. Since this problem did not appear to differ between diet treatments, the low digestibility of corn cannot solely be explained by these observations. Though this technique has been considered useful in determining digestibility of concentrate feeds, it is apparently not adequate when attempting to describe a rate of disappearance.

TABLE 9. EFFECT OF DIET ON IN SITU DIGESTIBILITY OF BARLEY CULTIVARS AND CORN

Item[a]	% digestibility
KLA	59.9[c]
ADV	63.9[c]
GUN	48.3[c]
TRON	57.3[c]
CORN	9.8[d]
SE[b]	7.24

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231
b Pooled standard error of the mean.
Means in column with different superscripts differ (P<.01)

The ranking of ruminal digestibility of starch resembles IVDMD to a much greater extent than the in situ digestion. Ewes fed the corn diet had ruminal starch digestibility of 82.3% which was lower (P<.01) than the digestibilities of all barley cultivars except Gunhild ANT 246 (87.0%) (Table 10). These results agree with Spicer et al (1986) who reported barley starch to be slightly more rumen degradable than corn starch when fed to steers. Morgan et al. (1989) reported 95-

97% ruminal starch digestion for barley and 92-97% digestibility for corn starch in ewes fed whole grain. While the ranking of barley cultivars with regard to ruminal starch digestion was not identical to the IVDMDR ranking, the most rapidly fermenting cultivars, Klages and Tron ANT 231 were also the cultivars with the most highly rumen digestible starch. Likewise, the two cultivars with the slowest IVDMDR, Advance ANT 534 and Gunhild ANT 246, were the two with less rumen digestible starch.

Starch which bypasses the rumen is hydrolyzed directly to glucose in the small intestine. Starch utilized in the small intestine is used more efficiently than starch fermented in the rumen as fermentation to volatile fatty acids results in the production of heat and gases (Waldo, 1973). This represents energy lost to the animal.

TABLE 10. EFFECTS OF TREATMENT DIET ON RUMINAL STARCH DIGESTION AND STARCH FLOW TO THE DUODENUM

Item[a]	Ruminal digestion (%)	Duodenal flow (g/d)	Duodenal flow as as % of intake (%)
KLA	92.9^{de}	5.9^{d}	1.7
ADV	89.3^{de}	7.6^{cd}	2.2
GUN	87.0^{cd}	9.5^{d}	3.0
TRON	93.8^{e}	8.1^{d}	1.8
CORN	82.3^{c}	14.6^{e}	3.8
S.E.[b]	0.73	0.70	

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231
b Pooled standard error
Means in columns with different superscripts differ, $P < .05$.

While the lower ruminal digestibility for corn starch was expected, this study demonstrates that barley genotype may influence the quantity of starch delivered to the small intestine and therefore influence the efficiency with which barley energy is used for growth by ruminants. The flow of starch to the duodenum was greater ($P<.05$) in ewes fed corn. The higher quantity of starch (14.6 g/d) in the duodenum of ewes fed corn was expected (Table 10). In ewes fed Klages (the normal barley) 5.9 g of starch flowed to the duodenum per day which was less ($P<.05$) than that in ewes fed the proanthocyanidin-free mutants Gunhild ANT 246 (9.5 g/d) and Tron Ant 231 (8.1 g/d) and Advance ANT 534 (7.6 g/d). It is particularly interesting to note that the Gunhild ANT 246 diet provided from 36-71 g/d less starch than the other barley diets, however the duodenal starch flow was numerically greater for the Gunhild ANT 246 diet than for all other barley diets (Table 10). This may be an indication of some degree of resistance to bacterial enzyme activity as ruminal starch digestibility of Gunhild ANT 246 was the least among the barley diets. Duodenal starch contents expressed as a percentage of starch fed further defines the numerical range of duodenal starch flow due to diets. Values ranged from 1.7% to 3.0% starch fed among barley treatments. Morgan et al.(1989) reported 3-5% intestinal digestion of starch from similar whole grain barley based diets.

In a comprehensive review of starch utilization by ruminants, Theurer (1986) noted that digestibility of protein and starch in cereal grains are related. Since starch granules are embedded to varying degrees in a protein matrix, the structure

of the matrix and the digestibility of the protein can affect access of the ruminal microorganisms to the starch granules. Both starch and protein of barley are more digestible than those of corn (Spicer et al.,1981). Differences in the starch composition of the cultivars used in this study may translate to differences in the susceptibility or resistance to rumen degradation.

Ruminal NH_3 concentration was similar for ewes fed the barley cultivars (Table 11). Ruminal NH_3 concentrations ranged from 28.2 mg/100 ml in ewes fed Klages to 37.8 mg/100 ml for corn fed ewes (P>.1). These results do not agree with Odle and Schaefer (1987) who found ruminal NH_3 concentrations to be greater for barley than for corn. Ruminal NH_3 values for barley diets in this study correspond to those of Hynd and Alden (1985) who reported a range of 24.5 to 63.4 mg/100 ml of ruminal fluid in barley fed sheep.

TABLE 11. EFFECTS OF TREATMENT DIETS ON RUMINAL AND DUODENAL AMMONIA (NH_3) CONCENTRATION (MG/100ML)

	Diets[a]					
Measurements	KLA	ADV	GUN	TRON	CORN	S.E.
Ruminal NH_3	28.2	35.0	33.7	33.4	37.8	4.8
Duodenal NH_3	6.5	7.3	8.0	6.7	6.8	0.6

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231

Ammonia is a product of protein degradation in the rumen and differences in NH_3 concentrations may be an indication of differences in the digestibility of the protein in the feed. Differences in the ruminal levels of NH_3 may also be indicative of

differences in NH_3 use by microorganisms in the rumen. It is important to note that each of the diets contains a small percentage of urea and that the amounts differ slightly, complicating the interpretation of ruminal NH_3 analysis data. The range of rumen NH_3 resulting from feeding the barley diets, though not statistically significant, is interesting because of the distribution. The in vitro phase of this project suggested negative influences of phenolic compounds on microbial yield. Ewes fed diets formulated with proanthocyanidin-free mutant cultivars maintained similar NH_3 concentrations, (35.0, 33.7, 33.4 mg/100 ml) compared to ewes fed the normal barley (28.2 mg/100 ml). The presence of proanthocyanidins in the normal barley may have rendered some of the protein unavailable for degradation and therefore resulted in lower NH_3 concentrations in the rumen. It is also possible that overall polyphenolic compound concentrations in the normal barley may have had an inhibitory effect on the proteolytic activity of the microorganisms in the rumen with the same result. Since ruminal NH_3 is the major nitrogen source for many species of rumen microorganisms, the level of NH_3 maintained as a result of feeding these diets is important. The values reported in this study are within the range considered to be adequate for maximum microbial growth (Odle and Schaefer, 1987).

Partitioning of the nitrogen fraction in the duodenum indicated differences in nitrogen flow to the small intestine in ewes due to diet fed (Table 12). Duodenal crude protein flow differed among ewes ($P<.05$) with values ranging from 101.7 g/day (Tron ANT 231) to 128.7 g/day (Gunhild ANT 246). Ewes fed corn and

Gunhild ANT 246 showed similar CP flow to the duodenum. Crude protein flow in ewes fed corn or Gunhild ANT 246 were greater ($P<.05$) than in ewes fed tron ANT 231 which were similar to the flow found in Klages- and Advance ANT 534-fed ewes. Morgan et al. (1989) reported values of 116.4 and 125.0 g/day in sheep fed high barley diets of whole grain.

Further analysis of the duodenal contents indicate similar ($P>.1$) concentrations of ammonia nitrogen (NH_3-N) (Table 11). Duodenal NH_3-N concentrations were numerically greater in the ewes fed two of the proanthocyanidin-free mutant barleys than for those fed the normal barley or corn. Concentrations of duodenal NH_3-N ranged from 8.0 mg/100 ml in ewes fed Gunhild ANT 246 to 6.5 mg/100 ml for ewes fed Klages.

Flow of microbial crude protein (MCP) per day was not significantly different ($P>.01$) among ewes as a result of the diet fed (Table 12). Daily flow ranged from 14.6 g MCP/day (corn) to 18.3 g/day (Gun ANT 246). Kay et al. (1972) and Spicer et al. (1986) reported greater bacterial CP in the abomasum of steers fed barley than those fed corn. In this study, ewes fed the barley cultivar, Tron ANT 231, produced numerically less MCP than the other barleys. Based on in vitro purine accumulations, the higher MCP flow for ewes fed Gunhild ANT 246 and Advance ANT 534 might have been predicted. Both cultivars produced the two greatest in vitro purine accumulations and also delivered equally high levels of MCP to the small intestine in the metabolism study.

The remaining nitrogen fraction in the digesta is assumed to be feed protein which escaped degradation in the rumen (ESCP). The amount of ESCP differed (P<.01) among treatments with ewes fed the corn diet receiving more (P<.01) ESCP than ewes fed the barley diets. Values ranged from 4.9 g/day (Advance ANT 246) to 30.5 g/day (corn).

TABLE 12. EFFECTS OF TREATMENT DIETS ON DAILY FLOW OF CRUDE PROTEIN (CP), ESCAPE PROTEIN (ESCP) AND MICROBIAL CRUDE PROTEIN (MCP) TO THE SMALL INTESTINE.

	Diets[a]					
Item	KLA	ADV	GUN	TRON	CORN	SE[b]
CP (g/d)	113.7[cd]	113.7[cd]	128.7[d]	101.7[c]	123.6[d]	6.8
ESCP (g/d)	9.0[e]	4.7[e]	12.6[e]	12.8[e]	30.5[f]	3.6
MCP (g/d)	17.1	18.6	18.3	14.9	14.6	1.5

[a] KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246 TRON = Tron ANT 231
[b] Pooled standard error
[cd]Means in rows with different superscripts differ, P < .05.
[ef]Means in rows with different superscripts differ, P < .01.

Microbial efficiency (MOEFF) differed (P<.01) among ewes fed the different diets (Table 13). Measured in g microbial protein produced per 100 g OM digested, microbial efficiency serves to characterize the ruminal environment created by a feed. MOEFF values ranged from 13.3 to 21.4. Microbial efficiency in ewes fed the Gunhild ANT 246 (21.4) barley diet was greater (P<.05) than MOEFF in ewes fed corn (13.3) or Tron ANT 231 (14.2). Ewes fed the Klages and Advance

ANT 534 diets had efficiencies of 17.6 and 21.0, respectively, compared with MOEFF associated with feeding corn (13.3).

TABLE 13. EFFECTS OF TREATMENT DIET ON MICROBIAL EFFICIENCY (MOEFF) (g MICROBIAL PROTEIN PRODUCED/100 g ORGANIC MATTER DIGESTED.)

	Diets[a]					
Item	KLA	ADV	GUN	TRON	CORN	SE[b]
MOEFF	17.6^{cde}	21.0^{de}	21.4^{e}	14.2^{cd}	13.3^{cd}	1.58

a KLA = Klages, ADV = Advance ANT 534, GUN = Gunhild ANT 246
TRON = Tron ANT 231
b Pooled standard error
Means in row with different superscripts differ (P<.05).

Efficiency associated with the feeding of Klages (17.6) and Tron ANT 231 (13.3) is as low (P<.05) as that associated with feeding corn (14.2). The microbial response to Gunhild ANT 246 and Advance ANT 534 is also noteworthy. It is possible that the lower rumen digestibility of the starch in Gunhild ANT 246 provides a sustained energy supply to coincide with protein degradation. Although Spicer et al. (1986) reported greater (P<.05) MN in the abomasum of steers fed barley diets than for corn diets, no differences in MOEFF were found.

Microbial efficiencies listed in NRC (1984) range from 7.7 to 27.0 g microbial protein/100 g OM digested, with higher values generally associated with roughage diets. Several studies in which barley was fed to sheep reported MOEFF values of 16.3, 11.3 and 8.1 MCP/100 g OM (Van Soest, 1982). Czerkawski (1986) summarized 65 individual studies according to methods of measurements of

microbial synthesis. Mean efficiencies ranged from 17.5 -22.4 MCP/100 g OM. This summary did not include information regarding types of diets. Hynd and Alden (1985) suggest MOEFF is more variable in sheep fed high concentrate barley and corn diets than forage diets.

In order to fulfill the objectives of this study, correlation coefficients were generated to describe the relationship between in vitro and in vivo results. Since results from the in situ DMDR evaluation were inconclusive, the association between in vitro DMDR and ruminal starch digestibility was evaluated. The correlation (r=.62) was not significant (P=.38). The association between in vitro purine accumulation and duodenal microbial nitrogen (r=.53) was not significant (P=.46). The variation associated with animal studies and many in vitro procedures is reflected in the lack of significance of these correlations.

In spite of the variation, this study serves to illustrate cultivar effects on the digestive physiology of barley when fed to ruminants. Within this group of cultivars, ranges of values were expressed for important physiological responses in the animals treated. This study demonstrates potential differences in the value of barley cultivars intended for ruminant feed.

CHAPTER 5

CONCLUSIONS

Based upon the results from the in vitro study, it can be concluded that differences exist among barley cultivars which may have a measurable effect upon the utilization of the grain by ruminants. The results of this study demonstrate effects of cultivars and/or chemical constituents on in vitro DMDR and estimated microbial yields.

Although in situ DMDR data from this was inconclusive, I believe the same procedure can be successfully used in future studies with some minor modifications. The frequent collection of bags necessary to describe a DMDR curve may have interfered with normal fermentation patterns. The number of bags initially placed in the rumen may have restricted free movement of the bags and the circulation of rumen fluid to the bags. Several steps might be taken to improve the procedure for sheep studies.

1. The bags should be moistened in McDougal's buffer before placing them in the rumen. This should decrease the time required for rumen fluid to enter the bags.

2. If one cultivar can be evaluated in two animals simultaneously, frequent bag collections could be made by alternating animals at each successive time interval. This should also allow incubation of fewer bags in each animal.

The use of cattle rather than sheep would allow suspending the bags farther apart in the rumen. This would improve circulation of fluid around the bags and would decrease the overall impact on the rumen of frequent collections. It is my feeling that the size of the sheep rumen is too small for such intensive sampling. I believe fermentation in the bags was prematurely ceased due to the creation of a micro-climate within the bag.

Although in vivo starch digestibilities appear to support in vitro DMDR, no significant correlation was found between the in vitro results and the results of the metabolism trial. In future studies, this correlation may be established.

Data from this study indicate that a range of values for the ruminal digestibility and intestinal flow of starch may be expected among barley cultivars. The range in duodenal starch flows as percentages of starch intake suggest that starch utilization by rumen microorganisms differs among cultivars. The results reported here suggest that some barley cultivars may be comparable to corn as sources of energy for ruminants. Further investigation into the specific nature of the starch in these cultivars may be of value in predicting how efficiently ruminants will utilize barley starch.

Site of protein digestion in barley cultivars was shown to differ. Although results from the in vitro study suggested that fermentation of barley cultivars results in varying microbial yield, no significant correlation was found between in vitro purine accumulation and duodenal MCP flow. Partitioning of the nitrogenous

contents of the small intestine indicated differences in the source of protein supplied to the animal for digestion and absorption at the small intestine.

The barleys used in this study were selected in part for the presence of genetic blocks preventing synthesis of proanthocyanidins. If the elevated ruminal NH_3 in the animals fed the mutant cultivars is assumed to be indicative of increased protein degradation, then it can be inferred that proanthocyanidins are interfering with the use of the protein in the normal barley, Klages. The MCP flow and the MOEFF data suggest, however, that the presence or absence of proanthocyanidins alone is not an adequate predictor of the influence of these compounds on protein use by ruminants.

The specific type of phenolic compounds found in these cultivars were not known. The in vitro study indicated significant effects of total polyphenolic compounds on microbial yield and perhaps on microbial activity. A more detailed description of the phenolic compounds present in the cultivars and their specific effects on the rumen microbial population in vivo may provide a basis for development of cultivars particularly supportive of rumen microorganisms.

Ultimately, adequately feeding ruminant animals requires first meeting the specific needs of the ruminal microorganisms. The MOEFF data in this study suggest not only that cultivars differ in their influences on ruminal microorganisms, but that some cultivars appear to have a more favorable effect on the ruminal environment than corn.

Finally, recognizing that this study utilized only a small number of cultivars from a wide and varied population, I believe this work serves to illustrate the potential of barley as a prime source of feed for ruminants. Further, I believe it is reasonable to continue this investigation in order to clarify the results reported here.

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APPENDIX

TABLE 14. ARRANGEMENT OF BARLEY CULTIVARS IN VITRO FERMENTATION RUN SCHEDULE

Run	Cultivars
1	Klages, CA 990, Gunhild 246, *
2	Advance ANT 534, *, *, *
3	Manker ANT 563, ANT 13/Rupal, Tron ANT 231, *
4	Klages, ANT 13/Nordahl, *, *
5	Klages, *, *, *
6	Advance ANT 534, Manker ANT 563, ANT 13/Rupal, ANT 13/Nordahl
7	Manker ANT 563, Klages, CA 990, Tron ANT 231
8	Klages, ANT 13/Nordahl, Gunhild ANT 246, Tron ANT 231
9	Advance ANT 534, ANT 13/Rupal, CA 990, Gunhild ANT 246

* = Cultivars not evaluated in this study.

TABLE 15. LEAST SQUARES ANALYSIS OF VARIANCE FOR RUMEN DIGESTIBILITY OF DRY MATTER AND ORGANIC MATTER

	Dry Matter			Organic Matter		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	149.3	0.01	4	55.7	0.11
Ewe	4	140.0	0.02	4	142.5	0.01
Period	4	134.0	0.02	4	109.2	0.02
Error	12	30.0		12	23.5	

TABLE 16. LEAST SQUARES ANALYSIS OF VARIANCE FOR DRY MATTER AND ORGANIC MATTER FLOW TO THE DUODENUM

	DM			OM		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	6325.5	0.13	4	15644.1	0.01
Ewe	4	15575.5	0.01	4	6544.4	0.12
Period	4	12489.8	0.02	4	12504.0	0.02
Error	12	2964.3		12	2881.3	

TABLE 17. LEAST SQUARES ANALYSIS OF VARIANCE FOR PH AND VISCOSITY OF RUMEN FLUID IN EWES

	pH			Viscosity		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	0.031	0.44	4	3.43	0.37
Ewe	4	0.051	0.22	4	3.93	0.31
Period	4	0.076	0.10	4	12.04	0.03
Error	12	0.031		12	2.94	

TABLE 18. LEAST SQUARES ANALYSIS OF VARIANCE FOR RUMINAL STARCH DIGESTION AND STARCH FLOW TO THE DUODENUM

	Digestion			Flow		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	219.0	<0.01	4	222.8	0.800
Ewe	4	120.1	<0.01	4	570.1	0.111
Period	4	154.4	<0.01	4	436.6	<0.01
Error	12	24.2		12	23.5	

TABLE 19. LEAST SQUARE ANALYSIS OF VARIANCE FOR FLOW OF CRUDE PROTEIN (CP) AND ESCAPE PROTEIN (ESCP) TO THE DUODENUM

	CP			ESCP		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	546.1	0.11	4	498.1	<0.01
Ewe	4	1227.4	0.01	4	86.5	0.30
Period	4	973.6	0.02	4	306.5	<0.01
Error	12	234.0		12	63.7	

TABLE 20. LEAST SQUARES ANALYSIS OF VARIANCE FOR FLOW OF MICROBIAL CRUDE PROTEIN (MCP) TO THE DUODENUM AND MICROBIAL EFFICIENCY (MOEFF)

	MCP			MOEFF		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	670.5	0.0	4	69.4	0.02
Ewe	4	2105.3	0.0	4	99.4	0.01
Period	4	1676.0	0.0	4	54.8	0.01
Error	12	440.0		12	12.5	

TABLE 21. LEAST SQUARES ANALYSIS OF VARIANCE FOR CONCENTRATION OF RUMINAL AMMONIA AND DUODENAL AMMONIA

	Ruminal NH_3			Duodenal NH_3		
Item	df	Mean Square	P	df	Mean Square	P
Treatment	4	60.3	0.73	4	1.9	0.45
Ewe	4	138.3	0.36	4	12.6	<0.01
Period	4	543.2	0.02	4	11.6	<0.01
Error	12	117.5		12	1.9	

TABLE 22. LEAST SQUARES ANALYSIS OF VARIANCE FOR IN VITRO DRY MATTER DISAPPEARANCE RATE (DMDR) AND IN VITRO PURINE YIELD OF BARLEY CULTIVARS

Item	df	IVDMD Mean Square	P	df	Purines Mean Square	P
Barley	7	1.9	.12	7	.66	.01
Run	8	21.9	<.01	8	6.10	<.01

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