

INTAKE, DIGESTION SITE, EXTENT OF DIGESTION AND DIGESTA KINETICS
IN GRAZING LACTATING COWS

by
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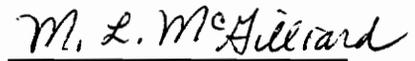
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(ABSTRACT)

Increasing feeding costs has led many dairy farmers to use pasture for lactating cows to reduce their production cost. Little is known about efficiency of nutrient utilization by lactating cows under grazing conditions. The objective of this study, therefore, was to investigate ruminal turnover, intake and site and extent of nutrient digestion in grazing lactating cows. Four dual cannulated (rumen and proximal duodenum) cows were randomly assigned to two groups to graze permanent pasture with no supplement or with 6.4 kg/d corn-mineral mix in a switchback design with three periods of two wk each. Solid and liquid markers (chromic oxide and Co-EDTA), used to estimate duodenal flow, fecal output, and ruminal turnover, were administered through the rumen cannula twice daily at 1100 and 2300 h. The supplemented cows had greater milk production (23.7 kg/d) than those on pasture only (19.5 kg/d) with an increase of .66 kg of milk production per kg of concentrate. Corn, however, depressed milk fat percentage resulting in a similar milk fat yield between the two diets. Supplemental corn reduced rumen ammonia-N (22 vs 17 mg/dl) and increased N recovery at the duodenum (86 vs 75% of N intake). True Nitrogen digestibility averaged 72% and microbial N flowing to the duodenum was 67% of the total N flow. This results indicated that grazing cows may benefit from concentrates containing undegradable protein. Daily OM intake was not different, but when cows were fed the supplemented diet, pasture OM was lower than when fed pasture only.

Organic matter, NDF and ADF digestibility in the rumen and whole digestive tract were greater when cows were fed. Cellulolytic activity may have been reduced by grain supplementation due to decreased ruminal pH (6.4 vs 6.2). The reduction in fiber digestibility may explain the decrease in forage intake when cows were fed supplemental grain. Ruminal rates of passage (kp) for solid (7.3 %/h) and liquid (18.2 %/h) markers were similar for both diets. The kp values observed for solid and liquid indicate that grazing cows may have a faster ruminal turnover than cows fed diets containing primarily hay or silage.

Dedication

This thesis is dedicated to my wife Grazia, for her patience, love and support without which my academic and research pursuits could not have been completed; to my parents Adriano and Lucia for their financial and moral support.

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Introduction

Research efforts in dairy production mainly have been directed toward increasing milk, fat and protein production. These efforts were justified by the fact that higher production generally resulted in higher profits. Milk prices were high enough to cover the feeding costs of large amounts of cereal grains and protein supplements required by high producing cows.

In the last few years, however, stagnation in milk prices and continuous increases in feeding costs have reduced the economic margin of dairy producers forcing some of them to go out of business. To decrease production costs, some dairy farmers have begun using alternative feeds, byproduct feeds in particular. Other farmers have returned to grazing and use of pasture for lactating cows where available. Often this returns attractive economic benefits. Despite the interest of dairy producers in pasture, very little research has been conducted recently in this country. Most of the available information on grazing is from studies conducted in other countries, much of it with sheep.

In order to improve efficiency of milk production with grazing, it is necessary to obtain information on nutrient utilization. It is known, for example, that well managed pasture may provide a highly digestible, highly palatable forage, with high N content that can support milk production of about 25 kg/d. However, there is little information on ruminal turnover, ruminal fermentations, and the factors that affect

nutrient flow to the duodenum. It is necessary to investigate the use of energy or protein concentrates, and their influence on utilization of nutrient in pasture

Literature Review

Herbage intake by grazing ruminants

Regulation of voluntary intake. The importance of feed intake on animal performance and efficiency of utilization of the forage has been recognized for a long time by the scientific community as indicated by the publications on this specific subject (Van Soest, 1965; Forbes, 1986; NRC, 1987). It is known that in diets based on forages the voluntary intake is limited by physical factors such as bulk of feeds, rumen volume and rate of disappearance of digesta from the digestive tract. The limitation of intake is often attributed to the structural fraction of the forage, the cell wall. It was observed by Van Soest (1965) that the cell wall content is negatively correlated to forage intake and digestibility. Other studies (Minson et al., 1964; Troelsen and Campbell, 1969) have pointed out that voluntary intake of roughage was highly correlated with the digestibility of the forage.

However, it is clear that physical constraints are not the only factors that determine voluntary intake. Conrad et al., (1964) and Baumgardt (1970) demonstrated that with highly digestible diets the voluntary intake was controlled by the energy requirement of the animal. As a consequence, it was postulated that intake was regulated by both physical and metabolic factors. When gut fill is the limiting factor, an increase of diet digestibility alleviates the physical constraints allowing the animal to increase voluntary intake to the point at which chemostatic mechanism take place;

intake is then limited by digestible energy intake to a constant value. Any further increment in digestibility results in a decrease in intake (Figure 1).

The change-over point between physical and metabolic factors represent the maximum DM intake and, for lactating cows, was thought to be at a DM digestibility of 67% (Conrad et al., 1964). It has been shown, however, that this point is not fixed as suggested by Conrad but moves along the digestibility scale in direct relationship to the energy demands by the animal (Van Soest, 1982).

Although, the dual mechanism of regulation of voluntary intake described above has been shown to operate on a wide variety of diets, it seems it does not apply to diets based on fresh grass (Freer, 1981). On cattle grazing perennial ryegrass, Hodgson (1977) found that herbage OM intake increased linearly as OM digestibility increased from 55 to 81%. No deflection point occurred to imply physiological regulation of intake. This result is in agreement with other studies (Minson et al, 1969) carried out either with sheep or cattle, suggesting that the intake of fresh grass is regulated only by physical factors. A stronger evidence in favor of the physical limitation was brought by Thornton and Minson (1973). In their study, conducted with sheep, the relationship between the physical constraints (digesta volume and retention time), voluntary intake and digestibility of grasses and legumes was evaluated. Intake of digestible OM was highly correlated ($r = .96$) with OM digestibility but was also correlated with the rumen retention time ($r = .95$) indicating that alleviation of rumen fill by the higher rate of disappearance of the digesta allowed higher intake. Moreover,

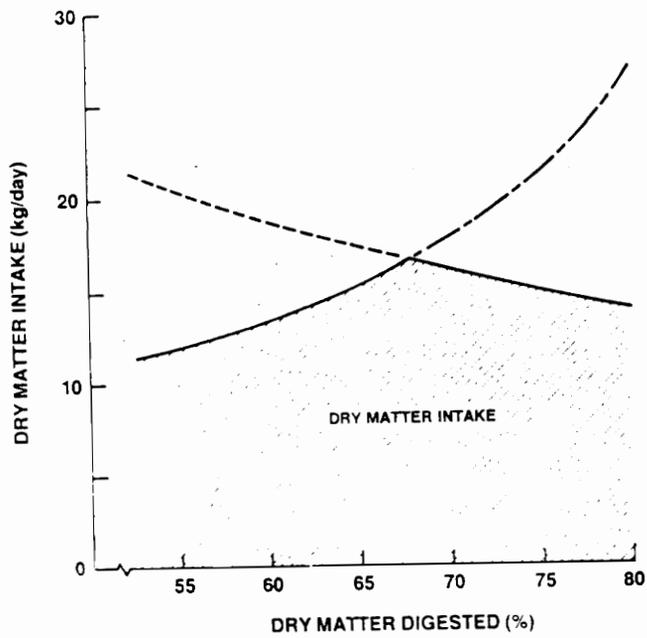


Figure 1. Relationship between DM digestibility of the diet and intake in ruminants (NRC, 1989).

the higher intake that is usually observed with legumes compared to grasses was explained by the lower retention time and higher DM content in the rumen. It is clear from the results found in the literature that in order to obtain high pasture intake and satisfy the nutrient requirement of high producing animals like dairy cows it is necessary to obtain highly digestible forage.

In addition to the factors discussed above, intake of grazing animals is strongly influenced by non-nutritional characteristics of the sward (Freer, 1981) such as herbage mass and structure that modify the capacity of the animal to harvest the available forage through changes in ingestive behavior (Allden and Whittaker, 1970; Hodgson, 1985). According to Allden and Whittaker (1970) intake in grazing animals (I) can be defined as the product of intake of herbage per bite (IB) times the rate of biting (RB) times the grazing time (GT):

$$I = IB \times RB \times GT.$$

This concept has provided a new framework in which researchers have studied the effect of sward characteristic on animal performance. In their study with sheep, Allden and Whittaker (1970) observed maximum intake when pastures contained at least 3000 kg of DM/ha. Decreasing herbage availability from 3000 to 2000 kg DM/ha reduced IB but was accompanied by a higher RB and GT resulting in a small decline in intake. An additional decrease of forage availability determined a sharp fall of IB without sufficient compensatory effect of RB and GT resulting in a marked reduction of intake. The capacity of the animal to compensate for the decrease in IB seems

restricted in dairy cows by a maximum biting rate of 60-70 bites/min (Mayne and Wright, 1988) and by a maximum GT of 10-12 h (Freer, 1981). Since the compensation of RB and GT is limited, IB is the variable most closely related to voluntary intake (Hodgson ,1985).

Intake and sward characteristics. Among the different sward characteristics (herbage mass , height, density, chemical composition, and digestibility), height is the variable most closely related to IB in temperate pasture while sward density is more important in tropical pasture (Hodgson , 1985). Therefore, in temperate forage characteristic of most of the eastern U.S., the measure of canopy height represents a simple but powerful tool that can be used under practical condition to manage the pasture for optimal animal performance. The values of herbage height that limit intake in dairy cows may vary with the grazing system adopted . Under continuous stocking, with higher tiller number, intake is reduced at a sward height of 8-9 cm while under continuous stocking, with lower tiller density, reduction of intake appears at a height of 10-12 cm (Mayne and Wright, 1988).

Estimation of herbage intake. One of the most difficult problems to solve in grazing studies is the measure of herbage intake. The simplest method is to feed fresh grass to housed animals recording the amount of herbage offered and orts. This technique, however, fails to consider herbage selectivity, effect of sward height and

social factors that greatly affect forage intake. There are several techniques to estimate fresh forage intake that can be classified in two major groups: sward methods and indirect animal methods (Meijes, 1981).

In sward methods, intake is calculated by difference between the quantity of forage present before and after grazing. Herbage mass is usually estimated by cutting representative areas at a fixed height and the grass quantity of those areas is applied to the whole grazing plot. In case of long grazing trials, methods are available to correct the estimated intake to account for herbage accumulation during the experimental period. With these methods, it is also possible to collect information on forage availability that allow calculation on sward utilization but they do not provide any information of intake by the individual animals.

All the indirect animal methods are based on the principle that intake can be calculated knowing fecal output and apparent DM digestibility (DMD) of the forage:

$$\text{Forage Intake} = \frac{\text{Fecal output}}{1 - \text{DMD}} \quad (1)$$

Fecal production can be measured by equipping the animals with collection bags.

However, this method is affected by several problems such as incomplete fecal collection, reduction of animal performance and alteration of animal behavior (Cordova, 1978). Alternatively, fecal output can be estimated using indigestible external indicators (markers) that are expected to be completely excreted through the feces. The marker needs to be administered once or twice daily at a constant dose and

fecal production can be calculated as the ratio of the marker daily dose and concentration in the feces. Fecal index technique based on N excretion are also available but the high variability of the estimated intake have discouraged its use.

The evidence that grazing animals actively select forage with higher nutritive value greatly limit the capacity to obtain representative *in vivo* DMD of the herbage from housed animal fed with cut grass. More frequently, representative pasture samples are obtained using esophageal cannulated animals and DMD is estimated by *in vitro* incubation or by internal marker present naturally in the forage (Cordova, 1978). The fact that the internal markers are not reliable indicators of DMD in fresh grass (Cochran et al., 1986b; Fahey and Jung, 1983) make the *in vitro* DMD the most reliable technique in pasture studies (Cordova, 1978).

Herbage protein

The crude protein (CP) content of fresh grass is highly variable depending on the species, stage of growth, fertilization and climate (Minson, 1990). Legumes are known to have greater N content than grasses and temperate grasses greater than tropical ones (Minson, 1981). Nitrogen content declines rapidly with stage of maturity mainly because of the increase in proportion of stem that have lower protein content and for a dilution effect due to the accumulation of carbohydrate with a generalized reduction of N in all plant fractions (Minson, 1990). Therefore, managing the pasture

to maintain the sward in an active growing stage would result in a more digestible, higher CP forage, more suitable for high producing animals like dairy cows.

Several studies have indicated that protein value of feeds can not be simply described by CP content and digestibility. In the new protein systems (ARC, 1980; NRC, 1985), feed proteins are evaluated on their ability to deliver protein to the duodenum as microbial and by-pass protein. Under this concept proteins are classified (NRC, 1985) either as rumen degradable (DIP), that supplies N for microbial growth, or rumen undegradable protein (UIP), that directly supplies N to the duodenum.

Based on in situ incubation, van Vuuren et al. (1991) estimated that the DIP of fresh perennial ryegrass ranged between 60 to 80% with the higher value obtained with immature grass and the lower with mature grass. In the same study, the increase of N applied as fertilizer from 0 to 700 kg/ha, raised the CP content but also CP degradability. This resulted in a reduction of estimated N recovery at the duodenum as percentage of N intake from 78 to 44%. The increase of CP after N fertilization can be explained by accumulation of NPN in the plant, with a decrease of the true protein content from a normal level of 75% to as low as 45% (Mangan, 1982). The high rumen degradability of herbage CP can result in an excessive production of ammonia (Beever et al., 1986b) with losses of N through the urine. Therefore, the supply of protein to the duodenum of animals grazing young growing grass heavily relies on microbial growth.

Protein requirement of high producing cows can not be satisfied by bacterial protein. Duodenal supply of proteins can be increased by using supplements rich in UIP (for example corn gluten meal, dried brewers grains or fish meal) or by protecting dietary proteins using chemical treatments or formaldehyde. Stobbs et al. (1977) fed casein treated or untreated with formaldehyde to cows grazing a pasture with a protein content of 20% and solubility of 40%. Milk production increased 2.5 kg/d with the treated casein and only .4 kg/d with the untreated one. This indicates that grazing lactating cows may highly benefit from a supplement that is high in rumen undegradable proteins.

Passage rate

Rumen passage rate (K_p), also called turnover, can be defined as the rumen outflow (L or kg per h) as percentage of the rumen content (L or kg) and is expressed as %/h. The inverse of K_p ($1/K_p$) represents the average time (h) the ruminal contents spend in the rumen and is called retention time (RT). The measure of K_p require the use of markers, which is discussed later.

Rumen content is not an homogeneous entity but can be partitioned into liquid and particle phases. Rumen K_p of the liquid portion is higher than the feed particles. This can be explained by the theory that rumen outflow is regulated by the dimension of reticulo-omasal orifice that selectively retain feed particles but does not retain liquids. As a result of this selectivity the liquid turnover in dairy cows may vary

between 6.0 and 12.0 %/h while particle turnover range within 2.5 - 5.2 %/h (Hartnell and Satter, 1979; Colucci et al., 1981; Erdman et al., 1986; Rode and Satter, 1988; Llamas-Lamas and Combs, 1990).

Various factors influence rumen Kp such as physical characteristics of the particles, level of intake, and dietary, animal and climatic factors (Faichney, 1986).

Particle size and density. Since the rumen outflow is determined by passage through the reticulo-omasal orifice, it is understandable that the size of feed particles must play an important role on rumen escape. Poppi et al. (1980) found that smaller feed particles have higher probability to escape the rumen than larger particles. The observation that less than 5% of the particular matter in abomasal and fecal samples of sheep was retained by a sieve size of 1.18mm supported the theory of the existence of a critical particle size under which particles escape the rumen. Particle comminution is carried out primarily by chewing during eating and rumination and secondarily by microbial breakdown (Ulyatt et al., 1986).

During the fermentation process, the decrease in particle size is accompanied by increase in particle density (Nocek and Kohn, 1987). Using inert particles varying in size (.32-1.27cm) and density (.91-2.37 g/ml), Ehle and Stern (1986) demonstrated that both physical characteristics affected particle Kp and that density had more importance than size. In a review on this subject, Lechner-Doll et al. (1991) calculated

that 87% of the variation in RT can be explained by particle characteristics, and that density accounted for 58% of the variability while size only 28%.

Intake. An increase of level of intake is generally accompanied by a decrease in RT for both liquid and particulate phases (Grovmum and William, 1977; Colucci et al., 1982). In Colucci's study, the increase in intake from 1.3 to 3.2 %/ BW in dairy cows, caused a reduction in RT of feed particles of about 80% inducing also a decrease in diet digestibility. The reduction of digestive utilization with increase of feed consumption is well documented and can be explained by the variation of K_p (see below).

According to the critical size theory, a raise in rate of particles comminution by rumination is expected when particles turnover increase. However, rumination time per g of feed ingested tend to decrease with higher feed intake resulting in escape from the rumen of larger particles (Van Soest, 1982), suggesting a modification of particle selectivity by the reticulum-omasum orifice.

The relationship between K_p and intake is widely accepted but some inconsistencies are reported in which the increase in intake did not affected particle turnover (Owens and Goetsch, 1986). The rumen can adjust to an increase of feed intake not only by an increase of rumen clearance (K_p) but also by an increase in rumen capacity (Van Soest, 1982). With this mechanism the rumen is able to augment its output to accommodate the higher intake minimizing the changes in rumen K_p .

An increase in liquid turnover is usually observed with the increase of feed intake. The fact that liquid K_p is highly affected by rumen infusion of osmotic solutions and not by water intake, indicate that change in salivation is the major cause of variation of liquid turnover (Owens and Goetsch, 1986). The increase in salivation is explained by the increasing time that the animal spend ruminating when fed at higher intake level (Van Soest, 1982).

Digestibility and site of digestion. It has been recognized that feed digestion is highly affected by the length of time it resides in the digestive tract. In particular, the longer the feed is retained the more completely it is digested. Hungate (1966), based on experience accumulated with continuous fermentors, was one of the first who tried to describe the ruminal digestion process as a mathematical function of the rumen turnover and the fermentability of the feed. However, because fermentability of feed was poorly described mathematically, Hungate did not develop a clear relationship between rate of passage and digestion.

Waldo (1972) recognized that digestion of the degradable portion of feed (D) followed first order kinetics and the digestion process of this fraction can be described by one parameter, the fractional rate of digestion (K_d). The combined action of digestion and passage determine the rate of disappearance of D from the rumen and the competition between these two process determine the portion of D that is fermented or subsequently escapes the rumen. Under steady state rumen conditions

(volume, outflow, K_p) the digestibility of D can be calculated as the digestion rate over the sum of rate of passage and digestion (Waldo, 1972):

$$\text{Rumen Digestibility} = \frac{K_d}{K_d + K_p} \quad (\text{II})$$

The in vivo validation of this equation necessitates firstly, knowing the fraction digested in the rumen. This may be determined by use of animals cannulated at the abomasum or proximal duodenum. Secondly estimating K_d with in situ or in vitro incubation of the feeds and thirdly measuring K_p employing indigestible markers. Despite the fact that all those techniques are available there is no study to date, that has attempted validation by all three methods. Nevertheless, equation II has been widely accepted based on its mathematical validity and largely applied to estimate rumen degradability of feedstuffs incubated in dacron bags (Orskov and McDonald, 1979; Nocek, 1988). This equation express an inverse relationship between K_p and rumen digestibility, that is, the fraction of feed fermented in the rumen will decrease with an increase of ruminal turnover.

The consequence of K_p variation on the utilization of the feed depends on the nutrient that is considered. The digestion of the cell wall fraction of feed (cellulose, hemicellulose and pectin) relies mainly on rumen microbial activity and, only marginally, on bacterial fermentation in the large intestine (Van Soest, 1982). According to equation II, an increase of K_p , would reduce rumen digestion and

decrease whole tract fiber digestion. The decrease of apparent digestibility of the diet that is commonly observed at higher intakes may be explained by the changes in Kp (Colucci et al., 1982; Tyrrell and Moe, 1975).

The non-fiber fraction (starch, proteins and fats) of the diet is potentially completely digestible and can be either fermented in the rumen or digested in the intestines. The escape of these nutrients from the rumen would shift the site of digestion from the rumen to the intestines with minimal reduction of whole tract digestibility. Because of the losses of energy as heat and methane during ruminal fermentation, the efficiency of energy utilization of non-structural carbohydrate, like starch, may be enhanced by an increase in ruminal escape (Nocek and Tamminga, 1991). The increase of dietary protein flow to the duodenum is particularly desirable in high producing cows if the ruminal degradable N is sufficient to fully support microbial fermentation (NRC, 1985).

Little can be done to change ruminal turnover. One of the few options available is to modify the particle size of the diet or of a specific supplement. Netemeyer et al. (1980), for example, evaluated the rumen degradability of soybean coarsely or finely chopped using cannulated steers. The fraction of N that bypassed the rumen increased from 45 %, for the coarse soybean to 61 % for the fine one. Although, Kp was not measured in that study, it is possible that the smaller particle size resulted in higher Kp and higher protein bypass.

Under practical conditions, however, changes in ruminal digestibility are obtained by choosing concentrates that vary in Kd. In the case of proteins, fish meal or dried distillers grain, for example, have a Kd of the protein fraction of 2.2 and 4.4 %/h and either would be more effective in increasing protein flow to the duodenum than soybean meal protein that has a Kd of 10.2 %/h (Nocek and Russell, 1988).

Ørskov and McDonald (1979) proposed use of equation II to predict protein digestion in the rumen by using the in situ technique to estimate Kd. This concept has, since, been applied to different feed components to estimate nutrient digestibility in the rumen (Nocek, 1988). In vivo studies with animals cannulated at the abomasum or proximal duodenum have yielded results of ruminal digestion that diverge from those estimated by equation II. Erdman et al (1987) reported that, in lactating cows, the predicted degradability of N calculated by simultaneous measurement of Kd and Kp of alfalfa hay and distillers dried grains were 17 percentage units lower than the in vivo values found in the literature. Overall, the predicted values overestimate N digestion of feed with lower N degradability and underestimate those of high degradability. Inconsistencies between predicted and observed do not question the validity of equation II, but indicate the necessity of more accurate techniques to estimate Kd and Kp.

Markers

Total fecal collection has been traditionally used to calculate diet apparent digestibility. The determination of digesta flow at different points along the gastro-

intestinal (GI) tract can be obtained with animals fitted with re-entrant cannulae (McRae, 1975) and with the simultaneous measurement of rumen digesta pools. Rumen digesta pools can be determined by manual evacuation or animal slaughter (Warner, 1981), then, ruminal turnover can be calculated. All these techniques, are time consuming and can be used only in animals confined in stalls where total fecal collection and digesta measures are possible. The use of indigestible indicators, also referred as markers, can simplify the measures of digestibility, digesta flow and rumen kinetics. Furthermore, this opens the possibility to obtain this information from grazing animals.

The ideal marker is a substance that is non-absorbable, does not interfere with the digestion process or the microbial population, it is physically associated or flow with the material of interest and it has a specific and sensitive analytical method (Faichney, 1975). Even though there is no marker that fully satisfies these criteria (Owens and Hanson, 1992), their use has improved understanding nutrient utilization in ruminants.

With continuous infusion of a marker, it is possible to determine digesta flow and fecal output. Assuming steady state conditions of digesta flow, a continuous or frequent marker infusion will result in steady marker concentration ($[M]$) in the digesta. At any given point of the GI tract, $[M]$ depends on the marker daily dose (M) and the digesta flow (F):

$$[M] = \frac{M}{F} \quad (III)$$

This equation can be solved for F resulting in

$$F = \frac{M}{[M]} \quad (IV)$$

that indicates that digesta flow can be calculated knowing marker daily dose and concentration.

Under practical conditions, one of the most common problems is to obtain a representative sample from the cannula. Digesta, in fact, is not an homogenous entity but can be partitioned into liquid and particle phases. Since these two phases have different physical characteristics, the passage through the cannula may result in partial separation of the two phases with samples having a proportion of liquids and particulates that differ from the digesta flowing through the cannula.

A solution to this problem is offered by the 'two marker' method described by Faichney (1980). With this method, two markers, one associated with the liquid and the other associated with particulate phases, are administered simultaneously. Their concentration is, then, determined in each phase which are separated by physical methods (filtration or centrifugation). Under steady state assumption the ratio of markers in the whole digesta is equal to the ratio of the two markers in the daily dose. Whole digesta sample can be mathematically reconstructed based on marker

concentration of the liquid and particulate phases with adjustment to the ratio of markers infused. Armentano and Russell (1985) proposed a mathematical method that calculates digesta flow based on the two marker technique but also accounts for markers which are not completely associated with a unique phase.

Rumen turnover rate and volume can be calculated based on changes of [M] in the duodenal or fecal sample after a marker pulse dose or after the continuous infusion of marker is withdrawn. Rumen turnover can be calculated with different mathematical methods. The simplest, is to fit the descending [M] curve either in duodenal or fecal samples with a linear regression after logarithmic transformation of [M]. Alternatively, K_p can be calculated by fitting marker depletion with a nonlinear model using specific computer software (Appendix Figure 1).

Marker fecal excretion following a pulse dose can be fitted by a two exponentials plus time delay model (Grovmum and Williams, 1973) (Appendix Figure 2). The two exponents represent the kinetics of the digesta in the rumen and large intestine, the two major compartment of the GI tract, with the rumen having the longest retention time (Grovmum and Williams, 1973). Others model have been proposed (Ellis et al., 1979) that differ in numbers of compartments and time dependency of the parameters estimated. None of the models have been shown to be superior and goodness of fitting seems to vary with diet and animals (Cochran et al, 1986a; Moore et al., 1992). Pulse dose and subsequent mathematical treatment offer the opportunity to determine digesta pools, ruminal turnover, total tract retention time

and fecal output from a single excretion curve. However, the estimation of fecal output with a marker pulse dose may be less accurate than with the continuous marker infusion (Hatfield et al., 1990).

Markers can be categorized as internal and external. Internal markers are indigestible compounds naturally present in plant tissues and because they are continuously supplied with the diet can be used only to determine digestibility and digesta flow. In grazing trials, since the intake is unknown, the only parameter that can be determined by internal markers is forage digestibility. Examples of internal markers are lignin, chromogen, acid insoluble ash and indigestible ADF (IADF) or NDF (INDF). Cochran et al (Cochran et al, 1986) compared in vivo digestibilities of cubed alfalfa hay, fresh tall fescue, tall wheatgrass hay supplemented with soybean, and prairie hay to the values estimated using as internal markers IADF, INDF, ADF after 10 d of cellulase incubation (ADFIC) and lignin. Digestibilities calculated using IADF and INDF were similar to in vivo determinations only for the diets based on alfalfa and wheatgrass. Digestibilities for fresh fescue and prairie hay were underestimated. All the internal indicators underestimated digestibility of fresh tall fescue by an average of 20 percentage units. Cordova et al (Cordova et al ,1978) concluded that more reliable digestibility values of pasture or fresh grass are obtained by in vitro incubation than using internal markers.

External markers are inert compounds that can be administered to the animal. Example are chromium sesquioxide (Cr_2O_3), chelated minerals (Co-EDTA, Cr-EDTA),

chromium mordanted fiber and rare earth mordants (Ce, La, Sm and Yb). The oldest and probably the most used marker in grazing studies to determine fecal output is Cr_2O_3 . It is undigestible, it can be analyzed by atomic absorption spectroscopy and it is easy to use. The major problem of Cr_2O_3 is its cyclic fluctuation in fecal excretion that may result in a biased Cr concentration in the feces. Hopper et al. (1978) identified precise diurnal patterns of Cr excretion of cows grazing fescue or fescue-legume pastures with maximum values at 0900 h and minimum at 2000 h. Fecal output were accurately estimated by collecting fecal samples at the same time the marker was dosed.

Fluctuation in the digesta and feces have been reported for markers other than Cr_2O_3 (Prigge et al., 1981). The deviation from steady state marker concentration may be explained by variation in feed ingestion during the day. Increasing the number of meals may reduce these fluctuation in stall fed animals, but these conditions do not apply to grazing animals. In the case of Cr_2O_3 , variation in marker excretion seems related to its physical properties. When dosed orally, Corbett (Corbett, 1955 cited by Owens and Hanson, 1992) observed that Cr_2O_3 did not completely mix with the rumen digesta content and in particular in the dorsal sac. Because of its high density (5.2 g/ml) Cr_2O_3 may rapidly sink and accumulate in the rumen or rapidly escape through the rumen-reticulum orifice. Mixing can be enhanced by impregnating Cr_2O_3 with paper (Owens and Hanson, 1992) and also by dosing the marker through the rumen cannula. Since fluctuation cannot be avoided, an increase of accuracy can be obtained

by increasing the number of samples and extending the sampling period. Klooster et al. (1969), for example, observed Cr_2O_3 recovery over the first 24 h of collection of 90% but it increased to 99% by sampling over a period of 72 h.

Metal chelates of ethylenediaminetetraacetic acid (EDTA) are the most used liquid markers. Metals such as Cr and Co form a stable complex with EDTA that easily dissolve in water and can be utilized as liquid phase markers. The association of Co and Cr to the liquid phase were maintained after Co-EDTA and Cr-EDTA were incubated with rumen fluid followed by acidification (Combs, 1985). Uden et al. (1980) also found that only 2-3% of Co and Cr are excreted in the urine, confirming the suitability of these substances as liquid phase markers.

Concentrate supplementation

It is common practice to supplement stall-fed dairy cows with energy concentrates to meet the energy requirement of the animal. When grazing cows are offered these supplements, however, they reduce herbage intake with a degree of substitution of forage for the supplement that vary depending on the herbage availability (Leaver, 1985). Meijs and Hoekstra (1986), in fact, found that in lactating cows with high herbage availability the average substitution rate of herbage by supplement was .5 while it decreases to .1 for cows at low forage availability. The magnitude of substitution depends also on the type of energy concentrate with a lower substitution rate for fibrous concentrates than for starchy ones (Meijs, 1986). Because

of the substitutive effect, milk response to supplementation of concentrates is generally low varying between 1 to .15 kg of milk per kg of concentrate (Leaver et al., 1968).

Objectives

The objectives of this study were to evaluate:

1. flow and turnover of rumen contents in grazing animals;
2. intake of fresh grass by grazing animals;
3. site and extent of OM, N, NDF and ADF digestion;
4. the effect of corn supplementation on forage digestion;

Materials and Methods

Experimental design and animals

A switchback design of three periods (each of two wk) was used in which the third period was the replication of the first one. Four cows, cannulated at the rumen and the proximal duodenum (T-shaped cannula), were used in this study. A fifth cow, rumen cannulated, was used to obtain representative pasture samples. The dual cannulated cows had an average body weight of 554 ± 49 kg and were 130 ± 25 d in milk. The animals were permitted to adapt to pasture for 3 wk during which 6.4 kg of corn-mineral mix (95% cracked corn, 4% dicalcium phosphate, and 1% trace mineral salt) was offered daily in two meals after each milking (0100 and 1300 h). The dual cannulated cows were randomly assigned to one of two groups and fed either pasture alone or pasture supplemented with 6.4 kg of corn mix per d. At the beginning of the second period one dual cannulated cow had to be replaced with another cow already adapted to grazing.

Pasture

The study was conducted at the Virginia Tech Dairy Center during June and July 1991. Four contiguous paddocks of about 2.3 ha were used in a rotational stocking scheme. Twenty four cows began grazing in April. Grazing was managed to

maintain an actively growing canopy. Because of the dry weather, liquid lagoon waste was spread during June and July following rotation of the animals to another paddock.

Pastures were visually evaluated for botanical composition according to the DAFOR scale (Brodie, 1985). With this method plant species are classified as dominant, abundant, frequent, occasional and rare.

On d 11 (700 h) of each period, ruminal contents of the rumen cannulated cow were manually emptied. After the animal had been allowed to graze for 15 to 30 min, grass masticates, representing pasture samples, were collected from the cardia region of the rumen and stored at -20 °C. After the samples were collected the rumen was refilled with the original contents.

Markers

Chromium oxide (Fisher scientific, Pittsburgh, PA) and Cobalt ethylenediaminetetraacetate (Co-EDTA) were used as particulate and liquid markers, respectively. The Co-EDTA was prepared according to Uden's procedure (Uden, 1980). Solution of Co-EDTA was prepared prior to the beginning of the study by dissolving Co-EDTA crystals into distilled water at a concentration of 62.5 g/L. From d 1 through d 10 of each period, 15 g Cr₂O₃ and 120 ml Co-EDTA solution (7.5 g Co-EDTA) were administered at 12 h intervals (1100 and 2300 h) through the rumen cannula. Single doses (15 g) of Cr₂O₃ were pre-weighed and placed into single paper

bags. The Cr_2O_3 was dosed by emptying the paper bag into the rumen and leaving the bag inside the rumen.

Sampling and storage

Milk production was recorded at each milking during the three periods. Milk samples were taken on d 11 at the am and pm milk.

For each cow and each period two series of whole duodenal digesta and feces samples were taken. The first set was taken on the last three days (from 7 through 10 d) that the markers were administered and were used to determine digesta flow at the duodenum and fecal output. The second series was collected during the 96 h (from 10 d to 14 d) following the last dose of markers, to follow marker depletion and estimate rumen turnover.

Six duodenal and fecal samples were collected on days 7 (1000 h), 8 (0200 and 1800 h), 9 (0600 and 2200), and 10 (1400 h). Duodenal samples were taken by opening the cap of the cannula, discarding the first surge of digesta and collecting about 1.5 L of digesta into a large beaker. The digesta were immediately mixed and subsampled by filling two 270 ml cups and stored at $-20\text{ }^\circ\text{C}$. Grab fecal samples were wrapped within plastic bags and stored at $-20\text{ }^\circ\text{C}$.

At the end of each period, whole duodenal and fecal samples were thawed at room temperature, mixed and composited by cow. Three 270 ml cups of whole duodenal composite were refrozen and stored at $-20\text{ }^\circ\text{C}$. The remainder of the digesta

was centrifugated at 3000 x g for 10 min to separate it into particulate and liquid phases. Particulate, liquid and fecal composites were lyophilized in pre-weighted cups.

Samples of duodenal digesta and feces to determine markers depletion were taken at 2, 4, 8, 12, 18, 24, 30, 36, 48, 60, 72 and 96 h after the last dose of markers. Samples were then freeze dried, ground and stored in a sealed container.

On d 11 at 0500 h, rumen fluid (2 L) was collected through the rumen cannula by suction through a plastic tube fitted with a filtering probe. Two subsamples (5 ml) were pipetted into two plastic tubes containing 1 ml meta-phosphoric acid or 1 ml of meta-phosphoric acid with 1 ml of internal standard (7 μ M/ml isocaproic acid), the former for NH_3 -N analysis, and the latter for VFA analysis. Ruminant fluid pH was determined by a glass electrode pH-meter within approximately 30 min using the remaining sample.

Rumen bacteria were isolated from the remaining rumen fluid by differential centrifugation. After centrifugation at 200 x g for 10 min for removal of protozoa and feed particles, the supernatant was centrifugated three times at 35,000 x g for 20 min. After each centrifugation, the supernatant was discarded and the pellet resuspended with deionized water.

Corn samples were collected weekly and composited across periods.

Laboratory analyses and calculations

Milk fat and protein percentages were determined by the Virginia Federation of DHIA laboratory.

Pasture, corn supplement, whole duodenal, particulate phase digesta and feces samples were freeze-dried, and ground through a 1-mm screen in a cyclone mill. Liquid phase digesta and bacterial isolate also were freeze-dried, but ground with a mortar and a pestle. Organic matter was determined by ashing at 600 °C in a muffle furnace for 6 h. The Kjeldahl method (AOAC, 1980) was used for nitrogen analysis. Neutral detergent fiber and ADF were determined by the procedure of Goering and Van Soest (1970).

In vitro OM digestibility (IVOMD) of five replicates of each pasture sample was determined by precisely following the procedure described by Goering and Van Soest (1970). The only changes from the original procedure were the use of a 50 ml plastic tube instead of an Erlenmeyer flask and gassing CO₂ only at the beginning of the incubation rather than continuous gassing. Each replicate (300 mg) was incubated at 40 °C in the plastic tube with 30 ml of buffered rumen fluid. The rumen fluid (pH = 6.5) was obtained from a rumen cannulated grazing cow, and was mixed with the buffer (pH = 6.9) in the ratio of 1 part of rumen fluid to 4 parts of buffer. Anaerobic conditions were obtained by gassing CO₂ and closing the tubes with a rubber stopper fitted with a Bunsen valve. Resazurin, a reducing indicator, was present in the buffer (.125 mg/L). The achievement of a reduced environment was indicated by the lack of

color in the solution. After 48 h incubation, toluene (1 ml) was added to stop bacterial activity. Samples were centrifuged at 3000 x g for 10 min, after which the supernatant was discarded and the pellet resuspended in an acid (pH 2 using 6 N HCl) pepsin solution and incubated for another 48 h. Tube contents were then filtered through pre-weighed ashless filter paper (Whatman 54, Whatman, Clifton, NJ). Residual DM and OM was determined by drying the filters overnight at 100 °C and ashing at 500 °C for 6 h. Organic matter disappearance was corrected for residual OM of blanks and digestibility calculated using the following equation:

$$\text{IVOMD}\% = \frac{\text{OMS} - (\text{OMR} - \text{OMB})}{\text{OMS}} \times 100$$

where IVOMD% = In vitro OM digestibility, %

OMS = OM Sample, g

OMR = OM Residue, g

OMB = Average OM residue in the blanks, g.

Fecal and digesta samples were wet ashed with nitric, perchloric, sulfuric acid and hydrogen peroxide (SAC, 1973) and marker concentrations were determined with a Varian AA-475 atomic absorption spectrophotometer (Varian Instruments, Palo Alto, CA). Approximately 300 mg of each sample were placed into volumetric digestion tubes to which two ml of perchloric and nitric acid were added in the evening. The next morning, two drops of hydrogen peroxide were added and the tubes were set in an aluminum block placed on a hot plate. Temperature of the block was raised to 100-

120 °C with formation of dark fumes. The temperature was maintained at 120 °C until white fumes evolved, usually taking about 4 h then one ml of sulfuric acid was added and the temperature increased gradually to 150 °C. The main purpose of adding sulfuric acid was to assure the perchloric acid did not reach dryness. Digestion was considered complete after the samples had turned a reddish color (Williams, 1962). Tubes were then allowed to cool overnight in the block. The best results, in terms of repeatability between duplicate and reproducibility among runs, were obtained by maintaining the block temperature within the limits indicated above with 12-14 h of digestion. Chromium and Co standards were also subjected to acid digestion as suggested by Rains (1991). Upon completion of digestion, samples were diluted to a Cr and Co concentration within the detection range of the spectrophotometer, filtered through Whatman 1 paper (Whatman, Clifton, NJ) and stored at 2 °C until analysis. Chromium was analyzed with an acetylene-nitrous oxide flame at 357.9 nm wavelength (slit width .5 mm) and Co was determined with an air-acetylene flame at 240.7 nm wavelength (slit width .2 mm).

Based on the two-marker technique (Faichney, 1980), OM flow was calculated by solving the following system of equations (Armentano and Russell, 1985):

$$M_{Cr} = F_p[Cr]_p + F_L[Cr]_L$$

$$M_{Co} = F_p[Co]_p + F_L[Co]_L$$

where: M_{Cr} and M_{Co} = daily dose of the marker (Cr or Co), mg

F_p and F_L	=	flow of OM associated with particulate and liquid phases, kg
$[Cr]_p$ and $[Cr]_L$	=	concentrations of Cr in particulate and liquid phases (ppm)
$[Co]_p$ and $[Co]_L$	=	concentrations of Co in particulate and liquid phases (ppm).

An example of calculation is shown in Appendix Table 2.

Rumen turnover rates were determined by fitting the descending portion of the Co and Cr depletion curves of the whole duodenal digesta with a non-linear model using the Marquardt method of the NLIN procedure of SAS (1988):

$$M = B * e^{(-K_p * t)}$$

where	M	=	Marker (Co or Cr) concentration at time t (ppm)
	B	=	Marker concentration at peak (ppm)
	K_p	=	Rumen turnover rate (%/h)
	t	=	time after peak.

Microbial contribution to OM and N flow at the duodenum was determined using cytosine as microbial marker (Broderick and Merchen, 1992). Rumen bacteria, liquid and particulate phases were analyzed for cytosine by high performance liquid chromatography using a Varian 2510 chromatograph (Varian Instruments, Palo Alto, CA) (Konig cited by Schelling, 1982). Microbial N as percentage of total duodenal N

flow was estimated by the cytosine:N ratio of rumen bacteria, liquid and particulate phases (Appendix Table 3):

$$\%PMN = \frac{MN}{MC} \times \frac{PC}{PN} \times 100$$

%PMN	=	microbial nitrogen of the individual phases, %
MN	=	microbial nitrogen, g/g OM
MC	=	microbial cytosine, μ moles/g OM
PC	=	cytosine of the individual phases, μ moles/g OM
PN	=	nitrogen of the individual phases, g/g OM

Apparent OM and N digestibility in the rumen were corrected for microbial contribution to calculate true digestibility.

Rumen fluid and whole duodenal digesta were centrifuged at 3000 x g for 10 min to remove particulate matter and filtered through a 45 μ Metrical filter (Gelman Sciences, Inc., Ann Arbor, MI).

Ruminal VFA concentration was determined on the supernatant of rumen fluid by a Varian Vista 6000 (Varian Instruments, Palo Alto, CA) gas chromatograph.

Ammonia was determined on the supernatant of rumen fluid and duodenal digesta samples using a spectrophotometer (Spectronic 1001, Baush and Lomb, Rochester, NY) following the procedure described by Weatherburn (1967) with sample incubation at room temperature for 18 h.

Nonammonia N (NAN) and nonammonia-nonmicrobial N (NANMN) flow at the duodenum were calculate subtracting NH₃-N flow to total N flow and subtracting microbial flow to NAN, respectively.

Statistics

All the results were subject to ANOVA by using the type III sum of squares of the GLM procedure of SAS (1989). The data from the cow that was replaced was discarded; thus ANOVA was performed using 11 records.

The data was analyzed with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + g_k + e_{ijk}$$

where

- Y_{ijk} = dependent variables
- μ = overall average;
- α_i = effect of diet i, i = 1,2;
- β_j = effect of period j, j = 1, 2, 3;
- g_k = effect of cow k, k = 1, 2, 3, 4;
- e_{ijk} = residual error (0, σ^2).

Differences were considered significant at a $P < .10$. All the results are reported as least squares means (see Appendix Table 1 for an example of an ANOVA table).

Validation Trial

The validity of the technique used to estimate herbage intake was verified in a following indoor trial using two lactating cows (BW 606 kg, milk production 18 kg/d) fistulated at the rumen and duodenum. The animals were adapted to grazing for 2 wk prior to the beginning of the trial. Cows were ad libitum fed chopped grass harvested daily (1030 h). The grass was weighed, sampled and fat 0200, 0800, 1400, and 2000 h, daily. During the day, the grass was stored in plastic cans at -20 °C. At 0130 and 1330 h, after each milking, cows were fed 4.5 kg corn-mineral mix composed of 95 % of cracked corn and 5 % of a salt-mineral supplement. This supplement contained adequate supplement of minerals, vitamins and sodium bicarbonate (18 %) (Table 1). Corn refusals were recorded after each meal before fresh grass was fed. Orts were collected, weighed, and sampled at 1200 h. Grass and ors samples were dried at 70 °C for 48 h. Administration of markers , sampling and analytical procedures, and calculations of fecal OM output and OM intake were identical to the previous trial.

Table 1. Mineral and vitamin content of the salt-mineral mix used in the validation trial.

Item	% of DM
Minerals	
Calcium, %	16.0
Phosphorus, %	6.5
Chlorine, %	5.8
Potassium, %	3.5
Sulfur, %	3.2
Magnesium, %	2.2
Zinc, ppm	1325
Manganese, ppm	1100
Iron, ppm	265
Copper, ppm	132
Selenium, ppm	5
Cobalt, ppm	3
Iodine, ppm	2
Vitamins	
A, IU/kg	110000
D3, IU/kg	44000
E, IU/kg	550

Results and Discussions

Botanical and chemical composition of pastures

The botanical composition of the pastures is shown in Table 2. Tall fescue (*Festuca arundinacea*) was the dominant species in the two paddocks used during the first two periods. In these periods, also, there was a good presence of legumes with ladino (*Trifolium repens* cv. *Ladino*) and white clover (*Trifolium repens*) and other grass species like bluegrass (*Poa pratensis*) and orchardgrass (*Dactylis glomerata*). In the third period, there was a prevalence of grasses with orchardgrass, bluegrass and fescue as dominant, abundant and frequent species, respectively. Because different paddocks were used in the three experimental periods, the differences in botanical composition observed have to be attributed to the differences among paddocks rather than a shifting in botanical composition during the trial. Even though fescue was the dominant species, it appeared that it was the least palatable. During the trial, it was observed that the animals, when introduced in a new paddock, preferentially grazed other grass and legume species rather than fescue. In order to maintain higher forage intake the animals were removed from a paddock before they were forced to graze fescue and moved to a new paddock. The intake of fescue, therefore, was limited and it was not representative of the abundance of fescue on the fields.

Crude protein content of the pasture was high across the three periods with values always above 20 % (Table 3). The highest CP content (28 %) was observed

Table 2. Botanical evaluation of the pastures according to the DAFOR scale

	Period		
	1	2	3
Dominant	Fescue <i>(Festuca arundinacea)</i>	Fescue <i>(Festuca arundinacea)</i>	Orchardgrass <i>(Dactylis glomerata)</i>
Abundant	Ladino Clover <i>(Trifolium repens cv. Ladino)</i>	Ladino Clover <i>(Trifolium repens cv Ladino)</i>	Bluegrass <i>(Poa pratensis)</i>
	Bluegrass <i>(Poa pratensis)</i>	Bluegrass <i>(Poa pratensis)</i>	
	Orchardgrass <i>(Dactylis glomerata)</i>	Orchardgrass <i>(Dactylis glomerata)</i>	
Frequent	White Clover <i>(Trifolium repens)</i>	White Clover <i>(Trifolium repens)</i>	Fescue <i>(Festuca arundinacea)</i>

Table 3. Chemical composition and in vitro OMD of the herbage and corn supplement

Item	Period			Corn ¹
	1	2	3	
	-----%/OM -----			
CP	24.5	22.6	28.3	9.3
NDF	61.1	58.7	68.1	8.7
ADF	36.1	40.9	37.6	2.7
In vitro OM digestibility	74.1	69.5	70.5	67.0

¹ In vivo OM digestibility (Tyrrell and Reynolds, 1988)

during the third period. Considering the botanical composition, a higher CP content of the forage was expected in the first two periods, when the pasture had more legumes rather than the third period. However, it has to be considered that the pastures were periodically irrigated with liquid lagoon during the trial. The paddock used in the last period was probably irrigated more times than in the previous periods. This may have increased the N content in the soil causing an accumulation of N in the plant tissues.

The NDF content of the forage was similar in the first two periods but was higher in the third one. Acid detergent fiber, however, was similar across periods. Considering that the pastures were maintained in an active growing stage, the fiber content was higher than expected. This probably can be attributed to the warm climate that characterized the months the trial was carried out. In vitro OM digestibility was about 70 % with a slightly higher value in the first period.

OM intake, flow, and digestion

Herbage OM intake was 3.2 kg higher for the pasture diet than the corn supplemented diet (Table 4). However, total OM intake had a tendency to be greater when cows were fed corn. This tendency also is shown when intake was expressed as percentage of the BW.

With lactating cow producing about 24 kg/d and grazing perennial ryegrass (*Lolium perenne*), Meijs and Hoekstra (1984) predicted that herbage OM intake would vary from 12.1 to 16.9 kg/d by increasing daily herbage allowance from 15 to 25

Table 4. Organic matter intake, flow to the duodenum and digestibility in response to grazing pasture with or without corn supplementation.

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
Intake:				
Pasture, kg/d	13.0	9.8	.8	.07
Corn, kg/d	.0	5.4		
Total, kg/d	13.0	15.2	.8	.16
Total, % of BW	2.4	2.8	.1	.18
Flow to the duodenum:				
Particulate, kg/d	5.4	7.3	.3	.02
Liquid, kg/d	1.2	1.3	.4	NS
Total, kg/d	6.6	8.6	.3	.02
Digested in the rumen:				
Apparent, kg/d	6.3	6.6	.6	NS
True, kg/d	8.3	8.9	.6	NS
Digestibility:				
Whole tract, %	71.9	69.9	.5	.08
Rumen apparent, %	48.9	43.3	1.6	.09
Rumen true, %	64.3	58.7	1.3	.06
Rumen, % of whole tract	68.1	62.3	2.4	NS

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

kg/cow. The herbage intake obtained in our study was near the bottom of the range found by Meijs and Hoekstra. In that trial, the herbage had lower NDF and higher IVOMD than our study and it may explain their higher intakes.

In a second study (Meijs, 1986), cows were supplemented daily with 5.4 kg of either a high starch (corn based) or high fibre (sugar beet pulp based) concentrate. Daily OM intake averaged 17.4 kg/d or 3.1% of BW. In this study, herbage quality (IVOMD and NDF) was similar to our study but they still obtained higher OM intake. It is possible that, in our study, intake may have been limited by low forage availability. Even though this parameter was not quantified, by visual evaluation, forage availability was not a limiting factor during our trial. A very warm climate characterized the months our trial was conducted. Average and average maximum temperatures were 20, 27 and 23, 30 °C, for June and July '91, respectively (Fig.2). Temperatures were higher than normally recorded during these months, with the maximum temperatures exceeding the comfort range for dairy cows. Shading was not available except at midday during feeding and milking, for approximately 3 h. Depression in food intake is a typical response to heat stress.

Since herbage intake was reduced 3.2 kg by feeding 6.4 kg of corn-mineral mix, mean substitution rate ($3.2/6.4$) was .50. This value is within the range of .39 to .64 reported by Meijs (1981) for lactating grazing cows. The cause of substitution rate is discussed later.

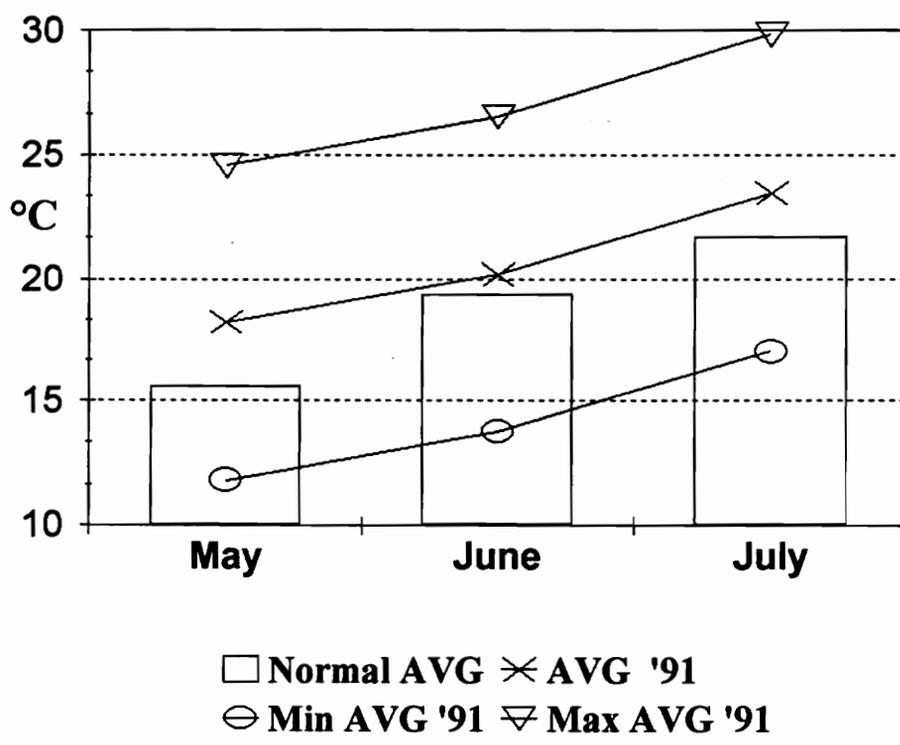


Figure 2. Average, maximum, and minimum temperatures recorded in Blacksburg during May, June, and July 1991 versus normal average temperatures for these months.

The higher OM flow associated with the particulate fraction in cows fed the corn supplemented diet resulted in a higher total OM flow than for pasture alone (Table 4). Whole tract digestibility of OM is the result of the technique utilized to estimate intake. Because the OM digestibility of corn (67%) was lower than the average IVOMD of the herbage (71.9%), the corn diet had an overall lower digestibility. Organic matter apparently (OMAD) and truly (OMTD) digested was lower when cows were fed the corn supplemented cows. The percentage of OM digested in the whole tract (OMDR) that disappeared in the rumen, however, was similar between diets.

Beever et al (1986b), with Friesian steers reported an OMAD in the rumen for perennial ryegrass and white clover of 64% and 62%, respectively. These values are higher than those found in our study. The herbage intake in Beever's study was only 1.8% of BW compared to 2.4% for the pasture diet in our trial. The higher level of intake of dairy cows may have reduced rumen retention time and therefore rumen digestibility. With lactating cows fed with fresh perennial ryegrass, eating about 13 kg/d of herbage OM, OMAD ranged between 64 and 68% (van Vuuren, 1992). Intake of fresh grass was similar to our study but OMAD was higher than our values. It is likely, therefore, that the lower digestibilities in our study are the result of the lower quality of the forage that had higher fiber content (NDF 63 vs 43 %) than that in van Vuuren's study.

There is also the possibility that the lower rumen digestibility observed was due to underestimation of forage intake and(or) overestimation of duodenal flow.

Considering the numbers of assumptions to be made, it is possible that forage intake was underestimated. The procedure used to estimate herbage intake was evaluated in the validation trial. Estimated OM intake was slightly greater (2.5 %) than the actual intake (Table 5). However, variability between the two cows was high. This may be the cause of the variation observed in the OM intake and rumen digestibility data in our trial. On the other hand, if the duodenal flow was overestimated not only OMAD would have been affected but also OMDR. For pasture, OMDR was 68%, which is similar to the values of 69 to 71% reported by Beever et al (1986a). It does not support the idea of an underestimation of duodenal flow in our study.

Milk production and composition

Although not a primary objective of this study, milk production and composition were compared for cows grazing pasture with or without corn supplementation (Table 6).

Milk yield was increased from 19.5 to 23.7 kg/d by corn-mineral mix supplementation. Milk production increased .56 kg per kg of supplement. Considering the NE_1 (NRC, 1989) of corn (1.84 Mcal/kg) and milk (.74 Mcal/kg) one would expect an increase production of about 2.5 kg of milk per kg of corn.

The difference can be mainly attributed to the reduction of herbage intake by the supplemented cows caused by a negative associative effect.

Table 5. Actual and estimated OM intake by cows fed fresh grass and supplemented with 9 kg of corn-mineral mix/d in the validation trial

Cow	OM intake		Estimated as % of Actual
	Actual	Estimated	
1855	6.8	6.5	95.5
2261	7.2	7.9	109.5
	Average		102.5 ± 7.0

Table 6. Production and composition of milk in response to grazing pasture with or without corn supplementation

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
Milk, kg/d	19.5	23.7	.6	.01
Fat, g/d	710	770	24.5	NS
Protein, g/d	550	680	24.5	.05
Fat, %	3.69	3.25	.09	.05
Protein, %	2.84	2.84	.01	NS

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

Leaver et al. (1968) summarizing several trials with cows producing 14-18 kg of milk/d, reported an average increase in milk yield of .32 kg for each kg of concentrate. Journet and Demarquilly (1979) found, that cows producing 25 kg/d, responded with a .4 kg. The response in this study (.56 kg) may be expected from high producing cows. In our study, milk response was higher than the average reported in the literature. The animals used in our study were producing 45 kg/d before the grazing trial started. Therefore, these cows had a great milk production potential, which may explain the greater milk production response.

Despite the increased milk output when corn was fed, fat yield was similar between diets. Fat percentage was lower for the supplemented diet than the pasture diet. A previous report indicated a depression in milk fat content due to corn supplementation, and the depression was even greater when higher amounts of corn were fed (Polan et al., 1985). This effect may be associated with changes in the VFA profile in the rumen. Supplementing corn did not affect milk protein content; therefore, the difference in protein yield directly reflects the differences in milk production. However, in other research, milk protein percentage increased as concentrate intake increased (Huber and Boman, 1966).

Ruminal parameters

Ruminal pH, VFA, NH₃-N and turnover are shown in Table 7. Rumen fluid pH had a tendency to be lower when corn was fed (6.2 vs 6.4), but both were in the

Table 7. Ruminal pH, VFA, ammonia-N, and ruminal turnover in response to grazing pasture with or without corn supplementation

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
pH	6.4	6.2	> .1	.14
VFA:				
Total, mmol/L	150	148	4	NS
Acetate, %	63.2	62.4	.5	NS
Propionate, %	18.7	19.1	.1	.08
Isobutyrate, %	1.4	1.3	.1	NS
Butyrate, %	12.9	13.5	.7	NS
Isovalerate, %	2.1	2.2	> .1	NS
Valerate, %	1.7	1.5	.1	NS
C2/C3 ⁴	3.4	3.3	< .1	.01
NH ₃ -N, mg/dl	22.4	17.1	1.6	.10
kp Cr ₂ O ₃ , %/h ⁵	7.5	7.1	.4	NS
kp Co-EDTA, %/h ⁵	18.2	18.5	.5	NS

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

⁴ C2/C3 = ratio of acetate to propionate

⁵ kp = Ruminal rate of passage

expected range. Fermentation of starch in the rumen may result in an increase in VFA concentration in the rumen and(or) production of lactic acid that would cause a reduction of pH. Since there was no difference in VFA concentration between the two diets, a higher production of lactic acid is possible. Conditions of sampling rumen fluid must not be overlooked. The concentrate was fed in two meals and rumen fluid was sampled 4 h after feeding, the time when maximum effect of concentrate fermentation might occur. However, one must be cautious with these conclusions. Immature pasture forages contain rather high concentrations of fermentable sugars, so perhaps corn starch is no better substrate. Also, VFA concentrations are not only the result of production, but of absorption also, so comparing absolute concentrations with no knowledge of VFA pools or turnover is risky.

Concentration of VFA was similar between diets and averaged 149 mmol/L. In cows grazing perennial ryegrass supplemented with either 1 or 7 kg of an energy concentrate, VFA concentration varied during the day between 110 and 160 mmol/L, (Van Vuuren, 1986). This value is higher than that normally observed in dry lot diets indicating rapid fermentation of fresh grass OM.

Molar proportion of acetate, butyrate, isobutyrate, isovalerate and valerate did not differ between diets. Propionate was increased modestly, but significantly ($P < .08$) by the supplement that also resulted in a decrease in the ratio of acetate to propionate. These changes are not unusual considering that propionate is the major endproduct of

starch fermentation. Acetate is the main lipogenic precursor in ruminants and its rumen production contribute directly to fat secretion in the mammary gland. Propionate, on the other hand, is the main glucogenic precursor that contributes hexose units for lactose synthesis. Because lactose production is directly correlated to milk yield, the supply of propionate may affect milk yield (Sutton, 1981).

According to Sutton (1981), milk fat changes would be the result of variations in fat secretion rate relative to lactose production. Fat depression therefore, can be caused by a reduction in acetate supply, and(or) an increase in lactose secretion via an increase in propionate supply. In our study, since acetate in the rumen did not change, the reduction of milk fat percentage may be interpreted as the result of the dilution caused by the increase in milk yield rather than a depression of fat output.

Ammonia-N concentration was lower with corn supplementation than pasture alone (17.1 vs. 22.4 mg/dl). High rumen $\text{NH}_3\text{-N}$ are common when an animal is fed with temperate fresh grass (Beever and Siddons, 1984). Cammel et al. (1983) reported values ranging between 28 and 59 mg/dl in steers fed with white clover. In grazing cows fed fresh ryegrass (van Vuuren et al, 1986), rumen $\text{NH}_3\text{-N}$ peaked at 30 mg/dl when supplemented with 1 kg of an energy concentrate daily and 20 mg/dl when 7 kg of supplement were fed.

Most rumen bacteria require ammonia as a major N source. Satter and Slyter (1974) indicated that microbial growth was maximized when $\text{NH}_3\text{-N}$ concentration reached 5 mg/dl. Lower concentration may result in uncoupled energy fermentation

(Nocek and Russell,1988) which reduces efficiency of microbial protein synthesis (EMPS). Higher $\text{NH}_3\text{-N}$ concentration would not improve EMPS and microbial flow to the duodenum would depend on fermentable energy available in the rumen. It appears, that in our study as well as in most grazing conditions, rumen $\text{NH}_3\text{-N}$ concentrations exceed bacteria requirement.

Under this condition, an increase of rumen fermentable energy in the supplemented diet may have stimulated microbial growth and lowered rumen $\text{NH}_3\text{-N}$ concentration. Also, lower $\text{NH}_3\text{-N}$ concentration when corn was fed may be explained by the lower herbage intake with a reduction in N intake.

As suggested by Owens and Hanson (1992), rumen turnover will be discussed in term of marker passage. Rate of passage (kp) of Cr_2O_3 and Co-EDTA were not affected by the supplement and averaged 7.3 and 18.4 %/h, respectively (Table 7). Therefore, corn supplementation had little or no influence on ruminal turnover in grazing cows.

Evans (1981), analyzing rumen kinetics data from either sheep or cattle found that particulate kp was increased by higher percentages of forage in the diet. This effect was not observed in our study. Instead, our data agree with the results of Colucci et al. (1990) in which the turnover of Cr mordanted to the fiber of the forage, was similar between high forage (forage 83%) and low forage (forage 32%) diets fed ad libitum to lactating cows.

Rumen kp of particulate and liquid markers in lactating cows fed a diet based on silages or hay varied from 2.5 to 5.2 and from 6.0 to 12.0 %/h (Colucci et al., 1982; Hartnell and Satter, 1979; Llamas-Lamas and Combs, 1990; Rode and Satter, 1988), respectively. Their values are considerably lower than those obtained in our trial with grazing cows. The reason may be due to the different markers used. The particulate markers used in the studies cited were either rare earth elements (cerium, lanthanum, samarium and ytterbium) or Cr labelled or mordanted to the fiber of the forage. The Cr_2O_3 used in our study may separate from feed particles (Owens and Hanson, 1992) and flow with the liquid phase. This would bias particulate turnover with a tendency to overestimate it. It is possible, therefore, that the higher particulate kp in our study is the result of differences in marker characteristics. In contrast, van Vuuren et al. (1992) reported that, rate of passage of Cr-mordanted fiber in grazing cows was of 6.7 %/h, similar to the kp of Cr_2O_3 in our study and higher than generally found in the literature.

Chromium or cobalt chelates of EDTA were the liquid markers used in the above studies. The kp of the two markers is similar (Uden et al., 1980); therefore, values among studies are comparable. The high kp of Co-EDTA (18.3 %/h) determined in our study agrees with the values of 17 to 22 %/h reported for grazing cows (van Vuuren et al., 1986, 1992) and indicated that grazing cows may have a faster liquid turnover than cows fed drylot diets.

N intake, flow, and digestion

Intake, duodenal flow and digestibilities of N are reported in Table 8. Forage N intake was lower for corn supplemented diet than pasture alone. This reflects the decline of herbage intake when corn was fed. Total N intake, however, was not different ($P > .10$). There were no statistical differences in N flow at the duodenum between diets for particulate, liquid, total, and NAN. As noted for OM, whole tract digestibility was higher for pasture than the supplemented diet. Intake N recovered at the duodenum was about 12 percentage units greater for the supplemented diet (87 vs 75%). Even though intake and duodenal flow were not statistically different, numerically corn supplementation caused lower N intake and higher duodenal flow that resulted in the greater N recovery at the duodenum. The NANMN was calculated by subtracting microbial flow from NAN flow. Considering a limited contribution of endogenous N, NANMN is similar to the flow of dietary protein that escape ruminal degradation. The NANMN expressed as percentage of N intake averaged 26% and was not different between diets. These results indicate that about 74 % of herbage protein were degraded in the rumen.

Beever et al (1986b) working with steers grazing perennial ryegrass or white clover, reported feed N degradations that varied from 64 to 87% with an average of 75 and 79% respectively for the grass and the legume. Cammel et al. (1983), with grazing steers, estimated N degradabilities above 90%. From rumen incubation with nylon bags, it appears that in fresh grass more than 90% of N compounds are

Table 8. Nitrogen intake, duodenal flow and digestibility in response to grazing pasture with or without corn supplementation

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
Intake:				
Pasture, g/d	522	391	37	.09
Corn, g/d	0	80		
Total, g/d	522	471	37	NS
Flow to duodenum:				
Particulate, g/d	274	281	16	NS
Liquid, g/d	112	127	8	NS
Total, g/d	386	408	15	NS
NAN ⁴ , g/d	371	396	14	NS
Microbial, g/d	243	273	14	NS
Microbial, % of NAN	65.2	69.1	2.0	NS
NANMN ⁵ , g/d	128	123	7	NS
NANMN, % of total	24.9	26.2	2.2	NS
EMPSAD ⁶	38.6	40.4	2.6	NS
EMPSTD ⁷	29.0	31.0	1.4	NS
Digestibility:				
Whole tract, %	78.8	71.9	1.0	.01
Duodenal recovery, %	75.3	86.7	3.7	.09

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

⁴ Nonammonia nitrogen

⁵ Nonammonia, nonmicrobial nitrogen

⁶ Efficiency of microbial protein synthesis per OM apparently digested in the rumen

⁷ Efficiency of microbial protein synthesis per OM truly digested in the rumen

degradable and that degradation rate may vary from 10 to 20%/h (van Vuuren et al., 1991; Beever and Siddons, 1984).

The rate of dietary CP degradation to $\text{NH}_3\text{-N}$ relative to the rate of $\text{NH}_3\text{-N}$ disappearance determine the level of $\text{NH}_3\text{-N}$ in the rumen. Three processes determine $\text{NH}_3\text{-N}$ disappearance: it can be absorbed through the rumen wall and enter the bloodstream, it can outflow to the small intestine, or it can be incorporated into microbial protein. When protein N is rapidly degraded, as in fresh grass, $\text{NH}_3\text{-N}$ may accumulate increasing the rate of absorption through the rumen wall and the excretion of N in the urine. Because temperate pastures are high in N content and degradability, losses of N in the rumen are expected. With sheep fed either ruani (*Lolium perenne*) or manawa (*multiflorum x perenne*) grassland or white clover, N losses in the rumen were 31, 11, and 22%, respectively. When ryegrass and white clover were fed in different proportions to lactating cows, N losses varied from 14 to 21% (Beever and Siddons, 1984).

Nitrogen losses in our study varied between 8 and 35% and agree with the values found in the literature. The losses were lower for corn supplemented diet and this was associated with a lower $\text{NH}_3\text{-N}$ concentration in the rumen. It is possible that the energy supplementation modified N kinetics in the rumen. It is known that energy supplements may reduce rate of forage degradation, decreasing release of N, reducing NH_3 accumulation in the rumen and raising dietary rumen outflow.

Ammonia-N was decreased when starch was supplemented to dairy cows fed with a diet based on alfalfa haylage and corn silage (Cameron et al., 1991). This was associated with an increase of dietary protein flow which implies a reduction of CP degradation in the rumen. In our study, however, NANMN was similar between diets suggesting that rate of in the rumen N degradation was unchanged.

Microbial N flow was 30 g/d higher (Table 8) when cows were fed corn supplement, but the difference was not statistically different ($P > .10$). The N fraction incorporated into microbial cells that flowed through the duodenum was similar for the diets and averaged 65%. Also, similar, was the efficiency of microbial synthesis (EMPS) either expressed as OMAD (average 40 g/kg) or OMTD (average 30 g/kg). Because EMPS was not different, the slight increase in microbial flow can be attributed to the higher OM digested in the rumen. The fact that the difference in amount of OM digested was small (600 g/d) and not significant might explain lack of a significant increase in microbial N flow.

Information on EMPS in grazing cattle is very limited. Beaver and Siddons (1984), in their review on pasture utilization, cited only one study where microbial N was determined. In that study, EMPS varied from 42 to 91 g/kg of OMAD and from 30 to 48 g/kg of OMTD (Cammel et al., 1983). In dairy cows fed fresh grass microbial N flow was of about 20 g/kg of OMAD (van Vuuren et al., 1992). Large variations in EMPS may be attributed to the different techniques used to determine microbial flow (Broderick and Merchen, 1992). Comparison among EMPS should be

made within a study or among studies that used similar techniques (Buttery and Lewis, 1982).

In previous studies conducted in our lab, where rumen ammonia was not limiting microbial growth (fish meal diet in Zerbini et al., 1988), the EMPS ranged between 20.7 and 40 g/kg of OMAD (Chapin, 1986; Zerbini et al., 1988; Wonsil, 1990, Seymour et al., 1992). In all these studies, diets were based on corn silage and alfalfa haylage and dietary N degradability averaged 60%. It seems therefore that during grazing, EMPS may be higher than that noted for more traditional diets. Walker et al. (1975) used radioactive sulphur (^{35}S) to measure microbial flow in sheep fed either with hay or fresh grass. EMPS and microbial turnover were higher for fresh grass than for hay, suggesting that the improvement in microbial flow was caused by the higher passage rate of digesta in the rumen. As demonstrated by Owens and Isaacson (1977), EMPS is maximized at high rate of turnover. Particulate and liquid turnover in the Zerbini and Chapin's studies were about 4.3 and 7.5 %/h, respectively. These values are much lower than those determined in our study suggesting that the higher rumen turnover in our study, may have determined higher EMPS.

NDF and ADF intake, flow, and digestion

Intake of NDF and ADF was higher for pasture alone, but was not statistically different (Table 9 and 10). Duodenal flow of fiber was similar between diets. Feeding corn, decreased whole tract digestibility of NDF, but only a similar trend existed for

Table 9. NDF intake, flow and digestibility in response to grazing pasture with or without corn supplementation.

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
Intake, kg/d	7.4	6.2	.5	NS
Flow to duodenum, kg/d	2.7	2.8	.1	NS
Digestibility				
Whole tract, %	70.4	64.5	1.0	.02
Rumen, %	62.0	53.6	2.4	.09
Rumen, % of whole tract	88.1	82.6	2.8	NS

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

Table 10. ADF intake, flow and digestibility in response to grazing pasture with or without corn supplementation

Item	Diet		SEM ²	P ³
	Pasture	Corn ¹		
Intake, kg/d	4.9	3.8	.75	.09
Flow to duodenum, kg/d	1.8	1.7	.1	NS
Digestibility				
Whole tract, %	71.6	67.9	1.2	.13
Rumen, %	62.6	55.5	3.0	NS
Rumen, % of whole tract	87.4	81.7	3.1	NS

¹ 6.4 kg of ground corn-mineral consumed daily

² Standard error of the least squares means for n = 5

³ NS = not significant ($P > .10$)

ADF. The difference were markedly greater (6% for NDF and 4% for ADF) than that observed for the OM (2%). This indicate that the supplement may have decreased cellulolytic activity in the rumen. Differences between diets were even higher (9% for NDF and 7% for ADF) for rumen digestibility. However, these differences were not statistically significant ($P > .10$).

It is known that readily digestible carbohydrates like starch may depress fiber digestion (Mould, 1988). The activity of cellulolytic bacteria may be directly affected by the presence of starch or indirectly through a decrease in ruminal pH (Grant and Mertens, 1992). In in vitro incubations with constant pH (6.8), the addition of starch increased the lag time of fiber degradation but did not affect the rate of digestion (Mertens and Loften, 1980). The authors, however, concluded that the increase in lag time would not explain the depression in digestibility that is usually observed in vivo. Feeding energy concentrate is usually associated with a decrease in ruminal pH.

Russell et al. (1979) showed that rate of bacterial growth decreased with a decrease in pH and this effect may be more prominent in cellulolytic strains. The kinetics of NDF degradation, at two pH values (5.8 and 6.8), was evaluated in vitro by Grant and Mertens (1992). The decrease in pH caused an increase in the lag phase but did not affect rate of digestion.

It is possible, therefore, that an increase in lag time may have occurred in our study. Because rumen retention time (i.e. rumen turnover) was similar between diets, a delay in the onset of fermentation would have caused a reduction in fiber digestibility.

Voluntary feed intake during grazing is regulated by physical factors. Under these constraints, a decrease in fiber digestibility would increase gut fill and explain the reduction in forage intake when cows were fed corn.

Summary and Conclusions

The results of this study indicated that grazed fresh grass is highly digestible and the main site of digestion is the rumen. Forage protein was subjected to extensive breakdown that resulted in high rumen $\text{NH}_3\text{-N}$ concentration and loss of 25% of N in the rumen. Nitrogen flow to the duodenum relied mainly on microbial N that represented 67% of the total N flow. It appears, therefore, that lactating grazing cows would benefit from supplements high in rumen undegradable protein.

The ruminal environment was characterized by high VFA concentration and high rate of passage of particulate and liquid markers. The fast turnover, by decreasing rumen retention time, may reduce rumen digestion of concentrate fed to grazing cows. However, the Cr_2O_3 used in this study is not an ideal marker for particulate kinetics. It is necessary, therefore, to verify these results with other particulate markers before any further conclusion are made.

Milk production increased by 4.2 kg/d by supplementing 6.4 kg/d of corn-mineral mix. The low milk response can be mainly attributed to the lower forage intake when cows were fed the supplement.

The corn supplement decreased NDF and ADF digestibility in the rumen and whole digestive tract. Because fiber is the major bulk component of the forage, lower fiber digestibility may have caused the reduction in intake. Marker turnover was not affected by the supplement. The reduction in fiber digestibility, therefore, can be

explained only by changes in kinetics of fiber degradation (lag phase and(or) rate of digestion) caused by the lower pH and possibly by a direct effect of starch on cellulolytic activity in the rumen.

The corn supplement decreased rumen $\text{NH}_3\text{-N}$ concentration and N losses in rumen. The increase in microbial N flow at the duodenum was only marginal and the decrease in N losses can be mainly explained by the lower forage N intake during supplementation.

The economic benefit of corn supplementation for grazing cows may be questionable due to the low milk production response to the concentrate. More research is needed to verify the effect of other energy supplements such as fibrous concentrates, protein supplements, and rumen protected fats that have a lower impact on rumen fermentation may be more effective in increasing milk production.

Even though, energy and protein supplementation may play an important role in increasing animal performance, the result of this study indicates that the major factor limiting milk production during grazing is forage intake. The capacity of the animal to harvest fresh grass is determined by the characteristics of the pastures. The success of pasture for lactating dairy cows, therefore, relies mainly on the ability of the farmers to manage the pastures for maximum forage intake.

The results in this study were characterized by high residual variability that could not be accounted for by the factors included in the statistical model. Because of the high cost and labor required in studies that use cannulated animals, it is not always

feasible to increase the number of observations (i.e. number of animals) of the study. It is necessary, therefore, to increase the accuracy of the techniques, in particular the analytical methods, markers, and the sampling procedures of cannulated animals.

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Appendix

Appendix Table 1. Example of ANOVA table

Source	Degree of Freedom
Model	6
Diet	1
Period	2
Cow	3
Error	4
Total	10

Appendix Table 2. Example of factors associated with calculations of OM intake and duodenal flow of cow 2287 when fed pasture supplemented with corn in the second period of the trial

Parameter	Abbreviation and Calculation	Result
Daily dose Co-EDTA	DCo	2045 mg/d
Daily dose Cr ₂ O ₃	DCr	19300 mg/d
Fecal OM Conc. of Cr ₂ O ₃	FCr	4077 ppm
Corn OM intake	CI	5.4 kg/d
Corn OM digestibility ¹	COMD	.67
Pasture in vitro OM digestibility ²	IVOMD	.695
Particulate phase Cr conc.	CrP	2738 ppm
Particulate phase Co conc.	CoP	41 ppm
Liquid phase Cr conc.	CrL	325 ppm
Liquid phase Co conc.	CoL	1155 ppm
Calculation of OM intake		
Fecal OM output total	FOT = DCr / FCr	4.73 kg/d
Fecal OM output from corn	FOC = CI x (1-COMD)	1.78 kg/d
Fecal OM output from pasture	FOP = FOT - FOC	2.95 kg/d
Pasture OM intake	PI = FOP / (1-IVOMD)	9.67 kg/d
Total OM intake	I = PI + CI	15.07 kg/d
Calculation of duodenal OM flow:		
$DCr = F_p \times CrP + F_l \times CrL \Rightarrow 19300 = F_p \times 2738 + F_l \times 325$		
$DCo = F_p \times CoP + F_l \times CoL \Rightarrow 2045 = F_p \times 41 + F_l \times 1155$		
By simultaneously solving these two equations		
Particulate OM phase flow	F _p	6.87 kg/d
Liquid OM phase flow	F _l	1.52 kg/d
Total OM flow	F = F _p + F _l	8.39 kg/d

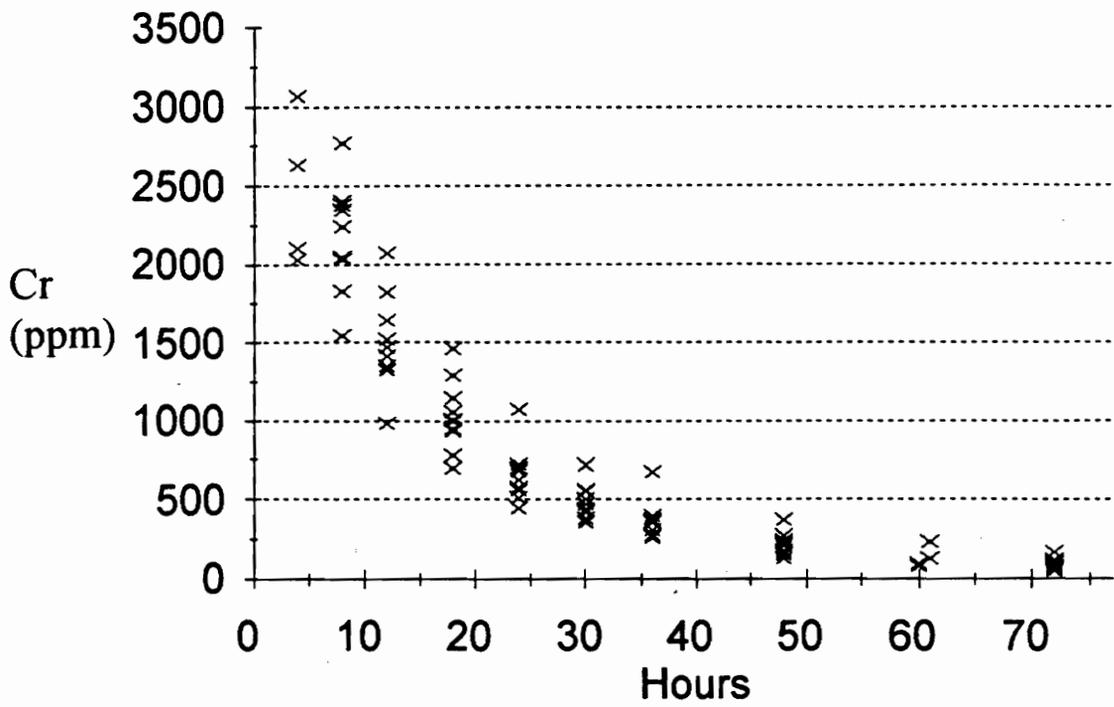
¹ Tyrrell and Reynolds (1988)

² Goering and Van Soest (1970)

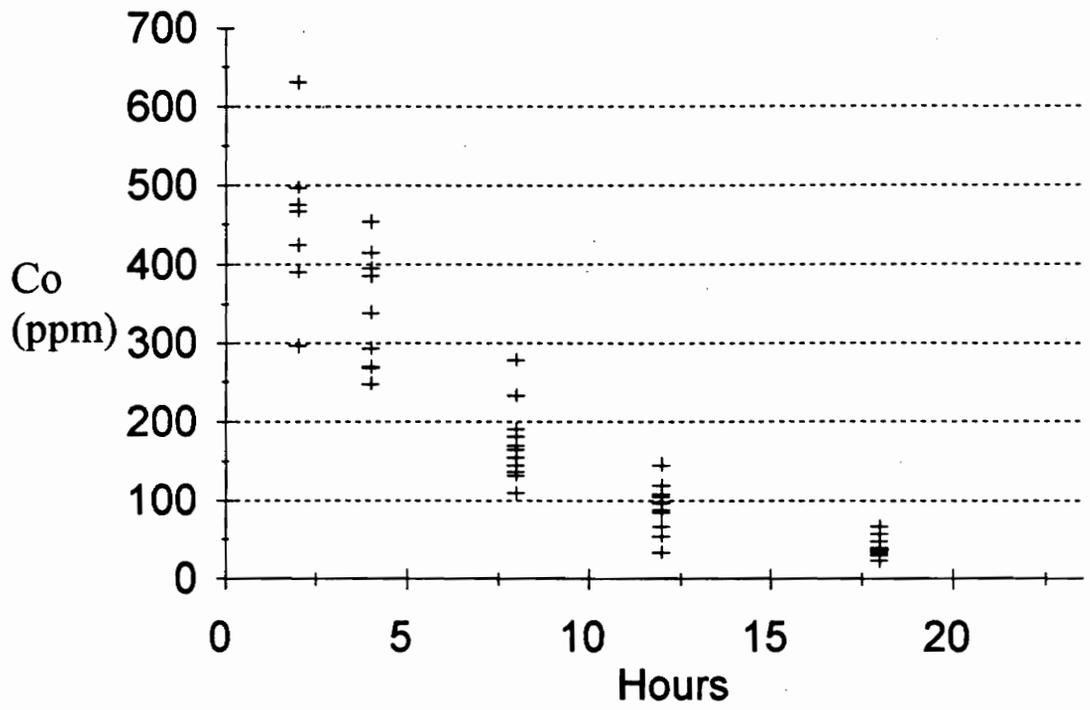
Appendix Table 3. Cytosine and nitrogen content of rumen bacteria on DM and OM bases and cytosine content of particulate and liquid phases at the duodenum of grazing lactating cows supplemented with or without corn.

Item	Pasture	SD	Corn ¹	SD
Mixed rumen bacteria:				
Cytosine, $\mu\text{mol/g DM}$	71.7	5.0	67.3	2.3
Cytosine, $\mu\text{mol/g OM}$	76.4	5.9	72.0	2.6
N, mg/g DM	116	3	110	5
N, mg/g OM	123	3	118	6
N:Cyt ratio mg/ $\mu\text{mol DM}$	1.62	.1	1.64	.1
N:Cyt ratio mg/ $\mu\text{mol OM}$	1.61	.1	1.64	.1
Cytosin in duodenal Samples:				
Particulate, $\mu\text{mol/g DM}$	19.4	2.6	17.0	2.7
Liquid, $\mu\text{mol/g DM}$	11.8	1.2	12.9	1.7
Particulate, $\mu\text{mol/g OM}$	21.7	3.0	18.3	3.0
Liquid, $\mu\text{mol/g OM}$	25.2	1.7	25.9	1.5

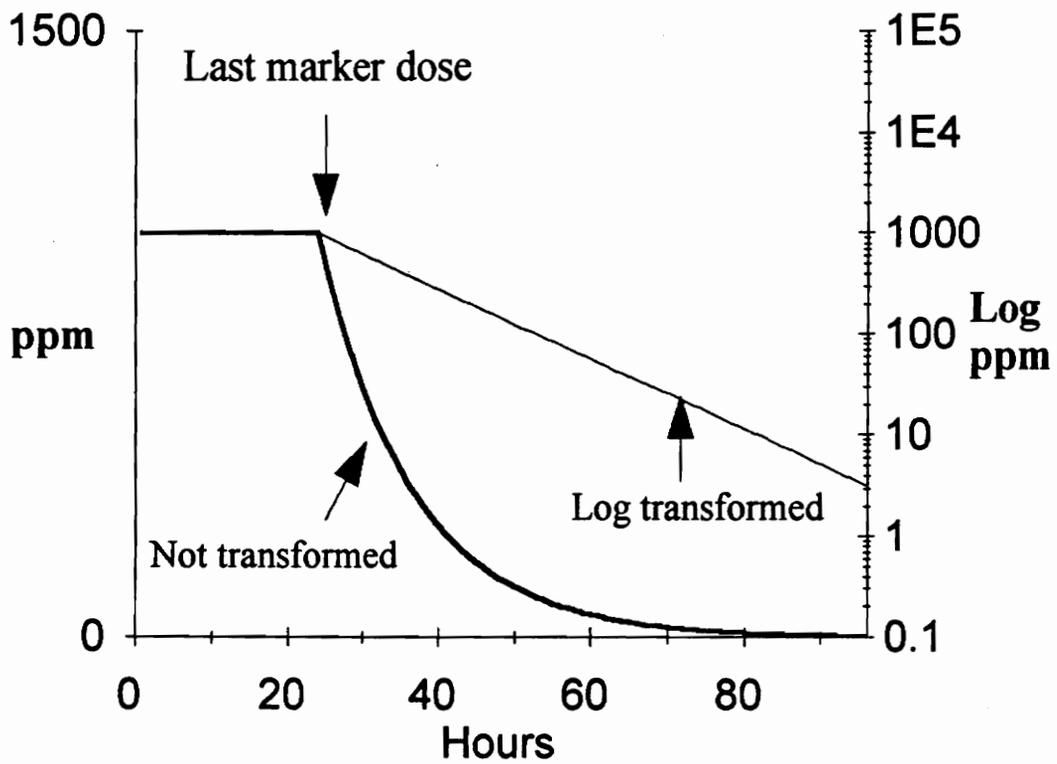
¹ 6.4 kg of ground corn-mineral consumed daily



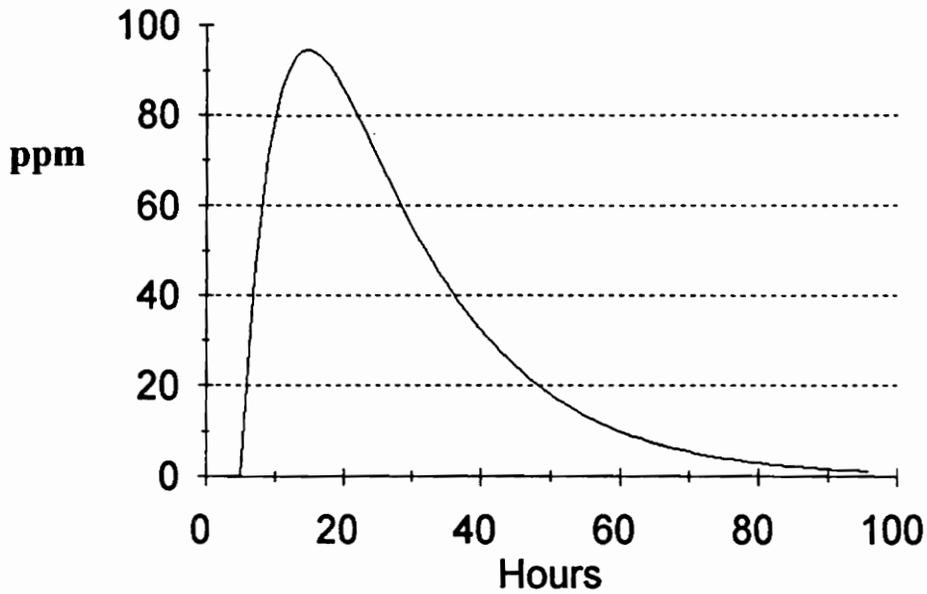
Appendix Figure 1. Depletion of Cr in the whole duodenal samples



Appendix Figure 2. Depletion of Co in the whole duodenal samples



Appendix Figure 3. Theoretical marker concentration [M] in duodenal or fecal samples after withdrawal of marker



$$[M] = B \times e^{-k_p (T-TD)} - B \times e^{-k_l (T-TD)}$$

- [M] = Marker (Co or Cr) concentration at time t (ppm)
 B = Marker concentration (ppm)
 k_p = Rumen turnover rate (%/h)
 k_l = Lower tract turnover rate (%/h)
 T = Time after dose
 TD = Time delay

Appendix Figure 4. Theoretical marker concentration [M] in the feces after a pulse dose of marker using the mathematical model of Grovum and Williams (1973)

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