Effect of Nonstructural Carbohydrates and Rumen Undegradable Protein on Intake, Growth, and Body Condition of Dairy Heifers.

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Science (Dairy)

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December 18, 1990

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Effect of Nonstructural Carbohydrates and Rumen Undegradable Protein on Intake, Growth, and Body Condition of Dairy Heifers.

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Dana J. Tomlinson
Robert E. James, Chairman
Animal Science (Dairy)
(ABSTRACT)

Three trials using 30, 32, and 51 dairy heifers evaluated response to diets varying in rumen undegradable intake protein (UIP). Intake, growth, and apparent feed efficiency were evaluated in a factorial arrangement of two levels of TDN (93 and 109%) and two levels of UIP (30, 40%) over a 105 d period. Initial BW were 142 to 242 kg. Daily intake was 12.1 g DM/kg BW⁻⁰.⁷ five lower on low TDN-High UIP treatment with BW gain (.91 kg/d) similar to high TDN treatments. Feed efficiency improved 2.8 and 10.0 Mcal DE/kg gain when DBG replaced SBM in low and high TDN treatments. A second trial was conducted to evaluate performance over a wider range of UIP with isocaloric diets for a 50 d period. Heifers were 213 to 231 kg BW. Treatments were four levels of UIP (31, 43, 50, 55% of total N) at 100% of NRC TDN. Diet UIP was raised by substituting blood meal for SBM. Dry matter intake declined 24.1 g/kg BW⁻⁰.⁷ with increasing UIP, while daily BW gain increased .129 kg/d. Increased UIP improved feed efficiency 6.7 Mcal DE/kg gain. Treatments showed a positive linear response in BW gain, but did not differ in wither height growth or urea space estimates of body composition. Previous work with lactating dairy cows showed response to UIP was dependent on diet nonstructural carbohydrate content, therefore a third 60 d trial was conducted. Treatments were all combinations of UIP (30, 40, 50%) and nonstructural carbohydrates (NSC) (17, 22, 26% of DM). Diet UIP content was verified by in situ dacron bag techniques.
Mean DM intake was 100.6 g/kg BW and did not differ. Gain varied from .259 to .694 kg/d and increased with UIP and NSC while feed efficiency improved from 42.2 Mcal DE/kg BW gain at 30% UIP to 30.0 at 40 and 50% UIP. Gain and feed efficiency increased with increasing NSC. Lowest positive change in empty body fat occurred at 21.1% NSC. Longissimus muscle area increased with UIP and NSC. Increasing dietary UIP to 50% supported high BW gains (.6 to .9 kg/d), and skeletal growth, with little change in body composition when energy concentration was below NRC standards. Trial results indicate increasing dietary UIP permits the use of lower energy diets to achieve acceptable BW gain (.6 to .9 kg/d) and growth in stature, while improving feed efficiency.
Acknowledgements

The author wishes to express his appreciation to the following individuals.

Graduate committee members M. L. McGilliard, G. S. Lewis, C. E. Polan, C. C. Stallings, and W. E. Vinson for their consideration of this work.

Mr. Lee Johnson for his aid in collection and sketching of ultrasonic scans.

Mr. Chuck Miller, Harold Nester, and calf barn workers at the Virginia Tech Dairy Cattle Center for their assistance in all phases of these trials.

Ms. Judy Baker, and technicians at the Virginia Tech Forage Testing Laboratory for performing forage analysis.

The graduate students in management and nutrition helping process all those in situ bags, and aiding in urea space procedures.

Ms. Donna Richardson for her love, friendship, support, and understanding throughout my years at Virginia Tech.

Dr. R. E. James for his guidance and support throughout my doctoral program.

To my parents Joyce and Wilbur Tomlinson for their love and support at all times.
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Introduction

Nutritional management of dairy heifers dictates that growth be adequate, economical, and not predispose the animal to factors which reduce lifetime producing ability. While nutritional strategies can improve growth performance, assessment of nutritional adequacy and growth should not be delayed until calving. Rate of growth prior to puberty affects lifetime milk producing ability. Rapidly grown heifers (1.1 kg/d ADG) produced less milk than conventionally-reared heifers (.8 kg/d ADG) in first, second, and third lactations (39, 147). Sejrsen (182) and Tucker (208) reported heifers raised on a high plane of nutrition (> .6 kg/d ADG), during the allometric phase of mammary development (prepubertal period, 90-325 kg BW large breeds), had less mammary secretory tissue than heifers raised at .4 to .6 kg/d. These workers suggested heifer growth rates be maintained below .6 kg/d until puberty with compensatory growth allowed to occur during the post pubertal period. Heifers on a high plane of nutrition during the prepubertal period were found to have decreased mammary growth due to decreased secretion of somatotropin and corticoids (182). Body weight gain and height measurements are not indicative of composition. Mitchell (134) reported that weight gain of growing animals varies in content of water, fat, and protein. Reid et al. (172) concluded body water content was inversely proportional to fat content while protein and mineral content in the fat free body were relatively constant. Preston and Kock (168) used dilution of an injected 20% urea solution as a means of estimating body water. Urea space estimates of body water, fat, and protein in live animals were developed by regression on carcass composition. Hammond et al. (75) concluded that urea space is a useful tool in prediction of body composition and provides more definitive information toward nu-
tritional effects than weight measures alone. Recently, improvements in real time ultrasonic scanning equipment have provided an additional means of assessing growth of soft tissues. Works by (202, 221) established ultrasound as a reliable means of estimating growth in longissimus muscle area and depth of backfat in beef and hogs. By combining compositional estimates provided by urea space with ultrasonic scans, estimates of nutritional effects on body compositional changes may be possible.

Incorporation of new feeding strategies can play a critical role in achievement of growth and management goals. These strategies entail intege diet of the degradable protein system as outlined by NRC (144) and a system considering carbohydrate degradability (151). The new protein system (144, 218) separates dietary protein into a ruminally degraded and an undegraded fraction.

In dairy calves and heifers, a number of growth and intake studies (24, 47, 84, 231) showed animals were most efficient in converting dietary substrates to tissue protein when diets contained >14% CP and ADF <18%. However, only (47) controlled protein degradability while adjusting ADF concentration.

Protein fractionation is supported by works showing improvement in growth rate and feed efficiency when a portion of the crude protein bypasses ruminal digestion (2, 47, 93, 231). Research by (2, 76, 93) showed incorporation of by-product feeds with highly undegradable protein fractions tended to depress dry matter intake while improving feed efficiency (feed:gain). Reports by (2, 76, 93) suggested microbial protein production was not sufficient to support growth of rapidly growing dairy replacements.

Response to undegradable protein should not be totally attributed to characteristics of dietary protein, but should include ruminal availability of dietary carbohydrates (151). The detergent system (70) fractionates feedstuffs into cell walls (neutral detergent fiber, NDF) and cell contents (neutral detergent solubles, NDS), thereby providing the foundation for ruminal carbohydrate availability. Nocek and Russell (151) proposed sub-
tracting protein, ether extract, and ash from NDS would leave nonstructural carbohydrates (NSC), a fraction readily digestible within the rumen. Matching ruminal degradabilities of proteins and carbohydrates should improve microbial growth and efficiency of feed utilization thus increasing production of volatile fatty acids and microbial protein. Research with lactating dairy cows has shown improved protein and carbohydrate utilization, nutrient flow to the small intestine, and milk production when ruminal degradability of proteins and carbohydrates were matched (79, 119).

To date, few studies have examined the effects of undegradable protein supplementation in conjunction with ruminal carbohydrate availability on intake, growth, body compositional changes and subsequent lactational performance of dairy heifers.

The objectives of these trials were:

1. Evaluate effects of diets varying in protein undegradability and energy concentrate on intake, daily gain, wither height growth, reproductive efficiency, and first lactation performance of Holstein heifers.

2. Evaluate response in growth, intake, feed efficiency, and body composition of Holstein heifers receiving isocaloric and isonitrogenous diets varying in rumen undegradable protein.

3. Evaluate effects of diets varying in concentrations of nonstructural carbohydrates and rumen undegradable protein on intake, growth, feed efficiency, and change in body composition of Jersey heifers.
Review of Literature

Heifer Management

Nutritional management of dairy heifers is one of the most neglected areas in dairy herd management. The goal of a dairy heifer management program should be to rear heifers capable of producing to their genetic potential at the lowest possible cost. As non-income producing animals, this group tends to be neglected nutritionally resulting in either excessive or deficient growth having a detrimental impact on lactation, reproductive performance, and herd life.

Growth management

The dairy heifer should be 40-45% of mature weight at time of breeding (152, 197). Etnes et al. (57) indicated large breeds (Holstein, Brown Swiss) should weigh 354-400 kg, Ayrshire and Guernsey breeds 290-336 kg, and Jerseys 250-282 kg at time of breeding. Others (39, 78, 217) make similar recommendations. These weights correspond with the recommended age of 14-17 months at breeding (57, 85, 140) and provide for calving between 23 and 26 months of age. Current age at first calving in Virginia DHI herds is 29 months. This age reflects problems with heifer nutrition and management, and is well above the standard of 24 to 26 months for optimal lactational potential and economic return (69). Norman et al. (152) reported first lactational milk yield increased substantially with increased calving age up to 26 months. Virginia DHI data shows highest first lactation yield at 26 mo with a plateau through 32 mo at which time it be-
gins to decline (B.G. Cassell, personal communication). These data showed increased rearing expenses incurred by calving later than 26 m offset by increased milk production by a more mature animal.

Holding replacements to calve at an older age and heavier weight can greatly affect profitability of the replacement enterprise. Researchers (3, 57) estimate that increased age at first calving, past the optimum, costs $20-$70/heifer/month for confinement reared heifers. Ely (55) found feed costs to be lowest when heifers were raised on pasture and bred to calve by 31 mos. Miller and Amos (133) suggested heifers be raised on permanent pasture and be permitted to calve at a later age. Their study reports a reduction in feed costs by 50% due to reduced feeding of concentrates and stored forages. They, however, creatively manipulated pasture feeding and animal acquisition costs resulting in the pasture system as a more economically feasible alternative. They indicated that good pasture management should provide ample forage, thus allowing heifers to gain adequately and calve at 25 months.

Effect of feeding level

Swanson (195, 196), Gardner (66), and Little and Kay (107) reviewed the effects of growth rate on lactational ability of dairy heifers. Swanson (195), using seven pairs of identical twins, determined that rapidly grown heifers produced less milk than conventionally grown heifers in first, second, and third lactations. Average fat corrected milk production of fattened heifers was 84.8% and 93% of control animals for first and second lactations. Gardner (66) presented similar results suggesting rapid growth and fattening was uneconomical, yet had no adverse affects on lifetime production, herd life or reproduction. Little and Kay (107) found similar depression in milk production and
first conception rate with accelerated rearing, but decreased dystocia in normally reared heifers.

Effect of feeding level on mammary growth, with observation of age at which animals reach puberty, was addressed by Foldager (59), Sejrsen (182), Gardner (66), and Little and Kay (107). Studies (66, 180, 196) indicate that appearance of puberty is directly and positively related to rate of growth and is dependent on size more than age. Heifers within a breed tend to reach puberty at similar body size regardless of feeding level (180). Gardner (66) reported heifers reached first estrus at 8.3 and 10.2 months when on accelerated and control growth programs, respectively. Dunnington et al. (51) suggested sexual maturity in chickens is dependent not only on age and weight, but also on body composition. They found that chickens could be of mature age and weight, but would remain nonlayers if body condition was inadequate. These findings suggest plane of nutrition as the most important factor in determining age of first estrus.

Increasing proportions of diet energy and protein concentrations can reduce age at first calving to less than 20 months (66, 107). Feeding this type of diet causes overconditioning (66, 197), decreased milk production (39, 195, 197), and calving problems (39, 107, 197). Others (59) suggest diet energy concentration or composition has no influence on mammary development and subsequent lactation, when plane of nutrition is the same. When plane of nutrition is increased, and gain is >.60 kg/d in the prepubertal period, mammary development is impaired (59). Swanson (195, 196, 197), and Foldager and Sejrsen (59) reported a decrease in milk production (300-500 kg) in rapidly grown, or flattened heifers, may be due to reduced parenchymal growth within the mammary gland. A critical period for mammary growth has been suggested to occur during the prepubertal period between 90 to 325 kg for large breeds (182, 208), and is 60 to 230 kg
for small breeds. During the allometric phase (3 to 9 mos.), growth of mammary parenchyma is as much as 3.5 times faster than body weight growth. It is during this phase that ductular development is most critical. Sejersen et al.(182) and Tucker (208) indicated heifers raised on a high plane of nutrition (> .80 kd/d), during the allometric phase of mammary development, had less secretory tissue in their mammary glands than heifers raised at a normal rate of growth (< .60 kg/d). In the pre-pubertal period hormonal concentrations within the animal are affected by plane of nutrition (182). Heifers on a high plane of nutrition were found to have decreased mammary growth due to decreased secretion of somatotropin and corticoids. Work by (59) indicated a positive relationship between parenchyma growth and somatotropin production, and a negative relationship with prolactin. Administdiet of exogenous somatotropin, to heifers on high planes of nutrition, was found to increase mammary parenchyma growth and decrease extraparenchymal tissue and total mammary weight (59). Thus, animals with higher circulating levels of somatotropin and corticoids may have greater potential for secretory tissue growth on high planes of nutrition. Grings et al. (73) injected heifers 13 to 16 mo of age for 5 mo with 41.2 mg bovine somatotropin and found greater structural growth at calving, but no difference in first lactation milk production.

Reports (182, 197, 208) indicate growth of secretory tissue is inversely related to extraparenchymal adipose tissue in the allometric phase. Therefore, animals on high planes of nutrition (gaining > .60 kg/d) may have greater production of adipose tissue, and increased growth of mammary fat pad with reduced secretory tissue growth. Kertz (92) determined that heifers in the 3 to 12 month age group can be fed high energy diets (those supporting > .8 kg/day gain) without adverse affects, when protein levels are also increased. Heifers grew at .9 to 1.0 kg/day and showed greater skeletal growth without
fattening. Effects on mammary development and subsequent milk production were not available.

At onset of puberty, and until pregnancy, growth rate of the mammary gland decreases to a level similar to the rate of body weight gain. Plane of nutrition during this period has little influence on hormone levels or mammary growth in proportion to bodily growth (59). Presence of adipose tissue in this, the post-pubertal isometric phase, is unrelated to growth of mammary tissue.

During pregnancy the fat pad and ducts again begin to grow at a rate faster than the body, with plane of nutrition being less critical than the prepubertal period. Foldager et al. (59) studied heifers gaining .40, .60, and .80 kg/d, from 325 kg live weight to three months prepartum. They concluded heifers gaining .40 kg/d had lower mammary gland weights than those gaining .60 or .80 kg/d, but no differences in parenchyma were found. Based on these results, heifers with higher live weights at calving tend to produce more milk due to greater bodily energy stores, lower growth requirements, and higher feed intake, rather than due to greater parenchymal growth. It can be concluded that feeding regimes should support ample growth in weight and stature without overfattening of the animal to support development of mammary secretory tissue.

While over-fattening during the pre- and postpubertal periods has proven to be detrimental to overall productivity (67, 180, 195, 197), underfeeding can also depress lactation performance. Heifers fed nutritionally inadequate diets tend to lack size by first calving (87, 197). Swanson et al. (195) studied the effects of feeding animals bred to calve at 2 yr at 75% of normal intake of TDN. Underfed animals exhibited increased dystocia, and 13% lower milk production in the first lactation. Underfeeding of heifers
can be corrected by feeding supplemental concentrates during first and second lactations (87, 197).

These studies indicate plane of nutrition during the pre-pubertal critical period has lasting negative effects on lactation performance. Growth rates during the pre-pubertal period affect levels of circulating hormones more than growth rates in the isometric phase. Circulating levels of somatotropin, insulin, and corticoids are reduced by a high plane of nutrition. A plane of nutrition supporting .40 kg/d has been suggested as providing for optimal secretory tissue growth during the allometric phase (59). Mammary development between puberty and pregnancy may not be affected by nutrition. A plane of nutrition during pregnancy resulting in daily gains of .70 kg/d, will result in increased secretory tissue growth and improved lactation performance. This suggests that growth standards be established so heifers are raised to achieve maximum secretory tissue growth, body weight gains and growth in stature providing for maximal lactation performance.

**Growth Standards**

Standards rates of growth are presented in Tables 1 and 2. Swanson (197) recommended that for optimal growth, performance, and age at first calving, heifers be fed in three stages of gain; .55 kg/d, weaning to breeding, .68 kg/d, breeding to 20 months, and .91 kg/d until calving. He suggested that under such a scheme animals will grow successfully to breeding age and size without problems associated with fattening. Post breeding growth rates allow for growth to mature size by 24 months, and prepare the animal for lactational levels of intake.
Table 1. Recommended body weights of Holstein heifers at various ages.

<table>
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<tr>
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Regression Equations:

Clapp (31) : \[ \text{BWT(kg)} = 42.30 + 22.52 \times \text{AGE (mos.)} \]
Eigen (41) : \[ \text{BWT(kg)} = 51.21 + 20.17 \times \text{AGE (mos.)} \]
Heinrichs (51) : \[ \text{BWT(kg)} = 47.66 + 20.30 \times \text{AGE (mos.)} \]
NRC (74) : \[ \text{BWT(kg)} = 29.74 + 19.12 \times \text{AGE (mos.)} \]
Table 2. Recommended body weights of Jersey heifers at various ages.

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Regression Equations:

- Clapp (31): $BWT(kg) = 24.226 + 15.287 \times AGE$ (mos.)
- Etgen (41): $BWT(kg) = 32.990 + 14.994 \times AGE$ (mos.)
- Heinrichs (51): $BWT(kg) = 22.294 + 17.180 \times AGE$ (mos.)
- NRC (74): $BWT(kg) = 13.834 + 14.770 \times AGE$ (mos.)
Previous works (133, 164) suggested rearing heifers at a slower rate, with compensatory growth periods, can optimize use of cheap feeds, increase growth and feed efficiency, and produce an animal with high lactation potential. Park (164) fed heifers averaging 7.6 mos. of age and 205 kg BW on a 4 stage schedule. Animals were fed 5 months at 85% of NRC (142) CP and TDN, 2 months at 140% of (142), 5 months at 85% of (142), and 2 months at 140% of (142). Animals were compared to control heifers gaining .45 kg/day. Heifers within the treatment regimen gained more, and consumed less feed resulting in substantial improvements in growth and feed efficiency. Growth during the maintenance phase was .25 kg/day, while growth was 1.9 kg/day during the compensatory phase. First lactation heifer performance improved by 10% when heifers were raised on the compensatory regimen.

Research to date indicates growth management of the dairy heifer has a considerable influence on production potential. It has been established that growth rates > .60 kg/d, during the allometric phase of mammary growth, depress parenchymal growth resulting in lower milk production. Of additional importance is diet nutrient concentrations as research suggests that high energy concentrations depress secretion of somatotropin and corticoids which influence growth of secretory tissue. In summary, growth management of the dairy heifer must be adequate for maintenance of continual structural growth, development of reproductive and mammary systems, and provide sufficient body condition for breeding by 13 to 15 mo and calving by 26 mo.
Body Composition

Introduction.

In many nutritional studies, measurement of intake and body weight change are used as criteria of nutritional response. These criteria are more susceptible to error in studies containing ruminants than non-ruminants. In ruminants, fill affects body weight and can result in considerable error with greatest impact on studies of short duration. In addition, the use of body weight change assumes that, regardless of treatment, weight gained or lost is of the same chemical composition. Mitchell (134) concluded that weight gains of growing animals vary in content of water, protein, and fat. The data of Callow (28) showed an inverse relationship between fatty tissue and muscle tissue, but suggested changes were far more complicated since alteration in water, lipids, carbohydrates, proteins, and minerals follow any alteration in the basic structural tissues of the body. The inverse relationship between body water and fat provides a useful tool for assessing body composition, both for research and as a management tool.

Concepts of body composition.

Current knowledge of body composition is dependent upon knowledge gained from animal studies where slaughter and analysis of the body were conducted. Lawes and Gilbert (101) performed the pioneer task of analyzing chemical composition of bodies of three steers, five sheep, and two pigs. Since that time, other workers have provided useful information concerning the influence of age (54, 162, 168, 172) and nutritional status (138, 139) on body composition. These studies established that fat animals con-
tained proportionally small amounts of water. Therefore, the hypothesis derived from early investigations was the animal body consists of two phases: the fat and the fat-free masses. Research indicates the water content of the fat-free mass varies between 71.8 and 74% (68, 162, 167, 172). Protein and mineral content of the fat-free fraction are relatively constant at 18.5 and 4.5% (172). All studies agreed that body water content varies inversely with fat content. These principles provide the basis for estimation of total body composition from either total water or fat determinations.

**Constancy of body components.**

**Water**

As indicated earlier, body water content is relatively constant after removal of the effects of fat. Murray (141) and Moulton (137) concluded that relative fatness of animals of the same species did not influence composition of the fat-free body. They did, however, find water content of the fat-free body decreased slightly with age. Garrett (68), Reid et al. (172), and Rule et al. (176) also reported slight decreases (1-5%) in body water in fat-free tissues with increasing age. Pearson (165) reported a range of 56.3 to 78.3 for percent water in the body of cattle. Reid et al. (172) suggested this range may be wider (e.g. 49.4 to 76.8) and reflected fat content of animals measured.

Body water tends to achieve constancy at about 73% in most mammalian bodies, with slight deviation from this range occurring between species (163). Hammond (75) and others (75, 168, 176) developed prediction equations for body composition for various species, breeds, and body types to allow for accurate estimation of treatment response.
Fat

There is a great deal of variation in fat content with changes in level of feeding (28, 139, 172) and age (137, 167, 172). Percentage of fat in the whole body of sheep was found to vary from 13.0 for the young to 21.5 for older animals (165). Reid (172) reported fat in the empty body of sheep ranged from 4.9 to 46.6. Similarly, the percentage fat content in cattle was extremely variable with values ranging from 1.97 to 21.57 for cattle over first 18 mo of life (225) and from 1.24 to 6.03 for calves less than 42 d. Reid et al. (172) reported fat content in whole empty bodies of cattle ranged from 1.8 to 29.2 with fat content of mature dairy cattle averaging about 12% of live weight. Fat content of beef animals was slightly higher at 16% of live weight. These findings support the premise that fat content varies with age and species.

Protein and Ash

The content of body protein and ash is much less variable than fat and water (165, 172). Bailey and Zobrinsky (12) reported the percentage distribution of proteins in various body parts relative to total in the empty-body was stable. They generalized that muscle comprises 50% of the total body protein, bone contains about 20%, blood 5%, and the remainder is approximately equally divided between skin, organs, and fatty tissues. Generally, protein and ash make up 12.4 - 20.6% and 3.0 - 6.1% of the whole empty body of cattle. In the fat-free, moisture-free body, protein and ash are practically constant at 80% and 20% (172). Murray (141) reported the ratio of percent protein to ash was constant in sheep, swine and cattle. He and Moulton (137) indicated non-fat matter is of the same composition regardless of degree of fatness, therefore chemical composition can be estimated when fat or water content is known.
Chemical maturity.

To date, there has accumulated a great amount of evidence supporting the generalization that percentage of water, protein, and mineral matter approach constancy in the fat-free body of animals after "chemical maturity" is attained (137, 137, 162, 172). Moulton (137) defined age of chemical maturity as the point at which concentration of water, protein, and mineral matter in the fat-free body become practically constant. The age at which chemical maturity was attained by swine and cattle was 150 to 300 d. Although animals achieve chemical maturity at varying ages, Moulton (137) showed animals are chemically mature after reaching 3.9 to 4.6% of their total life-span. Moulton (137) and Murray (141) concluded that within species the relative fatness of animals did not influence composition of the fat-free body. Therefore, the most pronounced effect of fattening on concentration of water, protein, and ash in the whole empty body is that of dilution.

Measures of body composition.

Prediction of body composition is often achieved by one of two methods: direct or indirect. The direct method, while providing very definitive data on body composition, is expensive, time consuming, and requires sacrificing the test animal. This method does, however, provide validation of indirect methods by comparison to carcass characteristics determined at slaughter. Indirect methods rely upon use of dilution techniques and predictive equations for estimation of body composition. Advantages indirect methods proclaim are: ease of data collection, require minimal time, relatively inexpensive materials, rapid and simple laboratory analysis, and do not require slaughtering trial animals.
Indirect methods of estimating body composition can be separated into two categories; 1) Invasive, those techniques requiring injection of a known substance with subsequent measurement of its dilution; 2) Non-invasive, techniques which rely upon electrical stimulation or magnetic fields for estimation of body components.

**Invasive Techniques**

**Body Water Diluents**  Dilution techniques involve introduction of a known tracer which becomes uniformly distributed throughout a compartment in the animal body (153, 168). A sample of the compartment (i.e. blood) is taken and tracer concentration is established. Test substances "tracers" must possess certain properties to be acceptable as measures of body water: (a) it should not be toxic or have physiologic effects; (b) must be metabolized; (c) it must diffuse homogeneously into all the volume to be measured; (d) it should not be selectively stored, secreted or metabolized; (e) an accurate and convenient estimation of its concentration in the plasma or blood should be available (168). Early research indicated antipyrine (95, 162) N-acetyl-4 aminoantipyrine (19, 163) and $^{35}$S-thiocyanate (161) and potassium 40 (162) could be used as estimators of body water. They were later deemed too variable to give good estimates of body water with much of their use discontinued (165).

Urea (15, 94, 168), tritiated water (TOH) (162, 163), and deuterium oxide ($D_2O$) (27, 115) were used successfully in prediction of body water. Deuterium oxide and urea have been used most extensively as they give more accurate results, and do not require the expense of disposal of radioactive wastes and animals.
Since urea is relatively inexpensive and procedures required for plasma urea-N analysis are minimal, this technique has found acceptance in research and may be applied in industry where interest in measurement of body composition during growth exists. Bartle et al. (16), Koch and Preston (94), and Rule et al. (176) reported correlation between urea space and fatness ranged from .63 to .92. These researchers agreed this technique works best with heavy animals having a relatively high degree of fatness, compared to lighter cattle with a low degree of fatness. Rule et al. (176) and Hammond et al. (75) noted fat content of 6-month-old steers was not predicted well by equations developed on 12 to 18-month-old steers. Hammond et al. (75) suggested predictive equations for estimating fat from urea space were different for dairy steers than beef due to differences in body type and fat deposition. Therefore, they developed predictive equations for thinner type (younger) cattle using Holstein steers.

Bartles and Preston (16) evaluated the effects of urea diffusion from the blood into the rumen or urine pools. They concluded that urea did not diffuse appreciably into the reticulo-ruminal water during infusion. Empty body water estimates was only inflated by the amount of urea lost in urine formed during the 12 min equilibration period.

Hammond et al. (75) concluded that urea space is a useful tool in prediction of body composition on a mass basis and provides more definitive information toward nutritional effects than weight measurements alone.

Byers (27) first reported on the use of D$_2$O dilution to directly predict body composition in growing beef cattle. Robelin (173) used D$_2$O to study 340 beef cattle for whole-body composition and found the weight of water and protein was relatively close in relation to fat-free mass. Due to the time required for equilibration, 2-3 h, variable diffusion into
water of the alimentary tract and complexity of dilution curve analysis, this method has found limited application in research and is too complex for industrial applications.

Non-invasive techniques

While dilution techniques give good estimates of body water for prediction of fat, Temple et al. (201) and Stouffer (193) suggested ultrasound as an accurate tool for measuring fat thickness and muscling in cattle. More recently, Terry et al. (202), Kempster and Owen (91), and Mersmann (125, 126) indicated this non-invasive technique of acquiring sequential measures on the same animal was particularly useful in measurement of growth and body composition in nutritionally manipulated animals.

Ultrasound scans are based upon the acoustic impedance of sound waves reflected by soft tissues of the body from a transducer back to itself. Sound waves are created by piezoelectric crystals housed within the transducer. When the sound beam encounters tissue interfaces of differing acoustic impedance, a portion of the sound beam is reflected back to the transducer which acts as a receiver. Echoes returning from soft tissues are converted to electrical impulses and displayed on an oscilloscope screen as a cross section of the tissue (202).

Wallace et al. (221), Miles et al. (132), and Terry et al. (202) concluded that subcutaneous fat thickness and longissimus muscle area could be reliably measured in cattle and swine with ultrasound. Wallace et al. (221) reported correlations of .77 and .58 to .77 between ultrasonic measurements of fat-thickness and longissimus muscle areas in cattle. Correlations between fat thickness and longissimus muscle areas in swine of .75 and .62 were reported by Terry et al. (202). Ultrasound has, however, been found to have limited application in determination of carcass composition in sheep (52, 90, 105).
Other non-invasive methods of estimating body composition are nuclear magnetic resonance (224) and electric impedance (104, 108). Nuclear magnetic resonance is capable of assessing total body water and fat content, but will find limited use in animal research due to size requirements of the imaging machine and cost. Lukaski et al. (108) reported electrical impedance as a viable means of estimating body composition in humans. Leymaster (104) and Sedensky and Klein (181) indicated this method provides minimal improvement in the predictability of body composition of sheep and dogs, therefore suggesting other means of body composition prediction should be utilized.

By applying techniques of estimating body composition, scientists have improved their assessment of nutritional treatments on animal response. Use of non-invasive methods provide reasonable estimates of body fatness or leanness with minimal animal disturbance. Furthermore, in addition to using body composition in evaluation of nutritional experiments this information could be used for breeding, management, and feeding decisions for the lactating herd, e.g. body composition estimates could be used in conjunction with body condition scoring.

Characterization of nutritional effects on changes in body composition isolates only a portion of the overall performance response. Nutritional variation can alter body composition as a result of intake and energetic efficiency. Therefore, factors affecting intake and growth response are of utmost importance in assessing response to nutritional variation.
Regulation of intake

Introduction

Feed intake of ruminants is regulated by many highly complex mechanisms. Three hypotheses have received the greatest focus in the past two decades. These include physical effect of gut distention in limiting voluntary intake, chemostatic or physiological mechanisms, and psychogenic inhibition of intake (89). Freer and Campling (63) suggest it is possible that the voluntary intake of some roughages might be regulated more by the limited capacity of the reticulo-rumen, during a meal, than by their rate of disappearance from this organ. This is supported by the findings of Campling et al. (30) in that amount of ingesta in the rumen at feeding time and at the end of the feeding period has some form of control on voluntary intake of forages. Montgomery and Baumgardt (136) found that ruminants adjust voluntary intake in relation to a physiological demand for energy as long as fill or rumen load is not limiting. Similar results were found (21, 50, 230) when proportions of concentrates in the rations, or energy density was increased, voluntary intake decreased so that available energy consumption was relatively stable. Psychogenic inhibition of intake involves the animal's response to environmental effects. These effects include the stresses associated with social interactions among animals, different feeding situations, and the animal's preference for specific feeds due to sight, smell, taste, physical form, or possibly location of the feed.

Physical regulation

The theory of physical limitation is that some restriction of capacity limits intake. It was suggested by Balch (13) that voluntary intake of forages is related to the amount
of digesta in the reticulo-rumen, which is a function of the rate of digestion of food particles and their rate of passage out of the rumen. Blaxter et al. (20) found that within the limits controlled by quality of the roughage used, the amount of forage consumed by sheep is determined by the capacity of the digestive tract, and physical factors. Conrad, Pratt, and Hibbs (42) using trials with 114 lactating dairy cows determined that intake begins to decrease when diets contain greater than 67% digestibility, suggesting metabolic control of intake. Intake of diets below this value is controlled by the amount of undigested residue per unit body weight and the animal’s body capacity (physical control). Montgomery and Baumgardt (136) using dairy heifers found that maximal DM intake was achieved at the point of 56% digestibility in the ration. Above this value heifers began to reduce intake to maintain a constant energy balance. They, as the previous authors, suggest that these digestion coefficients are arbitrary and should not be confused as being fixed maximal points where physical factors no longer limit intake. Differences in the digestion coefficients could be caused by differences in physical form of diets used in the studies. Conrad et al. (42) used diets composed primarily of alfalfa or timothy hays in the long or ensiled form, whereas the rations fed by Montgomery and Baumgardt (136) contained dehydrated alfalfa meal and ground shelled corn in the form of pellets. These results are in agreement with (25) who found lower digestibility but similar intakes between diets containing pelleted and chopped roughages. Studies conducted by Freer and Campling (63) using diets of hay, dried grass and concentrates concluded that cows ceased eating when the reticulo-rumen contained about 15.9 kg of DM. When roughages had a slower rate of disappearance from the reticulo-rumen than 8.2 kg of DM/day, eating ceased. Thus intake was regulated to maintain an amount of digesta in the rumen which could be reduced to 8.2 kg of DM immediately before the next meal. It was evident that the amount of digesta in the reticulo-rumen did not ap-
proach these limits before or after feeding when concentrates were offered ad libitum (63).

There is some evidence that on some diets animals do not eat to a constant rumen fill. When cows were offered hay and oat straw ad libitum, hay was consumed to contain 35 percent more DM in the reticulo-rumen than straw (30). Montgomery and Baumgardt (136) found similar results feeding long and chopped hay and straw to heifers. These results suggest that intake is not only controlled by the physical capacity of the rumen, but also by the structural carbohydrate components which affect rate and extent of digestion. Comparisons of hay and silage showed that more digesta was present in the reticulo-rumen with hay than silage suggesting that silage intake was not restricted by rumen capacity, but by factors inherent to the silage (220). Other workers (63) concluded that rumen fill was less and not limiting DM intake when diets consisted of highly digestible concentrates. Makela indicated that rumen size is limited by the abdominal cavity (17). He and Blaxter (20) suggest that space required by the growing fetus places limits on the voluntary intake of the pregnant animal. Forbes' (60) studies with pregnant ewes determined that until about the 120th day of gestation there was little change in rumen volume. After this time, rumen volume appeared to be depressed and may have caused a depression in roughage intake in late pregnancy. Forbes (60) also observed a negative relationship between volume of rumen contents and the volume of the uterus plus abdominal fat and abdominal organs. Others (29) indicated the reticulo-rumen volume of the lactating cow in mid-lactation is considerably greater than that of non-lactating animals. They suggest differences in cattle are not due to restriction by fetal growth, but by endocrine differences associated with onset of lactation.
Taylor (29) observed the amount of abdominal fat to be important in restricting intake of herbage by grazing cattle, possibly by restricting rumen capacity. The limits within which physical regulation of intake occurs are not yet clearly defined. Voluntary intake of poor quality forages with a low protein content such as straw is controlled partly by physical factors, and especially by the rate of digesta breakdown in the rumen, and by some metabolic factor arising from the nitrogen status of the rumen. Huber and Kung (82) reported that diets containing less than 12% protein had depressed DM digestibility, lower intake, and poorer energy utilization. In young ruminants which have less ability to digest large quantities of bulky, highly fibrous feeds, physical limitations on intake are more pronounced as the diet becomes less digestible (29).

It is evident that control of intake is not solely regulated by physical factors controlling the rate and extent of reticulo-rumenal digestion. While physical factors do affect the rate of microbial digestion and mechanical disintegration, voluntary intake is also controlled by various metabolic factors unique to the physiological state of the animal.

**Metabolic regulation**

The concept of physiological or metabolic regulation of intake is based on the theory that maintenance of energy balance is the force controlling intake. Baumgardt (17) used the term set point to describe the body weight and composition that the animal attempts to attain and maintain at all times. Simply stated, an animal eats to meet its energy requirement, however, degree of control is imprecise. First, is the energy requirement for maintenance. This is influenced by the inherent genetic metabolic efficiency of the animal to use energy to maintain body functions (129). This requirement can, however, be affected by ambient temperature. As temperature decreases energy required to maintain
body temperature increases, in addition to energy needed for basal activity. This requirement varies depending on the feeding system and the animal’s environment. Energy intake to meet needs for maintenance, production, and maintenance of tissue reserves can be affected by many factors. The first of these is diet energy concentration.

When concentrates or high quality forages make up a large portion of the diet it appears intake is regulated by factors other than rumen load or fill. Researchers (21, 50, 230) have found that as the amount of concentrate increased in the ration, voluntary intake decreased so that available energy intake was relatively constant. This suggests voluntary intake of concentrated diets is probably controlled by chemostatic or homeothermic regulatory mechanisms acting as satiety signals (136). Baumgardt (17) described a receptor system involved in the regulation of energy balance. Receptors, which exist in various tissues of the animal, are responsible for detection of feedback signals (e.g., physical distention, changes in metabolic concentrations of volatile fatty acids, free fatty acids, pH and temperature). These signals are relayed to the hypothalamus, which then regulates feed intake so that energy homeostasis occurs. Baile and Della-Fera (8) reported that a central nervous system (CNS) peptide, cholecystokinin octapeptide (CCK-OP), is a neural signal responsible for energy homeostasis. This peptide signal is produced by the hypothalamus when its receptors are activated. The CCK-OP then travels via the CNS to receptors within the digestive tract sensitive to CCK. The receptors are therefore responsible for the initiation of satiety and related digestive changes. Extensive research with rodents using chemical and electrical stimulation or lesioning of various hypothalamic areas has identified the ventromedial and lateral hypothalamus as important centers in the control of intake and regulation of energy balance. Lesions in the ventromedial hypothalamus (VMH) of goats had an effect on feeding, resulting in hyperphagia and obesity (9). The lateral hypothalamus (LH) seems
to be associated with the initiation of feeding, and lesions in this area result in aphagia and weight loss (8). Injections of neural depressant drugs into the cerebrospinal fluid have been successful in blocking the inhibitory action of the medial hypothalamus. Neural depressants caused feeding when injected directly into the medial hypothalamus of goats and sheep (10). Satiated goats, sheep, and calves eat vigorously during perfusion of the cerebrospinal fluid with pentobarbital. Therefore, it can be concluded that there are neural signals transferred from the neural centers to the digestive system which may affect intake. While the exact sources and compositions of these signals is not yet known, the control they elicit on the animal is quite definite.

Metabolites

In ruminants blood glucose concentration, arteriovenous differences of glucose, and glucose utilization rates show little relationship to feeding (8). In non-ruminants the glucostatic theory is represented by the response of satiation when there is an increase in glucose utilization in the ventromedial nucleus of the hypothalamus. Workers (17) have shown that intravenous injections of glucose do not depress intake in ruminants. Glucose utilization rates increased after intraperitoneal infusions of glucose into dairy heifers but had no effect on feed intake (185). Because ruminants have relatively low blood sugar levels, relative resistance to insulin, and lack of an alimentary hyperglycemia following feed intake, one would expect that glucose levels are not an important satiety signal (185).

In contrast, volatile fatty acids (acetate, butyrate, propionate, and branched forms) are important in the energy metabolism of ruminants. During and after feeding the concentration of VFA's in rumen and blood increase (36). Infusion of acetate, propionate,
and butyrate at 15% of the animal's estimated digestible energy requirement and a VFA mixture (60, 20, 20% of calories from acetate, propionate, butyrate) reduced voluntary intake of a pelleted diet in dairy cows (185). These findings are supported by Baile et al. (10) who observed depressed intake when solutions of acetate and propionate were injected intraruminally. Injections of acetate made in the dorsal area had a greater effect on intake than those made into the ventral rumen, reticulum, or abomasum (11). Exposure of as little as 5% of the rumen to high concentrations of acetate was sufficient to decrease feeding (114). In goats, injections of sodium acetate into the jugular vein depressed intake less than injections into the rumen. This suggests chemoreceptors on the luminal side of the rumen are not as sensitive as those in blood vessels to VFA concentrations. Receptors are probably not the same for all VFA's. Injection of propionate into the dorsal rumen, ventral rumen, reticulum, or abomasum depressed feed intake similarly. However, propionate injected into the ruminal vein was more effective in depressing intake than injections into the lumen of the rumen, or the mesenteric or portal veins, or carotid artery (11). This suggests propionate receptors may be present in the walls of the ruminal vein as well as the luminal side of the rumen. It can be concluded, that while blood glucose concentrations have little effect on intake, acetate, propionate, and butyrate can act as satiety signal compounds in intake regulation. Presence of chemoreceptor sites in the ruminal lumen, as well as the walls of the rumen vein, act in monitoring VFA concentrations in the rumen as well as the rumen vein. Signals from these receptors probably act upon the hypothalamus which then regulates feed intake to maintain energy balance.
Psychogenic inhibition or stimulation

The animal's feeding environment can be an important factor in altering intake. Stresses (in addition to disease or parasites) usually will reduce intake. Such stresses as crowding, noise and disturbances, and excessive hauling tend to keep animals excited and reduce feed consumption. Social interactions among animals may affect intake in certain feeding situations (129). Coppock et al. (43) reported cows fed in groups ate 7% more of a complete feed than individually stanchioned cows, without affecting milk yield or composition. Social interactions do not necessarily facilitate feeding, especially if there is insufficient space for all animals in a group to eat at once. When space is limiting, dominant animals will have first selection of feed and greatest opportunity for feed intake. This situation probably alters intake of less dominant animals due to reduced time allowed to eat and composition of feed remaining in the bunk after selection is usually more fibrous (129). In addition to social interactions, overall stress on the animal associated with management of the operation, feeding facilities, and feeding situation can depress intake. Proper design of feed bunks, mangers, and water supplies can encourage increased intake.

Palatability characteristics are related to the animal's perception of a feedstuff and the relish with which an animal consumes it. Palatability is essentially the result of many different factors sensed by the animal in the process of locating and consuming food. Consumption depends upon appearance, odor, taste, texture, temperature, and in some cases, auditory properties of the feedstuff (38). These factors are probably most pronounced in the intake inhibition associated with wet or fermented feeds that is not a function of moisture content. Such things as off flavors and aromas, mold or deteriodiet of the feeds all have an effect on palatability which may result in lower voluntary intake.
of feeds (129). Voluntary intake problems may gain partial or complete correction through improvements in feeding management. Such improvements as frequent feeding, overfeeding by 5-10% to allow for selection, and regularly cleaning of bunks and waterers may stimulate intake to achieve maximal performance (129).

In summary, social acclimation encourages feeding in dairy cattle, and animals eat more when kept in groups than when penned individually. Other psychogenic effects controlling intake are palatability of the feedstuff as well as their amounts and facilities in which they are provided.

Temperature

Environmental temperature is known to have an effect on DM intake of dairy heifers (143). This effect is most likely the result of changes in feedstuff digestibility due to differences in ruminal motility during thermal stress. Highest energy utilization occurs between 13-18°C, with little change in feed intake recognized within the range of 5-25°C (143). Magnitude of effect of extremes above or below the 5-25°C range is dependent upon feed type, quality of feed, humidity, hair coat, and growth rate.

Feed intake. The National Research Council (143) indicates normally growing dairy heifers need not have diets adjusted for extremes in heat or cold. Quigley (169) reported that dairy heifers appear to adjust DM intake when temperatures are above or below the thermoneutral zone. When ambient temperature was greater than 30°C heifers probably switch eating schedules to night feeding so that dry matter intake remained constant. Lactating dairy animals on a 60:40 roughage to concentrate diet increased intake 35% when subjected to constant temperatures of -20°C. This intake response to cold is likely
the result of increased reticulo-rumen motility and rumination activity. These digestive changes result in increased rate of passage and decreased apparent digestibility of feeds (143).

**Nutrient utilization.** Reduction of metabolizable energy (ME) values of feeds when fed to cold-stressed cattle and sheep has been reported (143). These reductions in ME values are apparently the result of increased fecal and urinary losses (143). Similarly, increases in feed utilization are associated with warmer temperatures, suggesting digestibility factors are related to changes in rumen motility associated with thermal stress. Animals may reduce daily gain during cold periods, but will compensate during warmer periods of the year. Increases in energy density of the diet are recommended for beef animals (during cold periods) to maintain gain, but increases in protein, vitamins, and minerals are not required (143).

The NRC (143) suggests high and low ambient temperatures can have mild to marked influence on feed intake and growth rate of heifers. Further studies (143) indicate that short-term growth suppression from high temperatures is compensated for during times of more moderate temperatures. It is probable that dairy heifers are able to regulate intake to adjust for changes in ambient temperature, and rely upon compensatory growth to account for losses due to thermal extremes.
Diet factors affecting intake

Moisture

Heifers fed silages voluntarily restrict their rate of intake below that of contemporaries fed hay. Research (203) suggests differences in DM content and/or other chemical constituents of silages may be factors in determining their rate of voluntary intake. Factors inherent in the moisture content of the diet are known to influence dry matter intake (72, 100, 174). Researchers (72, 100, 174) indicate that factors causing reduction of DM intake include moisture content, organic acid content, fermentation products, and silage pH. High moisture (< 60% DM) in diets may be advantageous for a variety of reasons. Adequate moisture in complete feeds (DM >40 and <60%) may prevent or reduce separation of ingredients. Silages or high moisture grains may be favored over drier feeds because of ease of preservation, reduced harvest losses, and increased quality. Increased moisture content may increase palatability by improving texture or may dilute undesirable flavors (100). In contrast, dairy cattle consuming high moisture diets may be unable to achieve maximum intake and production. Several researchers (72, 174, 203) reported cattle consumed more DM from hay than from wilted haylage (27 - 33% DM). Others (72, 80, 174) fed diets containing high-moisture silage (<65% DM), low-moisture silage (>65% DM), and hay (85% DM) to lactating dairy cows. Their studies conclude that method of preservation influences chemical composition of the forage when fed. Forages preserved as hay were lower in protein, ether extract and ash than those preserved as silage. Wilted silages generally contain a greater total acid content than low-moisture silage. Roffler reported butyric acid was the predominant acid present in wilted silages, while lactic acid predominated in low-moisture silages. From these trials it can
be concluded that variation in DM intake may be attributed more to organic acid, pH, and fermentation products, than to moisture content of the feed.

**Silage quality.** High-quality silage is characterized by low pH, low contents of butyric acid, acetic acid, and ammoniacal nitrogen and by high levels of lactic acid (72). Many studies (72, 80, 174, 203) have observed wilted or high-moisture silages to be higher in butyric acid and ammoniacal nitrogen. Ammoniacal nitrogen constituted a greater proportion of the total nitrogen in wilted silage than in low-moisture silage. This suggests more extensive protein breakdown occurs during the fermentation of wilted silage, as compared to low-moisture silage (72, 174). Total silage acid content is markedly higher in high-moisture silage, suggesting a more complete carbohydrate fermentation than occurs with low-moisture silage and may affect DM intake in dairy animals.

**Forage intake.** Reports (72, 80, 174, 203) indicated cows fed low-moisture silage usually consume more forage DM than cows fed high-moisture or wilted silages. Thomas et al. (203) found that heifers consumed more DM from haylage (43-50 % DM) than direct-cut silage (20-25% DM) when forages were harvested from similar fields and cuttings. They also found that addition of water to wilted silage, haylage, hay, or intraruminally did not change trends in DM intake. The addition of silo effluent to dry hay reduced DM intake by 2.9 lb./heifer/day (203). Addition of similar effluent directly into the rumen, via small fistulas, also depressed voluntary intake of hay. Lahr et al. (100) concluded that diets containing less than 60 - 65% DM depressed intake in lactating cows. This response occurred regardless of whether the diet DM content was reduced by ensiled feeds or the addition of water.
It is therefore concluded that forage moisture content at time of ensiling results in differences in fermentation. The relationship between DM content and intake may be more a reflection of the fermentation process and quality of fermented product than its actual DM content. Thus, animal intake responses are most-likely controlled by quality of fermented product which is a direct reflection of pre-storage DM content and proper storage rather than influence of moisture on animal intake.

\( pH \)

Variations in DM content of silages prior to ensiling changes not only the organic acid content, but also silage pH. Intake depression associated with ensiling of forages ranges from 4 to 50\% (72, 80). Shaver et al. (183) suggested silage moisture content is not the limiting factor in DM intake, but that low intake of silages is more likely due to end products of silage fermentation common to silages with low DM contents.

**Fermentation products.** Two major compositional changes occur during ensiling: degradation of plant proteins to nonprotein nitrogenous compounds and conversion of water-soluble carbohydrates to organic acids (204). Although ammonia and amines can be found in large quantities in silages, a direct correlation between their content and DM intake has not been established. Direct addition of ammonia and amines to ether hay or silage was not found to depress intake (154).

Addition of lactic and acetic acids to reduce pH of the diet has resulted in reduced DM intake (72, 226). Shaver et al. (183) conducted studies to determine if the response to addition of organic acids was due to their presence or a change in diet pH. Addition of sodium bicarbonate to neutralize corn silage, increased silage pH from 3.79 to 7.11 and
increased dry matter intake by .78 kg/day. Acidification of fresh whole corn with hydrochloric acid reduced pH from 5.20 to 3.66 and depressed organic matter intake 0.29 to 3.62 kg/day. Wilkinson et al. (226) conducted similar experiments involving addition of lactic and acetic acids to fresh corn plants. Their studies indicated DM intake was a direct reflection of diet pH and titratable acidity. They suggest depression of voluntary intake is caused by the acid load created by feeding ensiled feeds. This acid load affects the acid-base balance and nitrogen balance, thus causing acidification of the urine and increasing urinary ammonia excretion.

A trend toward decreased live weight gain, and reduced feed efficiency was found to exist when silage pH was reduced. Studies with heifers indicate gain was maximized when diet pH was increased to 5.78 with sodium bicarbonate (183).

It can be concluded that forage pH affects voluntary intake of corn silage and alfalfa haylage. It seems likely that total hydrogen ion content and not specific organic acid content is responsible for differences in voluntary intake of ensiled forages. This premise is supported by the addition of sodium bicarbonate increasing pH and intake, while hydrochloric acid addition decreased pH and voluntary intake of forages.

**Protein**

Protein nutrition in ruminants is a complex, dynamic process. Nitrogen is a critical nutrient in the ruminant, since it is the primary component in protein (amino acids) (144). Availability of nitrogen is dependent upon microbial intervention and a readily available carbohydrate source. Microbes convert dietary nitrogen and nonprotein nitrogen into bacterial protein. Bacterial protein is subsequently digested by the animal and used as
a supply of amino acids. It is this amino acid supply, along with the lesser amount of protein which is able to by-pass the rumen, which is used for production of milk, and animal or fetal tissues.

Various researchers (7, 24, 82, 84) have indicated varying levels of protein in the diet of growing dairy heifers may affect DM intake, gain, and skeletal growth. In two trials involving 44 dairy heifers, Bagg et al. (7) fed three levels of dietary protein (80%, 100%, 120% of (143)) to animals between 71 and 295 d of age. In the first trial, animals were fed according to requirements from 85 to 182 d. Animals were then assigned to diets containing one of the three protein levels and maintained on this diet to 295 d of age. Weight at 295 d was found to increase linearly with level of protein. Wither height and DM intake were unaffected by the level of protein in the diet. In the second trial, he assigned animals to treatment diets for the period of 71 to 182 d of age. Animals were rerandomized to either a medium or high protein diet and maintained on this diet till 295 d of age. At 181 d a quadratic effect was determined to represent weight and wither height response with the greatest response occurring from the medium protein (100% of (142)) diet. A wither height interaction between period one and two treatments indicated a response to high protein levels by those heifers previously receiving the low or medium protein diets. These authors summarized that level of dietary protein linearly increased protein digestibility, but had no effect on DM intake. Gains appeared to be more closely associated with DM and energy intake than amount of protein in the ration.

These findings are in agreement with Brown and Lassiter (24), and Gardner (66) who also found no significant effect of dietary protein on DM intake. However, low protein diets tend to depress DM intake (7). Protein concentrations less than 12% were re-
ported to depress DM digestibility, reduce intake, and cause poorer energy utilization (82). This response is likely the result of inadequate nitrogen availability for bacterial growth and thus a subsequent depression in fiber digestion is realized. Brown and Lassiter (24) indicated dairy calves grow at comparable rates on protein levels between 12 and 24%. However, calves fed 16% protein diets had slightly greater rates of gain than those fed higher or lower levels of protein. This suggests an optimal level of dietary protein may exist. These studies indicate protein levels greater than 12% should be sufficient to maintain bacterial growth. Feeding higher levels of dietary protein may exceed the amount required to maximize skeletal growth, and should therefore be avoided for economic reasons when formulating rations.

Within the normal range of dietary protein contents, voluntary intake should not be affected by protein content (60). These findings suggest dairymen feeding low protein forages to heifers can stimulate forage intake and digestibility by addition of supplemental protein. Supplemental protein can be in the form of high protein grains or urea as both will successfully increase DM digestibility and accelerate fiber digestion.

**Digestibility and Fiber**

It is often assumed that intake and digestibility of forages are directly related. Although they are somewhat interdependent, intake and digestibility of forages are separate parameters of quality. Intake is dependent upon structural volume and, therefore, cell wall content, while digestibility is dependent upon both cell wall and its availability to digestion as determined by lignification and other plant factors.
Digestibility is dependent upon two factors: the proportion of total forage made up of cell contents, and lignification of the fibrous residue (213). In terms of chemical composition, the only consistent effect that can be observed for all forages is that of the total fibrous fraction, i.e. NDF. As this fraction increases, voluntary intake declines with an increasing negative slope. In forages with low NDF, digestibility and intake apparently are not related. Legumes are characterized by a rapid burst of fermentation followed by a plateauing as the soluble cell contents are exhausted. Thus, due to a high cell content fraction legumes tend to have shorter fermentation time resulting in lower DM digestibility yet higher voluntary intake. In forages with a high NDF fraction intake is highly correlated with both chemical composition and amount of digestible DM. This suggests the relationship between digestible DM and voluntary intake depends on the proportion of digestible energy from cell-wall constituents. In contrast to legumes, grasses are observed to have a slower initial fermentation due to lower cell contents, yet fermentation continues steadily to yield equal or higher DM digestibility. For these reasons DM digestibility and voluntary intake of grasses are closely associated.

Van Soest (213) presented data which supports the theory of fibrous mass inhibiting intake in forages with high NDF. He suggests the point at which fiber mass appears to become limiting occurs when NDF is between 50 and 60% of the forage DM. It should be noted that level of NDF was based on all-forage diets using 121 different types of forage. Mertens (128) sought to validate these levels by comparison of diets based on alfalfa hay, Bermudagrass hay, and corn silage, balanced with concentrate to the same NDF content. Alfalfa diets contained lower amounts of concentrate and thus lower TDN (65%) or NEI (1.50 Mcal/kg) resulted in higher production of 4% fat corrected milk. Highest production of fat corrected milk for all diets occurred when diets contained 36% NDF. Cows consuming 36% NDF rations, based on alfalfa, produced an
average of 7 pounds per day more 4% fat corrected milk than cows on corn silage or Bermuda grass hay diets. This study suggests that while an optimal level of NDF may be possible, differences in digestibility of the NDF fraction affect animal performance. Research with dairy heifers indicated that when diet NDF content was above 42% it was negatively correlated (r = -.42) with DM intake, while below this level correlation was very low (r = -.03) (85). This suggests the effects of NDF on regulation of voluntary intake become increasing important as dietary NDF concentration increases. Variability in rate and extent of NDF digestion has sparked investigations into feasibility of using NDF to formulate rations varying in physical fill characteristics.

Using polyester bags suspended in the rumen of cannulated dairy cows, rate and extent of fiber degradation was determined for 22 feedstuffs (215). Varga et al. (215) determined that for forages, rate of NDF degradation was negatively correlated (r = -.98) with NDF content and extent of degradation. For all other feeds (concentrates) a low negative relationship (r = -.50) existed between NDF content and extent of degradation. This suggests that physical and chemical factors limiting rate and extent of cell-wall digestion of forages may not be similar to those associated with grains. This study also found that grouping of feeds into like characteristics (protein and/or energy sources) based on NDF was not possible because feeds within the same group differed in NDF content as well as rate and extent of degradation.

In a feeding trial, based on NDF rate and extent of degradation information, two diets with 39% NDF but varying in rate and extent of NDF degradation were used (215). Diets were formulated to be isonitrogenous, isocaloric, and similar in ADF and soluble protein content. Results indicated DM intake, fat corrected milk, daily fat production, and solids not fat did not differ between diets. Cows fed low fill (faster estimated NDF degradation) produced more milk (30.3 vs. 26.3 kg/d) and milk protein (.97 vs. .78 kg/d)
than diets with slower estimated rates of NDF disappearance. However, slower rate of 
disappearance diets had higher milk fat content (3.92 vs. 3.54%) than low fill diets. They 
concluded that low ruminal pH and/or other physiological mechanisms, such as higher 
VFA production, may have prevented cows on the low fill diet from consuming more 
DM than cows on the high fill diet. In addition, while similar in chemical composition, 
ingredient composition was different between diets. This also contributed to differences 
in nutrient utilization resulting in differences in milk production and composition.

Examination of pooled data from 20 experiments using early to mid-lactation Holstein 
cows (22) to determine relationships between NDF content of the diet and milk yield and 
DM intake indicated NDF has a greater effect on DM intake than on milk yield, and its 
use in formulating diets was limited to use within roughage sources. Limited application 
of NDF is due to lack of a critical threshold at which maximum DM intake and milk 
yield occur. Feeds similar in NDF content may differ in bulk and caloric density and 
due to these differences may differ in rate and extent of degradation.

In summary, intake and digestibility are separate estimates of quality or feed value of a 
feedstuff. Intake is dependent upon the volume, or fibrous cell wall components and is 
therefore directly related to the NDF fraction. Digestibility is dependent upon amount 
of cell wall lignification as well as physical form, rate of passage, and NDF content.

**Digestion and Metabolism**

As indicated earlier, protein plays a critical role in nutriture of the growing animal. 
Protein required by the ruminant is second in quantity only to energy, therefore it is 
imperative that one understand its digestion and utilization by the ruminant. A number
of recent publications have focused on the importance of nitrogen (N) metabolism and protein requirements of the animal (32, 33, 40, 82, 177, 199). Differentiation of requirements into degradable and undegradable protein fractions was emphasized. Of particular importance were characteristics of proteins within feedstuffs and assessing their rumen availability. Nitrogen compounds in feedstuffs consist of proteins and nonprotein N (NPN). Nonprotein nitrogen includes amino acids, peptides, nucleic acids, free ammonia, urea, Maillard products, and in an inorganic form, compounds such as ammonium salts and nitrates. Chemically, feed nitrogen can be fractionated into several components, but their biological significance to ruminants can not be assessed unless its changes in the gastro-intestinal tract are known (96). Chalupa (34) reported proteolysis, deamination of amino acids, and microbial protein synthesis in the rumen all affect availability of feed N and amino acid flow to the small intestine. Therefore, to evaluate the biological value of a given source of N for ruminants, it is necessary to know its potential as a N source for rumen microbes and as a source of amino acids available for absorption in the small intestine (96).

In 1985 the National Research Council subcommittee on N usage (144) described the separatrix of dietary protein into two fractions. These fractions were titled ruminannally “degradable” and “undegradable” protein. Degradable protein contains nitrogenous fractions of a feedstuff which are either soluble within the rumen liquor or degradable by rumen microbes within the time constraints of rumen turnover. Undegradable protein is dietary protein which has either resisted rumen microbial attack or evaded attack by passing out of the rumen without thoroughly mixing with rumen contents. The undegradable fraction resisting attack is typically termed “undegraded” while “bypass” describes the latter protein fraction.
Dietary protein entering the reticulo-rumen is often extensively degraded by bacteria or protozoa. Protein degradation occurs outside the bacterial cell by hydrolysis of peptide bonds (proteolysis) resulting in peptides and amino acids. These products are subsequently transported into the bacterial cell and peptides are hydrolysed further to amino acids. The amino acids are either incorporated into bacterial protein or degraded to volatile fatty acids, ammonia, carbon dioxide, methane, or they may serve as sources of branched chain fatty acids which act as growth factors for a number of bacterial species including the cellulosytics.

In spite of proteolytic capabilities of rumen microbes, substantial amounts of feed protein resist ruminal degradation thus bypassing to the small intestine (32). Satter and Roffler (177) concluded that as little as 40% or as much as 80% of dietary protein might normally be degraded within the rumen and be transformed into microbial protein. Figure 1 depicts a schematic of protein utilization in the ruminant as outlined by Chalupa (34).

Extent of protein degradation in the reticulo-rumen is dependent upon microbial proteolytic activity, access by microorganisms to the protein and the rumen environment. A number of factors influence protein degradation in the rumen including protein structure, rumen retention time, chemical treatment of dietary protein, level of feed intake, and physical form of the feedstuff (Tamminga 199). Thus many factors affect availability and degradability of a feedstuff, ultimately having an influence on amount of amino acids available to the animal.
Figure 1. Protein digestion and metabolism in lactating dairy cattle (34).
Protein Fractions

Several workers (96, 144, 166, 199) have described the delineation of feed N into three groups of biological significance: soluble NPN, true protein, and unavailable insoluble N. Pichard and Van Soest (166) designated these fractions as fraction A (soluble), B (slowly degradable), and C (unavailable). Fraction A is composed of NPN or soluble proteins which are degraded very rapidly within the rumen. The B fraction consists of proteins degraded at a rate similar to the rate of passage (.02 to .07/h). The C fraction is composed of bound or unavailable protein that is not degraded in the rumen. The C fraction is generally composed of lignified N, Maillard products, and tannin-protein condensates. Most often the greatest proportion of unavailable protein is the result of heat damage producing "artifact lignin" or Maillard products. Artifact lignin is most prominent in processed by-product feeds and low moisture silages. Goering and Van Soest (71) reported acid detergent insoluble N characterized this N fraction well.

At least five common feeds have potential for containing sizable portions of bound or unavailable protein. They are hay crop silages, dehydrated alfalfa, citrus pulp, corn distillers dried grains and brewers dried grains (144). Storage of feeds by ensiling often creates a shift in these N fractions with A and C increasing at the expense of the B fraction.

Generally, fraction B (true protein) is the only feed N fraction with degradation rates relevant to rumen N escape estimates. Pichard and Van Soest (166) subdivided the B fraction based on ruminal rate of degradation. A very rapidly degraded B fraction was labelled B₁, with a degradation rate of 8.29 %/h. A B₂ label described a more slowly degraded fraction with a degradation rate of .28 %/h. Van Soest et al. (214) extended
this system to include a subfraction $B_3$ which was very slowly degraded and usually ruminally undegradable.

Krishnamoorthy and coworkers (96) indicated that knowledge of pool sizes ($A, B, C$ fractions), degradation rates, and passage rates are imperative in quantitation of protein degradation in the rumen. They found pool sizes of the protein fractions varied considerably among feedstuffs. For example, timothy hay and beet pulp contained predominantly $B_1$ and $B_3$ fractions, brewers dried grains showed a single slow digesting pool ($B_3$), and oats exhibited $B_1$, $B_2$, and $B_3$ fractions. Soybean meal showed two faster degrading fractions ($B_1$ and $B_2$), while corn showed a single slow digesting pool ($B_3$).

Various methods, in vivo, in vitro, and in situ, have been developed for measuring the degradation of dietary protein in the reticulo-rumen. Waldo and Glenn (218) in comparing various protein systems emphasized the use of these techniques in sepadiet of the protein fractions for more accurate formulation of diets.

Measurement of Protein Degradability

Measurement of protein degradation by rumen microbes is a difficult task. Often, there is wide variation in protein degradability within and among feedstuffs. There are also differences among animals regarding rumen environment and retention time of feed within the reticulo-rumen. Despite these difficulties, in vivo measurement of protein degradation serves as the standard for comparison of all other means of estimating protein degradability. In vivo estimation of ruminally degraded and post-ruminally digested feed protein is expensive, time consuming, labor intensive, and requires use of surgically altered animals equipped with cannula in the rumen and abomasum or proximal
duodenum. Using these animals, dietary protein degradability can be estimated as the
difference between total and microbial protein entering the abomasum or small intestine,
after correction for microbial protein.

Microbial protein content can be estimated by use of specific markers such as nucleic
acids, 2,6-diaminopimelic acid (DAPA), 2-aminoethylene-phosphonic acid (EAP), or by
radioisotopes, $^3$S, $^3$P, or $^{15}$N (106). To achieve meaningful results, flow of digesta to the
lower gut must be corrected. Measurement of flow can be accounted for by use of in-
digestible solid and liquid phase markers, such as chromium oxide and polyethylene
glycol. Estimates of microbial protein flow are subject to errors inherent in the tech-
niques used. In practice, some investigators use microbial markers present only in bac-
teria, therefore ignoring protozoal protein contributions to the pool. Another
discrepancy in protein pool estimation involves estimation of endogenous protein. Fre-
cently, endogenous protein is ignored thus leading to overestimation of undegradable
protein when various techniques are employed (199).

Two common methods of estimating protein degradability in vivo are the 1) incremental,
and 2) difference methods. The incremental method allows estimation of degradability
for a given protein source by feeding increments of a feed with a basal diet which con-
tains adequate N for microbial synthesis. With this method microbial N flow is disre-
garded as the increase in protein flow is attributed to the test feed (144). The difference
method simply involves quantitating total non-ammonia N and microbial N in the
duodenal digesta. Feed protein escape is calculated as the difference between total
duodenal non-ammonia N and microbial N. Both techniques give good estimates of in
vivo protein degradation in the rumen. The incremental technique is most appropriate
for calculation of protein degradability of individual feedstuffs. The difference method
works best when determining protein degradability of complete diets.
In Vitro Techniques

In vitro methods are generally based on either the release of ammonia following incubation of a protein sample in rumen liquor, or the proportion of N which goes into solution after incubation in a solution for a fixed time. Estimating protein degradation from ammonia release has the disadvantage of microbial growth occurring simultaneously with protein degradation. Therefore, protein degradability may be underestimated as part of the released ammonia becomes incorporated into microbial protein. Various solutions have been applied as incubation media for the latter method. Such solutions as NaOH, artificial saliva, autoclaved rumen fluid, diluted solution of pepsin in .1N HCl, and water of various temperatures were discussed as effective agents in estimation of protein degradability (218). A general limitation of all in vitro methods is that, although they may yield a value for degradability, they do not yield data representing actual degradation in vivo. Broderick (23) attempted to overcome this problem by studying the kinetics of protein degradation resulting in a measurement of degradation rate. By use of degradation rates he was able to better separate the various protein fractions thus reducing error in prediction of degradability.

In Situ Techniques

An alternative to the multiple cannulated animals needed for in vivo studies is the in situ technique. This technique also known as in sacco, artificial fiber bag, or dacron bag technique can provide rapid and reliable estimates of feed degradability for a wide variety of feedstuffs. This method has found general acceptance (116, 124, 147, 160, 192) for characterization of dietary protein degradability. In this method a direct measurement
of protein degradation is achieved by incubating a sample of feedstuff enclosed in a dacron bag suspended in the rumen for varying periods of time. Following incubation, residues are washed, dried, ground, and analyzed for chemical components. Rate of component degradability is determined by regression of component disappearance on time. An important advantage of this method is that it yields a direct estimate of protein degradation not biased by inaccuracies of the estimation of microbial protein. Nocek (151) suggested there is no better way to simulate the rumen environment within a given feeding regimen (temperature, pH, buffer, substrate, enzymes). A drawback, however, is the feed sample is not subjected to the total ruminal experience: i.e. mastication, rumination, and passage. With increased popularity this technique has received extensive evaluation and criticism regarding factors influencing feed sample digestion (e.g. bag pore size, sample size, sample particle size, and animal and dietary effects)(123, 145, 147, 210).

**Bag Porosity**

Soluble and mechanical particle loss from bags can account for a considerable proportion of nutrient disappearance. Appropriate bag porosity is a compromise between limiting influx of rumen contents (i.e. microbial populations and foreign feed particles) while at the same time limiting efflux of undegraded sample particles. Therefore, feed particles lost from the bag prior to incubation, via solubility or mechanical loss, are considered part of the rapidly degradable and readily available pool, which may or may no be correct (111). Nocek and Grant (149) suggested a preincubation wash to quantitate and remove this questionable fraction as well as mimicking salivation by wetting the sample. They reported preruminal incubation (15 min in 39°C water) of soybean meal (2 mm grind) resulted in 15 and 27% loss of N and DM from bags ranging
in pore size from 6 to 59 μ. Pore size did not, however, affect N or DM washout. As pore size increased to 80 and 120 μ, N and DM washout increased by 30 and 14%, respectively.

Weakly et al. (223) observed lower disappearance of DM and N from soybean meal and distillers grains from 5 μ pore size bags than 52 μ dacron bags regardless of ruminal residence time. Uden and Van Soest (210) reported increased cell wall digestion of timothy with increasing pore size (20, 37, and 53 μ). These workers also indicated the increase in digestibility with pore size could not be totally explained by mechanical losses. Although extent of digestion is important, rate at which nutrients disappear from the bag in relation to rate of passage dictates the true extent of ruminal degradability. Limits of bag porosity are difficult to ascertain and are most likely dependent upon sample particle size and physical characteristics of the feedstuff in question. Nocek (151) suggested a bag porosity of 40 to 60 μ allows adequate microbial and content influx and digested material efflux for estimation of feedstuff digestibility.

**Particle Size**

Since feedstuffs included in in situ digestion studies are not masticated or ruminated, microbial fermentation and fractionation by ruminal activity are the only means for particle reduction. The question remains as to whether prepared materials, for in situ, should mimic the feed as fed or mimic its characteristics after mastication and ruminal passage. Ehle et al. (53) showed rate of N digestion for several feeds were not affected by particle size within feed sample (1180, 600, 300, 150 μ; 70-μ pore size; 20 g sample). Nocek (146) found no difference in DM or N digestion rates for soybean meal that was unground or ground (1,2,5 mm) and incubated in bags of 59 μ pore size. He later dem-
onstrated that grinding (5mm) various corn grain forms increased rate of DM, N, and non-N DM disappearance when compared to as fed form. There is a tendency toward greater losses of water-soluble and filterable material when either grains or forages are ground to particle lengths shorter than their as fed form. Nocek and Russell (151) suggested establishment of standards for feedstuff particle size prior to in situ incubation. His guidelines were 2-mm grind for protein supplements, by-product ingredients, and high energy grains. Roughages with DM > 60% should be ground to pass a 5 mm screen, and those < 60% DM should either be freeze dried or air dried to 60 to 70% DM prior to grinding or be ground frozen with dry ice. Reduction of feedstuff particle size may slightly alter digestibility estimates but also serves to establish uniformity and reduce variation in sampling and digestion rate.

**Sample size per bag surface area.**

Importance of sample size to bag surface area ratio is well recognized (146). The optimal sample size is that amount providing adequate residue following incubation for chemical analysis without delaying microbial attachment, which increases lag time and underestimates digestion rates. Uden and Van Soest (210) reported cell wall digestibility decreased from 54 to 38% when sample size increased from 6.5 to 50 mg/cm². Varga and Hoover (215) compared various bag sizes and sample weights, finding that 14.6 mg/cm² gave the best DM and NDF digestibility estimates when compared to in vivo estimates. Nocek (146) concluded 10 to 20 mg/cm² should be utilized for most forage and concentrate ingredients to achieve reliable digestion estimates and provide sufficient sample for nutrient analysis.
Dietary Effects

Diet is the major factor determining quantity and type of microbial population and thus rate and extent of dietary nutrient digestibility. Diet N and energy concentration fed the cannulated cow has shown to have a variable effect on in situ digestion results. Forage to concentrate ratio not only dictates microbial population make-up thus influencing fiber, carbohydrate, and N digestibility, but also affects mixing of bags with rumen contents. Weakley et al. (223) postulated that medium to high forage diets improved N digestion through abrasive action of the forage against the bag and differential pressures exerted on the feed sample thereby improving the mixing action. High grain diets seemed to be detrimental as they increased clogging of the bag’s pores from bacterial slime, promoted less sample mixing due to lower rumen motility, and depressed growth of cellulolytic bacteria. Several studies (146, 148) indicated digestibility estimates were most reliable when the cannulated animal received a diet similar to a treatment in question or contained the feedstuffs of interest during the in situ incubation period.

Animal Effects

In situ digestion techniques have been utilized in several species including cattle, sheep, goats, ponies, and rabbits (146, 210) and is limited only by the ability to establish a fistulae. The most often used species are cattle and sheep. Siddons and Paradine (184) reporting on sheep and steers fed similar diets, found sheep had higher ruminal ammonia, lower VFA, and similar pH and rumen fluid dilution rates than steers. While feedstuff degradability tended to differ slightly between species, ranking of feedstuff degradability was similar. Within species, differences related to sex or physiological status are sources of potential variation. Often differences are related to a specific type
of diet associated with a definite physiological status and may influence ruminal factors other than digestion (184). Nocek (148) recommends the animal type be identical to which results will be applied and to feed a diet containing the test ingredients to meet maximum performance requirements.

**Microbial Contamination**

Due to intimate contact of test feed particles with rumen microflora, potential contamination of residues remaining in the bag during in situ incubation creates additional digestibility variation (149). High levels of microbial contamination were observed with fibrous-low digestibility feedstuffs such as straw or late cut hay (147). Contamination was low for protein supplements and highly digestible forages (5 to 10% of residual N). Nocek and Grant (149) indicated less than 5% contamination of ground dried shelled corn with soybean meal having up to 19% contamination. However, this did influence rate of N digestion. They (149) showed reduced digestion, lag times, less non-digestible residue, and faster N digestion rates when forages were corrected for bacterial N contamination. Generally, it is recommended that low protein forages and coarse feedstuffs be corrected for microbial contamination.

**Kinetic Interpretation of Nutrient Fractions.**

As reported earlier, there are at least three fractions into which proteins can be delineated with respect to ruminal availability (i.e. soluble, degradable, and non-degradable) (144). These fractions are referred to as A, B, and C, respectively. In situ techniques can be used in quantification of each fraction yet are only capable of estimating digestion rate of B. Nocek (144) and others (192, 210) demonstrated that a portion of the
test feed escapes from bags prior to ruminal incubation and is considered to be soluble. Reports (45, 192) demonstrated soluble N was highly correlated with short-term (2h) ruminal incubation. This fraction is generally assumed to be readily available to rumen microbes and digested at a rate greater than 10 times the rate of passage. Since this fraction is soluble and therefore associated with the liquid phase of digesta, potential for ruminal escape is high. Nocek and Russell (151) concluded that removal of this fraction by soaking prior to incubation will reduce interference by this fraction when determining rates of insoluble nutrient components.

When determining degradability of a feedstuff it is important to: have an adequate number of time points to detect an observable lag time (1-h intervals); to detect multiple rate components within the potentially degradable fraction to allow meaningful regression interpretation (6 to 12-h intervals); and to detect an end-point of digestion. For most concentrates 48h and for forages 72h of incubation is adequate to detect a ruminal digestion end-point (148). Mertens and Lofsten (131) indicated the potentially degradable nutrient fractions could be described by first-order kinetics. Primary assumptions were that fractions in question were homogenous and substrates remaining were degraded as a linear (log transformed) function of time in the rumen. These basic assumptions are violated when soluble and undigestible pools are not determined and subtracted from the fermented residue. Nocek and English (148) applied several mathematical approaches to obtain digestion rates for feedstuffs with different digestion profiles. Orskov and McDonald (159) proposed a model for estimating the percentage of dietary protein degraded in the rumen. Potential degradability P is estimated by in situ techniques and is related to incubation time t by the equation:

\[ P = A + B (1 - e^{-\alpha}) \]
Constants A and B represent the protein fractions as described earlier, and c represents the constant rate of B fraction degradation. Armentano et al. (4) described a model using a monoexponential rate of disappearance to estimate residual protein (RP) remaining in the rumen at any given point in time. Using this model they estimated fraction B and its rate of degradation (kd).

\[ \text{RP} = B \ e^{-kt} + C \] (for \( t > 0 \))

Orskov and McDonald (159) calculated protein degradability in percent (D) with the following equation:

\[ D = A + \frac{(B \cdot k_d N)}{(k_d N + k_r)}. \]

where:

- \( D \) = protein degradability (%);
- \( A \) = readily degradable protein fraction (%);
- \( B \) = protein fraction degraded at a measurable rate (%);
- \( k_d \) = protein degradation constant of B fraction;
- \( k_r \) = rumen turnover rate (.05/h).

Orskov and McDonald (159) concluded that use of empirical models provides a close relationship between rumen availability estimates by the bag technique and in vivo degradation without the requirements of dual cannulated cows, and greater time and expense involved in in vivo studies.
Protein Solubility and Degradability.

Between 20 and 60% of N in many diets may be soluble in buffer (199). Studies by McDonald and Hall (120) it is often assumed that N solubility in buffer solution is synonymous with rumen feedstuff degradability. In general, proteins soluble in the rumen liquid phase are more rapidly and completely degradable than those that are insoluble. Solubility is not, however, a guaranteed percent nor a prerequisite for degradability (34). Leng and Nolan (103) pointed out that soluble proteins such as serum albumin, ovalbumin, chloroplast protein extract, and soluble proteins from soybean meal and rapeseed meal have variable resistance to degradation. Structural characteristics of proteins are an important determinant of degradability (103). Mahadevan et al. (111) reported proteins which have no terminal amino or carboxyl (i.e. ovalbumin) and those with disulfide cross-linking appear to be less accessible to proteolytic enzymes. Solubility of feed protein is partly determined by the relative amount of soluble albumins and globulins and the less soluble prolamins and glutelins. Wohlt et al. (229) showed feeds whose major protein fractions were albumins and globulins had higher solubility than feeds containing mainly prolamins and glutelins. Considerable variation was reported in soluble N in energy feeds (5.1 to 48.5% of total N) and protein supplements (2.7 to 93.2% of total N)(228). Pichard and Van Soest (166) indicated soluble protein may be degraded rapidly or slowly, and insoluble fractions include a rapidly degradable and a more slowly degradable fraction.

When considering various classes of feedstuffs, low solubility is not synonymous with low degradability as degradability is not always proportional to solubility. However, many laboratories report degradable protein fractions as soluble (113, 189, 179). For example, solubility of barley protein is low, ranging from 17 to 31% of total N, while
degradability determined in vivo is high, ranging from 86 to 100% (106, 117). To date, protein solubility is determined using many different methods with varying results (45, 219, 222, 228). These methods were discussed earlier in methods for in vitro digestibility estimates. At present, there is not a uniform or "best" method for determining protein solubility.

Animal response to varying protein solubility and degradability.

In ruminant feeding one must consider two protein requirements, requirements of the animal itself and requirements of the microbial population in the reticulo-rumen. Meeting the animal's requirements means supplying adequate blood levels of essential amino acids, and nitrogen, carbon and energy for synthesis of non-essential amino acids. To meet these requirements, sufficient protein must enter and be subsequently absorbed from the small intestine. Main sources of this protein will be undegraded dietary protein and microbial protein synthesized in the rumen. Selection of feedstuffs with low degradability may increase protein supplied to the small intestine and improve animal performance (49). The dietary level of soluble and/or degradable protein has received increased emphasis as a factor involved in digestion and metabolism of protein and non-protein N in ruminants (34, 61, 190, 229). Of primary concern with solubility is that high levels of rumen ammonia will result from rapid microbial degradation of the soluble portion of feed protein. Increased absorption across the rumen wall leads to decreased energetic efficiency and N utilization due to energy cost of converting ammonia to urea, and the loss of N in urine. A practical method of reducing N losses via high levels of urea hydrolysis would be to formulate diets using ingredients containing protein fractions slowly degraded within the rumen.
Limiting protein solubility may have its disadvantages. An insufficient supply of ammonia-N can depress growth of rumen microflora and fermentation. For maximal microbial growth, and rumen microbial protein production, a minimal ammonia concentration of 5 mg NH₃-N/100 ml of rumen fluid is required (178). Satter and Slyter (178) indicated that under normal feeding conditions this level of ammonia can be achieved with a diet containing 11 to 14% CP. Maximal rumen fermentation rates were achieved by Mehrez et al. (124) when ammonia concentration was 23.5 mg/100 ml rumen fluid. Tamminga (199) concluded a dietary crude protein of 13.4% would not sustain maximal microbial fermentation of dietary fiber, but had no effect on degradation of N-free extract. If dietary soluble N is inadequate for microbial growth, addition of a NPN such as urea is required. Alternatively, an increase in flow of dietary protein out of the rumen is possible by protecting the protein by heat treatment or treatment with chemical agents (i.e. aldehydes, tannins, or volatile fatty acids). Chemical treatment creates a reversible pH dependent chemical change that inhibits ruminal proteolysis, but allows proteolysis of protein at the much lower pH in the abomasum and proximal duodenum (Tamminga 199). Diet formulation using feeds low in degradability or solubility has proven successful (97, 157, 188) in some studies, but not in others (31, 56, 74).

Dingley et al. (49) reported a significant decrease in supply of essential and total amino acids to the udder of early lactation cows receiving increased levels of soluble protein. This study indicated that supply of amino acids to the mammary gland and overall N utilization were influenced by dietary protein solubility. Aitchison et al. (1) used urea as a completely soluble N source to formulate diets varying in solubility. Diets contained variable amounts of urea and corn in place of soybean meal which was added to a basal diet of corn silage. They conducted three N balance trials with diets of 12, 13,
and 15% CP (DM basis) fed to early lactation cows (30 to 45d postpartum). Diets ranged in solubility from 31.5 to 48.7% of total N. At 15% CP the low solubility diets had the highest milk (39.1 kg/d) and DM intake (22.4 kg/d). Highest milk yield at 12 and 13% CP was with diets containing 45 and 40% soluble protein, respectively. This study suggests protein solubility of 40% may provide adequate ruminal NH₃-N and microbial growth rates in the early lactating animal. Twenty Holstein cows in early lactation (8 to 10 wk) were used to study the effects of protein solubility on protein utilization (112). Two levels of protein (12.6 and 15.3% CP) and solubility (22 and 42% of total N) were formulated using common feedstuffs. Dietary protein solubility had no effect on intake of DM, CP, and net energy of lactation. Highest milk yield (27.7 kg/d) was obtained on the high protein low solubility diet. Janicki et al. (86) used 34 pluriparous Holstein cows to examine the effects of CP (13.6% vs. 15.3%, DM basis) and soluble protein (39.7 vs. 47.9% of total N) on digestibility, and energy and protein balances during early lactation. He concluded, reducing diet solubility improved energy intake, but resulted in no significant increase of milk yield or body tissue balances. Greatest digestive efficiency was reported for animals receiving the 15.3% CP and 39.7% solubility diet. In a companion study, Holter et al. (81) examined these effects over an entire lactation. Cows receiving the 15.3% CP diets produced 196 kg more milk than those on the low protein diets. Cows receiving the reduced solubility diets produced 347 kg more milk than those fed the high solubility diets. Income over feed costs for the lactation were highest and postpartum weight loss least for cows receiving the 15.3% CP and low solubility diets in early lactation.

Zerbini and Poian (231) fed fifty bull calves either a control diet with 11.6% CP or one of four treatment diets (15.5% CP) containing protein sources varying in degradability. Protein sources were soybean meal, corn gluten meal, cottonseed meal, or fishmeal.
Body weight gains were increased an average of 17% with added protein. Soybean meal and fishmeal diets showed the highest rates of gain .815 and .844 kg/d, respectively. The researchers concluded fishmeal and soybean meal were better protein sources than corn gluten meal or cottonseed meal for growth of ruminating calves. Quigley and Bearden (170) studied the effects of protein concentration (14 vs. 16% CP) and ruminal undegradable protein (35 vs. 45% of total N) in calf starters on intake and gain of 32 Holstein calves between 4 and 12 wk of age. Reduction of dietary protein concentration improved efficiency of protein utilization, but did not affect intake or daily gain. Their results indicated overfeeding protein and(or) undegradable protein may depress development, growth, and ruminal function of young dairy calves. A critical analysis of protein allowances for growing ruminants was conducted by Rohr et al. (175). They reported optimum supply of protein for dairy heifers (300 kg BW, .65 kg ADG) on high fiber diets (50-70% straw, 30-50% concentrates) was 550-600 g CP/d when fishmeal was the main protein source and the diet provided 150-200 g/d undegradable protein. Average daily gain decreased when soybean meal replaced fishmeal and declined further when urea was added to the diet. A study conducted at North Carolina State University (76) measured response in growing beef cattle when soybean meal was replaced by corn, urea, and(or) blood meal. Cattle receiving soybean meal or urea as protein sources gained .78 and .55 kg/d. Feed cost per kilogram of gain was lowest at 60.6 cents for soybean meal diets, while blood meal and urea were 64.6 and 80.5. Klopfenstein and Goedeken (93) measured the effects of diets supplemented with urea, soybean meal, corn gluten meal, or blood meal on growth and feed efficiency of beef calves. Their results indicated calves receiving diets containing blood meal required only 60% as much supplemental protein as those receiving soybean meal. Calves receiving either soybean meal or blood meal supplemented diets were intermediate in daily gain (.77 kg ADG) to those receiving a
corn gluten meal-blood meal mixture (.81 kg ADG) or urea supplementation (.68 kg ADG).

Erdman and Vandersall (56) fed diets containing two levels of protein degradability at 14.3% CP in diet DM to 24 early lactation Holstein cows. Concentrates were formulated to have low protein degradability (52.9% of total N) or high degradability (72.8% of total N). Solubility of low and high degradability diets were 31.6 and 40.3% of total N. Diets had no effect on DM intake, milk yield, or fat percent. These researchers questioned the efficacy of diet formulation based on protein degradability for the early lactating cow. Hawkins and Strength (77) reported milk yield did not differ when cows received diets varying in soluble protein from 29 to 42% of total N. Using heat-treated soybean meal to decrease soluble protein, Grummer and Clark (74) fed diets varying in solubility from 24.6 to 34.1% to 20 early lactation (2 wk postpartum) Holstein cows. No significant response was measured in milk yield or composition due to these treatments. Other studies have shown no effect on growth of cattle or N-metabolism in sheep when dietary protein solubility was reduced (26, 58, 64, 207).

Grummer and Clark (74) suggested a number of factors which may be responsible for the variable results seen when regulating dietary protein solubility or degradability. These factors were: 1) greater dietary N escaping degradation, 2) improved patterns of amino acids reaching the small intestine, 3) inadequate NPN intake, 4) greater soluble carbohydrate intake stimulating greater microbial protein synthesis.

Crooker et al. (46) suggested the failure to increase animal productivity through protection of dietary proteins against ruminal degradation could result from; a) factors other than absorption of essential amino acids limit productivity, b) the protected protein is of low biological value, c) the protein is protected inadequately, d) the protein is over-
protected, e) the protected protein is naturally resistant to microbial degradation, and f) microbial protein production in the rumen is decreased. Additional factors that may minimize animal performance responses to protected protein include pattern of amino acids absorbed from the intestine, energy effects of protein, feed consumption, and dietary energy concentration.

To date, research indicates that under most feeding conditions manipulation of dietary protein solubility and degradability affects lactational or growth performance of the ruminant animal. Nocek and Russell (151) and others (155, 156, 191, 200) suggest protein degradation is vitally important to the nutritional status of the animal. More importantly, however, is the synergistic response between protein and energy yielding nutrients within the rumen and within the ruminant body to the overall pattern of nutrient utilization. Nocek and Russell (151) indicated changes in rumen available protein appeared to have less dramatic effects on production performance than modifications of energy, but the two were highly interdependent.

**Carbohydrate Classification and Digestion**

Carbohydrates make up 70 to 80% of DM in a typical dairy cattle ration. Therefore, carbohydrate availability in the rumen and to the animal can greatly affect overall pattern of nutrient use. Although plant carbohydrates are composed of various molecular compounds, they can be classified as nonstructural and structural. Nonstructural carbohydrates are often water soluble and are composed of sugars, starches, and pectins. Pectins are often associated with the cell wall and are precipitated in neutral detergent solution, but are not covalently linked to the lignified portion of the wall. Galactans are unique to legumes and replace starch as a carbohydrate reserve, while fructosans are the
storage material in temperate grasses. Starches are mainly found as storage carbohydrates in grains. Free sugars or soluble carbohydrates are either present as monosaccharides or oligosaccharides containing two to six glucose units (118).

Structural carbohydrates are insoluble in neutral detergents and contain the cell wall material. Depending on maturity and plant species the ratio of cellulose, hemicellulose, and phenolic lignin compounds can vary greatly. These cell wall components comprise the insoluble digestible and nondigestible fractions, with the size of the nondigestible fraction partially related to lignin content (151).

All carbohydrates are composed of repeating units of sugars or sugar derivatives, but due to their complexity, little is known about the molecular structure of complex carbohydrates. The ability of individual sugars (i.e. glucose, xylose, arabinose, galacturonic acid, glucuronic acid) to form several types of bonds with other units and the inability of reagents to create sequential or specific degradation, have confounded the study of plant carbohydrates (6).

Degradation of carbohydrates differs between structural classes. Structural carbohydrate degradation requires a lag phase during which rumen bacteria adhere to insoluble substrate while appropriate enzymes are synthesized in sufficient concentrations (127). During this lag period an extra-cellular slime layer is formed which possibly facilitates adhesion of bacteria to feed particles. Development of this layer is promoted by soluble carbohydrates, which explains why small amounts of soluble carbohydrates promote digestion of structural carbohydrates (37). Nonstructural carbohydrates do not require a lag phase thus their degradation is rapid and starts immediately after feeding. It is assumed that free sugars are degraded almost instantaneously, followed by a rapid
degradation of other insoluble-degradable nonstructural carbohydrates such as fructosans and starches (200).

Proportion of carbohydrate degradation in the reticulo-rumen is dependent on amount of carbohydrate which is potentially degradable, rate of degradation, and rate of carbohydrate passage through the rumen. Tamminga (199) concluded that under normal conditions 90% of digestion of cellulose, hemicellulose, pectic substances, and sugars take place in the reticulo-rumen. The proportion of starch and fructosans digested was lower and ranged from 75 to 100% for starch, and 65 to 75% for fructosans.

Of the digestible structural carbohydrates, the proportion not digested in the rumen is thought to be degraded by microbial fermentation in the lower gut. Ulyatt et al. (211) found a negative relationship between proportion of structural carbohydrates totally digested and proportion digested in the hind gut. Digestion of nonstructural carbohydrates, particularly starch, is not restricted to microbial fermentation in the reticulo-rumen or large intestine, but may also occur in the small intestine, but not unlimited. Orskov (158) estimated the capacity of the small intestine to digest starch is limited to about 20% of metabolizable energy (ME) requirement in lambs, calves and steers, but to only 11% of ME required by lactating dairy cows. The absorptive capacity for glucose was estimated to be somewhat higher at up to 25% of ME required for lambs, calves, and steers, and up to 14% of ME for lactating dairy cows.

Tamminga (200) reported carbohydrates by-passing the rumen are subjected to enzymatic digestion in the small intestine and microbial degradation in the cecum and colon. Enzymatic digestion is practically limited to nonstructural carbohydrates. Limited ability to hydrolyse starch, as indicated by Orskov (158), may be the result of intestinal pH below the optimal for pancreatic alpha amylase. This decreased intestinal
starch digestion was not offset by increased fermentation in the hind gut, as a low intestinal pH resulted in high starch excretion in the feces.

Both structural and nonstructural carbohydrates may be digested via hind gut fermentation. Site of intestinal digestion and absorption affects the nutritive value of carbohydrates. Hind gut fermented carbohydrates result in volatile fatty acids as nutrients for the animal and fermentation losses (i.e. CH₄, microbial protein, NH₃) which lower the nutritive value of carbohydrates when compared with those digested and absorbed (mainly as glucose) from the small intestine.

Given differences in carbohydrate components and degradability it becomes apparent that through selection of various feedstuffs, growth of and degradation by rumen microflora may begin to be controlled. This control of carbohydrate degradation is, however, dependent upon accurate procedures capable of estimating carbohydrate fractions and degradability within the reticulo-rumen.

**Measurement of Carbohydrate Degradability**

Degradability of carbohydrates within the rumen has been estimated by both in vitro and in vivo methods. These methods fractionate carbohydrate components of feedstuffs into soluble, digestible, and undigestible fractions. Several studies (18, 35, 48) have reported on measurement of solubility of carbohydrates as indicators of ruminal availability. Soluble carbohydrate extraction methods included hot water extraction or acid hydrolysis of cold water extract and reaction with ferricyanide (18, 121). These techniques suffer the same limitations as those for determination of soluble protein, e.g. there are several carbohydrate fractions that vary in degree of ruminal degradation in relation to quantity of soluble carbohydrate. An additional problem is that chemical constitu-
ents of soluble carbohydrates vary considerably (6, 118). As previously indicated, different soluble carbohydrate sources are peculiar to specific plant and grain types. Therefore, no one chemical analysis, like N, will identify all the various carbohydrate fractions.

Enzymatic techniques have been utilized in fractionation and quantitation of both structural and nonstructural carbohydrates. Using Taka-diastase, Smith (186) measured total nonstructural carbohydrates in forages. Taka-diastase is an alpha-amylase derived from Aspergillus oryzae and is capable of more than 30 different enzymatic functions, not only amylolytic, but also proteolytic and lipolytic (151). Bacillus subtilis has a type A amylase specific for alpha-1 to 4 glucosidic linkages of polysaccharides (starch). Use of an enzyme from a source such as Bacillus subtilis may underestimate the quantity of readily fermentable carbohydrates in forages and certain by-products due to their high content of pectins (glucuronic acids) and reducing sugars and its inability to cleave their glucosidic linkages.

Use of enzyme procedures to simulate ruminal fiber digestion generally results in less solubilization of DM than does use of ruminal microbes (122). Nock and Hall (150) studied the effectiveness of cellulase, pectinase, and hemicellulase on digestion of soyhull cell walls. They observed lower cell wall digestion with enzyme combinations compared to in situ values. Hungate et al. (83) used cellulase, alpha-arabinosidase, xylanase, and polygalacturonase (PGase) to evaluate alfalfa cell wall digestion. When incubated separately, PGase was the most effective in digesting cell walls. Purified cellulase was relatively ineffective. When a combination of PGase and alpha-arabinosidase were used, amount digested exceeded the sum digested by the two separately. These studies suggest a synergism exists between cell wall digesting enzymes and that mixtures of enzymes may be necessary to simulate ruminal fiber digestion.
Enzymatic digestion techniques may be more suitable to measuring relative differences between feedstuffs than providing absolute digestibility values. Accuracy of prediction seems to depend upon chemical structures of the forage or feedstuff in question, and synergism between enzymes used in the incubation.

**In Vitro and In Situ Methods of Carbohydrate Fractionation**

Methods incorporating the use of rumen fluid offer potential in estimating ruminal substrate digestion. The single or two stage Tilly and Terry (205) system is the most commonly used in vitro digestion system. The two stage system consists of a 48h digestion period with rumen fluid to estimate ruminal digestion followed by a second 48-h digestion period using pepsin and weak acid to simulate postruminal digestion. Nocek (146) reported a correlation of .81 between in vivo nylon bag digestibility estimates and those from in vitro by (205). Van Soest (214) indicated major disadvantages to this method were number of steps and length of time required for analysis.

The sequence for all in vitro rumen procedures is an anaerobic fermentation of a sample substrate with rumen liquor followed by an end-point measurement. End-point procedures include measurement of gas production, volatile fatty acid production, cellulose disappearance, residual DM, the residue after pepsin digestion, and neutral detergent residue (214). Sepadiet of the various end-point products can give estimates of various nutritive components within carbohydrates and proteins.

In situ bag techniques are probably the most popular means of estimating feedstuff digestibility. Nocek and English (148) discussed the use of this procedure for fractionation of carbohydrates into ruminally soluble, degradable, and undegradable
components. With this technique, they were able to quantitate the various fractions as well as determine rate of digestion of the degradable fraction. Errors associated with in situ dacron bag techniques are the same for estimation of carbohydrate digestion as for protein digestibility (i.e. bag porosity, sample size, particle size, and animal and dietary effects). Nocek and Russell (151) concluded these factors create similar variability in estimates of soluble, degradable, and undegradable carbohydrate disappearance as was found for protein. Mehrez and Orskov (123) reported the most important factor creating variability in disappearance from artificial fiber bags was the sample size in relation to bag size. They also reported considerable variability between animals and day of incubation in their study, but found this technique to be satisfactory as a simple and fairly rapid means for measuring nutrient disappearance from the rumen.

In vivo techniques are most commonly used for determining ruminal availability of proteins, but can be used for carbohydrates (151). In vivo determinations of nutrient availability have generally been considered as the standard to which other techniques are compared. This method is by far the closest to physiological conditions. As with other methods of estimating digestibility, several disadvantages exist. Digesta flow and microbial markers are extreme sources of variation (151). MacRae (119) indicated a coefficient of variation of 5 to 20% existed for digesta flow measurements, and that 50 to 95% of the variation could be attributed to between animal differences. Sutton and Oldham (194) determined that to detect a 10% difference in treatment response using in vivo procedures, two 6x6 Latin square design experiments would need to be conducted. It therefore becomes readily apparent that potential for error in digestibility estimates is high when determined using in vivo procedures.
Nocek and Russell (151) introduced a simple method of estimating the soluble carbohydrate fraction of feedstuffs. They suggested the following equation for estimating nonstructural carbohydrate content:

\[ \text{NSC} = 1 - (\text{NDF} + \text{CP} + \text{EE} + \text{ASH}) \]

where:

\( \text{NSC} \) = Nonstructural carbohydrate content, (%);

\( \text{NDF} \) = Neutral detergent fiber, total structural carbohydrate, (%);

\( \text{CP} \) = Crude protein, total N content, (%);

\( \text{EE} \) = Ether extract, total fat content, (%);

\( \text{ASH} \) = Sample mineral content, (%);

Using this equation, they concluded that one should be able to formulate diets accounting for the synergistic response in rate of protein and carbohydrate availability. Expanding upon this premise, they developed an equation for estimating ruminal total carbohydrate availability;

\[ \text{RAC} = \frac{[.9 \times (\text{NDS} - (\text{CP} + \text{EE})) + (\text{NDF} \times \text{NDF availability})]}{[(\text{NDS} - (\text{CP} + \text{EE})) + \text{NDF}]} \]

where:

\( \text{RAC} \) = Rumen available carbohydrate, (%);

\( \text{NDS} \) = Neutral detergent solubles, \((100 - \text{NDF})\), (%);

\( \text{NDF} \) = Neutral detergent fiber, (%);

\( \text{CP} \) = Crude protein, (%);

\( \text{EE} \) = Ether extract, (%);
Based on unpublished work from their laboratory, they reported the nonstructural carbohydrate fraction was almost completely digested in the rumen by 24h, thus the correction of available carbohydrate by .9. Using this equation, they reported RAC values varied greatly within and between feedstuffs. Concentrate feeds were reported to vary between 47.4 and 90%, while forages varied between 27.3 and 90.4%. These authors indicated that using a dynamic model which incorporates in situ and in vitro techniques may help to expand the database needed to clarify the balance between protein and carbohydrate digestibility.

As shown in the discussion on measurement and feeding of different proportions of protein fractions, carbohydrate degradability and solubility in the rumen are important factors which need careful considered diet when formulating diets for ruminants. At this time, no one procedure is capable of fractionating carbohydrates into components important to formulating diets on a protein and carbohydrate rate of digestibility basis. Equations presented by Nocek and Russell (151) provide a basis for formulating diets accounting for carbohydrate availability. By considering carbohydrate and protein availability, diet formulation accounting for the interrelationships between these components should become possible.

**Protein and Energy Interrelationships**

To this point, milk production and growth responses to protein and carbohydrates have been discussed separately to emphasize their individual importance. However, the interrelationships between protein and energy yielding nutrients both within the rumen and the ruminant-body can have profound effects on overall pattern of nutrient use. A number of review articles (151, 155, 156, 200) reported on the importance of accounting...
for the synergistic response between protein and carbohydrate digestion when formulating rations. Oldham (155) suggested energy and protein needs are linked together in a formal, predictive manner. The link or synergism is the prediction of microbial protein yield from intake of ruminally degraded N and organic matter. Oldham (155) reported that DM intake may be responsible for the positive relationship between protein intake and performance. Natural protein supplements, especially those low in soluble N, stimulate feed intake above diets containing urea or highly soluble N (88, 99, 151). Others (41, 45, 191, 200) demonstrated that increasing dietary protein improved milk yield and daily BW gain (13, 24, 84) by increasing energy intake. A slight or lack of response resulted when an increase in dietary protein intake did not result in increased energy intake (151, 155, 200). Tyrrell (209) reported that increasing crude protein concentration of isocaloric diets from 11 to 20% of diet DM increased digestibility of the diet by overriding the expected depression of digestibility due to increased intake. Nocek and Russell (151) indicated the stimulating effect of protein appeared to involve a cycle of improved efficiency, increased dilution rate, increased feed intake, and subsequently, increased energy intake. He concluded that other factors such as increased amino acid supply to the small intestine and improved amino acid balance probably play a lesser role to energy intake. Certain nutrients (i.e. NH₃, amino acids, sulfur, micro-nutrients, co-factors) may limit microbial growth (200). If supply of nutrients is adequate, microbial growth is limited by energy supply. Even under conditions of an adequate nutrient supply, energetic efficiency of microbial growth varies (191). Reports (151, 155, 156, 200) indicated that source and digestibility characteristics of both diet protein and carbohydrate fractions affect efficiency of rumen biomass formation. Microbial growth requires energy (ATP) for two processes. First, ATP is needed for maintenance processes such as motility, turnover, production of extracellular macromolecules (proteins, carbohydrates), active transport and lysis, and resynthesis of macromolecules constitut-
ing the various cell components. Rate at which ATP becomes available controls the ratio of ATP use for maintenance and synthesis and thus overall growth rate. Critical factors to availability of ATP genediet are the availability of carbohydrate and nitrogenous compounds, ruminal turnover rate, and availability of carbohydrates and proteins in relation to each other (151, 155, 191, 200). Oldham and Alderman (156) reported mixtures of starch and cellulose supported higher efficiencies of microbial growth than either substrate alone. Similarly, mixed roughage-concentrate rations were found to maintain higher efficiencies than either high concentrate or high roughage diets (151, 191, 200). Oldham and Alderman (156) concluded that for effective capture of degraded N in the rumen it is necessary to match rate of release of N-substrate with rate of availability of energy from rumen fermentation. It is, therefore, the combination of source of dietary N and dietary carbohydrate which is important in determining net outflow of amino acids from the rumen (155).

**Protein and Energy Interactive Response.**

In an attempt to maximize ruminal microbial protein synthesis, efficiency, and flow of protein to the small intestine, Casper and Schingoethe (31) fed lactating dairy cows diets varying in ruminal carbohydrate solubility and protein source. Soluble carbohydrate sources were corn, barley, and dried whey, while protein sources were soybean meal and urea. Rations were fed totally mixed and were isonitrogenous at 16% CP. Milk production was similar for corn and dried whey diets (30.0 and 29.5 kg/d) but lower (27.9 kg/d) for cows fed barley. Solubility of protein did not affect milk production (32.2 and 31.5 kg/d, SBM vs urea) or 4% fat corrected milk. Intake of DM was lowest for cows fed barley (18.8 kg/d vs. 20.4 and 20.5 for corn and dried whey) with intakes similar between protein treatments (19.9 kg/d). They concluded that matching carbohydrate and
protein solubility did not improve substrate utilization and hence milk production. Herrera-Saldana and Huber (79) used a 2x2 factorial arrangement of grain and protein sources varying in rumen degradability to study response of carbohydrate and protein degradability on lactating cow performance. Rations were composed of barley, cottonseed, barley-brewers dried grains, milo-cottonseed, and milo-brewers dried grains. Rumen degradable protein levels were 59.5, 43.7, 55.9, and 35.4% and rumen degradable starch was 74.7, 69.9, 62.3, and 48.3% for the four treatments as listed. Milk production was highest for the barley-cottonseed diet (37.4, 34.9, 34.2, and 34.6, respectively) while milk fat was higher on milo than barley diets (3.1, 2.9, 3.4, and 3.6% BF). These researchers concluded: 1) productivity of dairy diets containing similar protein and available energy can be altered by ruminal degradability of protein and starch; 2) protein of higher degradability does not always adversely affect milk production provided there is synchronous release of starch; 3) rumen escape of slowly degradable protein and starch may be compensated for by increasing the amount of natural protein and starch available for digestion and absorption in the small intestine.

McCarthy et al. (119) investigated effects of source of protein (fishmeal vs. soybean meal) and carbohydrate (corn and barley) on ruminal fermentation, nutrient flow to the small intestine, and animal performance. Treatments were in a 2x2 factorial arrangement. Dry matter and starch intakes were greater when corn was fed rather than barley. Barley-based diets were more extensively degraded in the rumen, thus providing more energy for microbial growth than corn-based diets. However, passage of amino acids and starch to the duodenum was greater for corn-based diets due to higher intake and lower ruminal degradability. Microbial protein contributed a greater portion of the total N and strongly influenced post ruminal amino acid pattern when compared to either fish or soybean meals. Results indicated corn-based diets stimulated higher milk production.
(35.9 and 35.2 vs. 32.4 and 32.6 kg/d) than diets containing barley as the carbohydrate source, regardless of protein supplement.

Stern et al. (191) examined effects of nonstructural carbohydrate (NSC 49.0, 32.6, 19.6% of DM), urea, and soluble protein levels on microbial protein synthesis in continuous culture. In their first experiment they found ADF digestibility increased as starch content decreased in isonitrogenous diets (19.2, 18.8, 18.7% CP in DM), while DM digestibilities were not affected by treatment. Although digestion coefficients for crude protein (94 to 98%) were not affected by level of starch supplementation, substitution of starch for cellulose decreased ammonia levels and increased microbial protein synthesis. For their second trial, the high starch diet of trial one (49% NSC) was used as a basai diet. Four isonitrogenous diets (17.7% CP) were formulated to vary in level of dietary urea and protein solubility (36.1 and 51.2% soluble N with urea, 22.7 and 36.0% soluble N without urea). Protein solubility was varied by using heated and unheated peanut meal. They concluded ADF digestibilities were not affected by urea feeding or protein solubility. Dry matter digestion coefficients were slightly lower for urea diets than non-urea diets, with ammonia levels higher on urea diets. Urea and protein solubility did not affect microbial growth when expressed as grams CP synthesized per 100 g digestible organic matter. However, grams microbial protein synthesized daily was significantly lower for urea containing diets. Therefore, Stern et al. (191) concluded that source of carbohydrate and its rate of availability to rumen microbes play an important role in nitrogen utilization.

While a number of studies have evaluated protein-energy interrelationships in lactating animals, few studies have applied this concept to growing animals. An early study by Brown and Lassiter (24) monitored growth and intake of dairy calves receiving diets varying in CP (14, 16, 18% of DM) or protein-to-energy ratio (1:46, 1:48, 1:50, %
CP: Estimated Net Energy. Protein levels were adjusted by supplementing urea or soybean meal, while energy levels were adjusted by incorporating animal fat. Calves receiving isocaloric diets varying in CP showed very little difference in growth. The ratio of protein-to-energy appeared to influence growth rates as body weight gains decreased (.689, .685, and .581 kg/d ADG) with increasing protein-to-energy ratios. Average daily gain of calves receiving the 1:50 ratio were significantly below gains of calves receiving the other ratios. Feed efficiency was also lowest on diets containing the 1:50 protein-to-energy ratio. These results suggest protein level may not be as critical in formulating diets for growing calves as availability of the protein in relation to energy availability.

Jahn and Chandler (84) reported on performance of calves receiving diets varying in protein and fiber. Diets were formulated with four levels of protein (9.0, 11.5, 14.5, and 17.5% CP) and three levels of ADF (11, 18, and 25% of DM) with response measured on intake, body weight gain, and body composition. Significant protein by fiber interaction for body gains, corrected for digestive fill, indicated response to added protein depended on the amount of diet fiber. Protein intakes for maximum live weight gains for 11, 18 and 25% ADF were .47, .66, and .57 kg/d, producing gains of .97, .91, and .76 kg/d. Increasing protein from 9 to 14.5% increased DM intake at all fiber percentages while decreasing DM intake as protein was further elevated in 11 and 25% ADF diets. Efficiency of transformation of dietary protein into tissue protein (g CP/g tissue protein) was 33.8, 31.7, and 25.4 for 11, 18, and 25% ADF treatments. Tissue accumulation increased markedly at all fiber levels as dietary protein was increased from 9 to 17.5% for the 18% ADF ration, while maximum energy accumulation occurred at 14.5% CP for the 11 and 25% ADF treatments. This study showed calves were most efficient in converting dietary substrates to tissue protein when diets contained 14.5% CP, or higher, and contained high nonstructural carbohydrate as reflected by the low ADF content.
Amos (2) evaluated the balance of protein and energy supplied to growing heifers and steers on growth and feed efficiency. He utilized a 2x2 factorial arrangement of rumen undegradable protein (36 or 70% of total N) and dietary energy concentration (95 and 105% of NRC 1978) as treatments. He concluded that increasing energy density of the diet and protein escaping rumen degradation resulted in equal or higher rates of daily gain with a marked improvement in feed efficiency. Feed requirement per unit of gain was approximately .6 kg/d less for diets high in undegradable protein. Performance was consistently lowest for animals receiving diets high in energy and low in undegradable protein. Apparently, increasing energy intake without a shift toward greater protein undegradability created a shift in microbial efficiency and availability of substrates to the animal. Various workers (98, 109, 116, 123, 187, 212) have studied the effects of varying protein and carbohydrate degradability on sheep, dairy heifers, and lactating dairy cattle. These workers concluded that the synergistic response between rumen degradable protein and energy has a significant effect on microbial growth efficiency and overall performance of the animal.

Feeding Management

There are a number of feeding management strategies which when implemented should optimize ruminal and animal efficiency and performance.

1. Formulate diets for adequate amounts and types of carbohydrates and proteins. Nutritionists must begin to think in terms of providing a variety of carbohydrate types which degrade at different rates in the rumen. Carbohydrate degradation must be synchronized with protein types and amounts so that both can be most efficiently utilized by the microbes.
2. Total Mixed Rations. Blending of feed ingredients is the best method of ensuring animals receive a proper blend of carbohydrate and protein at each feeding.

3. Forage:Concentrate Ratio. By altering this ratio one can effectively shift the concentration and form of soluble, degradable, and undegradable carbohydrates and proteins.

4. Particle Size. The particle size of both forages and concentrates affect digestion and passage rate from the rumen. Incorporation of long fibers, such as long stem hay, within the diet tends to increase starch escaping rumen degradation and helps to buffer rumen pH.

5. Feeding Frequency. Increasing feeding frequency of a TMR often shows limited improvements in production. This result is reflective of the constant variety of carbohydrate and protein forms available to the rumen. However, when forages and concentrates (nonstructural carbohydrates) are offered separately "shocking" the microbial population is likely to occur. Feeding more frequent meals of each component may serve to stabilize the rumen environment and increase the efficiency of substrate utilization. Computerized feeding strategies offer the flexibility of matching carbohydrate and protein degradabilities to each other and as complements to the forages.

6. Feeding protein and nonstructural carbohydrate sources together. Application of this strategy provides the microbial environment with nitrogen, carbon skeletons, and energy for efficient microbial protein synthesis.
To date, no studies have established standards for matching carbohydrate and protein availability. However, animal response indicates a synergism exists between these substrates with marked improvements in performance and efficiency when diets are formulated to contain moderate amounts of nonstructural carbohydrates and ruminally undegraded protein. Future nutritional significance of this phenomena will be dependent upon greater experimental evidence, availability of rapid and simple methods of determining rumen carbohydrate and protein degradability, and need for improved production and growth efficiency.
A number of factors served as the basis for this research. Prior to completion of my Masters degree research the National Research Council released new guidelines recommending formulation of diets for growing dairy replacements using the degradable/undegradable protein system outlined by (NRC 1985). Recommendations for undegradable intake protein (UIP) were not, however, practical or achievable using common feedstuffs (i.e. 200 kg BW, 60 kg/d ADG, recommendation: 16% CP, 287 g UIP). Our interest was upon the influence of increasing dietary UIP on heifer intake, growth, and feed efficiency.

It was also at this time that an independent data set was needed to validate a DM intake prediction equation (for dairy heifers) developed as part of my Masters degree research. Therefore, Trial 1 was conducted with 2 objectives; 1. Investigate the validity of undegradable protein supplementation for growing dairy heifers by replacing soybean meal with dried brewers grains in conjunction with two levels of energy concentration; 2. Continue management of feeding trial heifers as one group up to and through first lactation evaluating possible effects of dietary treatment during the prepubertal period on subsequent lactation performance.

Results from Trial 1 were somewhat unexpected. We found that heifers receiving low energy diets (93% of NRC TDN) supplemented with dried brewers grains had depressed DM intake yet grew at rates comparable to heifers on high energy diets (109% of NRC TDN). These heifers were also more efficient in apparent conversion of DE to gain than their counterparts receiving soybean meal. When Trial 1 was completed, A. J. Heinrichs and L. D. Muller at Pennsylvania State University approached Dr. James and me about
conducting a simultaneous study within the objectives of NC-119 (North Central Region Research Project).

Therefore, Trial 2 was conducted to evaluate heifer performance and change in body composition (water, fat, and protein) due to a wider range of dietary UIP. Diets were formulated to be isocaloric and isonitrogenous isolating any differences in response on protein undegradability. Heifer performance in intake and growth was similar to Trial 1. No differences were found in body composition due to treatment effects. Upon evaluation of my Masters research and Trials 1 and 2, it became apparent that response to undegradable protein may be affected by ruminal availability of nonstructural carbohydrates (NSC). In addition, recent research with lactating animals suggested a synergistic response existed between the degradable fractions of protein and NSC. Trial 3 was conducted in the interest of evaluating response to varying levels of UIP and NSC. To achieve this goal, three concentrations of UIP (30, 40, 50% of total N) and three concentrations of NSC (17, 22, 26% of diet DM) were utilized. Urea space and real time ultrasonic scans were used to estimate change in body composition of water, fat, and protein, and growth in longissimus muscle area and backfat thickness.
Trial 1

Diet TDN and degradable protein impact on intake, daily gain, and subsequent lactation of Holstein heifers.

Abstract

Thirty Holstein heifers were assigned to one of four groups based on initial BW for the 105 d feeding trial. Average ages were 259, 221, 190, 160 d for four treatments and mean initial weights were 242, 196, 157, 142 kg. Treatments consisted of all combinations of two levels of TDN (95%, 115% of 1978 NRC) and two levels of rumen undegradable intake protein (UIP)(30%, 40%, estimated undegradability). Total mixed rations were composed of corn silage, chopped orchardgrass hay, high moisture corn, soybean meal, dried brewers grains, and minerals and formulated for .68 kg/d ADG. Treatment UIP levels were achieved by replacement of soybean meal with dried brewers grains. Mean DM intake (g/kg BW\(^{0.75}\)) was 96.6, 96.1, 94.5, 84.0, for low UIP-high TDN, high UIP-high TDN, low UIP-low TDN, high UIP-low TDN, respectively. Daily gain (kg/d) was .94, .94, .73, and .91. While DM intake was lower in the high UIP-low TDN treatment, performance was similar to high TDN treatments. Megacalories of DE intake/kg BW gain were 27.0, 24.2, 25.1, 15.1, reflecting an apparent improvement in feed efficiency in the high UIP-low TDN treatment. First lactation peak FCM yield was 31.5, 34.6, 32.8, 32.1 kg/d. Mature equivalent (ME) FCM was 10365, 10057, 9532, 9733 kg. Somatic cell scores were 2.10, 2.14, .55, 1.81. Treatments did not differ for SERV, Peak FCM, ME FCM, or somatic cell score. Protein undegradability did not appear to
influence intake, growth, or lactation of heifers receiving high TDN rations. Increased UIP content improved daily gain and ME FCM of heifers receiving low TDN treatments. Culling as heifers or during first lactation was not different between treatments.
Introduction

The objective of replacement dairy heifer feeding regimes should be maintenance of desirable growth rates to provide a mature animal at time of calving capable of producing to her genetic potential. To achieve optimum production potential, Holstein dairy heifers should obtain 40-45% of mature weight at time of breeding (18)(References on pg. 96) and weigh 550 to 600 kg prior to calving at 24 mo (22). Heinrichs and Hargrove (8) reported Holstein heifers in herds with a rolling herd average (RHA) of 7312 kg averaged 515 kg while those in herds with a RHA of 8056 kg averaged 526 kg BW at calving (24 mo). Their data support the positive association between heifer growth and herd production. Kertz (9) described and recommended a feeding program supporting higher daily BW gains (.9 to 1.0 kg/d) producing heifers weighing about 635 kg before calving at 24 mo.

Rapid growth rates of pre-pubertal heifers may not, however, be advantageous when considering life-time milk producing ability. Studies (3, 18) determined rapidly grown heifers (1.1 kg/d ADG) produced less milk than conventionally grown heifers (.8 kg/d ADG) in first, second, and third lactations. Sejrsen et al. (17) and Tucker (20) indicated heifers raised on a high plane of nutrition (> .6 kg/d ADG), during the allometric phase of mammary development, had less mammary secretory tissue than heifers raised at a lower rate of growth (.4 to .6 kg/d ).

Recent NRC (14) recommendations suggest increasing undegradable intake protein (UIP) to meet the needs of faster growing dairy replacements. Zerbini and Polan (23) found dairy bull calves receiving diets varying in crude protein (11.6 and 15.5 % of DM) and protein source, showed equal body weight gains with improved feed efficiency on
lower ruminally degradable protein sources. Bagg et al. (2) and Cummins et al. (4) suggested that dairy replacements receiving diets with increased levels of total protein (120% of (12)) or UIP > 40% of CP had average daily gains equal to or greater than heifers receiving conventional diets (100% of NRC CP or UIP ≤ 30% of CP). Replacements receiving higher UIP diets also tended to have lower body condition scores and improved feed efficiency.

The objective of this study was to measure effects of diets varying in protein undegradability and energy concentration on intake, daily gain, wither height growth, initial reproductive efficiency, and first lactation performance of Holstein heifers.

**Materials and Methods**

**Feeding Trial**

Thirty Holstein heifers were assigned to one of four groups based on initial BW. Mean initial BW were 242, 196, 157, 142 kg. For the 105 d trial period, heifers were housed in a counter-slope total confinement facility containing four 3.6m x 9.1m pens, each fitted with a Pinpointer 4000B computerized feeder (UIS., Inc. Cookeville, TN). Feeders contained a large hopper capable of storing one day's equivalent of total mixed ration. Each feeder was used to record individual heifer daily as-fed intakes within a treatment group. Prior to the feeding trial, heifers were permitted 10 to 14 d to become acclimated to the Pinpointer facility and trial rations. Heifers were permitted access to feeders at all times except during pen cleaning and and animal weighing. Treatments (Table 1) consisted of all combinations of two levels of TDN (95 and 115% of (12)) and two levels of rumen undegradable intake protein (UIP)(30 and 40% of CP). Total mixed rations were composed of corn silage, ground orchardgrass hay (mean length = 7 cm), high
moisture corn, soybean meal, dried brewers grains, and a mineral premix. Diets were formulated for .68 kg/d ADG according to (12). Treatment UIP levels were achieved by replacing soybean meal with dried brewers grains. Protein undegradability estimates (% of total N) for ingredients were: corn silage (27%), alfalfa haylage (25%), orchardgrass hay (42%), high moisture corn (60%), soybean meal (26%), dried brewers grains (48%), and corn gluten meal (57%) (13). Diets were provided daily at 1350 h for ad libitum intake and refusals of 5 to 15%/d. Treatment diets and ingredients were sampled 4 times per week and stored at 4°C prior to compositing for weekly analysis in the Virginia Tech Forage Testing Laboratory. Diets and ingredients were analyzed for DM, CP by (1), ADF, and NDF according to (7). Total digestible nutrients (TDN) was predicted from ADF as reported by (19). Digestible energy intake was predicted from TDN: 1kg TDN = 4.409 Mcal DE (12).

Body weight and wither height (WH) were recorded at 930h, 2 d consecutively at beginning and end of the feeding trial, and biweekly during the trial. An index of change in body condition (INDEX) between the beginning and end of study was the difference between ending and beginning ratios of BW/WH.

Compensatory Period

Following the feeding trial, heifers receiving low energy treatments (95% of (12)) were fed 120% of (12) CP and TDN for 7 wk to promote compensatory growth. Heifers receiving high energy diets (115% of (12)) during the feeding trial were fed 100% of (12) TDN and CP for the 7 wk period. Body weight and WH were measured biweekly. After 7 wk, heifers were moved to a loose housing facility and managed as one group to maintain BW gain of .4 to .6 kg/d ADG. For the period between the feeding trial and calving, heifers received corn silage, and orchardgrass hay, with access to
orchardgrass/foxtail pasture. Services per first conception were recorded. Heifers calved between August and November 1989.

**First Lactation**

Upon calving, heifers continued to be managed as one group receiving the diet ad libitum as outlined in Table 2. Daily milk yield was measured electronically with monthly fat test, somatic cell score (SCC), mature equivalent milk (ME MILK), mature equivalent fat corrected milk (ME FCM), peak milk production (PEAKM), and days to peak production (PEAKD) obtained from DHI.

Statistical analysis of data was conducted by least squares regression procedures as outlined in (10, 16). Analysis of treatments was by orthogonal contrasts among least squares means. Contrasts were: SBM/HTDN vs. DBG/HTDN, SBM/LTDN vs. DBG/LTDN, and the average of LTDN vs. HTDN treatments. Tests for differences in dependent variables DM intake, DE intake, and daily BW and WH gain were determined using the following model:

\[ Y_{ijk} = \mu + W_i + T_j + H_{(j)k} + (WT)_{ij} + E_{ijk}, \text{ where:} \]

- \( Y_{ijk} \) = Dependent variable of heifer k in treatment j for week i.
- \( \mu \) = Population mean.
- \( W_i \) = Fixed effect of week i, \( i = 1..15, 15..22 \).
- \( T_j \) = Fixed effect of treatment j, \( j = 1..4 \).
- \( H_{(j)k} \) = Random effect of heifer k in treatment j.
- \( (WT)_{ij} \) = Fixed effect of interaction between week i and treatment j.
- \( E_{ijk} \) = Random residual.
Differences among treatments in heifer response variables were tested using Hk(j) as the error term. Heifer response differences in INdex, services per first conception, and SIRE PDS were analyzed as a completely randomized design by ANOVA.

Treatment differences in least squares means of PEAKM, PEAKD, MEMILK, MEFCM, and SCC were analyzed by analysis of covariance as a completely randomized design by ANOVA, using SIRE PDS as a covariate.

All significant differences were for $P < .05$.

**Results and Discussion**

Nutrient composition of treatment diets is in Table 1. Diets were relatively consistent in CP providing similar amounts of total N to all animals. Calculated UIP% were close to initial formulations, thus providing more rumen available N to heifers receiving soy-based diets than those receiving dried brewers grains as the protein supplement. Treatment differences in TDN were not as wide as initially formulated (95 and 115% of (12)) with diets containing 93 and 109% of NRC (12). Low TDN treatments contained greater amounts of ADF and NDF reflecting the decrease in energy concentration by incorporating more orchardgrass hay and less high moisture corn.

Initial and final BW (Table 3) were different between heifers within high TDN treatments and the average of high TDN vs low TDN. As intended, differences were the result of heifer assignment to groups making group BW as homogenous as possible. Average age at the beginning of the trial was 259, 221, 190, 160 d, for the four treatments. Therefore, treatment differences in BW were reflective of age differences between
groups. Daily gain in BW did not differ between UIP treatments when TDN was high, but did differ between low TDN treatments. Heifers receiving the DBG/LTDN diet gained 180 g/d more than their counterparts receiving similar energy supplemented with soybean meal. Zerbini and Polan (23) reported increased ADG of dairy bull calves receiving fishmeal, above those receiving soybean meal. They suggested an improvement in daily gain resulted from greater total protein flow to the small intestine when calves received a ruminally undegradable protein supplement.

Initial WH measures reflected similar differences between treatments as BW. Final WH were similar due to greater rates of WH growth in both DBG treatments and SBM/LTDN. Greater growth in stature on DBG/HTDN and both LTDN diets was probably the result of heifer age. Daily growth in stature (WH cm/d) was greater on LTDN diets than HTDN. This response was expected, but not to the extent as in DBG/LTDN, as heifers were younger. Our observations in WH growth are supported by a Pennsylvania study (8) showing rate of WH growth declined as heifers increased in age. Within TDN levels increasing UIP increased WH growth response .022 to .035 cm/d for high and low TDN, respectively. This suggests increased dietary protein flow to the small intestine may stimulate skeletal growth due to improved N retention and amino acid profile.

Change in the ratio of BW/WH from end of the trial to beginning was used as an indicator of body condition (2). A positive change in INDEX indicated heifers increased more in BW without subsequent growth in WH. All treatments except SBM/LTDN were nearly identical in INDEX change indicating similar growth in BW in relation to WH. Heifers in the SBM/LTDN treatment showed a lower change in INDEX suggesting a reduction in body condition. The response by the SBM/LTDN group was a reflection of lower BW gain (.73 vs >.91 kg/d) and possibly reflected greater tissue
deposition as muscle. Waldo (21) reported lower empty body fat and improved energy deposition as protein in heifers grown at .76 kg/d when compared to .97 kg/d. Bagg et al. (2) reported an interaction between WH growth and protein intake. They reported high dietary protein (120% of NRC (12)) did not improve skeletal or BW growth over diets at 100% of (12) but did show improvement above heifers receiving 80% of (12). Their findings suggest digestible protein was adequate at 100% of (12) resulting in an improvement in DM intake and possibly undegraded protein flow from the rumen. Our body measurements indicate replacement of soybean meal with dried brewers grains may improve N availability to the animal resulting in improved N utilization for muscle and skeletal growth.

Dry matter intake (g/kg BW^{0.75}) was not affected by protein degradability when energy concentration was high. Intake of DM was, however, depressed in low energy diets when UIP% was increased. This suggests DM digestibility may have been depressed due to lower ruminal N availability thereby reducing rate of ruminal turnover and intake. Unlike our study Zerbini and Polan (23) reported improved DM digestibility (6%) in 9 wk old calves receiving less degradable protein supplements in diets containing 75% TDN. Cummins et al. (4) and Bagg et al. (2) indicated increasing UIP depressed DM digestibility but improved efficiency of N utilization. Therefore, increasing UIP may depress fiber digestion due to inadequate supply of readily degraded protein thus reducing DM intake.

Megacalories of DE/kg BW gain was used as an estimate of feed efficiency. Greatest efficiency of conversion of DE to gain occurred on the DBG/LTDN treatment. Remaining treatments were similar in apparent feed efficiency. Similar efficiencies of DE conversion to BW gain were reported by Tomlinson et al. (19) when heifers received low and high energy diets (<65% or >68% TDN) low in UIP%. While DM intake was
depressed on the DBG/LTDN treatment, apparent feed efficiency was improved by providing a slowly degraded N source coupled with slowly degrading carbohydrates (i.e. lower nonstructural carbohydrates). However, this improvement in feed efficiency may not have improved skeletal growth in relation to BW as indicated by the change in INDEX. Nocek and Russell (15) suggested animal performance could be improved by coordinating rates of protein and carbohydrate degradation within the rumen. However, their report dealt with lactational responses and did not define changes in bodily growth due to alteration of ruminal protein and carbohydrate availabilities.

During the compensatory period, heifers from HTDN treatments maintained BW gain at .94 kg/d. Heifers, having received LTDN, gained 1.03 and 1.02 kg/d ADG after switching to feeding levels of 120% of (12). With height growth for this period was .089, .070, .085, and .104 cm/d for the four groups. As mentioned earlier, differences in rate of WH growth were expected due to increasing age of heifers. Bagg et al. (2) reported rate of skeletal growth did not change with increased protein supplementation above (100% of (12)) as heifers increased in age.

No differences were found in SERV between treatment groups. Little and Kay (11) found heifers on accelerated rearing programs (.8 kg/d ADG) for 182 d (13 through 39 wk of age) had lower first conception rates and increased dystocia when compared to more slowly reared heifers (.6 to .8 kg/d ADG). In our study, variation in growth rates and length of the feeding trial may not have been great enough to create differences in reproductive performance.
Lactation Results

Twenty six of the original 30 heifers calved with 20 completing records of 250 d or more. Number of heifers completing 250 d records were 4, 7, 4, and 5 for the SBM/HTDN, DBG/HTDN, SBM/LTDN, DBG/LTDN treatments. Average age at calving (mo) and BW (kg) were 25.2, 525, 24.5, 510, 25.3, 463, and 24.8, 492 for the four groups. Culling as heifers or during first lactation did not appear related to treatments. Reasons varied from foot rot and reproductive failure to culling for low production. After calving, heifers were managed as one group and any reference to groups refers to treatments during the feeding trial period.

Lactation performance measures are in Table 4. Peak fat corrected milk (PEAKM) was higher in heifers having received DBG/HTDN than those receiving SBM/HTDN. Energy level during the feeding trial had no effect on lactational measures. Days to peak milk (PEAKD) were not different between groups. However, DBG/LTDN heifers tended to be lower in PEAKD as one heifer peaked at 37 d and all heifers from this group peaked before 92 d.

Heifers did not differ in MEMILK or MEFCM suggesting growth performance differences during the prepubertal period did not affect milk producing ability. These findings disagree with Gardner et al. (6) who reported rapid growth (1.1 kg/d) depressed milk production in the first three lactations. In addition, Foldager and Sejrsen (5) and Little and Kay (11) indicated excessive growth (ADG > .8 kg/d) and fattening impaired mammary development and future productivity. It is likely our feeding trial was conducted only in the latter stages of the critical growth period (90 to 325 kg BW for large breeds (17)) thus having only a limited impact on parenchymal tissue development and lactational potential.
Somatic cell scores did not differ between groups, but a trend was observed toward lower scores in heifers having received LTDN treatments. Little and Kay (11) reported incidence of mastitis was lower in heifers with low milk yield but occurred in rapidly reared heifers. Heifers from the SBM/LTDN treatment were from sires with lower predicted difference dollars (PDS) thus lowering the average of LTDN animals to a level different than HTDN. It is possible the reduced genetic potential of SBM/LTDN heifers had an influence on BW growth potential and may influence lifetime producing ability.

Results of this trial demonstrated differences in daily BW gain, WH growth, DM intake, and apparent feed efficiency when low energy diets (95% of (12)) were supplemented with a rumen undegradable protein source. This trial did not, however, reflect feeding trial differences in lactational and reproductive performance of heifers receiving diets varying in UIP% or TDN%. These results suggest variation in growth rates may not have been wide enough or occurred too late in the critical allometric phase of mammary growth to have a lasting effect on milk producing ability. Future studies must evaluate the effects of more widely differing growth rates (< .4 to > .8 kg/d ADG) and concentrate on animals between 90 and 325 kg BW (large breeds) to characterize effects of growth on lifetime milk producing ability.
Table 1. Trial 1 experimental diet formulations and analysis.

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<th>Ingredient</th>
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<th>DBG/HTDN</th>
<th>SBM/LTDN</th>
<th>DBG/LTDN</th>
<th>% of DM</th>
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¹ Least square means, (%).
² Calculated from (13).
Table 2. Diet formulation and analysis for first lactation heifers.

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<td>CP (%)</td>
<td>17.4</td>
</tr>
<tr>
<td>NE(_i) (Mcal/kg)</td>
<td>1.58</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>21.0</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>32.6</td>
</tr>
<tr>
<td>UIP(^2) (% of CP)</td>
<td>34.6</td>
</tr>
<tr>
<td>Ca(^3) (%)</td>
<td>.55</td>
</tr>
<tr>
<td>P(^3) (%)</td>
<td>.27</td>
</tr>
</tbody>
</table>

\(^1\) Mineral mix; 16% Ca, 6.5% P, 4.25% NaCl, 2.2% Mg,
3.2% S, 3.5% K, DM basis.

\(^2\) Estimated rumen undegradable protein (%),
calculated from (13).

\(^3\) Calculated using Virginia state average analysis.
Table 3. Least squares means of heifer performance measures of BW, WH, DM intake, DE intake, INDEX (BW/WH).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>SBM/HTDN</th>
<th>DBG/HTDN</th>
<th>SBM/LTDN</th>
<th>DBG/LTDN</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>Initial BW (kg)</td>
<td>242</td>
<td>7.8</td>
<td>196</td>
<td>7.5</td>
<td>157</td>
<td>7.5</td>
<td>142</td>
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<tr>
<td>Final BW (kg)</td>
<td>331</td>
<td>10.1</td>
<td>288</td>
<td>8.7</td>
<td>227</td>
<td>9.3</td>
<td>231</td>
</tr>
<tr>
<td>BW GAIN (kg/d)</td>
<td>.97</td>
<td>.04</td>
<td>.95</td>
<td>.04</td>
<td>.73</td>
<td>.04</td>
<td>.91</td>
</tr>
<tr>
<td>Initial WH (cm)</td>
<td>111</td>
<td>1.2</td>
<td>104</td>
<td>1.1</td>
<td>100</td>
<td>1.1</td>
<td>98</td>
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<tr>
<td>Final WH (cm)</td>
<td>120</td>
<td>1.3</td>
<td>117</td>
<td>1.1</td>
<td>110</td>
<td>1.2</td>
<td>113</td>
</tr>
<tr>
<td>WHGRO (cm/d)</td>
<td>.106</td>
<td>.01</td>
<td>.128</td>
<td>.01</td>
<td>.120</td>
<td>.01</td>
<td>.155</td>
</tr>
<tr>
<td>INDEX$^1$</td>
<td>.57</td>
<td>.03</td>
<td>.59</td>
<td>.03</td>
<td>.47</td>
<td>.03</td>
<td>.59</td>
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<tr>
<td>DM intake$^2$</td>
<td>96.6</td>
<td>2.8</td>
<td>96.1</td>
<td>1.9</td>
<td>94.5</td>
<td>2.1</td>
<td>84.0</td>
</tr>
<tr>
<td>DE intake$^3$</td>
<td>27.0</td>
<td>2.5</td>
<td>24.2</td>
<td>2.3</td>
<td>25.1</td>
<td>2.5</td>
<td>15.1</td>
</tr>
</tbody>
</table>

$^1$ Change in BW/WH index.

$^2$ DM intake, (g/kg BW·75).

$^3$ Digestible energy intake, (Mcal/kg BW gain).

Contrasts

A = SBM/HTDN vs DBG/HTDN
B = SBM/LTDN vs DBG/LTDN
C = AVE HTDN vs AVE LTDN

* Treatments differ, p<.05
Table 4. Least squares means of lactation performance measures of peak milk, days to peak milk, mature equivalent milk production, somatic cell score, and sire predicted difference dollars.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>SBM/HTDN</th>
<th>DBG/HTDN</th>
<th>SBM/LTDN</th>
<th>DBG/LTDN</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>PEAKM²</td>
<td>31.5</td>
<td>1.89</td>
<td>34.6</td>
<td>1.34</td>
<td>32.8</td>
<td>1.95</td>
<td>32.1</td>
</tr>
<tr>
<td>PEAKD²</td>
<td>118</td>
<td>28.1</td>
<td>111</td>
<td>19.9</td>
<td>112</td>
<td>29.0</td>
<td>60</td>
</tr>
<tr>
<td>MEMILK²</td>
<td>9612</td>
<td>.04</td>
<td>9849</td>
<td>.04</td>
<td>9532</td>
<td>.04</td>
<td>9733</td>
</tr>
<tr>
<td>MFECM²</td>
<td>10355</td>
<td>398</td>
<td>10057</td>
<td>282</td>
<td>9532</td>
<td>411</td>
<td>9733</td>
</tr>
<tr>
<td>SCC³</td>
<td>2.10</td>
<td>.53</td>
<td>2.14</td>
<td>.37</td>
<td>.55</td>
<td>.54</td>
<td>1.81</td>
</tr>
<tr>
<td>SIRE PD$⁴$</td>
<td>131</td>
<td>30.0</td>
<td>128</td>
<td>21.2</td>
<td>58</td>
<td>26.0</td>
<td>163</td>
</tr>
</tbody>
</table>

1 Peak fat corrected milk, (kg).
2 Day of peak milk.
3 305 d 2x mature equivalent milk, (kg).
4 Mature equivalent 3.5% fat corrected milk, (kg).
5 Somatic cell count score, (log scale, 1...10).
6 USDA sire predicted difference dollars, July 1989.

Contrasts:

A = SBM/HTDN vs DBG/HTDN
B = SBM/LTDN vs DBG/LTDN
C = AVE HTDN vs AVE LTDN

* Treatments differ, p<.05
References


Trial 2

Influence of ration protein undegradability on intake, daily gain, feed efficiency, and body composition of Holstein heifers

Abstract

Thirty two Holstein heifers between 213 and 231 kg BW were randomly assigned to one of four treatments for the 50 d trial. Treatments consisted of four levels of rumen undegradable intake protein (UIP) (31, 43, 50, 55% of total N) at 100% of 1989 NRC TDN and CP. Total mixed rations composed of corn silage, ground barley straw, soybean meal, blood meal, urea, and minerals were formulated for .60 kg/d ADG. Ration UIP% was varied by shifting protein sources. Mean DM intake (g/kg BW⁻⁰.⁷⁵) was 97.6, 84.4, 77.8, 73.5 for 31% UIP (soybean meal), 43% UIP (blood and soybean meal), 50% UIP control (blood meal with urea), and 55% UIP (blood meal) treatments. Daily gain was .835, .892, .910, .964 (kg/d). Digestible energy intake (DEI Mcal/kg BW⁻⁰.⁷⁵/d) was .279, .292, .242, .214, respectively and feed efficiency measured as Mcal DE/kg BW gain was 20.6, 16.1, 15.2, and 13.3. Treatments differed in DM intake (g/kg BW⁻¹), DE intake (Mcal/kg BW⁻¹), and DE Mcal/kg BW gain, daily BW gain, and hip height. Treatments did not differ in growth in wither height, or heart girth. Change in % empty body fat as estimated by urea space procedures was 6.73, 4.67, 6.67, 7.32 and did not differ. These results indicate increasing ration UIP% for growing heifers results in improved feed efficiency and daily gain.
Introduction

Recent advances in protein nutriture of growing dairy heifers have lead to NRC's differentiation of protein recommendations into degradable and undegradable fractions. Findings by Zerbini and Polan (29)(References on pg. 111) and Jahn and Chandler (8) indicated performance of young growing dairy replacements may be improved by rations containing higher total protein. Research by Bagg et al. (2) supported this work showing a quadratic response to dietary protein supplementation above 100% of 1978 NRC. Bagg and coworkers (2) reported NRC CP recommendations were adequate for calves to 6 mo of age but were too generous thereafter. Further evaluation of these works indicate increasing total CP may influence animal performance by increasing flow of undigested protein from the rumen.

Separation of protein into degradable and undegradable fractions is supported by works showing improvement in growth when a portion of the crude protein by-passes digestion in the rumen (1, 7, 23, 27, 29). Research by (23, 25, 29) demonstrated incorporation of UIP levels > 40% of CP tended to depress DM intake, yet supported equal or greater BW gain. Others (4, 15) reported no change in intake or daily gain with increasing UIP%. Reports by (1, 9, 25, 29) indicated feed efficiency (Mcal DE/kg BW gain) was improved when fishmeal, dehydrated alfalfa, bloodmeal, or corn gluten meal were added to diets of growing dairy heifers and feeder cattle, even though DM intake was lower. Various researchers (1, 12, 22) suggested quantities of microbial protein synthesized within the reticulo-rumen may be inadequate to support optimal and/or economical growth in rapidly growing dairy replacements. It therefore seems apparent that total protein needs could be reduced by providing a mix of degradable and undegradable sources.
The objective of this study was to evaluate response in growth, intake, feed efficiency, and body composition of dairy heifers receiving diets varying in rumen undegradable protein.

**Materials and Methods**

Thirty two Holstein heifers between 213 and 231 kg BW were randomly assigned to one of four treatment groups. Mean initial BW were 223, 215, 231, 217 kg. For the 50 d trial period, heifers were housed in a counter-slope total confinement facility as described by (16, 26). Treatments (Table 1) consisted of four isonitrogenous, isocaloric diets formulated to differ in rumen undegradable protein (UIP)(31, 43, 50, 55% of total N). Range of UIP was established as that achievable with soybean meal as the protein supplement, to the level recommended by NRC.

Total mixed rations were composed of corn silage, ground barley straw (mean length = 7 cm), soybean meal, blood meal, urea, and a mineral premix. Rations were formulated for .60 kg/d ADG according to (13). Diets were formulated to contain adequate protein for microbial growth while minimizing potential of degradable protein flow from the rumen (12). Protein undegradability estimates of ingredients were obtained from (12). Diets were provided daily at 1350 h for ad libitum intake allowing for 5 to 15% refusals. Treatment diets and ingredients were sampled and analysed as in (26). Total digestible nutrients (TDN) was predicted from ADF as reported by (26). Digestible energy intake was predicted from TDN using the formula 1 kg TDN = 4.409 Meal DE (NRC). Ration UIP% was calculated on a weekly basis using individual ingredient CP analysis and NRC (12) undegradability estimates.
Heifers were acclimated to the counter-slope facility for 12 d prior to the feeding trial. During the acclimation period, heifers received the 31% UIP ration to adjust heifers to the TMR containing chopped straw. Heifers had access to feeders at all times except during pen cleaning and animal weighing. Daily as fed intakes were recorded using Pinpointer 4000B computerized feeders (UIS., Inc. Cookeville, TN).

Body weight, wither height (WH), hip height (HIP), and heart girth (HEART) were recorded at 1100 h, 2 d consecutively at beginning and end of the feeding trial, and weekly during the trial.

**Urea Space Measurement**

Body composition of water, fat, and protein were estimated using urea space procedures according to Preston and Koch (14). These procedures are based on the concept of constant proportions of water (71.8 to 74%), protein (18.5%), and ash (4.5%) in the fat-free body mass (17). Prior to estimation of body water, heifers were fasted for a minimum of 12 h to reduce rumen volume. Heifers were secured in a head-catch chute and weighed to establish shrunk weight. A solution containing 20% urea dissolved in .9% saline was administered through a 12 ga needle into the right jugular vein. Volume injected was calculated to provide 130 mg urea/kg BW. Blood samples (10-15 ml with 200 µl sodium heparin) were collected prior to infusion and 12 min post infusion from the left jugular vein and immediately placed on ice. Twelve minutes was established as the length of time needed for solution equilibration with body water by (14). Blood was centrifuged at 5000 x g for 15 min and plasma frozen (-20°C) for subsequent urea analysis (3). Urea space was estimated as follows:

\[
\text{Urea space} = \frac{\text{Volume infused} \times \text{concentration of solution}}{\text{change in PUN}}
\]
where:

\[
\text{Volume infused} = .65 \text{ ml/kg BW};
\]
\[
\text{Conc. of Soln.} = 93.4 \text{ mg Urea-N/ml solution};
\]
\[
\text{Change in PUN} = \text{difference in plasma urea nitrogen taken from blood sample prior to and after urea infusion (mg Urea-N/dl)}
\]

Urea space as a percent of BW was calculated by multiplying urea space by 10 and dividing by BW (kg). Body water, fat, and protein were predicted on an empty body basis using equations by (5).

\[
\% \text{Empty body water} = 83.5 - .16 \times \text{urea space\%} - .032 \times \text{BW}
\]
\[
\% \text{Empty body fat} = -5.9 + .14 \times \text{urea space\%} + .030 \times \text{BW}
\]
\[
\% \text{Empty body protein} = 16.6 - .009 \times \text{urea space\%} + .005 \times \text{BW}
\]

Statistical analysis of data was conducted by least squares regression procedures as outlined in (10, 20). Analysis of treatments was by linear and quadratic orthogonal polynomial contrasts (20). Tests for differences in dependent variables of intake, and heifer growth were determined using the following model:

\[
Y_{ijk} = \mu + W_i + T_j + H_{(ijk)} + (WT)_{ij} + E_{ijk}, \text{ where:}
\]
\[
Y_{ijk} = \text{Dependent variable of heifer k in treatment j for week i.}
\]
\[
\mu = \text{Population mean.}
\]
\[
W_i = \text{Fixed effect of week i, } i = 1..8.
\]
\[
T_j = \text{Fixed effect of treatment j, } j = 1..4.
\]
\[
H_{(ijk)} = \text{Random effect of heifer k in treatment j.}
\]
\[
WT_{(ij)} = \text{Fixed effect of interaction between week i and treatment j.}
\]
\[
E_{ijk} = \text{Random residual.}
\]

Differences among treatments in heifer response variables were tested using \(H_{(ijk)}\) as the error term. Heifer response differences in change in body composition estimates were analyzed as a completely randomized design by ANOVA.
All significant differences were for P<.05.

Results and Discussion

Nutrient composition of trial rations is in Table 1. Diets were consistent in DM, CP, ADF, and NDF thus providing similar amounts of carbohydrates and total protein to all animals. Calculated UIP% were similar to initial formulations, thus creating a wide variation in rumen available N received by heifers as the protein supplement shifted from soybean meal to blood meal. Similarities between treatments in ADF and NDF suggest rumen fill characteristics should have been similar for all heifers. Montgomery and Baumgardt (11) reported intake by dairy heifers was not only a function of physical rumen capacity but also carbohydrate components which affect rate and extent of digestion. Given that diets were similar in energy concentration, heifer response differences should be a reflection of protein availability within the rumen.

Measures of heifer growth are in Table 2. As planned, treatments did not differ in initial BW. Daily BW gain showed a positive linear effect of 57, 75, and 129 g/d as dietary UIP% increased. Growth in WH and heart girth were not different. Growth in hip height showed an unexplainable quadratic effect with increasing UIP%. General trends in BW and growth in stature indicate replacement of soybean meal with blood meal stimulated greater structural growth. Studies by (6, 9, 19) indicated replacement of a portion of the degradable protein fraction with an undegradable source improved N utilization and growth rate. Klopfenstein and Goedeken (9) reported an increase in BW gain of 90 to 135 g/d in beef calves fed diets supplemented with corn gluten meal or blood meal as compared to urea. Harvey et al. (6) found similar improvement in BW
gains of 240 kg beef steers and heifers by replacing urea with blood meal. Rohr. et al. (19) conducted a critical analysis of protein allowances for growing ruminants and reported a depression in average daily gain when soybean meal replaced fishmeal and a further decline when urea was added to trial diets. Others (15, 23) reported no change in daily gain as diets increased in UIP%. Lack of response in these studies may have resulted from excess total protein masking an effect of an undegradable source. Researchers (6, 9, 28, 29) suggested growth response as diets increase in UIP% was a reflection of increased amino acid flow to the small intestine and possible overall improvement in amino acid absorption and composition of amino acids absorbed.

As diets increased in estimated UIP%, DM intake (kg/d) declined linearly (Table 3). All intake measures showed a significant linear effect to increasing UIP%. Relative to 1989 NRC estimates of DM intake, intakes on diets ≤ 43% UIP were nearly equal or greater than NRC (13) estimates of 4.9 kg/d. Diets with > 50% UIP were lower in DM intake than (13) yet performance in BW gain exceeded gains formulated according to (13). Depression in DM intake may reflect depressed microbial growth and fermentation of organic matter. Tamminga (24) concluded maximal microbial fermentation occurred when dietary crude protein was > 13.4%. Given that dietary CP was approximately 12% in all diets and DM intake depression occurred when UIP% exceeded 40%, it is possible rumen degradable N may have been inadequate to support maximal microbial fermentation of organic matter. Higher DM intake on the 50% UIP (blood meal + urea supplement) vs 55% UIP (blood meal supplement) treatment suggests soluble N was inadequate on the diet supplemented with blood meal alone. This is supported by Harvey et al. (6) who reported greater daily gains and feed efficiency when blood meal was supplemented with urea.
Treatments differed in CP intake with lower CP intake as UIP% increased. Rohr et al. (19) reported optimum supply of protein for dairy heifers (300 kg BW, .65 kg ADG) was 550-600 g CP/d and 150-200 g/d undegradable protein. Recommendations by (13) are for 783 g CP/d and 282 g UIP/d for large breeds (300 kg BW, .65 kg ADG). According to (13) trial diets were inadequate in total grams CP (Table 3) and only those supplemented with blood meal supplied sufficient undegradable protein when compared to (13).

Apparent feed efficiency (Mcal DE/kg BW gain) showed a declining linear response to increasing UIP%. As diets increased in UIP% feed required per kg BW gain declined. Similar efficiencies of DE conversion to BW gain were reported by (26) when dairy heifers received diets high in UIP% (> 40% of total N) and intermediate in energy concentration (65% TDN). Studies by (1, 6, 9, 29) showed increased feed efficiency of 20 to 30% when diets were supplemented with rumen undegradable protein sources such as corn gluten meal, blood meal, fishmeal, and dried distillers grains. Improved efficiency, in these trials, was attributed to an improvement in amino acid profile supplied to the small intestine. Blood meal and corn gluten meal contain high levels of lysine and methionine, respectively (21). Richardson and Hatfield (18) reported ruminant diets were deficient in methionine and lysine. Therefore, supplementation with these highly undegradable protein sources should improve availability of these amino acids through increased flow to the lower gut.

Urea space estimates of body composition are in Table 4. No differences were found between treatments in empty body water, fat, or protein. Treatments differed in change in estimated body water and fat indicating a quadratic response to increasing UIP%. Estimates of body composition agree with (5, 17, 28) suggesting urea space estimation of body water is a viable non-invasive method of evaluating nutritional effects on body
composition. Comparative slaughter techniques are superior to urea space yet were not viable under our conditions. Results from this trial suggest heifers may not have received trial rations for long enough to create measurable changes in body composition. However, similarities in body composition between treatments indicates response in intake and feed efficiency were not reflected in body fat or protein content.

Heifers receiving diets with higher UIP% (> 40% of total N) had lower DM intake, and DE intake while gaining 59 to 129 g/d more than heifers on control diets (30% UIP) without differing in body composition. This trial suggests supplementation of dairy heifer diets with blood meal may improve amino acid profile supplied to the lower gut and therefore enhance overall animal performance.
Table 1. Trial 2 experimental diet formulations and analysis.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Treatment classification (UIP %)</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31</td>
<td>43</td>
</tr>
<tr>
<td>Corn silage</td>
<td>57.3</td>
<td>59.3</td>
</tr>
<tr>
<td>Straw</td>
<td>28.5</td>
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<tr>
<td>Soybean meal</td>
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<td>Urea</td>
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<td>-</td>
</tr>
<tr>
<td>Mineral premix</td>
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<td>1.6</td>
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<table>
<thead>
<tr>
<th>Chemical analysis² x</th>
<th>x</th>
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<tbody>
<tr>
<td>DM</td>
<td>54.2</td>
<td>52.9</td>
<td>53.5</td>
<td>52.9</td>
</tr>
<tr>
<td>CP</td>
<td>11.8</td>
<td>11.7</td>
<td>12.2</td>
<td>12.7</td>
</tr>
<tr>
<td>TDN</td>
<td>64.4</td>
<td>63.6</td>
<td>63.4</td>
<td>63.9</td>
</tr>
<tr>
<td>ADF</td>
<td>33.0</td>
<td>34.0</td>
<td>34.5</td>
<td>32.5</td>
</tr>
<tr>
<td>NDF</td>
<td>57.1</td>
<td>58.6</td>
<td>58.2</td>
<td>55.6</td>
</tr>
<tr>
<td>UIP³ (％ of CP)</td>
<td>28.2</td>
<td>41.9</td>
<td>50.6</td>
<td>55.0</td>
</tr>
</tbody>
</table>

¹ From (Ruminant Nitrogen Usage (12))
² Least square means, n = 7.
³ Calculated from ingredient UIP%.
Table 2. Least squares means of heifer performance measures of growth in BW, WH, HIP height, HEART girth.

<table>
<thead>
<tr>
<th>Treatment classification (U1P %)</th>
<th>31</th>
<th>43</th>
<th>50</th>
<th>55</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW (kg)</td>
<td>223</td>
<td>215</td>
<td>231</td>
<td>217</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>Final BW (kg)</td>
<td>264</td>
<td>259</td>
<td>276</td>
<td>264</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>GAIN (kg/d)</td>
<td>.835</td>
<td>.892</td>
<td>.910</td>
<td>.964</td>
<td>.058</td>
<td>*</td>
</tr>
<tr>
<td>Initial WH (cm)</td>
<td>111</td>
<td>109</td>
<td>111</td>
<td>110</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Final WH (cm)</td>
<td>116</td>
<td>113</td>
<td>117</td>
<td>115</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>WHGRO (cm/d)</td>
<td>.111</td>
<td>.114</td>
<td>.119</td>
<td>.108</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Initial HIP (cm)</td>
<td>116</td>
<td>115</td>
<td>117</td>
<td>115</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>HIPGRO (cm/d)</td>
<td>.098</td>
<td>.084</td>
<td>.090</td>
<td>.119</td>
<td>.01</td>
<td>*</td>
</tr>
<tr>
<td>Initial HEART (cm)</td>
<td>142</td>
<td>140</td>
<td>142</td>
<td>140</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>HEARTGRO (cm/d)</td>
<td>.158</td>
<td>.153</td>
<td>.158</td>
<td>.160</td>
<td>.02</td>
<td></td>
</tr>
</tbody>
</table>

SE is appropriate for n=7.  

Polynomial contrasts

L = Linear treatment effect.  
Q = Quadratic treatment effect.  
* Contrast significant, p<.05
Table 3. Least squares means of heifer performance measures of DM intake, CP intake, and DE intake Mcal/kg BW gain.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Treatment classification (U1P %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31</td>
</tr>
<tr>
<td>DM Intake (kg/d)</td>
<td>5.97</td>
</tr>
<tr>
<td>DM Intake (g/kg BW^3)</td>
<td>96.6</td>
</tr>
<tr>
<td>DE (Mcal/kg BW^3)</td>
<td>.276</td>
</tr>
<tr>
<td>CP intake (g/d)</td>
<td>701.5</td>
</tr>
<tr>
<td>CP Intake (g/kg BW^3)</td>
<td>11.4</td>
</tr>
<tr>
<td>UP intake (g/d)</td>
<td>198.0</td>
</tr>
<tr>
<td>DEG (DE Mcal/kg Gain)</td>
<td>21.3</td>
</tr>
</tbody>
</table>

* SE is appropriate for n=56.

Polynomial contrast:
L = Linear effect.
Q = Quadratic effect.
* Significant contrast, p<.05.
Table 4. Least squares means of urea space estimates of body water, fat, and protein in growing Holstein heifers.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>31</th>
<th>43</th>
<th>50</th>
<th>55</th>
<th>L</th>
<th>Q</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEG %EBW</td>
<td>70.97</td>
<td>72.10</td>
<td>69.75</td>
<td>72.83</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEG %EBF</td>
<td>5.06</td>
<td>4.07</td>
<td>6.13</td>
<td>3.44</td>
<td>1.1</td>
<td></td>
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<tr>
<td>BEG %EBP</td>
<td>15.90</td>
<td>15.96</td>
<td>15.83</td>
<td>15.80</td>
<td>.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHNG %EBW</td>
<td>-7.09</td>
<td>-4.75</td>
<td>-6.99</td>
<td>-7.75</td>
<td>1.2</td>
<td></td>
<td></td>
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<tr>
<td>CHNG %EBF</td>
<td>6.73</td>
<td>4.67</td>
<td>6.67</td>
<td>7.32</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHNG %EBP</td>
<td>1.39</td>
<td>1.48</td>
<td>1.47</td>
<td>1.47</td>
<td>.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. SE is appropriate for n=7.
2. Beginning %Empty body water.
3. Beginning %Empty body fat.
4. Beginning %Empty body protein.
5. Change in %EBW, ending %EBW − beginning %EBW.
6. Change in %EBF, ending %EBF − beginning %EBF.
7. Change in %EBP, ending %EBP − beginning %EBP.
8. Treatments differ from 31% UIP treatment, p<.05.

Polyomial Contrast
L = Linear effect.
Q = Quadratic effect.
* Significant contrast, p<.05.
References


Trial 3

Effect of varying levels of nonstructural carbohydrate and rumen undegradable protein on intake, growth, feed efficiency, and body condition of Jersey heifers.

Abstract

Fifty one Jersey heifers between 110 and 300 kg BW were assigned to one of nine treatments for a 60 d trial. Treatments consisted of all combinations of three levels of rumen undegradable intake protein (UIP)(30, 40, 50% of total N) and three levels of non-structural carbohydrate (NSC)(17, 22, 26% of DM). Total mixed rations composed of alfalfa haylage, ground orchardgrass hay, ground straw, high moisture corn, barley, soybean meal, blood meal, and a mineral premix were formulated for .60 kg/d ADG. Ration UIP% was varied by adjusting protein sources while NSC was varied by adjusting forages and concentrates. Ration UIP content was verified by in situ dacron bag techniques. Mean DM intake was 5.51 kg/d and did not differ between treatments. Daily gain varied from .259 to .694 kg/d and increased with increasing levels of UIP and NSC. Feed efficiency measured as Mcal DE/kg BW gain improved from 42.2 at 30% UIP to 30.0 at 40 and 50% UIP. Daily gain and feed efficiency differed between diets. Urea space and ultrasound were used to estimate body fat, protein, longissimus muscle area, and backfat thickness. Treatments differed in change in body water, fat, and protein with lowest change in estimated body fat occurring at 21.1% NSC. Treatments differed in growth in longissimus muscle area with muscle area increasing with UIP and
NSC. Results indicate that within the ranges of UIP and NSC, increasing dietary UIP had a greater influence on heifer growth and apparent feed efficiency than dietary NSC.
Introduction

Incorporation of new feeding strategies can play a critical role in insuring adequate growth toward maturity. These strategies involve integration of the degradable protein system as outlined by NRC (22) and a system considering carbohydrate degradability (26).

The NRC (22) has suggested improved growth rates and efficiency can be achieved by including greater amounts of ruminally undegradable protein (UIP). Research by Swartz et al. (40), Tomlinson et al. (42, 43), Amos (1), and Klopfenstein and Goedeken (14) supports these recommendations showing improved growth of dairy replacements and beef steers receiving diets supplemented with UIP.

In a review, Nociek and Russell (26) suggested response to UIP should not be attributed totally to characteristics of dietary protein, but should also include digestibility or ruminal availability of carbohydrate fractions. The nonstructural carbohydrate (NSC) fraction represents a readily digestible portion of feedstuffs containing mainly sugars, starch, and pectins. Galactans and fructosans replace starch as the carbohydrate reserve material in legumes and grasses, respectively (24). Due to greater ruminal degradability, NSC often comprise a larger proportion of the carbohydrates in lactating animal diets (24).

Studies conducted by Herrera-Saldana and Huber (10) and McCarthy et al. (19) attempted to match carbohydrate and protein ruminal degradability in diets of lactating dairy cows. They indicated that matching substrate degradabilities improved substrate utilization, nutrient flow to the small intestine, and animal performance. Stern et al. (39) examining effects of NSC, urea, and soluble protein levels on microbial protein synthesis in continuous culture concluded that source of carbohydrate and rate of availability to rumen microbes are important in nitrogen utilization.
In dairy calves and heifers, a number of growth and intake studies (4, 6, 11, 48) showed animals were most efficient in converting dietary substrates to tissue protein when diets contained more than 14% CP and contained low dietary ADF content. However, only Cummins et al. (6) controlled protein degradability while shifting levels of ADF. Others controlled dietary CP amounts without regard to CP degradability. Klopfenstein and Goedeken (14) and Tomlinson et al. (42, 43) reported improvement in daily gain and DE Mcal/kg BW gain of beef steers and dairy heifers receiving diets high in UIP (> 40% of total N) with ADF 27 to 35% of DM.

Amos (1) found that increasing dietary energy concentration and UIP resulted in equal or higher rates (159 g/d) of daily gain with marked improvement in feed efficiency. However, energy concentrations were increased by adding tallow, not by varying NSC content.

Various workers (16, 17, 18, 20, 37, 45) concluded the synergistic response between UIP and energy had a significant effect on microbial growth efficiency and overall performance of sheep, dairy heifers, and lactating dairy cattle. Isolating on CP and ADF, previous studies failed to focus on the synergistic response between protein and carbohydrate degradability within the rumen of growing animals.

Therefore, objectives of this study were to investigate effects of varying levels of non-structural carbohydrates and rumen undegradable protein on intake, growth, apparent feed efficiency, and change in body composition of growing dairy heifers.

**Materials and Methods**

Fifty one Jersey heifers were assigned to one of nine treatment diets with 26 assigned to more than one treatment resulting in 77 heifer records in the final data set.
Preliminary period

Prior to the feeding trial, heifers were housed in a total confinement fence-line feed bunk facility. Heifers received a diet of 27.2% (DM basis) alfalfa haylage, 21.6% ground orchardgrass hay (mean length=7 cm), 14.5% ground straw (7 cm), 18.6% high moisture shelled corn, 14.5% ground barley, 3.2% blood meal, and .4% mineral premix for .60 kg/d ADG according to (23). Diets contained 13.3% CP, 40% UIP (estimated from NRC (22)), 34.6% ADF, 50.5% NDF, and 63.7% TDN. Ingredients were chosen to reflect those contained in trial diets to familiarize animals to the feeds and allow for adaptation of rumen microbial populations.

Heifers were moved to the feeding trial facility 14 to 21 d prior to trial periods and continued to receive preliminary period diet. This period allowed animals to become familiar with the counter-slope facility and Pinpointer 4000B computerized feeders (UIS, Inc. Cookeville, TN). The counter-slope facility and feeding system were described by (33, 44). During the acclimation period, heifer body fat content was estimated by urea space procedures according to (32).

Feeding trial

For the 60 d feeding trial, heifers were assigned to trial diets based on BW and estimated body fat so that treatments were as homogeneous as possible. Treatments consisted of all possible combinations of UIP (30, 40, 50% of total N) and NSC (17, 22, 26% of DM)(Table 1). Range of UIP was from (43) with NSC representing concentrations with limited readily available carbohydrate to a concentration available when diets are formulated according to NRC TDN. Treatments were randomly assigned to one of three periods (SETS). Sets were required as the feeding trial facility contained four pens each having one feed station. Treatment assignments were: SET1 = 1,3,5,8; SET2 = 2,5,7,9; SET3 = 4,5,6. Treatment 5 was repeated in each set as it contained mean concent-
trations of UIP and NSC. Repeating this diet also served as an indicator of seasonal effects on heifer response. Due to availability of the feeding trial facility, time interval between SETS was 189 and 31 d.

Total mixed rations contained alfalfa haylage, ground orchardgrass hay, ground straw, high moisture shelled corn, ground barley (.375 mm screen), 44% soybean meal, blood meal, and a mineral premix. Rations were formulated for .60 kg/d ADG (23). Protein undegradability estimates were obtained from (22) for ration formulations. Protein undegradability estimates were verified by in situ dacron bag techniques. Undegradability estimates were established for ingredients within each SET with ration UIP% verified using individual ingredients. Ration NSC content (% of DM) was estimated using the equation: 100 - (NDF% + CP% + EE% + ASH%)(26).

Ingredient NSC content was calculated and used to formulate rations. Diets were provided daily at 1350 h for ad libitum intake allowing for 5 to 15% refusals. Treatment diets and ingredients were sampled, stored, and analyzed as in (43).

In situ techniques

Dry matter, N, and NDF degradability of individual ingredients were estimated by in situ bag techniques (30). Two non-lactating Holstein cows (675 ± 23 kg BW) fitted with rumen cannula were used to estimate degradability of ingredient composites from each of 3 sets of treatments. One cow participated in estimation procedures for two sets thus a total of five cows were used for the in situ procedures. Cows received a diet (DM basis) of 30.2% alfalfa haylage, 26.2% ground orchardgrass hay, 17.5% ground straw, 8.7% high moisture corn, 13.1% ground barley, 3.5% blood meal, and .8% mineral premix on a DM basis. Diet analysis was 13.9% CP, 40.7% UIP, 33.0% ADF, 56.3% NDF, 23.2% NSC, 60.5% TDN, .6% Ca, .3% P. Cows were acclimated for 14 d prior to inserting bags. Diets were provided at 1130 and 2330 h, ad libitum. Bags of spun
polyester dacron (10 x 20 cm) with a defined pore size of 59 μ were filled with 5g feedstuff DM giving a feed exposure of 14.9 mg/cm². Bags were suspended in the rumen for 0, 2, 6, 12, 18, 24, and 72 h, with bag placement in reverse order starting with 72 h. Samples were in duplicate and each ingredient SET was replicated once allowing cows 7 d between replicates. All bags were removed from the rumen at the same time, washed and their contents dried at 60°C (25). Dried residuals were ground through a 1-mm screen and assayed for N (3) and NDF (8).

Using composition and weight of original samples, percent disappearance of DM, N, and NDF for each feedstuff was calculated for each SET of treatment ingredients. Measured components (DM, N, NDF) were assumed to be composed of three fractions: fraction A (rapidly degraded, “soluble”), fraction B (degraded at a measurable rate, “degradable”), and fraction C (undegraded 72 h residual, “undegradable”), as proposed by (2, 25). Fraction A was calculated by: 1 - (B + C). Component degradability in percent (D) was calculated using the equation of Orskov et al. (30):

\[ D = A + \frac{(B \times k_d B)}{(k_d B + k_r)} \]

where

- \( D \) = DM, N, or NDF degradability, (%);
- \( A \) = fraction readily degraded, (%);
- \( B \) = fraction degraded at a measurable rate, (%);
- \( k_d \) = component degradation constant of B fraction, (1/h)
- \( k_r \) = rumen turnover rate, (.05/h).

**Animal measures**

Intakes were recorded daily. Body weight, wither height (WH), hip height (HIP), and heart girth (HEART) were recorded at 830 h, 3 d consecutively at beginning and end of the feeding trial, and weekly during the trial. Body composition of water, fat, and protein were estimated according to (9, 32) 1 wk prior to heifer assignment to treatment diets and on the last day of the feeding trial. Procedures for urea space and equations used for estimation of body composition were outlined in (43).
Ultrasound procedures

Measurement of longissimus muscle area (loineye) and backfat thickness were made using an Aloka 210 real time ultrasound machine with a 3 MHz general purpose probe (Corometrics Medical Systems, Inc. Wallingford, CT). Ultrasound was used to estimate subcutaneous fat thickness over the longissimus muscle between the 12th and 13th ribs and to measure LOINEYE area at the same location. Heifers were restrained in a head-lock squeeze chute with ultrasonic measures made on the animal's left side. Mineral oil was applied at the measuring site to insure good contact between the probe and skin of the animal.

Ultrasound scans digitally displayed depth from the face of the probe to the fat-lean boundary in centimeters. Scans were recorded on a standard VHS video recorder for sketching. Loineye areas were traced directly off a video monitor onto acetate paper and the area determined using a spatial digitizer (Autosketch, Autodesk Inc., Sausalito, CA 94965). Backfat was also measured and adjusted for scaling of the video display. Ultrasonic measures were made in duplicate on one of the first three and last three days of the feeding trial. Average of beginning and ending loineye and backfat measurements were used for statistical analysis.

Statistical analysis

Statistical analysis of data was conducted by least squares regression procedures as outlined in (13, 36). Treatment response differences in dependent variables of intake, heifer growth, and feed efficiency were determined using the model in Table 2. Treatment differences were tested using mean square of heifers within treatments as the error term.
Treatment 5 differences between SETS were analyzed to determine differences in intake and growth which may have been associated with seasonal differences. Changes in estimated body composition and ultrasound measures were analyzed by analysis of variance for a completely randomized design. Pearson product-moment correlation coefficients between urea space and ultrasound estimates were computed to determine degree of colinearity in estimating changes in body composition, within heifers.

**Results and Discussion**

Nutrient composition of trial rations is in Table 1. Diet variation in DM reflected shifts in forage and concentrate ingredients to achieve UIP and NSC levels. Dietary CP content increased above the desired 13% as alfalfa haylage increased in protein content from initial analysis. Nutrient concentrations of UIP and NSC were consistent with objective formulations. Concentration of TDN reflected NSC content, therefore range of NSC content was limited by the criterion of keeping diets nearly isocaloric. Formulation of diets to vary in NSC and be isocaloric while also varying in UIP was not possible using common feedstuffs. Similarities between diets in ADF and NDF suggest feed characteristics should be similar for all diets.

In situ estimates of fraction A, B, and C, degradation rate ($k_d$) of fraction B, and rumen degradability of DM, N, and NDF for individual feedstuffs are shown in Table 3. Forages were lower in DM digestibility than concentrate ingredients. Low A fractions in orchardgrass and straw reflected lack of soluble sugars and high content of cellulose and hemicellulose. Due to very high quality, alfalfa haylage contained considerably high soluble (A) and degradable (B) fractions. High moisture corn and barley did not differ as widely as expected in DM degradability or rate of degradation. Nocek and Russell
(26) reported high moisture corn DM degradability of 9.4 %/h with barley at 7.9 %/h. Differences between previous works and ours are likely the result of high DM (79.7%) content of corn resulting in less fermentative breakdown of crystalline starches and sugars. Differences in barley DM digestibility may be attributed to variety or growing season differences as indicated by (5).

Fractional rates of N digestibility were consistent with (12, 26, 27). Ruminal N degradabilities of alfalfa haylage, soybean meal, and high moisture corn were higher than reported by (26). Differences from NRC (22) were not large, therefore use of book values in ration formulation proved acceptable under these circumstances.

Fractional rates of NDF digestibility agreed with Nocek and Russell (26) with the exception of orchardgrass hay and straw. Delayed harvest of orchardgrass hay in 1989 may account for high NDF content (76% of DM) and low digestibility. Low digestibility of straw may be a result of climatic conditions prior to grain harvest and its characteristically high NDF content (82% of DM).

Overall, feedstuff digestibilities were slightly lower than NRC (22, 23) and other reported values. Results probably reflect the use of poor quality (high ADF, NDF) feeds, and marginal availability of NSC and degradable protein, thus limiting rate of microbial fermentation.

**Heifer response**

Measures of heifer growth are in Table 4. As planned, treatments did not differ in initial BW. However, variation in initial BW was created as heifer assignment was also dependent upon body fat content. Although final BW did not differ, treatment BW differences began to reflect differences in daily gain. With the exception of treatments 2 and 9, as diets increased in UIP and NSC, daily BW gain increased. This response was more pronounced within levels of UIP as NSC increased. Results suggest availability
of readily degradable carbohydrates may have limited microbial growth and production of substrates needed for rapid heifer growth.

One heifer in treatment 2 gained an average of .04 kg/d thus depressing overall group response. However, with her data removed group ADG was .30 kg/d reflecting poor response by all. All heifers in treatment 9 grew poorly as standard deviation of the mean was only .08 kg. Separation of treatment 5 responses by SET indicated SET 2 BW gain was lowest at .403 kg/d while SETS 1 and 3 were .623 and .484 kg/d.

Given that treatments 2, 5, 7, and 9 all occurred within SET 2 it appears heifer response may be confounded by seasonal effects or possibly health. Ambient temperature during SET 1 (July - Sept. 1989) ranged from a high of 22 to 35°C to a low of 12 to 19°C. Range in temperature for SET 3 (April - June 1990) were similar to SET 1 at about 5° lower. However, SET 2 occurred during the period of January through March 1990 with daily highs of -3 to 22°C and lows of -11 to 12°C. Therefore, heifers receiving treatments within SET 2 may have utilized a greater portion of DE for maintenance of body temperature thus reducing energy available for gain. The NRC (23) indicates nutrient concentrations of heifer diets do not need adjustment when animals are subjected to thermal stress. Reports by (21, 33) indicate heifers adjusted DM intake to account for increased rumen motility and feedstuff digestibility due to cold weather. Apparently, in this study nutrient concentrations were too low and fiber content too high for heifers to make appreciable changes in intake and thus DE intake to off-set poor climatic conditions.

Growth in stature (WH and HIP) and heart girth reflected increased levels of NSC within diets. Regardless of UIP level, growth in WH, HIP, and HEART increased as NSC increased from 17 to 26%. Within levels of NSC, as diets increased in UIP the trend was toward greater structural growth. Skeletal growth did not, however, show as severe a depression as BW for treatments in SET 2. This indicates heifers continued to partition nutrients into structural components while reducing deposition and adipose
tissue. Tomlinson et al. (43) showed a positive response in structural growth of Holstein heifers while receiving diets of 30 to 50% UIP. These findings suggest UIP levels > 40% of CP may provide a more optimal protein profile when dietary NSC content is 20% or greater. Oldham (28) reported concentration of protein > 12% (of DM) in diets of nonlactating ruminants had little effect on DM digestibility. He indicated that response to higher protein was more often a result of reduced protein degradability improving amino acid flow to the small intestine. Brown and Lassiter (4) reported on protein:energy ratios showing dairy calves grew faster as the ratio decreased from 1:50 to 1:46, CP:ENE.

Dry matter intake in kg/d or g/kg BW<sup>0.75</sup> did not differ between treatments (Table 5). Intake did, however, differ between weeks with a significant week by treatment interaction. Weekly differences in intake were expected as a result of growing animals increasing nutrient intake. Week by treatment interaction may reflect ruminal adaptive changes over time and their influence on intake and growth. Dry matter intake tended to increase with increasing NSC. This trend suggests diets low in NSC were inadequate in maintaining microbial growth thus depressing fiber digestibility and intake. Nocek and Russell (26) suggested lack of fermentable carbohydrate could depress microbial growth, subsequently affecting supply of end-products (VFA's and microbial protein) and animal performance. Comparison of heifer response (treatment 5) for differences in DM intake between SETS indicated seasonal differences did not alter intake of heifers within this treatment.

Treatments did not differ in CP intake. Protein intake averaged 775 g/d which was 8% above NRC recommendations for .60 kg/d ADG while DM intake was 11% higher than (23). Quigley et al. (33) and Tomlinson (44) reported confinement reared Holstein heifers with ad libitum access to rations consistently ate 3 to 19% more DM than NRC estimates.

Trial 3  

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As planned, undegradable protein intake increased significantly as dietary UIP increased from 30 to 50% of total N. According to (23) only diets with > 50% UIP provide adequate undegradable protein (287 g/d) to sustain BW gains of .60 kg/d. Results from this study indicate comparable growth can be maintained at 30 and 40% UIP when NSC content is 21% of DM or greater. Oldham (28) indicated that increasing the supply of energy yielding nutrients improved protein utilization and overall animal performance. Our study supports his work because as NSC increased, utilization of degradable protein improved and thus heifer growth.

Due to differences in NSC and TDN content, treatments differed in DE intake (Mcal/d) and reflected DM intake variation between treatments (Table 5). Apparent feed efficiency (DE Mcal/kg BW gain), with the exception of treatment 2, improved with increasing dietary UIP. Even though apparent efficiency improved with UIP, more DE was required per kilogram BW gain than reported by Tomlinson (42, 43), and Klopfenstein and Goedeken (14). Heifers receiving diets with > 40% UIP required 30% less DE Mcal/kg BW gain than those on 30% UIP diets. Studies by (1, 14, 48) showed similar improvement in feed efficiency of 20 to 30% when diets were supplemented with rumen undegradable protein sources. Figure 1 shows heifer response in DE intake and BW gain with increasing levels of NSC at three levels of UIP. At low levels of NSC, UIP concentration had little relation to DE intake yet BW gain was greater for heifers receiving 50% UIP. Differences in BW gain diminished as dietary NSC content increased yet began to widen as NSC increased above 23% even though DE intake differences between UIP levels remained relatively constant.

Figure 2 shows the quadratic response in DE Mcal/kg BW gain to increasing levels of UIP and NSC reflecting equations from Table 6. As shown in Table 5 and Figure 2, as diets increased in UIP fewer megacalories of DE were required per kilogram BW gain. Improvement in apparent efficiency with increased NSC was greatest at 30% UIP, sup-
porting the hypothesis of (26, 28) that inadequate degradable carbohydrate depresses protein utilization and animal performance. Research by Sniffen and Hoover (38) and Orskov and Chen (29) suggested feed efficiency can be improved by supplementation of diets with high quality protein sources such as blood meal or corn gluten meal. They indicated these products contain high levels of lysine and methionine which were determined to be limiting in rapidly growing animals. Studies by Klopfenstein and Goedeken (14) and Richardson and Hatfield (35) verified these limitations of amino acids by replacing urea and soybean meal with blood meal, dehydrated alfalfa haylage, and corn gluten meal, showing an average improvement in gain of 30%. Even though increasing levels of NSC improved DE efficiency at 30 and 40% UIP, apparently the amino acid profile provided at 50% UIP was superior and could not be masked by increased energy intake and utilization of more degradable proteins.

**Body composition estimates**

Urea space and ultrasound estimates of body composition are in Table 7. As planned, beginning empty body water and empty body fat did not differ between treatments. Estimates of empty body water, fat, and protein were consistent with those reported by Hammond et al. (9) for dairy steers, but differed from reports by (7, 15) for beef animals. Significant negative change in empty body water indicated heifers increased in body fat content. Significant differences in change in empty body fat seemed to reflect poor growth response of treatments 2, 5, 7, and 9 of SET 2 more so than response to dietary UIP or NSC of remaining treatments.

Polynomial regression of DE intake and change in empty body fat percent on NSC and UIP is in Figure 3. As diets increased in UIP and NSC, deposition of energy as fat tended to increase. When dietary NSC fell below 17%, UIP content appeared to have little influence on energy deposition as fat. Waldo et al. (46) reported heifers retained
more energy in protein as total energy intake increased. They indicated that while rapidly growing heifers deposit more energy as protein it is proportionally less relative to fat. This study shows similar response in protein deposition to Waldo's work as heifers received increasing levels of NSC. Apparently diets containing NSC < 22% did not provide adequate readily fermentable carbohydrate to support appropriate levels of microbial growth and substrate utilization, thus affecting VFA patterns and energy absorbed from the gut.

Ultrasonic estimates of loineye area (longissimus muscle) differed between treatments. Taking the first derivative of loineye change with respect to UIP and NSC showed loineye area changed .081, .275, and .469 cm²/d as UIP increased from 30 to 50% at 22% NSC. Results suggest that as more energy became available a greater proportion of protein was deposited as muscle. Therefore at low NSC concentrations protein may have served as an energy source at the expense of muscle accretion.

Treatments did not differ in ultrasonic backfat measures. High standard errors were the result of young Jersey heifers having very little backfat which was difficult to measure from ultrasonic scans. Reports by Terry et al. (41) and Wallace et al. (47) concluded measurement of backfat thickness and loineye area could be accomplished reliably with ultrasound. They, however, utilized feeder cattle and hogs which contained backfat thickness measurable in centimeters. Correlations between backfat thickness and loineye areas were .58 to .77 (41, 47).

Correlations between urea space and ultrasound measures were of interest with respect to explaining similar changes in body composition. Correlations between empty body water and fat were high (r = -.99) as fat content was predicted from water. Body protein content was not significantly correlated to body water (r = .03) thus supporting the theory by Reid (34) of constancy of body protein content. High standard errors in change in urea space estimates reflect problems associated with prediction of body water
in very lean animals. Hammond et al. (9) indicated animal variation was high, with repeatability of body compositional estimates variable, when body fat content was low (<10%), similar to our study. High correlation between beginning and ending body protein estimates ($r = .73$) again substantiates the constancy of body protein. In addition, correlations between duplicate ultrasonic scans for loineye area and backfat were $r = .94, .84$. Correlation between beginning and ending loineye and backfat estimates were $r = .72$ and $.56$ suggesting loineye measures were more repeatable and consistent over time than backfat. Correlations between loineye area and body water were low ($r = -.32$ to -.53) when compared to empty body protein ($r = .68$ to .79) suggesting change in empty body protein was reflected by growth in longissimus muscle area.

Within the context of this study, these measures appear to be repeatable and provide sufficient information toward explaining body compositional differences due to treatment effects. High correlations between beginning and ending estimates by ultrasound and agreement of urea space estimates with those found in the literature, indicate estimation of body compositional changes by ultrasound and urea space may provide accurate information when evaluating nutritional effects on animal performance.

Varying dietary UIP and NSC content resulted in different gain responses without significantly affecting DM intake. Results indicated efficiency of DE conversion to BW increased as dietary UIP content increased from 30 to 50% of total N. Regardless of dietary UIP content, as dietary NSC content increased apparent deposition of dietary substrates as protein increased as reflected by increased empty body protein content and growth in loineye area. Change in body fat responded positively to increasing levels of NSC with response greatest on high UIP treatments. Results of this study indicate Jersey heifer growth can be maintained at .60 kg/d on low NSC diets when supplemented with rumen undegradable protein sources such as blood meal. Results also indicate
NRC recommendations for CP and UIP may be too generous for Jersey heifers between 119 and 300 kg BW.
Table 1. Trial 3 experimental diet formulations and analysis.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Treatment classification % of ration DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H  H  H  M  M  M  L  L  L</td>
</tr>
<tr>
<td>Alfalfa haylage</td>
<td>8.9 18.2 6.0 7.2 28.2 7.0 22.8 47.0 26.2</td>
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<tr>
<td>Orchardgrass hay</td>
<td>74.5 10.0 – 42.9 25.2 – 52.5 33.5 24.1</td>
</tr>
<tr>
<td>Straw</td>
<td>– 34.0 36.3 25.3 15.6 35.1 7.0 – 6.1</td>
</tr>
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<td>High moisture corn</td>
<td>10.7 13.5 11.5 4.3 13.1 12.0 4.5 – 2.1</td>
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<tr>
<td>Barley</td>
<td>– 17.2 40.1 10.8 16.1 38.5 3.5 19.1 36.2</td>
</tr>
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<td>Soybean meal</td>
<td>– 1.1 – 7.4 – 5.0 9.3 – 3.7</td>
</tr>
<tr>
<td>Blood meal</td>
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</tr>
<tr>
<td>Mineral premix</td>
<td>.4 .4 .4 .4 .4 .4 .4 .4 .4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical analysis¹</th>
<th>LS Means²</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of DM</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>84.6</td>
<td>72.9</td>
</tr>
<tr>
<td>CP</td>
<td>12.8</td>
<td>15.7</td>
</tr>
<tr>
<td>UIP³</td>
<td>60.6</td>
<td>49.7</td>
</tr>
<tr>
<td>TDN</td>
<td>58.5</td>
<td>61.4</td>
</tr>
<tr>
<td>ADF</td>
<td>36.1</td>
<td>33.2</td>
</tr>
<tr>
<td>NDF</td>
<td>61.5</td>
<td>54.7</td>
</tr>
<tr>
<td>NSC</td>
<td>17.6</td>
<td>21.9</td>
</tr>
<tr>
<td>EE</td>
<td>1.59</td>
<td>1.29</td>
</tr>
<tr>
<td>ASH</td>
<td>6.54</td>
<td>6.36</td>
</tr>
</tbody>
</table>

¹ Calculated from individual ingredient analysis.
² Least square means, n = 9.
³ From in situ N digestibility.

UIP H = 50%, M = 40%, L = 30%
NSC H = 26%, M = 22%, L = 17%
Table 2. Analysis of variance for heifer response in dry matter intake

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments¹</td>
<td>8</td>
<td>20.6</td>
<td>1.39</td>
</tr>
<tr>
<td>Weeks</td>
<td>8</td>
<td>17.8</td>
<td>50.50**</td>
</tr>
<tr>
<td>Tmt x week</td>
<td>64</td>
<td>.85</td>
<td>1.85**</td>
</tr>
<tr>
<td>Heifer(tmt)</td>
<td>68</td>
<td>19.3</td>
<td>54.91**</td>
</tr>
<tr>
<td>Residual</td>
<td>544</td>
<td>.35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>692</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Treatments tested using mean square of heifer(tmt).
** P < .01.
Table 3. Fractional rates (\%/h) of ruminal dry matter, crude protein, neutral detergent fiber digestion for alfalfa haylage, orchardgrass hay, straw, high moisture corn, barley, soybean meal, and blood meal.

<table>
<thead>
<tr>
<th>Component fraction</th>
<th>AH¹</th>
<th>OH</th>
<th>ST</th>
<th>HMC</th>
<th>BG</th>
<th>SBM</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
</tr>
<tr>
<td>A</td>
<td>48.4</td>
<td>13.0</td>
<td>12.8</td>
<td>1.4</td>
<td>3.5</td>
<td>1.2</td>
<td>20.1</td>
</tr>
<tr>
<td>B</td>
<td>31.7</td>
<td>7.1</td>
<td>45.3</td>
<td>1.7</td>
<td>36.2</td>
<td>4.7</td>
<td>78.4</td>
</tr>
<tr>
<td>C</td>
<td>19.9</td>
<td>5.9</td>
<td>40.9</td>
<td>2.9</td>
<td>60.2</td>
<td>3.6</td>
<td>1.6</td>
</tr>
<tr>
<td>k(\text{d} )²</td>
<td>10.4</td>
<td>1.2</td>
<td>4.9</td>
<td>.5</td>
<td>3.6</td>
<td>.4</td>
<td>8.7</td>
</tr>
<tr>
<td>D⁴</td>
<td>62.0</td>
<td>3.1</td>
<td>51.0</td>
<td>3.8</td>
<td>37.4</td>
<td>2.1</td>
<td>85.1</td>
</tr>
</tbody>
</table>

Fractional CP degradability

<table>
<thead>
<tr>
<th>Component fraction</th>
<th>AH</th>
<th>OH</th>
<th>ST</th>
<th>HMC</th>
<th>BG</th>
<th>SBM</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
</tr>
<tr>
<td>A</td>
<td>69.9</td>
<td>12.8</td>
<td>15.2</td>
<td>1.2</td>
<td>12.1</td>
<td>4.4</td>
<td>23.8</td>
</tr>
<tr>
<td>B</td>
<td>22.1</td>
<td>10.5</td>
<td>57.4</td>
<td>2.2</td>
<td>54.0</td>
<td>10.7</td>
<td>74.2</td>
</tr>
<tr>
<td>C</td>
<td>8.0</td>
<td>2.3</td>
<td>27.4</td>
<td>1.3</td>
<td>33.9</td>
<td>7.6</td>
<td>1.9</td>
</tr>
<tr>
<td>k(\text{d} )²</td>
<td>14.7</td>
<td>3.2</td>
<td>6.8</td>
<td>1.3</td>
<td>6.7</td>
<td>1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>D⁴</td>
<td>86.6</td>
<td>4.0</td>
<td>48.0</td>
<td>3.2</td>
<td>43.2</td>
<td>5.7</td>
<td>63.0</td>
</tr>
</tbody>
</table>

Fractional NDF degradability

<table>
<thead>
<tr>
<th>Component fraction</th>
<th>AH</th>
<th>OH</th>
<th>ST</th>
<th>HMC</th>
<th>BG</th>
<th>SBM</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
<td>( % ) SD</td>
</tr>
<tr>
<td>A</td>
<td>8.1</td>
<td>3.8</td>
<td>4.4</td>
<td>2.5</td>
<td>.9</td>
<td>1.0</td>
<td>6.2</td>
</tr>
<tr>
<td>B</td>
<td>48.8</td>
<td>5.9</td>
<td>51.3</td>
<td>7.7</td>
<td>37.7</td>
<td>5.2</td>
<td>81.7</td>
</tr>
<tr>
<td>C</td>
<td>43.1</td>
<td>6.2</td>
<td>44.2</td>
<td>1.9</td>
<td>61.5</td>
<td>6.0</td>
<td>12.1</td>
</tr>
<tr>
<td>k(\text{d} )²</td>
<td>7.5</td>
<td>4.4</td>
<td>4.9</td>
<td>1.5</td>
<td>3.2</td>
<td>1.1</td>
<td>3.3</td>
</tr>
<tr>
<td>D⁴</td>
<td>37.5</td>
<td>6.4</td>
<td>29.0</td>
<td>4.6</td>
<td>14.5</td>
<td>.4</td>
<td>40.4</td>
</tr>
</tbody>
</table>

¹Ingredients, AH = alfalfa haylage, OG = orchardgrass hay, ST = straw, HMC = high moisture corn,
BG = barley, SBM = soybean meal, BM = blood meal.

²Fractions of DM, CP, NDF are A = rapidly solubilized, B = degraded at a measurable rate, C = undegraded residue after 72 h ruminal incubation.

³k\(\text{d} \) = degradation rate of fraction B, (\%/h).

⁴D = estimated percent rumen degradability of component at rumen turnover rate of 0.05/h.
NE = non-estimable component.
Table 4. Least squares means of heifer performance measures of growth in BW, WH, HIP height, and HEART girth.

<table>
<thead>
<tr>
<th>Treatment classification</th>
<th>Treatment variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>SE&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>UIP;</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td>362</td>
</tr>
<tr>
<td>NSC;</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td></td>
<td>232</td>
</tr>
<tr>
<td>Trial variable</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE (d)</td>
<td>362</td>
<td>440</td>
<td>357</td>
<td>310</td>
<td>378</td>
<td>318</td>
<td>397</td>
<td>373</td>
<td>387</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Initial BW (kg)</td>
<td>195</td>
<td>214</td>
<td>191</td>
<td>160</td>
<td>201</td>
<td>164</td>
<td>208</td>
<td>195</td>
<td>206</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>Final BW (kg)</td>
<td>232</td>
<td>230</td>
<td>233</td>
<td>178</td>
<td>230</td>
<td>200</td>
<td>221</td>
<td>231</td>
<td>235</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>GAIN (kg/d)</td>
<td>.609</td>
<td>.259</td>
<td>.694</td>
<td>.310</td>
<td>.467</td>
<td>.605</td>
<td>.215</td>
<td>.605</td>
<td>.489</td>
<td>.014&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Initial WH (cm)</td>
<td>106</td>
<td>107</td>
<td>105</td>
<td>100</td>
<td>105</td>
<td>104</td>
<td>104</td>
<td>104</td>
<td>109</td>
<td>2.96</td>
<td></td>
</tr>
<tr>
<td>Final WH (cm)</td>
<td>110</td>
<td>110</td>
<td>109</td>
<td>103</td>
<td>109</td>
<td>105</td>
<td>108</td>
<td>108</td>
<td>109</td>
<td>2.68</td>
<td></td>
</tr>
<tr>
<td>WHGRO (cm/d)</td>
<td>.075</td>
<td>.061</td>
<td>.079</td>
<td>.070</td>
<td>.072</td>
<td>.082</td>
<td>.054</td>
<td>.068</td>
<td>.071</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>Initial HIP (cm)</td>
<td>109</td>
<td>110</td>
<td>109</td>
<td>103</td>
<td>108</td>
<td>104</td>
<td>108</td>
<td>108</td>
<td>107</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td>HIPGRO (cm/d)</td>
<td>.056</td>
<td>.051</td>
<td>.067</td>
<td>.061</td>
<td>.060</td>
<td>.076</td>
<td>.042</td>
<td>.040</td>
<td>.073</td>
<td>.004&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Initial HEART (cm)</td>
<td>132</td>
<td>135</td>
<td>130</td>
<td>119</td>
<td>131</td>
<td>121</td>
<td>131</td>
<td>132</td>
<td>131</td>
<td>5.39</td>
<td></td>
</tr>
<tr>
<td>HEARTGRO (cm/d)</td>
<td>.110</td>
<td>.054</td>
<td>.132</td>
<td>.088</td>
<td>.109</td>
<td>.112</td>
<td>.103</td>
<td>.098</td>
<td>.104</td>
<td>.005</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> SE appropriate for n=7.
<sup>2</sup> Daily gain effects; N, NS, NU, NSU.

N = Nonstructural carbohydrate effect.
NS = Squared effect of nonstructural carbohydrate.
U = Undegradable protein effect.
US = Square effect of undegradable protein.
NU = Effect of interaction between NSC and UIP.
NSU = Effect of interaction between NSC squared and UIP.
Significant treatment effects, p<.05.
Table 5. Least squares means of heifer performance measures of DM intake, CP intake, undegradable protein intake, DE intake, and DE intake per kilogram BW gain.

<table>
<thead>
<tr>
<th>Trial variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>SE^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM Intake (kg/d)</td>
<td>5.47</td>
<td>5.84</td>
<td>5.01</td>
<td>4.28</td>
<td>5.52</td>
<td>5.12</td>
<td>4.92</td>
<td>6.19</td>
<td>6.25</td>
<td>.075</td>
</tr>
<tr>
<td>DMI (g/kg BW^0.75)</td>
<td>97.0</td>
<td>99.4</td>
<td>108</td>
<td>90.8</td>
<td>99.3</td>
<td>102</td>
<td>88.9</td>
<td>112</td>
<td>108</td>
<td>1.29^N</td>
</tr>
<tr>
<td>CP Intake (g/d)</td>
<td>707</td>
<td>919</td>
<td>762</td>
<td>587</td>
<td>787</td>
<td>671</td>
<td>737</td>
<td>849</td>
<td>954</td>
<td>12.6</td>
</tr>
<tr>
<td>UP Intake (g/d)</td>
<td>419</td>
<td>456</td>
<td>405</td>
<td>231</td>
<td>304</td>
<td>251</td>
<td>222</td>
<td>233</td>
<td>273</td>
<td>4.4^US</td>
</tr>
<tr>
<td>DEG (Mcal/kg Gain)</td>
<td>23.24</td>
<td>40.15</td>
<td>24.79</td>
<td>31.20</td>
<td>35.18</td>
<td>25.30</td>
<td>58.85</td>
<td>30.04</td>
<td>37.79</td>
<td>.49^U</td>
</tr>
</tbody>
</table>

^1 SE appropriate for n=63.

N = Nonstructural carbohydrate effect.
NS = Squared effect of nonstructural carbohydrate.
U = Undegradable protein effect.
US = Squared effect of undegradable protein.
NU = Effect of interaction between NSC and UIP.
NSU= Effect of interaction between NSC squared and UIP.
Significant treatment effects, p<.05.
Table 6. Regression coefficients of heifer response variables on ration nonstructural carbohydrate and rumen undegradable protein content.

<table>
<thead>
<tr>
<th>Response</th>
<th>Intercept</th>
<th>NSC</th>
<th>NSC&lt;sup&gt;2&lt;/sup&gt;</th>
<th>UIP</th>
<th>UIP&lt;sup&gt;2&lt;/sup&gt;</th>
<th>NSC*UIP</th>
<th>NSC&lt;sup&gt;2&lt;/sup&gt;*UIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM&lt;sup&gt;2&lt;/sup&gt;</td>
<td>13.92211</td>
<td>.48423</td>
<td>-.00931</td>
<td>-.09243</td>
<td>.00107</td>
<td>4.93E-05</td>
<td>-8.54E-06</td>
</tr>
<tr>
<td>DMG&lt;sup&gt;3&lt;/sup&gt;</td>
<td>18.48239</td>
<td>9.58755</td>
<td>-.20228</td>
<td>-.00884</td>
<td>.01143</td>
<td>-.11249</td>
<td>.00285</td>
</tr>
<tr>
<td>DEG&lt;sup&gt;4&lt;/sup&gt;</td>
<td>124.20951</td>
<td>-4.31022</td>
<td>.06093</td>
<td>-2.56153</td>
<td>.00379</td>
<td>.13828</td>
<td>-.00258</td>
</tr>
<tr>
<td>CPR&lt;sup&gt;5&lt;/sup&gt;</td>
<td>26.51339</td>
<td>41.22449</td>
<td>-.37213</td>
<td>-10.2379</td>
<td>.00716</td>
<td>1.04269</td>
<td>-.03342</td>
</tr>
<tr>
<td>UIP&lt;sup&gt;6&lt;/sup&gt;</td>
<td>-175.97805</td>
<td>-.00497</td>
<td>.17386</td>
<td>6.12181</td>
<td>-.06348</td>
<td>.83785</td>
<td>-.02192</td>
</tr>
<tr>
<td>GAIN&lt;sup&gt;7&lt;/sup&gt;</td>
<td>-1.02372</td>
<td>.17717</td>
<td>-.00392</td>
<td>.01402</td>
<td>.00034</td>
<td>-.00406</td>
<td>.00010</td>
</tr>
<tr>
<td>HIPURO&lt;sup&gt;8&lt;/sup&gt;</td>
<td>1.10258</td>
<td>-.10994</td>
<td>.00275</td>
<td>-.02007</td>
<td>-3.77E-06</td>
<td>.00208</td>
<td>-.511E-05</td>
</tr>
<tr>
<td>CHEBF&lt;sup&gt;9&lt;/sup&gt;</td>
<td>24.58066</td>
<td>-2.48817</td>
<td>.05767</td>
<td>-.36047</td>
<td>-4.04E-05</td>
<td>.03697</td>
<td>-.98E-04</td>
</tr>
<tr>
<td>CHLDIN&lt;sup&gt;10&lt;/sup&gt;</td>
<td>22.85883</td>
<td>-1.34206</td>
<td>.02472</td>
<td>-1.12418</td>
<td>.00403</td>
<td>.07191</td>
<td>-.00135</td>
</tr>
</tbody>
</table>

<sup>1</sup>Model Y = b<sub>0</sub> + b<sub>1</sub>X<sub>1</sub> + b<sub>2</sub>X<sub>2</sub> + b<sub>12</sub>X<sub>1</sub>X<sub>2</sub> + b<sub>12</sub>X<sub>1</sub>X<sub>2</sub> + b<sub>6</sub>X<sub>1</sub>X<sub>2</sub>

where; Y = response, X<sub>1</sub> = % nonstructural carbohydrate, X<sub>2</sub> = % undegradable protein.

<sup>2</sup>Dry matter intake, (kg/d).

<sup>3</sup>Dry matter intake, (g/kg BW-75).

<sup>4</sup>Digestible energy intake, (Mcal/kg gain).

<sup>5</sup>Crude protein intake, (g/d).

<sup>6</sup>Undegradable protein intake, (g/d).

<sup>7</sup>Daily BW gain, (kg/d).

<sup>8</sup>Daily HIP growth, (cm/d).

<sup>9</sup>Change in estimated empty body fat, (%).

<sup>10</sup>Change in loin eye area, (cm).
Table 7. Least squares means of urea space estimates of body water, fat, and protein, and ultrasound estimates of loineye area and backfat thickness.

| Treatment classification | H | H | H | M | M | M | L | L | L | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | SE¹ |
| BEBW²                   | 69.51 | 68.97 | 69.83 | 72.18 | 69.10 | 70.48 | 67.28 | 69.74 | 67.02 | .92 |
| CHEBW²                  | -3.49 | .33 | -1.83 | -1.14 | .27 | -.09 | -.01 | -2.77 | 1.28 | 1.14 |
| BEBF⁴                   | 6.70 | 7.16 | 6.30 | 4.27 | 7.05 | 5.79 | 8.64 | 6.46 | 8.87 | .85 |
| CHEBF⁴                  | 3.12 | -.23 | 1.70 | 1.29 | -.18 | .15 | .06 | 2.50 | -1.03 | 1.00 |
| BEBP⁶                   | 17.02 | 17.00 | 16.96 | 16.88 | 16.98 | 16.88 | 16.86 | 16.91 | 16.85 | .11 |
| CHEBP⁶                  | .06 | .20 | .22 | .10 | .22 | .26 | .19 | .12 | .34 | .01US |
| BLON⁸                   | 22.78 | 27.42 | 19.73 | 22.47 | 25.90 | 24.93 | 28.07 | 21.92 | 30.05 | 2.74 |
| CHLON⁹                  | 6.40 | 3.06 | 8.32 | - .28 | 3.34 | 4.09 | .88 | 3.61 | 1.37 | 1.74 |
| BBFAT¹⁰                 | 3.11 | 2.80 | 3.17 | 2.15 | 2.77 | 2.61 | 2.30 | 3.10 | 2.39 | .27 |
| CHBFAT¹¹                | .43 | .36 | .23 | .28 | .20 | -.20 | .37 | .35 | .38 | .22 |

¹ SE appropriate for n=7.
² Beginning Empty Body Water estimate, (%).
³ Change in Empty Body Water, ending – beginning estimate.
⁴ Beginning Empty Body Fat estimate, (%).
⁵ Change in Empty Body Fat, ending – beginning estimate.
⁶ Beginning Empty Body Protein estimate, (%).
⁷ Change in Empty Body Protein, ending – beginning estimate.
⁸ Beginning Loineye area estimate, (cm²).
⁹ Change in Loineye area, ending – beginning estimate.
¹⁰ Beginning Backfat estimate, (mm).
¹¹ Change in Backfat estimate, ending – beginning estimate.
¹² Daily Heart girth growth, (cm/d).
US = Squared effect of undegradable protein.
Figure 1. Second order polynomial regression of DE intake (Mcal/d) and daily gain on nonstructural carbohydrate at three levels of rumen undegradable protein.
Figure 2. Second order polynomial regression of DE intake (Mcal/kg BW gain) and daily gain (kg/d) on nonstructural carbohydrate at three levels of rumen undegradable protein.
Figure 3. Second order polynomial regression of undegradable protein intake and change in loineye area (cm) on nonstructural carbohydrate at three levels of rumen undegradable protein.
References


Protein and energy supply for high production of milk and meat. Pergamon Press, NY.


Summary

Trial 1.

1. Increasing dietary UIP% by replacement of soybean meal (SBM) with dried brewers grains (DBG) decreased DM intake 12 g/kg BW\(^{.75}\) d.
2. Replacing SBM with DBG at 93% of NRC (142) TDN (LTDN) improved daily gain .18 kg/d. Daily gain of heifers receiving DBG and LTDN was comparable to heifers receiving 109% of NRC (142) TDN (HTDN), regardless of protein supplement.
3. Growth in wither height increased .022 and .035 cm/d for HTDN and LTDN treatments when DBG replaced SBM.
4. Apparent feed efficiency (Mcal DE/kg BW gain) was similar for heifers receiving SBM at both HTDN and LTDN, and DBG at high TDN (27.0, 25.1, 24.2), but was improved (15.1) for heifers receiving DBG and LTDN.
5. Heifers did not differ in services per first conception, peak FCM, ME FCM, or somatic cell scores.
6. Culling as heifers or during first lactation was not attributed to treatment effects.

Trial 2.

1. Increasing dietary UIP% by replacing SBM with blood meal or a blood meal/urea mixture depressed DM intake 24.1 g/kg BW\(^{.75}\) d
2. Increasing UIP% did not effect daily BW gain, growth in wither height, hip height, or heart girth.
3. Apparent feed efficiency was improved (20.6, 16.1, 15.2, and 13.3 Mcal DE/kg gain for 31, 43, 50, 55% UIP).
4. Estimates of change in empty body fat as determined by urea space were not different between treatments.

Trial 3.

1. Different combinations of dietary nonstructural carbohydrate (NSC) and UIP content did not affect DM intake, although trends were toward lower intake as UIP increased and higher intake as NSC increased.
2. Daily gain varied from .259 to .694 kg/d and increased with increasing concentrations of UIP and NSC.
3. Apparent feed efficiency improved from 42.2 Mcal DE/kg gain at 30% UIP to 30.0 at 40 and 50% UIP.

4. Daily gain and feed efficiency differed between treatments, but differences may have been partially attributable to seasonal effects.

5. Change in empty body fat differed between treatments and was lowest at 22, 21.1, 20.2% NSC for 30, 40, and 50% UIP.

6. Longissimus muscle area increased with increasing UIP and NSC, with growth in square centimeters differing between treatments.

7. In situ procedures were used to verify N, DM, and NDF degradabilities of feedstuffs. Results were slightly lower than previous estimates (145, 148) and may reflect differences in quality of feeds in an animal receiving low levels of NSC and degradable protein.
Conclusions

Increasing ruminally undegradable protein in the diet resulted in lower DM intake while improving apparent feed efficiency. Apparently, diets supplemented with either dried brewers grains or blood meal were inadequate in soluble and readily degradable protein to support optimal rumen function and fiber digestion. In Trial 2 when blood meal was supplemented with urea, DM intake increased .41 kg/d supporting the premise that soluble protein may be lacking in high UIP diets (>50% of CP). Improvement in apparent feed efficiency was related to lower DE intake and higher daily BW gains shown in Trials 1 and 2. As UIP increased, DE intake declined as a consequence of lower DM intake. However, utilization of protein and energy may have been more efficient as a result of improved amino acid flow to the small intestine and lower losses of protein as \( \text{NH}_3 \), thereby reducing expenditure of energy for recycling or excretion of urea. One could speculate that increased feed efficiency may be the result of improved coupling between NSC and protein sources, along with the reduced loss of energy used in N recycling.

Wither and hip height improved slightly in most cases with increasing UIP%. In Trial 3, growth in stature increased with increasing NSC concentrations regardless of UIP%. These results disagree with Trial 1 which showed no differences between HTDN and LTDN. The positive response in Trial 3 may be due to lower dietary energy concentration (by design) and total carbohydrate content available to the heifer. Therefore, increasing NSC provided more readily degradable substrate to the microbial population thereby improving substrate utilization. Stimulation of growth by increasing NSC may reflect a more ruminally acceptable match between the degradabilities of protein and
carbohydrates. It may also identify the point at which microbial growth begins to become limited by an inadequate supply of readily degradable carbohydrates within the diet.

Urea space estimates indicated heifers did not change appreciably in body composition. Lack of body compositional changes may reflect inadequate length of trials to result in meaningful differences due to treatment effects. Slight changes in body composition were not detectable by urea space as estimates are considerably more variable than slaughter procedures. Ultrasonic scans provided an additional estimate of body composition. Growth in longissimus muscle area increased with increasing UIP and NSC. Possibly more energy have become available to the rumen in conjunction with increased flow of amino acids to the small intestine, thus increasing total substrates available for tissue deposition. High variability of backfat estimates predisposed this measure to the same short comings of urea space.

Lactational and reproductive performance as a latent response to nutritional treatment during the prepubertal period suggested little correlation existed between the two. A number of factors may account for a lack of treatment differences in our study. Heifers were well beyond the initial weight of 90 kg and were approaching the upper limit of the critical BW range for mammary development when the began consuming experimental diets. Growth differences between LTDN and HTDN were not appreciably different, thus limiting potential for differences in parenchymal tissue deposition. Length of exposure to trial diets may have been inadequate to establish physiologic differences in heifers.

Results from these trials indicate heifers will gain .6 kg/d or greater on diets with energy concentrations below NRC guidelines and UIP levels of 40% or greater. Diets containing 50% UIP and 85 to 95% of NRC (145) TDN supported high BW gains (.6 to .9 kg/d), skeletal growth, and growth in longissimus muscle area, with little change in body

Conclusions
fat. While trial results did not show UIP to influence milk production it seems probable
that under conditions of an extended study, UIP may have a positive effect. Increasing
dietary UIP improves feed efficiency, therefore heifers require a lower energy diet to
grow at recommended rates of gain. Low planes of nutrition may stimulate mammary
parenchymal growth by increasing secretion of somatotropin and corticoids. Therefore,
UIP supplementation should help to reduce feed costs, improve feed efficiency, and
possibly improve milk production by stimulating parenchymal growth.

These studies suggest NRC recommendations for total dietary protein, UIP, and energy
may be too generous for heifers raised in total-confinement facilities. Achievement of
UIP recommendations in very young heifers (100 kg BW) are beyond practical limits and
not achievable using feeds available to dairies in most of the U.S. Formulation of
diets to account for differences in UIP and NSC indicate overall heifer performance was
greatest at 50% UIP and 26% NSC.
Bibliography


Protein and energy supply for high production of milk and meat. Pergamon Press, NY.


Appendix A. Materials and Methods

In Situ Degradation Procedures

The in situ dacron bag technique was utilized in determination of DM, NDF, and protein degradability of alfalfa haylage, orchardgrass hay, straw, barley grain, high moisture corn, blood meal, and soybean meal. Five nonlactating Holstein cows (675 ± 23 kg) fitted with rumen cannula were used to measure degradability of feeds used in the Jersey heifer study. Since the heifer trial was divided into three SETS and degradability of feeds from each SET was of interest, two cows were used per heifer study SET. One cow participated in feedstuff degradability estimation for the first two SETS, thus the total of five cows for the three SETs. Cow assignment was dictated by the availability of nonlactating cannulated cows. All cows received a standard diet (Table 1) formulated to resemble those used in heifer feeding trials. In situ procedures were conducted in duplicate on feeds from each SET of treatments giving six in situ replicates. Cows were acclimated for 14 d to the standard diet and received the TMR at 1130 and 2330 h, ad libitum.

Spun polyester dacron bags (10 x 20 cm) with a defined pore size of 59 μ were filled with 5 g feedstuff DM giving a feed exposure of 14.9 mg/cm² in each bag. Prior to placement in bags, feedstuffs were prepared as follows: alfalfa haylage and high moisture corn were frozen at -20°C then ground with dry ice through a Wiley mill fitted with 6 mm screen. Orchardgrass hay (mean length = 7 cm) was ground through a Wiley mill with 6 mm screen. Barley grain was shattered in a hammer mill fitted with a 3.175 mm screen. Soybean and blood meals were used in their original form.
Table 1. Standard diet fed to cannulated dry cows during in situ feedstuff degradability estimation.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of ration DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa haylage</td>
<td>30.2</td>
</tr>
<tr>
<td>Orchardgrass hay</td>
<td>26.2</td>
</tr>
<tr>
<td>Straw</td>
<td>17.5</td>
</tr>
<tr>
<td>Barley</td>
<td>13.1</td>
</tr>
<tr>
<td>High moisture corn</td>
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</tr>
<tr>
<td>Blood meal</td>
<td>3.5</td>
</tr>
<tr>
<td>Mineral premix(^1)</td>
<td>.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis(^2)(^3)</th>
<th>% of ration DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>13.9</td>
</tr>
<tr>
<td>UIP(^4)</td>
<td>40.7</td>
</tr>
<tr>
<td>ADF</td>
<td>33.0</td>
</tr>
<tr>
<td>NDF</td>
<td>56.3</td>
</tr>
<tr>
<td>NE(_j) (Mcal/kg DM)</td>
<td>1.34</td>
</tr>
<tr>
<td>TDN</td>
<td>60.5</td>
</tr>
<tr>
<td>NSC</td>
<td>23.2</td>
</tr>
<tr>
<td>Ca(^5)</td>
<td>.60</td>
</tr>
<tr>
<td>P(^5)</td>
<td>.30</td>
</tr>
<tr>
<td>Vitamin A (IU/kg)</td>
<td>5,868</td>
</tr>
<tr>
<td>Vitamin D (IU/kg)</td>
<td>1,010</td>
</tr>
</tbody>
</table>

\(^1\) Mineral mix; 16% Ca, 6.5% P, 4.25% NaCl, 2.2% Mg, 3.2% S, 3.5% K, % DM
\(^2\) Ingredient analysis performed by Virginia Tech Forage Testing Lab.
\(^3\) Analysis determined from combination of individual ingredient analysis.
\(^4\) Estimates taken from NRC (144).
\(^5\) Values taken from NRC (145) and adjusted for content of mineral mix.
Two bags were used for each feed and time of incubation. Bags were suspended in the rumen of both cows for 0, 2, 6, 12, 18, 24, and 72 h, tied shut using plastic cable ties and grouped according to length of incubation. Groups of bags were attached to 3-4 link segments of 3.2 mm chain using cable ties. A 60 cm segment of nylon cord connected the chain segment and fistula cover. Bags were placed at the bottom of the ventral sac of the rumen.

Prior to incubation, bags containing samples were soaked for 15 min in 39°C H₂O to remove readily soluble and 59μ filterable materials. This reduced lag time for wetting in the rumen (231). Bags were placed in reverse order beginning with 72 h placed at 1500 h. All bags were removed from the rumen at the same time and immediately washed with cold tap water to halt further microbial fermentation. Bags were separated from the chains and rinsed in cold running water until rinse water was clear. Bags were next placed in a Maytag (tm) washing machine, washed for 6 min, rinsed, and spun dry. The rinsing procedure served as a means of removing ruminal contaminants from the interior and exterior bag surfaces. Nocek (146) indicated that thorough rinsing removes any material (except attached rumen microbes) able to influx the bags and any feed material which is soluble or extensively degraded. Bags were then suspended in a 60°C forced air oven and dried to a constant weight. Bags containing dried residue were opened and weighed to determine dry matter disappearance. Dried residues were next removed from the bags, composited for each incubation time, and ground through a Cyclone mill fitted with a 1 mm screen. Residues were analyzed for total nitrogen by Kjeldahl procedures (5) and neutral detergent fiber (70). Using the composition and weight of original samples, percent disappearance of DM, N, and NDF for each feedstuff was calculated for the various incubation periods.
Degradability Calculations

Ruminal degradation of DM, N, and NDF was evaluated as a function of time and most often the data fit a general model containing three pools or fractions (144). The general model is most suited to the N component:

A - Nonprotein nitrogen or true protein degraded very rapidly, generally considered soluble with a disappearance rate considered infinity.

B - Protein with a rate of degradation similar to the rate of passage \( (k_d = .02 \text{ to } .07/h). \)

C - Bound or unavailable protein with a rate of degradation considered zero.

Only fraction B is considered to be affected by relative rates of passage and degradation within the rumen at any point in time. Estimation of fractions A, B, and C were made for each feedstuff in each set of treatments as outlined by Zerbini (231). These procedures are as follows. A linear rate of disappearance was used to describe degradability of fraction B and to estimate its value at time zero. Degradation constants \( (k_d) \) were estimated by regressing the natural logarithm \( (\ln) \) of the percent feedstuff remaining minus the C fraction \( (\ln (% \text{ remaining } - \text{ C})) \) over time. The antilog of the intercept represented slowly degraded fraction B with the slope equal to rate of B fraction degradation \( (k_{\text{subd}}). \) Residual material present after 72 h was expressed as a percent of original sample and reported as fraction C. The rapidly degraded or "soluble" fraction \( (A) \) was the quantity: \( A = 1 - (B + C). \) The B fraction was calculated by the equation:

\[
B = e^{-\text{intercept}} \quad \text{where} \quad e = 2.71828.
\]

Feedstuff DM, NDF, and N degradability in percent \( (D) \) was calculated using the equation of Orskov and McDonald (159):
\[ D = A + (B \cdot k_d B)/(k_d B + k_r), \]

where:

- \( D \) = Component degradability (%);
- \( A \) = Fraction readily degraded (%);
- \( B \) = Fraction degraded at a measurable rate (%);
- \( k_d \) = Component degradation constant of B fractin (1/h);
- \( k_r \) = Rumen turnover rate (.05/h).

Mathematical procedures as adapted from Zerbini (231) are outlined in Figure 2. This model was also used in determination of the A, B, and C fractions within the DM and NDF components. While the A fraction may not represent a truly soluble fraction within NDF, it may represent an amount of structural carbohydrate which is more easily degraded within the rumen.

**Urea Space Procedures**

**Infusion procedure**

Change in body water, fat, and protein content were estimated using urea space dilution techniques as outlined by Prestion and Kock (168). Prior to the infusion procedures heifers were fasted a minimum of 12 h to reduce rumen fill. Fasting began at 1550 h with infusion procedures following at 0750 h. Immediately preceding infusion, heifers were secured in scales with a head-lock mechanism and weighed. A 10-15 ml blood sample was collected via venipuncture of the left jugular vein (14 ga needle) into a heparinized tube (200 μl Na-Heparin, 1000 units/ml) and immediately placed on ice. This was used to establish baseline blood urea concentration. Heifers were infused with 130 mg/kg BW of 20% urea solution dissolved in .9% saline solution into the left jugular. Heifers were released from the head-lock to move freely within the scales while the urea solution equilibrated with body water. A second blood sample was obtained 12 min post-infusion from the right jugular vein and placed on ice. This sample provided
### In Situ Bag Technique

#### Total Dry Matter

<table>
<thead>
<tr>
<th>Total Nitrogen</th>
<th>=</th>
<th>A</th>
<th>+</th>
<th>B</th>
<th>+</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NDF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Rumen Availability

- Soluble
  - Readily degraded
- Insoluble
  - Degraded at a measurable rate
- Indigestible
  - Not degraded

#### Degradation Rate

- \( k = \text{infinite} \)
- \( k = 0.02 - 0.07/h \)
- \( k = 0 \)

#### Calculation of Fraction

- \( A = 1 - (B + C) \)
- \( B = e^{-\text{intercept}} \)
- \( C = \frac{72h \text{ residual wt}}{\text{original wt}} \)

#### Diagrammatic presentation of degradability estimation

![Diagram](image)

**Figure 1.** Summary of the mathematical model used to determine rumen degradability of DM, N, and NDF.

**Appendix A. Materials and Methods**
an estimate of body water established by movement of urea solution into the body water pool. Upon completion of infusion procedures, samples were transported to the lab and spun at 5000 x g for 15 min. Plasma was removed and stored at 5°C until deproteinization the following day.

**Deproteinization procedure**

Nine milliliters tungstic acid solution (9 parts .083N H₂SO₄ and 1 part sodium tungstate 10% w/v) was added to 1 ml plasma to deproteinize. The combination was vortexed and allowed to stand 15 min. Samples were spun at 5000 x g for 10 min to remove precipitated protein from solution. Supernatant was filtered through 42 Whatman filter paper into clean tubes. Deproteinized filtrate was stored at -20°C until analysis for urea-N. Sample preparation for urea-N analysis was according to Coulombe and Faureau (44).

**Urea-N analysis**

Absorbance of standard urea-N solutions was established by pipetting duplicate 200μl of working standards into 16x100 mm glass test tubes. Two tubes containing 200μl distilled H₂O served as reagent blanks. Working standards were prepared by dilution of stock solution (2.142 g urea (46.7% N) in 1 L distilled H₂O) to concentrations of 1 to 5 mg/100 ml. In separate tubes, 200μl aliquots of deproteinized filtrate were pipetted. Colorimetric reagent was prepared by combining two parts diacety-monoxime thiosemicarbazide with ten parts phosphoric acid. Five milliliters of reagent mixture was
added to each assay tube. Tubes were vortexed followed by incubation in a dry bath block incubator at 90°C for 20 min.

Following incubation, tubes were cooled to room temperature in tap water. Colorimetric concentration of the red complex formed between urea and diacetyl-monoxime was read on a spectrophotometer (Spectronic 1001, Bausch and Lomb) at 540 nm. Results were corrected for 10% dilution factor and reported as mg urea-N per 100 ml sample.

Urea Space Calculation

Urea space as a percent of BW was calculated as suggested by Preston and Kock (168). Their equations rely upon change in plasma urea-N following injection of 20% urea solution for estimation of body water, fat, and protein content. The following equations were used in calculating urea space as a % of BW:

\[
\text{Stock solution (20% urea)} = 200 \text{ mg Urea/ml} \\
\text{Urea} = 46.7\% \text{ N} \\
200 \text{ mg Urea} \times 0.467 (\% \text{ N}) = 93.4 \text{ mg Urea-N/ml solution} \\
\text{Urea space} = \frac{(\text{mg Urea-N infused})}{(\text{change in PUN mg/dl})}
\]

Therefore: Urea space % BW = \(\frac{\text{Ureaspace}}{\text{LiveBW}}\) \times 10

Hammond et al. (75) found dairy steers and heifers did not have the same distribution of body water and fat as beef animals. Therefore, they developed the following equations for estimation of body composition in growing dairy animals. These equations were used to estimate proportions of body water, fat, and protein of heifers in Trials 2 and 3.

Appendix A. Materials and Methods
\% Empty Body Water = 83.5 - 0.16 \times \text{US \% BW} - 0.032 \times \text{BW} \\
\% Empty Body Fat = -5.9 + 0.14 \times \text{US \% BW} + 0.030 \times \text{BW} \\
\% Empty Body Protein = 16.6 - 0.009 \times \text{US \% BW} + 0.005 \times \text{BW}
Appendix B. SETS 1-3 In Situ estimates.
Table 1. Trial 3 - SET 1. Fractional rates (%/h) of ruminal dry matter, crude protein, neutral detergent fiber degradation.

<table>
<thead>
<tr>
<th>Component fraction²</th>
<th>AH¹</th>
<th>OH</th>
<th>ST</th>
<th>HMC</th>
<th>BG</th>
<th>SBM</th>
<th>BM</th>
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<tr>
<td>A</td>
<td>32.3</td>
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<td>B</td>
<td>40.9</td>
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<td>67.2</td>
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<td>C</td>
<td>29.6</td>
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<td>56.6</td>
<td>2.1</td>
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<td>kₐ³</td>
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<td>8.5</td>
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<td></td>
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</tbody>
</table>

| Component | Fractional CP degradability |
|-----------|
| A         | 52.2| 13.8| 6.4 | 30.3| 19.8| NA  | 7.4|
| B         | 36.7| 54.8| 68.7| 68.1| 73.6| NA  | 30.8|
| C         | 11.1| 27.4| 24.9| 1.6 | 6.6 | NA  | 61.3|
| kₐ        | 18.5| 5.9 | 7.8 | 6.0 | 10.2| NA  | 1.1|
| D         | 81.1| 45.6| 48.2| 67.4| 69.3| NA  | 13.0|
|           |     |     |     |     |     |     |    |

| Component | Fractional NDF degradability |
|-----------|
| A         | 2.9 | 4.3 | 2.3 | 5.4 | 2.4 | NA  | NE |
| B         | 43.7| 51.9| 43.0| 73.4| 55.7| NA  | NE |
| C         | 53.4| 43.8| 54.7| 20.2| 41.9| NA  | NE |
| kₐ        | 7.2 | 2.8 | 1.9 | 3.1 | 4.8 | NA  | NE |
| D         | 28.8| 22.8| 14.0| 34.5| 29.7| NA  | NE |

¹ Ingredients, AH = alfalfa haylage, OG = orchardgrass hay, ST = straw, HMC = high moisture corn.

BG = barley, SBM = soybean meal, BM = blood meal.

² Fractions of DM, CP, NDF are A = rapidly solubilized, B = degraded at a measurable rate, C = undegraded residue after 72 h ruminal incubation.

³ kₐ = degradation rate of fraction B, (%/h).

⁴ D = estimated percent rumen degradability of component at rumen turnover rate of .05/h.

NA = not available
NE = non-estimable component.
Table 2. Trial 3 - SET 2. Fractional rates (%/h) of ruminal dry matter, crude protein, neutral detergent fiber degradation.

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<tr>
<th>Component fraction</th>
<th>AH</th>
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<th>HMC</th>
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<tr>
<td>C</td>
</tr>
<tr>
<td>kd</td>
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<tr>
<td>D</td>
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<thead>
<tr>
<th>Fractional NDF degradability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>kd</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

\[1 \) Ingredients, AH = alfalfa haylage, OG = orchardgrass hay, ST = straw, HMC = high moisture corn, BG = barley, SBM = soybean meal, BM = blood meal.

\[2 \) Fractions of DM, CP, NDF are A = rapidly solubilized, B = degraded at a measurable rate, C = undegraded residue after 72 h ruminal incubation.

\[3 \) kd = degradation rate of fraction B, (%/h).

\[4 \) D = estimated percent rumen degradability of component at rumen turnover rate of .05/h.

NE = non-estimable component.
Table 3. Trial 3 - SET 3. Fractional rates (%/h) of ruminal dry matter, crude protein, neutral detergent fiber degradation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Fractional DM degradability</th>
<th>Fractional CP degradability</th>
<th>Fractional NDF degradability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1H</td>
<td>OH</td>
<td>ST</td>
</tr>
<tr>
<td>A</td>
<td>48.7</td>
<td>10.9</td>
<td>3.1</td>
</tr>
<tr>
<td>B</td>
<td>30.8</td>
<td>44.6</td>
<td>37.9</td>
</tr>
<tr>
<td>C</td>
<td>20.5</td>
<td>44.5</td>
<td>59.0</td>
</tr>
<tr>
<td>k_d</td>
<td>11.1</td>
<td>5.5</td>
<td>3.7</td>
</tr>
<tr>
<td>D</td>
<td>69.9</td>
<td>34.3</td>
<td>19.1</td>
</tr>
</tbody>
</table>

1 Ingredients, AH = alfalfa haylage, OG = orchardgrass hay, ST = straw, HMC = high moisture corn, BC = barley, SBM = soybean meal, BM = blood meal.

2 Fractions of DM, CP, NDF are A = rapidly solubilized, B = degraded at a measurable rate, C = undegraded residue after 72 h ruminal incubation.

3 k_d = degradation rate of fraction B, (%/h).

4 D = estimated percent rumen degradability of component at rumen turnover rate of .05/h.

NE = non-estimable component.
Vita

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Publications:
  Tomlinson, D. J., R. E. James, and M. L. McGilliard. Effect of
  varying levels of NDF and TDN on intake and growth of Holstein heifers.

Abstracts:
  Tomlinson, D. J., R. E. James, and M. L. McGilliard. 1987. Effect
  of neutral detergent fiber and total digestible nutrients on dry matter
  intake of dairy heifers. J. Dairy Sci. 70 (Suppl. 1):142.

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  Tomlinson, D. J., R. E. James, and M. L. McGilliard. 1989. Effect
  of TDN and degradable protein on intake, daily gain and subsequent

  Tomlinson, D. J., R. E. James, and M. L. McGilliard. 1990. Effect
  of ration protein undegradability on intake, daily gain, feed


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