Effect of Energy and Undegraded Intake Protein on Growth and Feed Efficiency of Growing Holstein Heifers.

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirement for the degree of

Masters of Science

in

Dairy Science

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September 30, 1994

Blacksburg, Va

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(ABSTRACT)

Two trials using 32 heifers each evaluated response to undegraded intake protein (UIP) (30 or 50% CP), energy (supporting .59 or .91 kg ADG), and source of UIP (blood meal or combination protein supplement). Trial one was a 2x2 factorial, with two levels of energy and UIP. High UIP was achieved with blood meal supplementation. From 6-13 mo of age (phase I), high energy increased ADG and DMI, and high UIP decreased DMI. DM efficiencies (kg DMI/kg BW gain) improved with high energy and high UIP, and TDN efficiencies (kg TDN/kg BW gain) improved with high UIP. From 13 mo until calving (phase II), heifers were housed together and fed a common diet. Low energy, high UIP treatment had the highest ADG (1.01 kg/day) for phase I, but the lowest for phase II (.33 kg/day), and low energy, low UIP treatment had the lowest ADG (.62 kg/day) for phase I, but the highest for phase II (.53 kg/day). Overall ADG from 6 mo until calving averaged .59 kg/day, and was not affected by energy or UIP. In trial 2, two levels of energy and two sources of UIP were compared, resulting in four treatments: low

energy, high UIP with combination protein supplement; low energy, high UIP with blood meal; low energy, low UIP with soybean meal; and high energy, low UIP with soybean meal. Combination protein supplement contained blood meal, corn gluten meal, and fish meal. Trial was 300 days long, and began at 6.5 mo. of age. Dry matter intake and ADG were increased with high energy, but not affected by UIP. Overall DM efficiency was not affected by UIP or energy level. Results of both trials indicate UIP may improve feed efficiency of growing Holstein heifers.

Acknowledgements

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

Graduate committee members M. L. McGilliard, R. L. Nebel, and C. E. Polan for their guidance and consideration of this work.

Chuck Miller, Harold Nester, Curtis Caldwell, Robert Maxey, and workers at the Virginia Tech Dairy Center for their assistance in all phases of the trials.

Wendy Wark for her assistance with urea space procedure and plasma urea-N analysis.

Lee Johnson for his aid in collection of ultrasound scans.

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

Graduate students Amin Amadzadeh, Curtis Baughman, Sue Pandolfi, Luis Rodriguez, Billy Walker, and Brian Wonsil for their aid in urea space procedure and collection of blood samples.

Dr. R. E. James for his guidance, support, and friendship.

My parents, Wilson and Gail Bethard, for their love and support.

To my wife, Rachel, who assisted in all phases of these trials, from blood collection to computer data entry. Her many sacrifices have made this degree possible. Most of all, I am grateful for her love, understanding, and friendship.

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Introduction

Heifer management is critical to the success of a dairy farm, since heifers are the future of the dairy herd. Specific goals for large breed dairy heifers include achieving 340-385 kg breeding weight at 13-14 mo. (Etgen et al., 1987; Nebel, 1993; Perkins, 1993) and 544-567 kg calving weight at 24-25 mo (Gill and Allaire, 1976; Keown and Everett, 1986). These goals have been correlated with optimum profit per d of herdlife (Gill and Allaire, 1976).

To achieve breeding weight goals, heifers must gain .8 kg/d from birth until 13 mo. Early calving at 21 mo. requires .98 kg ADG to reach breeding weight by 11 mo. Excessive prepubertal gains (>.9 kg/d for large breeds) have been associated with impaired mammary development and decreased 1st and 2nd lactation milk yield (Foldager and Sejrsen, 1982; Harrison et al., 1983; Sejrsen et al., 1982; Swanson, 1960). Therefore, rapid rearing to achieve early calving can be detrimental to mammary development and milk production.

The most recent NRC (National Research Council, 1989) provides undegradable intake protein (UIP) and degradable intake protein (DIP) recommendations for growing dairy heifers. Below 200 kg BW, NRC recommends UIP levels >50%. These UIP levels are difficult to achieve using common feed ingredients. Tomlinson (1990) reported

improved feed efficiency as UIP increased from 31 to 55% of CP, using blood meal as a source of UIP. Similarly, Thonney et al. (1986) reported that replacing cottonseed meal with fish meal in the diet of growing Holstein steers improved feed efficiency but not ADG. Zerbini et al. (1985) found fish meal fed to Holstein bull calves improved ADG but did not alter DMI compared to soybean meal. Swartz et al. (1991) reported high UIP diets supplemented with blood meal improved feed efficiency from 14-25 weeks of age but not from 1-13 weeks of age compared to a low UIP soybean meal supplemented diet. Other researchers (Heinrichs et al., 1993; Heinrichs and Garman, 1992; Mantysaari et al., 1989b) found no response in ADG or feed efficiency with high UIP diets

The inconsistent response to UIP might partially be explained by protein source. Use of a single UIP source can result in an amino acid (AA) deficiency. Amino acids absorbed in the small intestine originate from protein of microbial and dietary origin. Microbial protein is an excellent source of AA (Chandler, 1989; Mantysaari et al., 1989a), and is relatively constant in AA content (Purser and Buechler, 1966). However, most supplemental protein sources are deficient in one or more AA. Therefore deficiencies in the AA profile of the bypass fraction will result in an AA deficiency unless microbial protein meets the AA requirements of the animal. For example, blood meal is deficient in Met, implying diets with blood meal as the only UIP source result in a Met deficiency. A combination of protein sources high in UIP from different origins would favor a balanced profile of essential AA.

Few studies involving UIP and dairy heifers have been conducted for an extended period of time (Mantysaari et al., 1989b; Swartz et al., 1991). Many studies were less than 100 d long (Tomlinson et al., 1990; Zerbini et al., 1985) or involved heifers less than 6 mo of age (Heinrichs et al., 1993; Heinrichs and Garman, 1992; Swartz et al., 1991). In addition, some studies evaluated UIP response using diets that varied by less than 10% UIP (Mantysaari et al., 1989b). Therefore, the objectives of these trials were:

1. To evaluate the effect of widely divergent levels of UIP and energy on growth of Holstein heifers from 6-16 mo of age.

2. To determine if rapid gains (>.9 kg/d) from 6-14 mo of age influence calving age and calving weight.

3. To determine the effect of providing a UIP source with a balanced profile of essential AA.

DAIRY HEIFER GROWTH

Swanson described the optimum growth pattern for dairy heifers as the regimen that will develop in the heifer her full lactational potential at a desired age and at a minimum of expense (Swanson, 1967). Since lactation is the ultimate goal of a dairy animal, growth patterns that influence lactation potential are important. Body weight gains alone are meaningless unless they improve the lactational ability of the heifer.

Growth Standards For Dairy Heifers

Heifer performance is routinely measured by BW and wither height. In addition, wither height index (kg BW / cm wither height) can be calculated for a crude estimation of body condition. Performance measures for Holstein heifers from 1 - 24 mo are in Tables 1,2, and 3. Data from Heinrichs and Hargrove (1978), Matthews and Fohrman (1954), and Hoffman (1993) are from field collection, while the values of Etgen et al. (1987) are recommendations. Etgen et al. and Matthews and Fohrman reported similar BW throughout the growth cycle, while weights reported by Heinrichs et al. were heavier for heifers >18 mo., and weights reported by Hoffman were heavier for all ages. Matthews and Fohrman recommendations are dated (1954), possibly not reflecting genetic change over time. Etgen et al. recommendations are conservative, indicating a desire to avoid excessive gains. Date from Heinrichs et al. was from 163 commercial dairy herds in

Table 1. Average Body Weights (kg) of Holstein heifers from 1 - 24 months.

Age (months)	Heinrichs[1]	USDA[2]	Hoffman[3]	Etgen[4]
Birth	------	43.6	------	42.22
1	62.9	53.5	------	52.2
2	83.9	73.1	76.3	72.6
3	100.9	96.7	------	------
4	126.7	123.5	------	122.6
5	145.3	152.1	154.8	------
6	171.0	179.8	------	177.1
7	195.5	206.6	------	------
8	211.0	230.6	224.7	231.5
9	236.8	253.8	------	------
10	264.7	276.5	------	276.9
11	286.8	298.7	327.3	------
12	305.7	324.1	------	317.8
13	332.0	335.9	------	------
14	353.3	351.4	385.4	354.1
15	375.6	365.5	------	------
16	386.4	381.8	------	385.9
17	411.0	396.8	463.5	------
18	429.9	414.0	------	413.4
19	443.4	429.5	------	------
20	462.6	447.2	533.4	444.9
21	491.2	465.4	------	------
22	515.6	------	------	476.7
23	516.9	------	610.2	------
24	525.9	------	------	513.0

[1] Heinrichs, et al., 1978
[2] Matthews and Fohrman, 1954
[3] Hoffman, 1993
[4] Etgen et al., 1987.

Table 2. Average Wither Heights (cm) of Holstein heifers from 1 - 24 months.

Age (months)	Heinrichs[1]	Hoffman[2]	Etgen[3]
Birth	------	------	73.7
1	81.0	------	78.7
2	86.5	85.1	86.4
3	90.2	------	------
4	94.4	------	99.10
5	97.4	99.1	------
6	102.0	------	106.7
7	105.5	------	------
8	107.9	109.2	111.8
9	111.4	------	------
10	113.9	------	116.8
11	116.7	119.4	------
12	118.5	------	121.9
13	120.9	------	------
14	122.4	124.9	124.5
15	124.1	------	------
16	125.3	------	127.0
17	126.8	130.6	------
18	128.2	------	129.5
19	129.5	------	------
20	130.2	132.8	132.1
21	131.5	------	------
22	133.2	------	134.6
23	133.8	136.7	------
24	132.8	------	137.2

[1] Heinrichs et al., 1978
[2] Hoffman, 1993
[3] Etgen et al., 1987

Table 3. Calculated Wither Height Index[1] of Holstein heifers from 1 - 24 months, using the data from tables 2 and 3.

Age (months)	Heinrichs[2]	Hoffman[3]	Etgen[4]
Birth	------	------	.57
1	.78	------	.66
2	.97	.90	.84
3	1.12	------	------
4	1.34	------	1.24
5	1.49	1.56	------
6	1.68	------	1.66
7	1.85	------	------
8	1.96	2.06	2.07
9	2.13	------	------
10	2.32	------	2.37
11	2.46	2.74	------
12	2.58	------	2.61
13	2.75	------	------
14	2.89	3.08	2.85
15	3.03	------	------
16	3.08	------	3.04
17	3.24	3.55	------
18	3.35	------	3.19
19	3.42	------	------
20	3.55	4.02	3.37
21	3.73	------	------
22	3.87	------	3.54
23	3.86	4.47	------
24	3.96	------	3.74

[1] Wither Height Index = kg BW / cm WH
[2] Heinrichs et al., 1978
[3] Hoffman, 1993
[4] Etgen et al., 1987

Pennsylvania. The higher BW of older heifers could be reflective of the high percentage of confinement-reared heifers in Pennsylvania. Hoffman BW were taken from the top producing herds in Wisconsin. Large differences in BW between Hoffman and others could be due to management in high producing herds. If the cows are milking well, it means they are fed well, therefore it is likely the heifers are fed well. Calculated wither height indexes reflect this - Hoffman heifers had the highest index.

Even though growth charts for Holstein heifers vary, most researchers agree that heifers should be bred at 340 - 385 kg (Etgen et al., 1987; Nebel, 1993; Perkins, 1993). If heifers are to reach this weight in 13 mo, they need to gain about 0.8 kg / d. Breeding at 13 mo should allow for an average first calving age of 24 mo. Recommended weight at first calving for Holstein heifers ranges from 485 kg to 615 kg (Hoffman, 1993; Etgen et al., 1987). These recommendations do not state if the weight is before or after calving. The lower part of the range would be adequate after calving, and the upper part would be adequate before calving. In a large field study, Keown and Everett (1986) determined 544 - 567 kg is the optimum freshening weight for Holstein heifers. They analyzed all Holstein records processed at the Northeast Dairy Records Processing Laboratory between July 1980 and August 1984. Heifers calving at these weights had higher first lactation milk yields than those that calved heavier or lighter. F values were higher for BW than for age at first calving, implying heifers should be bred by weight and not age. Gill and Allaire (1976) utilized lifetime production and reproduction data on 933 cows to develop a profit

function for Holstein cows. One aspect of the profit function was age at first calving. A conservative reference rearing cost of $535 was used for heifers calving at 24 mo. Individual rearing costs were adjusted plus $20 for each mo above or minus $20 for each mo below 24 mo age at first calving. They determined that 22.5 - 23.5 mo was the optimum age at first calving for total lifetime performance, but 25 mo was the optimum age for profit per d of herdlife. The findings of Keown and Everett (1986) and Gill and Allaire (1976) emphasize the importance of calving heifers early (24 mo) that have adequate but not excessive BW (544 - 567 kg for Holsteins).

Consequences of Inadequate Growth in Dairy Heifers

Severe nutritional deficiencies slow or stop growth in all body tissues except the brain (Widowsen et al., 1991). Inadequate growth in dairy heifers results in delayed maturity, delayed breeding, and delayed calving, which all are costly to dairymen. Webb (1986) reported total rearing costs changed with different ages at first calving as follows: 23.3 mo, $1074.41; 26.4 mo, $1167.17; 31.7 mo, $1295.07; 33.9 mo, $1366.06. Smith (1993) reported costs of calving a heifer beyond 24 mo to be $50.04 per mo over 24 mo. Webb assumed feed costs/d increased with earlier calving, but labor costs/d remained constant. Smith did not change costs/d, regardless of calving age. Since it is of economic importance to calve heifers at or near 24 mo, lifetime ADG until calving should be 0.64 -

0.77 kg/d to achieve 570 kg BW at calving. Growth rates below .64 kg/d require higher gains during other periods of the growth cycle. Heifers gaining below .45 kg/d may show less intense estrus (ovulation occurs, but signs of heat are not visible) and may develop smaller mammary glands (Head, 1992).

To evaluate effects of underfeeding dairy heifers, Swanson and Hinton (1964) fed nine identical twin pairs of mostly Jersey breeding either a normal diet containing alfalfa-grass hay ad lib and concentrates (up to one year), or a restricted diet (66% of the normal TDN) from 4 to 24 mo of age. Three of the nine pairs had to be removed because the heifers on the restricted diet had breeding difficulty or had calving problems. Heifers on the restricted diet were lighter at calving and produced less milk during first lactation (2154 vs 1870 kg FCM). During first lactation, the restricted heifers changed in BW from 78% of the normals to 95% of the normals. Second and third lactation performance was similar between restricted and normal heifers. Work of Swanson and Hinton (1964) showed that underfeeding heifers effected first lactation performance, but did not influence later performance.

Consequences of Excessive Growth in Dairy Heifers

Although delayed calving beyond 24 mo is costly (Webb, 1992), it is not always

beneficial to calve heifers at less than 24 mo of age. Heifers need to gain .83 kg/d to achieve a 1st calving weight of 570 kg at 21 mo of age. Gains in excess of .9 kg/d are achievable, but they may impair mammary development. Impaired mammary development can decrease 1st and 2nd lactation milk yield (Swanson, 1960). If the dairy producer's sole interest was rapid weight gains, wither growth, and low age at puberty and breeding, then accelerated ADG's would be appropriate. Kertz et al. (1987) found that Holstein heifers fed a high plane of nutrition had increased ADG, wither heights, and heart girths compared to heifers raised on a low plane of nutrition. Heifers on a high plane of nutrition received a 20.3% CP supplement (3.60 Mcal/kg DE) plus alfalfa hay ad lib, and those on a low plane of nutrition received a 17.8% CP supplement (3.48 Mcal/kg DE) plus alfalfa hay ad lib. They found that wither heights and heart girths increased in proportion to BW. Milk yields were not monitored, so correlation between growth rates and milk yield could not be made. Although the performance traits Kertz et al. measured are desirable, a dairy replacement heifer does not return money until lactation, therefore rearing conditions that favor high lifetime milk yield are desirable.

To evaluate to effects of rapid rearing on lactation performance, Swanson (1960) monitored seven identical twin pair small breed heifers through first and second lactation. The twins were divided into two groups. One of each pair was reared on a high plane of nutrition (nearly all of the concentrate they would readily consume, plus mixed hay), while the other was reared on a low plane of nutrition (hay and silage, limited concentrates for

12 mo.) . At 24 mo of age, the heifers fed high energy diets weighed 408 kg, while low plane heifers weighed 310 kg. All pairs except for one calved at comparable ages. The lighter heifers had 18% higher milk yields during the first lactation, and 8% higher milk yields during the second lactation.

Foldager and Sejrsen (1982) reported that 83 Swedish Red dairy heifers yielded on average 14.6% more FCM in 1st lactation when fed for low gains (.562 kg/d) rather than for high gains (.842 kg/d) from 150-300 kg BW. Similarly, they reported 49 British Fresian dairy heifers yielded 62% and 51% less FCM when reared rapidly (.997 and 1.124 kg/d, respectively) compared to slow rearing from 150-300 kg BW (.553 kg/d). Although Swanson (1960) and Foldager and Sejrsen (1982) demonstrated that rapid rearing is detrimental to 1st lactation milk yield, a correlation between rapid gains and mammary development could not be made, since mammary composition was not analyzed.

Harrison et al. (1983) measured mammary composition of heifers gaining in excess of 1.0 kg/d. Nineteen British Fresian heifers were reared rapidly (1.1 kg / d) or conventionally (.74 kg /d) from 3 to 15 mo of age. Rapidly reared heifers were fed a barley based diet ad lib, while conventionally reared heifers were offered pasture in the summer and hay plus concentrates in the winter. Heifers reared rapidly had smaller mammary glands (1590 versus 2210 g) and less mammary secretory tissue (8790 versus 1480 g) at 273 d of pregnancy than heifers reared conventionally. The influence of

excessive gains from 3 to 15 mo of age was still evident near parturition In a separate study, Harrison et al. (1983) fed British Fresian heifers for three levels of ADG (.57, .76, and 1.18 kg/d) until 11 mo of age, at which time they were slaughtered. The rapidly reared heifers had the largest gland weight (1940 g), but the lowest amount of secretory tissue (170 g). Conversely, heifers with the lowest ADG had the lowest gland weight (450 g), but the largest amount of secretory tissue (290 g). The second study by Harrison et al. links prepubertal and early postpubertal growth with mammary development.

Sejrsen et al. (1982) attempted to determine if growth during the prepubertal or postpubertal period had the most influence on mammary development. Mammary growth during the prepubertal growth period is allometric, meaning the mammary gland grows at a faster rate than other body tissues. Postpubertal mammary growth is isometric, meaning growth rate is similar to that of other body tissues (Akers, 1990). To evaluate the correlation between mammary development and BW gains during isometric and allometric growth, Sejrsen et al. fed prepubertal and postpubertal Holstein heifers a restricted or an ad libitum diet. During the prepubertal period, heifers fed ad lib had higher gains (1.271 kg / d) than those on the restricted diet (.637 kg / d). During the postpubertal period, heifers fed ad lib also had higher gains (1.164 kg / d) than those on the restricted diet (.588 kg / d). Prepubertal heifers fed the ad lib diet had larger mammary glands (2203 versus 1686 g) than the restricted heifers, but also had less secretory tissue (495 versus 642 g), less DNA (1061 versus 1562 mg), and more adipose tissue (1708

versus 1040 g). Postpubertal heifers fed the ad lib diet had higher daily gains (1.164 versus .588 kg), but there were no significant differences in total gland weight, secretory tissue content, and adipose tissue content between the two postpubertal treatments. Results of Sejrsen et al. (1982) results identify the prepubertal period as the critical phase when excessive gains likely impair mammary development.

Stelwagen and Grieve (1990) reported rate of gain influenced mammary development, but not 1st lactation milk yield. They fed 47 6-8 mo old Holstein heifers one of three diets for 279 d to achieve ADG of .611 kg/d, .737 kg/d, and .903 kg/d. Total mammary gland weights were greater ($p<.01$) for the high treatment (2136 g) than for the low (1106 g) or medium (1583 g) treatment, as were total g of fat (1552 versus 703 and 1096). DNA content was not significantly different between the treatments, although there was a trend for higher DNA content for the medium treatment. There were no significant differences in first lactation milk yield between the three treatments. The group with the highest rate of gain in this experiment did not gain excessively (.903 kg/d) for Holstein heifers, so differences between treatments weren't large. If gains were > 1 kg/d, a decrease in mammary DNA content or first lactation milk yield might have occurred.

Compensatory Growth

Dairy heifers exhibit compensatory growth when a period of underfeeding and slow gain is followed by a period of overfeeding (energy and CP above NRC requirements). Hogg (1991) defined compensatory growth as the growth rate of an animal fed ad libitum after a period of nutritional stress. He defined nutritional stress as a limitation in the quantity and quality of food required by the animal which will not allow it to express it's genetic potential for growth. Diets high in energy, protein, and minerals (15-40% greater than NRC) need to be fed to achieve compensatory growth (Head, 1992). Growth during the compensatory period is more efficient (kg BW gain per kg DM intake), and gains of 1.6 - 2.1 kg/d are possible (Park et al., 1987).

Park et al. (1987) attempted to improve the growth efficiency of Holstein heifers by utilizing compensatory growth. They used a stair step nutrient regimen, meaning heifers were exposed to alternating periods of underfeeding and overfeeding. Beginning at 7.6 mo of age, a stair step nutrient regimen was used, with alternating 5, 2, 5, 2 mo periods. Treatment heifers were fed 15% below NRC (1978) requirements for energy and protein during the 5 mo (maintenance) periods, and 40% above NRC for the 2 mo (compensatory) periods. Control heifers were fed 100% of NRC throughout the trial. For the 14 mo test period, treatment heifers averaged .28 kg ADG and 6.89 kg DMI/d during the maintenance periods, and 1.91 kg/d gain and 9.12 kg DMI/d during the compensatory

periods. This resulted in improved growth, energy, and protein efficiency during the compensatory periods compared to the maintenance periods. These workers used the following equations to predict efficiency:

Growth Efficiency = kg gain x 100 / kg DMI;

Energy Efficiency = kg gain x 1,000 / (kg DMI x metabolizable energy)

Protein Efficiency = kg gain x 100 / kg protein intake

Compared to control heifers, treatment heifers consumed less DM (7.52 versus 9.36 kg/d, $p<.001$) and had greater gains (.98 versus .68 kg/d, $p<.001$), resulting in improved growth, energy, and protein efficiency. In addition, treatment heifers had higher first lactation milk yields than the control heifers (23.4 versus 21.3 kg/d).

Park et al. (1989) used a similar stair step nutrient regimen to monitor mammary composition and milk yield. Holstein heifers, averaging 5.5 mo of age, began the first period (3 mo) on a restricted diet (15% below NRC), which was followed by 2 mo on a realignment diet (40% above NRC). The final four period lengths were 5, 2, 5 , and 2 mo respectively, and alternated from the restriction diet to the realignment diet. A control group was fed a diet to meet NRC (1978) recommendations. In two separate studies utilizing this stair step rearing method, Park et al. (1989) found increased ($p<.001$) milk yield for the test group (8715 kg versus 7913 kg for study I; 9251 versus 8533 kg for study II). Milk yields for studies I and II covered four and three lactation records,

respectively, for each cow. In addition, the test group had 20% more mammary DNA and 200% more mammary RNA than the control group. The increase in mammary RNA could indicate increased concentration of mRNA, specifically casein mRNA. Park et al.(1988) postulated that increased concentration of mammary mRNA alters the expression of milk protein gene, thereby increasing milk protein secretion.

Park et al. (1989, 1987) have shown compensatory growth is substantial and worth consideration. It would be difficult under most practical conditions to rear heifers utilizing several compensatory periods similar to Park et al. However, one or possibly two compensatory periods could be practical. The idea of compensatory growth also alleviates the need for excessive gains prepubertally, as long as heifers reach breeding weight at the proper age. Slower gaining heifers can catch up during a compensatory period when gains are more efficient.

GROWTH

From a physiological perspective, animal growth is defined as the increase in size and the change in functional capabilities of the various tissues and organs within the animal. A simpler definition is the net accretion of protein and fat in respective tissues, controlled by nutrition, environment and the genetic capacity to grow (Beever et al., 1992). The process of growth involves hyperplasia (increase in cell number) and hypertrophy (increase in cell size). Tissue DNA content is an estimation of cell numbers, while the ratio of tissue protein to DNA is an estimation of cell size. In all farm animals, early prenatal growth is accomplished by hyperplasia. In pigs, sheep, and cattle, growth during the early post-weaning period involves hyperplasia and hypertrophy. Later growth is achieved mainly by hypertrophy. Postnatal growth (age versus BW) can be fitted to a sigmoid curve. All tissues exhibit sigmoidal growth patterns, but they do not all develop at the same time. Vital organs are well developed at birth, but continue to grow until the later stages of growth. Skeleton and muscle growth slows dramatically after puberty, while fat growth increases at the same time. The high prenatal and prepubertal growth rates of vital organs, skeleton, and muscle emphasize the importance of proper nutrition during these stages of growth. The order of development of these three body components is skeleton, muscle, and then fat. Nutrition has the most important influence on growth and development. Peak growth rates of tissues are dependent on plane of nutrition. A low plane of nutrition (low energy and protein) can delay maturity, which effects the

development of adipose tissue, since adipose tissue growth occurs in late postnatal growth (Grant and Helferich, 1991).

Adipose Tissue Growth

Adipose tissue is primarily an energy reserve for animals, but it also provides insulation (subcutaneous adipose tissue), protection, fuel for certain muscle fibers, and is necessary for mammary gland growth (Vernon, 1992). The dominant cell of adipose tissue is the adipocyte. Although adipocyte numbers increase during postnatal growth, the majority of the increase in fat deposition is from accumulation of lipid within the adipocyte (hypertrophy) (Grant and Helferich, 1991). The primary lipid in adipose tissue is triglyceride. Therefore, the many factors that effect triglyceride uptake and utilization (uptake of triglycerides from the blood, fatty acid synthesis, lipolysis, reesterification of fatty acids into triglycerides within the adipocyte, fatty acid oxidation) influence fat deposition. In ruminants, fatty acid synthesis occurs in adipose tissue, with acetate being the major precursor (Vernon, 1992).

Muscle Growth

Muscle tissue is the most abundant tissue in the animal body. In cattle, it represents about 44% of the total live weight at weaning, and 39% at maturity. Next to water, protein is the major component of muscle tissue (Bergen and Merkel, 1991). Net protein deposition, or protein accretion, is the difference between protein synthesis and protein degradation (Grant and Helferich, 1991). Measuring net protein deposition is a method of evaluating growth. Differential slaughter (slaughter and chemical analysis of half carcass) and dilution (diluting a substance in the body water to estimate quantity of body water, which can predict body protein) methods are means of measuring whole body protein, which when measured at different intervals in the growth period, indicate lean muscle growth or net protein deposition. Nitrogen retention is another method of measuring protein deposition, but is not as accurate as the dilution or differential slaughter methods (Bergen and Merkel, 1991).

Muscle tissue is not homogenous - it consists of muscle fibers and many other cell types such as fibroblasts, capillary endothelial cells, adipocytes, and mononucleate satellite cells. Muscle fibers account for the majority of the muscle protein. In placental mammals, muscle fiber number is fixed at birth. During postnatal growth, length and width of muscle fibers increase (hypertrophy), as does DNA content (hyperplasia) (Harper and Buttery, 1992). Hyperplasia occurs through proliferation of satellite cells. Satellite cells proliferate

in response to external stimuli such as injury, and then fuse with existing muscle fibers. Although muscle fiber numbers do not increase, it is considered hyperplasia because nuclei number increase (Grant and Helferich, 1991). Satellite cells are abundant in the young animal and decrease with maturation (Harper and Buttery, 1992).

Body Composition

Animal bodies consist of fat and non-fat components, and the proportion of these two components varies depending on age, stress, nutrition, and genetic capabilities. The non-fat portion consists of water, protein, and ash. Fat content increases with age, but is widely variable. Murray (1922) reported fat content to vary from 3.64% to 60% of the bovine body. For this reason, body composition is often measured on a fat-free basis, since the non-fat components of the body are not as variable as the fat component. In addition, fat content does not influence the ratio of non-fat components. The water:protein:ash ratio is constant for an individual animal regardless of body fat content.

From conception until about 5 mo of age, or about 435 d in cattle, body composition on a fat-free basis changes dramatically. Percent ash and nitrogen increase steadily until about 5 mo of age, at which time the change becomes very slow. Protein does not appreciably increase after 5 mo of age, while ash changes very little. Water is the

inverse, decreasing steadily until 5 mo of age, at which time the change slows. Therefore, Moulton (1923) defined 5 mo of age, or the point at which the concentration of water, proteins, and ash becomes comparatively constant on a fat free basis, as chemical maturity for dairy cattle. After this time, the ratio of non-fat components varies little. In early gestation soon after conception, a developing bovine embryo is almost all water (>95%), with very little ash or protein (<1%). At birth, the fat free composition of cattle is 76.3% water, 18.4% protein, and 4.56% ash. After chemical maturity, water decreases to 72-75%, and protein increases to 22% (Murray, 1922; Robelin, 1984).

Measurement of Body Composition

Methods of estimating body composition include visual appraisal, live animal measurements, slaughtered animal measurements, ultrasonic probes, ^{40}K, and dilution techniques. Although all methods work satisfactorily under particular conditions, each has a distinct disadvantage. Visual appraisals (body condition scores) are subjective, therefore difficult to compare between studies or between evaluators. Heifers are particularly difficult to score, considering long hair and the small variation among heifers. Live animal measurements such as BW and wither heights are easy to obtain, but they do not provide a good indication of fat, protein, or water content. Slaughter and subsequent chemical analysis involves grinding all or part of the carcass to determine chemical composition. A

half carcass is typically analyzed, but the soft tissue around the 9-10-11 ribs can be used (Swartz et al., 1991). Slaughtered animal measurements are the most accurate measurement of body composition, but are costly and impractical in many situations. Ultrasonic probes measure muscle growth, not body composition. Indirectly, muscle growth can indicate body composition differences between groups of heifers. Estimation by ^{40}K is not practical because of equipment costs (Belyea et al., 1978; Belyea et al., 1986). Dilution is not as accurate as slaughter for determining body composition, but it does provide an easy, inexpensive *in vivo* method of measuring body composition.

Dilution methods rely on the assumption that body protein and fat can accurately be predicted from body water. Reid et al. (1955) reported that body composition (fat, protein, ash) can be predicted from water content (Table 4). Reid et al. concluded the key to the accuracy of these equations is the accuracy with which the water content can be measured. Data used to develop the equations was derived from the slaughter-analysis technique or the carcass specific gravity technique. These techniques were not practical on a large scale basis. Accurate dilution techniques to estimate body water would make Reid et al. equations useful. Even though such techniques were not available, Reid et al. showed that body water can predict body composition. The authors noted that error in prediction is greater in small, thin animals than in large, fat animals.

Urea Space. Findings of Reid et al. (1955) prompted a need for an inexpensive, in

Table 4. Equations to Predict Body Composition from Body Water Content.

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1) $Y = 355.88 + .355*X - 202.906 * \log X.$

2) $100 - (X+Y) =$ % of fat free dry matter in empty body.

3) $P = 80.93 - .00101*Z.$

4) (Fat free, dry matter (%)) - P = A

5) P*(Fat free dry matter (%)) = P_1

6) A*(Fat free dry matter (%)) = A_1

Where

Y= fat in whole empty body (%)

X = water in whole empty body(%)

P = protein in fat free dry matter (%)

Z = age of animals(days)

A = ash in fat free dry matter (%)

P_1 = protein in whole empty body (%)

A_1 = ash in whole empty body (%)

(Reid et al., 1955)

vivo technique to estimate body water and composition. Dilution techniques used to determine body composition in the live animal are based on the relatively constant relationship between empty body water and other body components (Bartle, et al., 1987). A marker that dissolves in water is introduced into the body water. Body water is estimated by the change in marker concentration after the marker is equilibrated throughout the body water pool. A marker should have the following properties: 1) even and rapid distribution throughout the body water; 2) no toxic effects; 3) not selectively stored, secreted, or metabolized; 4) accurate and convenient estimation of it's concentration in blood; and 5) a substance that is not foreign to the body (Preston and Kock, 1973). Urea exhibits all of these qualities (San Pietro and Rittenberg, 1953).

Preston and Kock (1973) infused urea into the jugular vein of beef steers weighing 169-492 kg. The infusion solution contained 20% urea dissolved in .9% saline, and was infused to achieve a dosage of 130 mg/kg live weight. Live weight was determined after a 12 h fast. The volume of water with which urea equilibrates, or urea space, was calculated by dividing the amount of urea injected by the change in plasma urea concentration from immediately prior to infusion and 9, 12, and 15 minutes post - infusion. These times were chosen based on unpublished preliminary work by Preston and Kock that showed urea equilibrated in the bovine body somewhere between 9 and 15 min. Urea space was expressed as a percentage of live weight or empty BW (live weight minus the refuge - i.e. the contents of the stomach, intestines, and urinary bladder). Cattle were

slaughtered three d after urea infusion, and body composition was estimated from carcass specific gravity. The 12 and 15 min sampling was more highly correlated with measured body composition than was the 9 min sampling. However, the 12 min time was preferred over the 15 min time by the authors because it decreased sampling time. Also, due to the effect of gastrointestinal tract fill (up to 15% of live weight) on the error involved with urea space as a % of live weight, empty BW were used. The following equations were developed:

Empty Body Fat = 73.01 - .976 (% urea space as a % of empty BW)

Empty Body Water = 18.25 + .7413 (% urea space as a % of empty BW).

These equations worked well for six of the twelve steers that were heavy, but for the remaining six light steers, the correlation between empty body fat and urea space was not significant. Therefore, the equations of Preston and Kock (1973) should not be applied to dairy cattle, but rather to heavier beef cattle. The equations could work with heavy dairy cattle, but the error of prediction would likely increase .

In another similar study, Kock and Preston (1979) estimated the body composition of 113 beef steers using the urea space procedure as outlined above. They determined that urea space measured 12 minutes after urea infusion was more highly correlated with rib soft tissue composition and carcass specific gravity than was urea space measured at 9,

15, and 18 min post infusion. Using the 12 min time, urea space was highly correlated with fat % of the rib (.79) for both heavy (243 kg) and light cattle (160 kg) . The equation to predict rib fat % from urea space was:

Rib Fat % = 102.2 - 1.5368 (% urea space).

However, carcass specific gravity was highly correlated with urea space for heavy cattle (.79), but not light cattle (.47).

The results of Preston and Kock (1973) and Kock and Preston (1979) indicated urea space was not highly correlated with fat content for light cattle when specific gravity was used to determine fat content. They used a procedure for determining specific gravity outlined by Garret and Hinman (1969). The procedure called for slaughter and separation of the carcass into halves. Garret and Hinman used 48 beef steers (mostly Hereford) in their study. The right side of the carcass was chilled for 48 h, then cut in half again and weighed under water to determine specific gravity. The water temperature was recorded so that carcass density could be corrected to 4^0 C. The entire right side was then chemically analyzed for fat, nitrogen, and water content. Carcass specific gravity determined by this method was found to be a good predictor of body fat, nitrogen, and water. Preston and Kock (1973) and Kock and Preston (1979) used the procedure of Garret and Hinman for determining specific gravity, and had poor prediction with light steers. Garret and Hinman utilized heavy animals, therefore their results should not be applied to dairy cattle. Considering the findings of Preston and Kock (1973) and Kock

and Preston (1979), it appears that carcass density is not a good predictor of body composition in dairy cattle. Therefore, studies involving dairy cattle where urea space is evaluated by comparison to carcass density should be interpreted with caution.

Hammond et al.. (1990) attempted to predict the body composition of Holstein steers using urea space. Procedures were similar to those outlined by Preston and Kock (1973), except full fed BW were used instead of 12 h fasted BW to calculate urea dosage. Chemical composition of steers was determined by analysis of the total ground non carcass and ground half carcass. Using previous equations of Hammond et al.. (1984, 1988) based on beef steers, the authors determined that prediction of empty body components of dairy steers from urea space and empty BW were not accurate using beef steer equations. These equations underestimated empty body water and overestimated empty body fat, demonstrating the problem in using beef cattle equations for dairy cattle. The authors concluded that prediction equations may be different for different species of cattle, and are certainly different between dairy and beef cattle. Therefore, new equations for dairy steers were developed (Table 5). For prediction of % empty body water, addition of urea space (% BW) as a model term reduced the standard error. It also increased R^2 slightly for the %empty body water and %empty body fat equations, which is expected when model terms are added, regardless of their worth. For the kilogram empty body fat and kilogram empty body water equations, addition of urea space to the model decreased standard errors and increased R^2. It can be concluded from these equations that

Table 5. Prediction equations of Hammond et al. for prediction of body composition of Holstein steers weighing 143 - 404 kg.

	Equation	SE	R^2
%EB Water	= 76.5 - .035 BW	1.4	.72
	= 83.5 - .16 US%BW - .032 BW	1.3	.75
%EB Protein	= 17.0 + .005 BW	.7	.17
	= 16.6 - .009 US%BW + .005 BW	.7	.17
%EB Fat	= .33 + .033 BW	1.6	.62
	= -5.9 + .14 US%BW + .030 BW	1.6	.64
EB Water, kg.	= 1.71 + .53 BW	6.7	.96
	= 6.7 + .37 US + .33 BW	5.9	.97
EB Protein, kg	= -7.4 + .18 BW	2.3	.96
	= -5.7 + .13 US + .10 BW	2.0	.97
EB Fat, kg	= -24.9 + .17 BW	5.2	.81
	= -22.1 + .21 US + .054 BW	4.9	.84

SE = Standard error of the estimate.
EB = empty body.
US%BW = urea space as a % of body weight.
BW = full fed body weight.

(Hammond et al., 1990)

that urea space calculation only slightly improves the prediction of body composition in dairy steers, but more so in mass equations (kg empty body water, kg empty body protein, kg empty body fat) than in percentage equations (% empty body water, % empty body protein, % empty body fat) .

Swartz et al. (1991) conducted an experiment similar to that of Hammond et al. (1990), except that dairy heifers were used in addition to dairy steers. They found Hammonds equations (% empty body fat, protein and water) adequately predicted body composition compared to chemical composition of the half carcass and 9-10-11 rib. They also developed their own equations (Table 6), but found that urea space did not improve predictions over BW alone.

Although there are many studies involving the prediction of body composition from urea space, there are few that utilize dairy steers, and even fewer that utilize dairy heifers. All of the equations that have been developed to predict body composition from urea space have problems when light beef cattle or dairy animals are used. Body weight appears to be about as good at predicting body composition as urea space. Future research involving urea space and dairy heifers could improve upon current prediction equations.

Deuterium Oxide. Another marker that can be used to estimate body water by

Table 6. Prediction equations of Swartz et al. for prediction of body composition for 25 wk.old Holstein male and female calves.

	Equation	SE	R^2
Protein, kg	= 16.3 + .025 US	2.6	.06
	= 3.17 + .09 BW	1.8	.53
	= 3.03 + .10 BW - .02 US	1.9	.54
Fat, kg	= 6.6 + .02 US	1.6	.15
	= -2.4 + .06 BW	.93	.72
	= -2.5 + .07 BW - .006 US	.98	.72

US = urea space.
SE = Standard error of the estimate.

(Swartz et al., 1991)

dilution is deuterium oxide (D_2O). Deuterium oxide has in vivo kinetics and metabolism similar to water (Byers, 1979), making it a useful marker. Similar to the urea space procedure, a baseline sample is taken and then a dose of D_2O (.22 kg D_2O/kg BW) is given. For urea space, only one more sample is needed to calculate urea space. For D_2O, samples are taken at 20, 30, 40, 50, 60, 90 min and 2, 4, 12, 24, 36, 48 and 72 hr post infusion (Maslanka et al., 1994). In addition to the large number of samples, other practical problems limit the use of D_2O as a marker. First, ~3 h are needed for deuterium oxide to equilibrate in water (Byers, 1979). Added time becomes significant when many animals are studied. Second, D_2O concentration is determined by infrared spectroscopy (Byers, 1979), adding equipment costs to the procedure. Third, the equations involved to determine body composition from D_2O are complex (Byers, 1979; Martin and Ehle, 1986). One important advantage D_2O has over urea is that it is not metabolized by the body. The complexity of the procedure, however, limits the usefulness of D_2O as a marker.

Ultrasound. Apart from dilution techniques, ultrasound is another live animal method of estimating body composition that is practical and useful. Ultrasound uses high frequency sound waves to produce images of soft tissue and internal organs. Liquids do not reflect sound waves, while dense objects such as bone reflect many sound waves. Therefore, liquids appear black on the screen, and bone appears white. Other tissues appear in varying stages of gray, depending on their ability to reflect sound waves (Pierson et al., 1988).

Ultrasound is used for live estimates of longissimus muscle area in cattle. Longissimus muscle area can be used to compare body condition between groups of animals. Estimation is convenient and inexpensive, provided an ultrasound machine is available. The scan is made between the 12th and 13th rib, and then sketched via computer software (Animorph, Woods Hole Educational Ass.). Waldner et al. (1992) reported ultrasonic scans of the longissimus muscle of Brangus bulls were not accurate ($p<.05$) in predicting longissimus muscle area compared to slaughter measurements at 4, 8, 16, 20, or 24 mo. of age. Predictions were accurate for bulls at 12 mo. of age. They also evaluated the effectiveness of an inexperienced versus an experienced operator. No correlation between experience and accuracy was found.

DIETARY CONSIDERATIONS FOR GROWTH

Energy and nitrogen are the primary substrates needed for microbial growth (Smith et al., 1989), therefore they must be consumed in sufficient quantities by the ruminant animal. Energy is the first limiting substrate for microbial growth and the most abundant nutrient in the diet of dairy heifers. Inadequate energy levels retard growth and development, and inadequate protein levels result in reduced mature body size and increased carcass fat content (Owens, 1992). This emphasizes the importance of balancing energy and protein in the diet of growing dairy heifers. The remainder of this literature review, however, will focus on protein and AA.

PROTEIN STRUCTURE AND FUNCTION

Proteins are high molecular weight polypeptides and their derivatives. They are fundamental components of all structures in an organism, and account for 15-18% of the BW of mature animals. Approximately 50% of total body proteins are in muscle. A polypeptide is a chain of AA linked by peptide bonds between each pair of AA. A peptide is a short chain of a few AA. A polypeptide chain containing more than 50 AA is usually called a protein (Eggum, 1989).

The most common measurement of protein in feeds is crude protein (CP). Protein contains 16% nitrogen, therefore, CP is the N content of a feed X 6.25. Crude protein includes true protein and non protein nitrogen (NPN). The Kjeldahl procedure used to determine nitrogen content or CP does not consider the type of protein or how it is utilized by the animal.

Protein Fractions

In the ruminant, dietary form of N is an important determinant of it's metabolism. Protein can be described in terms of it's solubility or rumen degradability. These terms are not synonymous and do not have any direct relationship. Rumen degradability refers to the extent to which the protein is degraded by rumen microorganisms. Soluble protein will dissolve in rumen fluid. For example, soybean meal and blood meal are both low in solubility (24.0 and 3.0 % of total N) but soybean meal is high in degradability (72% of CP), while blood meal is low (20% of CP) (Clark et al., 1987). Solubility can be measured in the lab consistently. Measurement involves soaking feed in a rumen buffer, filtering and washing after one h, and determining residual nitrogen (Pichard and Van Soest, 1977). Rumen degradability can not be easily determined in a lab, rather it is determined through *in situ* or dacron bag studies that involve incubating a feedstuff in a dacron bag suspended in the rumen (Nocek, 1988; Stern and Satter, 1984).

In the broadest sense, dietary nitrogen can be classified into three fractions based on solubility: A - soluble nitrogen , B - insoluble nitrogen, and C - unavailable insoluble nitrogen. Fraction A is water soluble, largely consisting of non protein nitrogen (NPN), and is degraded very rapidly in the rumen. Fraction B is considered true protein, and is degraded at a rate similar to the rate of passage (National Research Council, 1985). Fraction B also contains some NPN, from AA residues of protein. Fraction C is rumen undegradable and intestinally unavailable, and can be estimated by acid-detergent insoluble nitrogen (ADIN) (Pichard and Van Soest, 1977).

Although protein solubility is relatively easy to measure, it does not provide an accurate picture of how protein is utilized by the animal. Rumen degradability is determined not only by solubility, but also by microbial activity, microbial access to the protein, structural characteristics of the protein, and the nature of the feed particle in which the protein resides (Satter, 1986). Therefore, protein classification by solubility alone is impractical because of the inability of solubility to accurately predict rumen degradability. Pichard and Van Soest (1977) accounted for this in part when they further divided insoluble fraction B into fractions B_1 and B_2 based on rumen degradability. The fraction B_1 represented rapidly degradable insoluble nitrogen, while B_2 represented slowly degradable insoluble nitrogen. Digestion rate (K, estimated by regressing natural log of the residual degradable nitrogen upon time) was slower for fraction B_2 than B_1 (.28 vs

8.29), while B_2 had a longer half life than B_1 (4 h vs 10 min.) (Pichard and Van Soest, 1977).

Chalupa et al. (1991) proposed a model that added a B_3 fraction in addition to A, B_1, B_2, and C fractions. Although this model does not clearly distinguish rumen undegradable from rumen degradable protein, it is more useful than the Pichard and Van Soest (1977) model. A protein comprised of fraction A or B_1 is almost entirely degradable, while a protein comprised of fraction B_2 is mostly degradable. Fraction B_3 is mostly rumen undegradable and intestinally available protein, and fraction C is undegradable but unavailable protein. Table 7 shows rumen degradabilities and intestinal digestibilities of the fractions in the Chalupa et al. (1991) model.

The Chalupa et al. (1991) model predicted alfalfa silage N to be 58% fraction A, 0% B_1, 24% fraction B_2, 8% fraction B_3 and 10% fraction C, indicating a high solubility of alfalfa protein, plus some bound protein due to heating during ensiling. Soybean meal protein was predicted to have 3% fraction A, 17% fraction B_1, 77% fraction B_2, 2% fraction B_3 and 1% fraction C, indicating soybean meal is slowly but almost completely rumen degradable. Blood meal was predicted to have 2% fraction A, 2% fraction B_1, 55% fraction B_2, 38% fraction B_3 and 2% fraction C, indicating blood meal is not very rumen degradable, but intestinally available (Chalupa et al., 1991).

Table 7. Rumen degradability and intestinal digestibility of protein fractions.

Fraction	Rumen Degradability	Intestinal Digestibility
	(%/hr)	(%)
A	instantaneous	--------
B_1	200-300	100
B_2	5-15	100
B_3	1-1.5	80
C	0	0

Source: Chalupa et al., 1991

Protein Degradability

In addition to being difficult to measure, degradability and undegradability are difficult to define compared to solubility. Degraded intake protein (DIP) is dietary protein that is degraded in the rumen by rumen microorganisms. Undegraded intake protein (UIP) passes through the rumen to the omasum, as opposed to protein synthesized by rumen microbes from DIP and endogenous protein secretions. Intake protein that bypasses the rumen to the omasum has two fractions. Fraction 1 is protein that resists microbial attack in the rumen, and fraction 2 is protein that evades attack in the rumen and passes to the omasum without thoroughly mixing with ruminal contents. Fraction 2 would include protein flushed out of the rumen at feeding time and passing through the esophageal grove. Therefore, undegraded protein would describe fraction 1, and bypass protein would describe fraction 2. *In vivo* measurements account for both fractions, while in vitro measurements only account for fraction 1 (National Research Council, 1985). The undegraded portion of protein is important to quantify because it always bypasses the rumen. It remains constant for a given feed from animal to animal. The bypass portion of a protein varies depending on rate of passage, feeding time, and age of the animal. Therefore, the bypass portion of a feed protein will vary from animal to animal. For the remainder of this literature review, UIP refers to the sum of undegraded and bypass fractions.

Using the protein fractions A (soluble), B (insoluble), and C (insoluble and rumen undegradable), NRC (1985) defined protein degradability in terms of the degradation rate of fraction B. Using k_d to represent degradation rate, the fractional degradation rates are:

k_{dA} - fractional degradation rate of A, at least 10 times greater than rate of passage;

k_{dB} - fractional degradation rate of B, between 10 times greater and one-tenth the rate of passage; and

k_{dC} - fractional degradation rate of C, one-tenth the rate of passage.

Fraction A is considered entirely degraded, so k_{dA} is infinite for all practical purposes. Fraction C is considered entirely undegradable, so k_{dC} is zero for all practical purposes. Using k_{pB} to represent the relative rate of passage, the fraction of protein degraded is: $A + k_{dB}B/(k_{dB} + k_{pB})$, and the fraction of protein passed or undegraded is: $k_{pB}B/(k_{dB} + k_{pB}) + C$.

Ruminal protein degradation occurs in two steps. The first step is hydrolysis of peptide bonds to form peptides and AA, and the second step is deamination and degradation of released AA. The rate limiting step appears to be hydrolysis of peptide bonds. The extent of protein degradation depends on physical and chemical properties of the feed protein, the animal consuming the protein, the rumen environment (pH, rate of passage), and composition of remaining feed in the diet (Clark et al., 1987).

The four major types of protein in cereal grains and protein supplements are

albumins, globulins, prolamins, and glutelins. Albumins and globulins are soluble proteins, which are generally more degradable than insoluble proteins. Prolamins and glutelins contain disulfide bonds, making them less susceptible to proteolytic enzymes, thereby reducing rumen degradation. Therefore, proportionality of these four proteins effects rumen degradability. For example, corn protein is predominately prolamin and glutelin, making corn protein relatively insoluble and undegradable. Soybean meal, on the other hand, contains largely globulins that are degradable in the rumen. Albumins and globulins generally have a more desirable AA profile than prolamins and glutelins, which is unfortunate since prolamins and glutelins are less rumen degradable (Clark et al., 1987).

Processing effects rumen protein degradability in a number of ways. Meat and bone meal is an example of a feed moderate to low in degradability (51%) (National Research Council, 1989). During processing, the soluble, rapidly degradable protein fractions are lost in water or are converted to an insoluble form, thus reducing rumen degradability. In other feeds such as blood meal and roasted soybeans, heating is a common processing method that also reduces rumen degradability. During heating, some protein is denatured and coagulated, making the protein insoluble. After heating, cooling causes a random relinkage of chemical bonds and shrinkage of the protein molecules, both of which decrease rumen degradability (Clark et al., 1987).

Proteins in animal by-product feeds (meat meal, meat and bone meal, fish meal,

blood meal) are generally resistant to microbial degradation. These feeds may contain large amounts of collagen (connective tissue) or hair. Collagen Lys content is low, possibly resulting in a shortage of trypsin-sensitive bonds because trypsin cleaves the peptide bond of a Lys residue. Clark et al. (1987) suggested that microbial protease activity is "trypsin like", partly because Lys losses are greater than the average loss of other AA. Protein in hair has extensive disulfide bonds, which resist proteolytic enzymes. Therefore, feeds high in collagen or hair are generally less susceptible to rumen degradation.

Dry matter intake also appears to effect protein degradability in the rumen. At very low levels of DMI (1% BW), protein degradability in the rumen is high due to increased retention time. As DMI increases, rate of passage increases, retention time decreases, and degradability decreases. Owens (1986) calculated that protein degradability decreased from 83.1% to 72.8% as DMI increased from 1 to 2% of BW At higher levels of DMI (>2.5% BW), increases in DMI probably would not have the pronounced effect on degradability Owens reported.

Low rumen pH reduces protein solubility and to an extent protein degradability. Forage to concentrate ratio, feed particle size, and dietary fiber levels influence rumen fermentation and rumen pH by altering the rumen microbial population (Clark et al., 1987). Owens (1986) reported that increasing forage to concentrate ratio from 40:60 to

70:30 decreased protein degradability from 71% to 78%.

In light of the many factors influencing degradability, it is evident that a given feed does not have constant degradability, i.e. it varies from animal to animal and farm to farm. Therefore, "book" values or published values for degradability of a given feed protein are estimates only, and vary considerably. Chemical composition is a determining factor in the degradability of a feed, but it does not tell the whole story.

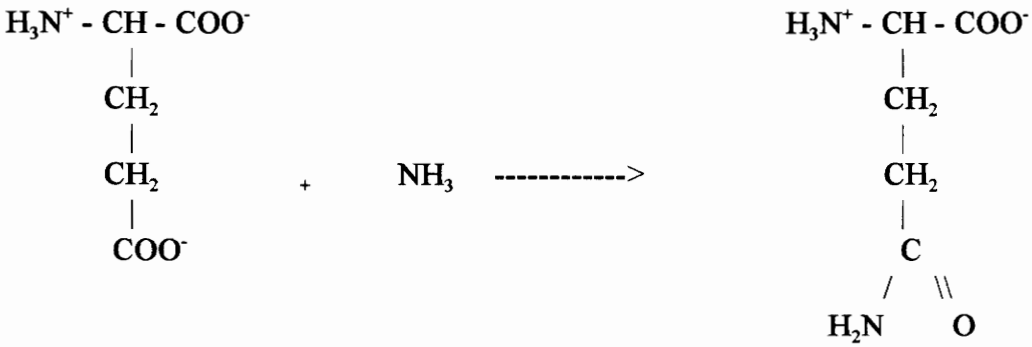
Non Protein Nitrogen (NPN)

Non protein nitrogen (NPN) includes nitrate, ammonia, amines, nucleic acids, and free AA (Pichard and Van Soest, 1977). The majority of NPN consumed by ruminants is rapidly degraded in the rumen to ammonia. Ammonia is of central importance in rumen metabolism since it is the primary source of nitrogen for rumen bacteria (Chalupa, 1972). Some rumen bacteria utilize ammonia as their only N source, therefore these bacteria have an absolute requirement for ammonia. (Smith, 1989). In addition to NPN from common feeds, NPN can be supplemented in the form of urea. Urea is a very common NPN supplement, containing 46% N and no AA. Two amino groups in urea contribute nitrogen. The chemical structure of urea is:

The concern with feeding NPN involves the rapid release of ammonia. Although ammonia is utilized by rumen bacteria, a compound such as urea can release ammonia faster than bacteria can utilize it, resulting in high rumen ammonia levels.

Under normal conditions in body tissues, glutamine is a carrier of ammonia N to the liver through the reaction catalyzed by glutamine synthase:

glutamate + ammonia ---------> glutamine



Glutamine is a non toxic, neutral AA that can readily pass through cell membranes. Once in the liver, the reaction is reversed and catalyzed by glutaminase,

glutamine ----------> glutamate + ammonia

and ammonia is released. Once in the liver, glutamate eventually forms urea. This process is called the urea cycle. In sheep, the complete urea cycle is functional in significant quantities only in the liver (Emmanuel, 1980). Enzymes involved in the urea cycle (arginine synthetase, arginase, ornithine transcarbamylase, carbamyl phosphate synthetase) are found in the heart, kidney, lung, spleen, and rumen epithelium, but at low concentrations (Emmanuel, 1980). Urea is passed through the bloodstream to the kidneys and excreted into the urine, or is recycled to the rumen through saliva or through the rumen wall where rumen bacteria convert it back to ammonia. Ruminants fed nitrogen deficient diets recycle the majority of urea formed in the liver back to the rumen, with little secreted in the urine (Van Soest, 1982).

If mechanisms of nitrogen excretion fail to utilize excess ammonia not utilized by rumen bacteria, ammonia toxicity can result. Symptoms of ammonia toxicity include depressed DMI (Kertz et al., 1982), increased rumen pH (Bartley et al., 1981; Davidovich et al., 1977; Webb et al., 1972), increased blood ammonia (Bartley et al., 1981; Webb et al., 1972), decreased numbers of rumen protozoa (El-Kabani et al., 1985), muscle tetany (Davidovich et al., 1977; Sutiak and Sutiakova, 1988), listlessness (Sutiak and Sutiakova, 1988), hypersalivation (Sutiak and Sutiakova, 1988), decreased VFA concentrations (Ahuja et al., 1989), and death (Bartley et al., 1976; Sutiak and Sutiakova, 1988).

Ammonia toxicity is more likely if a readily fermentable carbohydrate source is not available (Smith, 1989). Webb et al. (1972) reported that adding molasses (5.5 g/g total diet N) to the diet of Jersey cows supplemented with urea (100 mg N/kg BW) reduced the rise in rumen pH and blood ammonia compared to cows only supplemented with urea. Bartley et al. (1976) found high rumen ammonia concentrations may exist without ammonia toxicity if a readily fermented carbohydrate is fed, and rumen pH is below 7.4. Once ammonia toxicity occurs, removal of rumen contents alleviates symptoms and prevents death (Bartley et al., 1976; Davidovich et al., 1977).

The more common problem with overfeeding NPN is nitrogen waste and wasted feed dollars. Roffler et al. (1976) determined through an *in vivo* study that 5 mg ammonia N per 100 ml of rumen fluid was sufficient to support maximum growth rates of rumen microbes. Satter and Roffler (1976) studied ammonia concentrations and microbial protein synthesis using a continuous culture fermentor charged with ruminal contents of steers fed either a protein-free purified diet, a corn-based all concentrate diet, or a forage concentrate diet infused with urea to maintain various concentrations of ammonia. They found that protein synthesis in the fermentors increased with addition of urea until a threshold was reached where further additions of urea did not increase protein synthesis. This threshold occurred at the point where ammonia began to accumulate. The authors concluded that ammonia concentrations greater than 5 mg ammonia N per 100 ml of fluid did not improve protein synthesis. They developed the following equation to predict

rumen ammonia concentration: ammonia nitrogen (mg/100ml) = 38.73 - 3.04 %CP + .171 $\%CP^2$ - 49% TDN + .0024% TDN^2 ; r^2=.85. TDN was a factor in the equation because the amount of fermentable energy available influences growth of rumen microbes and quantity of ammonia utilized by microbes. Using this equation, rumen ammonia levels decrease within a given protein level with increasing energy levels. The authors suggest that NPN will not be effective when CP levels exceed 13-14% when TDN concentration is 60-65%. The practical problem with conclusions of Satter and Roffler (1976) are that rumen ammonia N levels often exceed 5 mg/100 ml (Davidovich et al., 1977; Emmanuel, 1980). In addition, their equations do not account for type of carbohydrate - only the TDN value. Rapidly fermented carbohydrates will enable rumen bacteria to utilize ammonia quicker. If the energy source is fat, very little ammonia will be utilized. Type of carbohydrate can influence rate and extent to which ammonia is utilized by rumen bacteria. If Satter and Roffler (1976) are correct, any ammonia past the 5 mg level is wasted. It is possible that under varying production levels and *in vivo* conditions, excess ammonia could be utilized by the rumen microbes.

Rations containing NPN frequently require additional sulfur supplementation (Shirley, 1986). Rumen microbes require sulfur to synthesize sulfur containing AA Met and cystine. True protein contains these AA, but NPN, with the exception of free AA, does not. Therefore, true protein supplies sulfur to the microbes. Diets low in true protein require sulfur supplementation, regardless of CP content. Thomas (1951)

observed that lambs fed diets containing urea lost weight and were in negative energy balance, while lambs fed diets containing urea and added sulfur were in positive energy balance. In both diets, urea was the only protein supplement. The remainder of the diet consisted of starch, dextrose, cellulose, wheat straw, lard, and a mineral premix that did not contain sulfur. With very little true protein in the diet, the lambs not supplemented with sulfur were severely deficient in sulfur. As a result, 3 out of the 4 sulfur deficient animals died, while all four of the sulfur supplemented animals lived. Bolsen et al. (1973) fed 84 yearling mixed breed steers (316 kg BW), 72 yearling Hereford heifers (273 kg BW), and 45 Corriedale lambs (39 kg BW) ground corn / corn cob based rations. Hereford heifers were the only group to receive forage (grass hay). Soybean meal or urea was supplemented to all diets with or without ammonium sulfate. No benefits were evident from supplying sulfur to any diets, suggesting that sulfur levels were adequate in these diets. It appears from the results of Bolsen et al. and Thomas that sulfur will be deficient only when NPN accounts for most of the dietary protein in a low protein diet.

Microbial Protein

Rumen microbes may supply 60-80% of the AA absorbed in the small intestine in the form of bacterial CP (BCP) (National Research Council, 1985). BCP includes essential AA that the animal can not synthesize. Quantity of BCP available to the small

intestine depends on microbial growth, microbial protein synthesis, and rumen liquid turnover (washout). Rumen microbial growth is determined by substrate available for fermentation in the rumen (carbohydrates and nitrogen), environmental conditions (pH), and rate of substrate availability (Mantysaari et al., 1989a). Energy and nitrogen are the primary substrates needed for microbial growth, with energy being first limiting. Rumen microbial yield can be predicted from the amount of ATP produced from a given amount of organic matter fermented (Smith, 1989).

Many factors affect the ability of rumen microbes to synthesize BCP. Purser (1970) concluded in a review of nitrogen metabolism in the rumen that protein solubility, composition of microbial population, nutrient availability within the rumen, timing of nutrient availability, and digestibility of specific AA influence microbial protein synthesis. Rumen microbes obtain nitrogen for BCP synthesis from NPN and dietary proteins they degrade to AA, ammonia, and carbon dioxide (Shirley, 1986). Some rumen bacteria require ammonia as an N source, while others require AA or peptides. (Abou Akkada and Blackburn, 1963; Baldwin, 1970; Nolan and Stachiw, 1979). Ammonia, however, is the major source of nitrogen for rumen bacteria (Nolan and Stachiw, 1979).

Actively growing rumen bacteria contain approximately 65% CP. Protozoa CP content is lower due to a higher polysaccharide content (Hungate, 1966). All of the microbial CP or N entering the small intestine is not available to the animal. NRC assumes

80% digestibility for microbial protein in the small intestine. Microbial protein is 80% AA N and 20% nucleic acid N, so roughly 64% of microbial protein is absorbed as true protein (Polan, 1992). Nucleic acid is of limited nutritional value because of it's low availability (Chalupa, 1972). Using these calculations, rumen microbes contain approximately 41.6% (.64 x 65) true protein that is absorbed in the small intestine.

Amino Acids

There are twenty basic AA from which proteins, polypeptides and peptides are constructed. From basic AA, many more are synthesized within the body. The ruminant cannot synthesize ten of these AA (Phe, Val, Trp, Thr, Ile, Met, Arg, His, Leu, and Lys), so they are considered essential and must be supplied by the rumen microbial population or feed protein that escapes rumen degradation. The majority of AA are utilized by the body to form proteins and synthesize other AA. In addition, AA can be oxidized to yield energy or enter glycolysis to form glucose. Heitmann et al. (1973) infused labeled glutamate and serine into the jugular vein of mature sheep. A total of 8% of plasma glucose and 6% of expired CO_2 originated from the labeled AA. They concluded that AA serve as a direct energy source, and are an important source of glucose.

Amino Acid Requirements

Crude protein requirements for dairy animals have been established by NRC (National Research Council, 1989), but AA requirements are not known. This makes it difficult to balance diets for AA. Amino acid needs for lactating dairy cows can be estimated by evaluating the AA content of milk, the major product of lactating cows (Chandler, 1989). For growing animals, lean tissue would be an appropriate base to

estimate AA requirements.

Amino Acids and Protein Quality

Amino acids entering the small intestine are from BCP, UIP, and endogenous protein sources (Schwab, 1989). Endogenous secretions contribute little, so the majority of AA are from BCP and UIP sources. The AA profile of these protein sources (BCP and UIP) can be compared to milk protein or lean tissue to predict if there is a possible AA deficiency (Table 8).

Regardless of diet, AA content of BCP is consistent. Purser and Buechler (1966) analyzed 22 strains of rumen bacteria for AA composition. These 22 strains were thought to represent some of the predominant organisms found in the rumen when either concentrates or roughage are fed. They found little variation in AA content between the 22 strains of rumen bacteria. Microbial protein AA content is not only consistent, but balanced, meaning it is not severely deficient in any AA. Comparing microbial protein to milk protein, Chandler (1989) calculated an essential AA index for rumen microbes. The index used chemical scores for each AA (relative to milk protein) of a feed protein, with no score exceeding 100. A chemical score of 100 for an AA implied the concentration of that AA is equal to or greater than the concentration of that AA in milk protein. Chemical

Table 8. Essential amino acid composition[1] (g/100 g protein) of tissue, milk and microbial true protein.

Amino Acid	Tissue	Milk	Microbes
Arginine	6.80	3.70	6.96
Histidine	3.00	2.70	2.69
Isoleucine	5.50	6.00	5.88
Leucine	7.20	10.0	7.51
Lysine	8.20	8.30	10.46
Methionine	2.70	2.70	2.68
Phenylalanine	4.60	5.30	5.16
Threonine	4.60	4.60	5.59
Tryptophan	1.20	1.40	1.63
Valine	5.20	6.70	6.16

[1] Mantysaari et al., 1989

scores for ten essential AA were corrected for metabolic utilization efficiency of each individual AA, and then calculated to an index. Rumen microbes had a score of 82 (on a scale of 1 to 100, milk protein=100), meaning they had about 82% of the essential AA content of milk protein. The top scoring feed was soybean meal, with an essential AA score of 71 (Chandler, 1989). Similarly, Schingoethe (1991) scored rumen microbial protein and feeds relative to milk protein. As opposed to using all ten essential AA to determine an index like Chandler, the single most limiting AA content was used relative to milk protein to calculate Schingoethe's index. On a scale of 0 to 1 (milk protein = 1), rumen microbial protein scored .78, while the highest scored feed was fish meal (.75). Mantysaari et al. (1989a) analyzed tissue, milk and microbial protein for AA content, assuming microbial CP is 80% true protein, and rumen microbes are 80% bacteria and 20% protozoa. The results showed microbes were deficient only in Leu, Ile, and Val. It is clear that microbial protein is an excellent source of AA, although they are not equal to milk protein. However, feed protein does not have the balanced AA content that microbial protein does. The index's of Chandler (1989) and Schingoethe (1991) showed microbes to be a better balanced AA source than any feed (Table 9).

Amino Acids and Undegraded Intake Protein

Feed protein that bypasses the rumen or escapes rumen degradation may not have

Table 9. Chemical scores of various feedstuffs relative to milk protein using index of Chandler (1989), and Schingoethe (1991).

Feed	Chandler	Schingoethe
Alfalfa meal, dehy.	65	----
Blood meal	60	.42
Canola meal	----	.68
Corn gluten meal	52	.21
Corn distillers grains w/solubles	54	.32
Cottonseed meal	----	.46
Dried brewers grains	67	.40
Feather meal	34	.19
Fish meal	68	.75
Meat meal	53	----
Meat and bone meal	51	.43
Soybean meal	71	.46
Sunflower Meal	----	.62
Rumen microbial protein	82	.78

the same AA content pre and post ruminally. In other words, the non microbial protein reaching the small intestine may be different than the original protein. The rumen environment, rate of passage, degradation rate, and available substrate (energy and nitrogen) for microbial growth can potentially alter the AA content. Chandler (1989) reported a 12% increase in the concentration of Leu, Ile, and Val in the escape protein compared with that of the original feed protein. However, Met, Trp, and His content did not change, but Phe, Thr, Ala, and Lys all were reduced, with Lys reduction the greatest at 7%. Clark et al. (1987) suggested Lys losses might be attributed to the "trypsin like" activity of microbial protease. Trypsin cleaves proteins at a Lys residue (Lehninger, 1993), meaning some Lys will be lost. Erasmus et al. (1994) found branched chain AA and, in particular, Leu and Ile to be more resistant to microbial degradation in the rumen. Degradation of Met was dependent on feedstuff, being more resistant in alfalfa hay and sunflower meal than in peanut meal, corn grain, blood meal, and soybean meal. Erasmus (1994) found Lys concentration to be lower in the escape protein than in the original protein, supporting the findings of Chandler (1989).

Even though microbial protein is more desirable than most feed protein, providing supplemental UIP is important for two reasons. First, microbial protein does not supply the needed quantities of all AA to the animal, even though it is a very balanced source of AA. Compared to milk protein Leu, Val, and Ile are the most limiting AA in microbial protein (Chandler, 1989). Escape or undegraded protein can supplement microbial protein

and supply a more balanced AA profile to the animal. Secondly, during periods of high nutritional requirement (growing heifer, high producing cow) or depressed intake (early lactation cow) microbial protein may not supply sufficient quantities of AA to meet the requirements of the animal. In these two situations, UIP can be beneficial. Selecting the right feed source to supply UIP is not easy, due to lack of knowledge regarding AA requirements and imperfect knowledge regarding AA content of escape portion of feed protein.

Limiting Amino Acids

An essential AA that is not supplied in sufficient quantities by the diet or the rumen microbes is a limiting AA. Animals require AA, not proteins, for milk production and growth. Insufficient quantities of one AA can result in decreased production (milk or growth), regardless of the quantity of remaining essential AA. For this reason, many researchers have attempted to determine limiting AA for growth and milk production in dairy cattle. Theoretically, if no single AA is limiting, dietary CP levels and nitrogen waste can be decreased.

Peptides as an Amino Acid Source

A peptide is a chain of two or more AA covalently joined by peptide bonds (Lehninger, 1993). A di-peptide contains two AA residues, and a tripeptide three. Traditionally, it was thought that proteins were hydrolyzed to free AA before absorption in the small intestine could occur. Small peptides were thought to be too large for uptake by the small intestine.

Recent work by Koeln et al. (1993) has shown substantial peptide flux across the gastrointestinal tract (GIT) and liver of growing Holstein steers (136 kg). In addition to peptide flux, they measured flux of plasma free AA and free AA in blood cells across the GIT and liver. Cannulas were surgically implanted in the abdominal aorta, a distal mesenteric vein, the portal vein, and a hepatic vein to determine AA flux across the GIT and liver. Steers were fed a ground corn, orchardgrass hay, and soybean meal based diet once every h via automatic feeders. Sampling occurred 9-12 d post surgery at 0930 h. Twenty-four hours after first sample, steers were deprived of feed for 72 h, then sampled again. Flux of free AA in blood cells was negative across the GIT and positive across the liver, resulting in no net splanchnic output. There was a flux of plasma free AA across the GIT, but the rate was reduced with feed deprivation. Hepatic flux of plasma free AA was positive, and increased with feed deprivation. In all steers, peptide AA accounted for the greatest concentration of AA in arterial blood. Across the GIT, peptide AA flux was 2.5

fold (fed steers) and 7.2 fold (unfed steers) the flux of plasma free AA. Overall, net splanchnic output of peptide AA was 7 fold the net splanchnic output of plasma free AA. Small peptides (500 - 1500 Da) accounted for the largest peptide flux.

McCormick and Webb (1982) studied various body protein pools in Holstein steers (147 kg BW). Similar to Koeln et al. (1993), McCormick and Webb (1982) fed hourly to simulate "steady state" metabolism. Plasma free, erythrocyte free, and plasma peptide AA concentrations were measured in arterial blood. Diets were similar to Koeln et al. The peptide fraction accounted for the greatest absolute amount of AA in blood, while erythrocyte free AA accounted for the least absolute amount of AA. Work by Danilson et al. (1987a, 1987b) on Holstein steers (130 kg) showed similar results. Housing and feeding management was similar to McCormick and Webb (1982) and Koeln et al. (1993), but diets were different in composition (corn starch, glucose, wood pulp, isolated soy protein, urea, refined corn oil, and minerals), yet similar in nutrient content (Webb, 1986). The total concentrations of peptide AA in whole blood was 2.7 times the free AA in whole blood.

The work of Danilson et al. (1987a, 1987b), Koeln et al. (1993), and McCormick and Webb (1982) clearly show peptides play a role in AA transport and utilization by young Holstein steers. The role of peptides appears to be significant, but the results must be interpreted with caution. First, all of the studies were conducted at the same laboratory

under similar housing and feeding management conditions. Second, young Holstein steers (<200 kg) were used in all cases. Sex, age, maturity, and lactation could influence findings. Therefore, these findings may not be applicable to growing dairy heifers or lactating dairy cows. At minimum, however, it appears that peptides play a role in AA absorption and transport, although the extent of their role is not certain.

Amino Acid Infusion - lactating cows

Researchers have attempted to determine limiting AA by post ruminal infusion of AA and by feeding rumen protected AA. Post ruminal infusion allows more accurate prediction of AA available for absorption by the animal than by feeding escape proteins. Most early postruminal infusion studies involved infusion of casein, because casein is the major protein in milk. Clark (1974) reviewed twelve studies involving post ruminal infusion of casein to lactating dairy cows. Post ruminal infusion of casein increased milk yield 1 - 4 kg/d compared to control infusions of water, saline, or isonitrogenous, isocaloric mixtures of urea, monosodium glutamate, and glucose. The largest response was for cows producing in excess of 20 kg/d. Only two studies failed to show a milk yield response to casein infusion. Lack of response was attributed to low producing cows and intake fluctuations. Casein infusion studies showed that supplementing AA that escape rumen degradation can improve milk production, and high producing animals are more

likely to respond to escape protein than low producing animals.

The AA Met and Lys are often implicated as limiting AA for lactating dairy cattle. Schwab, et al. (1976) conducted five trials of Latin square design, abomasally infusing mixtures of the ten essential AA to Holstein cows, and compared them to positive controls of sodium caseinate or a mixture of AA that represented proportions of essential AA in milk protein. Dietary energy requirements were met for all diets (1.21 - 1.46 Mcal/kg DM). Crude protein requirements were not met, (10.7 to 11.5% DM), so addition of AA should have elicited a response. Abomasal infusion of Met had no effect on milk, protein and fat secretion. Infusion of Lys accounted for 16% of the total response in milk protein yield that was obtained with the positive controls, while the combination of Met and Lys accounted for 43% of the total milk protein yield response. This indicated that Lys and Met were first and second limiting or co-limiting for milk protein synthesis.

Rumen Protected Amino Acids

Feeding rumen protected AA is another method of supplying known quantities of AA to the intestine of dairy animals. It is obviously more practical and less costly than infusion. Protection of AA is such that they are almost entirely undegraded in the rumen, yet are available post ruminally. One method of protection involves coating an AA core

with a pH sensitive copolymer. The coating protects the AA in the rumen, but under the more acidic conditions of the abomasum and duodenum, the AA is released. Polan et al. (1991) evaluated this method of rumen protection on Met and Lys. Protection and release were reported to exceed 95% for both Lys and Met. Similarly, Papas et al. (1984) compared dietary addition of encapsulated Met against a control of no added Met. Diets with encapsulated Met delivered more Met post ruminally and increased plasma Met levels over the control diets.

Chemical treatment is another method of protecting AA or proteins from rumen degradation. Certain chemical agents form reversible cross linkages with amino groups which decrease solubility of proteins at the pH of the rumen. The more acidic abomasum destroys these linkages (Chalupa, 1975). Formaldehyde is a widely studied chemical agent that provides rumen protection, but it is a carcinogen (Waltz and Stern, 1989; Erfle et al., 1986; Mir et al., 1984). Any other coating or encapsulation that is pH sensitive or somehow prevents deamination or cleavage of peptide bonds in the rumen would result in rumen protection. In addition, heat processing can increase rumen protection (Chalupa, 1975).

Waltz and Stern (1989) evaluated different methods of rumen protecting soybean meal, including treatment with sodium hydroxide, ethanol, formaldehyde, propionic acid, and lignosulfonate, plus expeller processing and extrusion. Formaldehyde treatment and

expeller processing increased total and essential AA flow to the duodenum, and had the largest reduction of protein degradation among the treatments. Erfle et al. (1986) reported formaldehyde adversely affected microbial AA content. In particular, Lys and Tyr content were reduced with formaldehyde treatment. Mir et al.(1984) successfully protected soybean and canola protein with sodium hydroxide, whole fresh blood, and fish hydrolysate without influencing protein digestibility.

Studies involving lactating cows. Armentano et al. (1993) supplemented rumen protected Lys and Met simultaneously at two levels of CP degradability in a 2 x 2 factorial arrangement. Early and mid-lactation multiparous Holstein cows were supplemented with 5.6 g of rumen protected Met (DL-Met) and 16.6 g of rumen protected Lys (L-Lys). Degradable protein was supplied to meet 85% or 100% of NRC recommendations. The difference in degradable protein was achieved by the addition of urea. In early lactation, Lys and Met supplementation increased milk protein concentration by 1 g/kg milk ($P < .05$) and milk protein yield by 37 g/d ($P < .05$), and did not interact with urea. Mid-lactation cows responded with similar increases in milk protein concentration ($P < .10$), but not yield, with AA supplementation. Urea addition depressed milk protein yield ($P < .05$) in mid-lactation cows, but this negative effect was prevented with addition of Lys and Met . Milk yields were not different for any treatment with the exception of significantly lower milk yields from the addition of urea in mid-lactation cows. In a similar study, Kincaid and Cronrath (1993) added 5 g rumen protected Lys (Zn Lys) and 5 g Met

(Zn Met) to the diets of multiparous Holstein cows. Unlike Armentano et al. (1993), Kincaid and Cronrath (1993) reported the addition of Met and Lys significantly increased 3.5% FCM and protein and fat yield ($P < .05$).

Polan et al. (1991) reported a six university field study using 304 Holstein cows fed corn silage based diets supplemented with soybean meal, corn gluten meal, or corn gluten meal plus rumen protected Met, Met + Lys, or Met + (2 x Lys). Cows were fed treatment diets from 21 d postpartum to 280 d postpartum. Replacement of soybean meal with corn gluten meal resulted in decreased DMI, milk protein yield and %, milk fat %, and milk yield for the total trial period. All of the CP in the corn gluten meal diet was contributed by corn or a corn by-product, therefore negative production responses could reflect AA imbalances or deficiencies. Adding Met decreased DMI during early lactation and increased plasma Met levels for the total trial period, but had no significant effect on any production responses compared to the soybean meal diet. It appeared that Met was not limiting in the soybean meal diet. Adding Lys increased milk yield, milk protein % and yield, and plasma Met, Lys, Arg, Thr, and His during early lactation compared to the soybean meal diet. It appears that Lys was limiting in the soybean meal diet. Illg et al.. (1987) supplemented an alfalfa hay, corn silage , and soybean meal diet fed to primiparous and multiparous cows with 15 g of rumen protected Met (DL-Met). An increase in DMI, milk yield, 4% fat corrected milk, and solids corrected milk was reported with the addition of Met, indicating Met was limiting in the control diet.

Amino Acid Infusions - growing cattle

Although the AA requirements are not known for lactating cows or growing cattle, they are certainly not the same for both. Profile of AA from milk and tissue, the major products of lactating cows and growing cattle, are not the same (Mantysaari et al., 1989a). In addition, primiparous cows would most likely have different requirements than multiparous or growing cattle since they are growing and lactating.

Traditionally, steers have been utilized in AA infusion studies of growing cattle. Hill et al. (1980) postruminally infused Lys and Met to growing steers averaging 230 kg BW. Increments of Met (0, 4, 8, 12 g/d) were infused with 25 g/d of Lys. The negative control was no supplementation of Lys or Met, and the positive control was 12 g/d Met, 24 g/d Lys, and 140 g/d sodium caseinate. Nitrogen retention increased with infusion of Lys or Lys/Met combination compared to the negative control. Infusion of Met at any level did not alter nitrogen retained compared to Lys infusion alone. This implies Lys was limiting to these steers, and Met was not. This would be expected considering the diet was 65% shelled corn, 30% cottonseed hulls, 1.2% urea, .16% sulfur, plus molasses and minerals. The majority of protein reaching the small intestine from this diet was most likely microbial protein. Some of the corn protein would bypass the rumen, and corn is low in Lys content. This would indicate a possible deficiency of Lys. Added sulfur should assure Met synthesis by rumen microbes. Infusion of Lys increased plasma Lys,

indicating Lys may not be the only limiting AA. Plasma Met increased linearly with incremental infusions of Met compared to the negative control, indicating Met was not limiting in this diet. Plasma urea nitrogen increased linearly with increments of Met infusion, again indicating that extra Met was not needed.. Addition of sodium caseinate improved nitrogen retention over all other treatments, indicating individual AA other than Lys were limiting.

Findings of Richardson and Hatfield (1978) support the conclusions of Hill et al. (1980). Feeding a corn starch and cottonseed hull diet to Holstein steers, they found Met, Lys, and Thr (in order of limitation) to be the first three limiting AA when microbial protein was the major protein source available to the animal. In a series of studies, Lys, Met, Thr, and Trp were infused. Infusion of a combination of Lys, Met and Thr resulted in the greatest nitrogen retention. In addition, Trp was determined not to be limiting. Plasma levels of each individual AA were increased when that individual AA alone was infused. However, when Lys or Met plus Lys was infused, plasma Met levels decreased compared to Met infusion alone. This indicates that Met alone was not limiting, but rather Lys and Met were co-limiting. The conclusions of Hill et al. regarding sulfur could explain why Richardson and Hatfield (1978) found Met limiting, while Hill et al. did not. By not supplementing sulfur, Richardson and Hatfield (1978) may have prevented microbial production of the sulfur containing AA, Met and Cys.

Amino Acids from Undegradable Intake Protein

In addition to infusion and rumen protection, AA can be supplemented by providing feeds high in undegradable intake protein. Table 10 contains estimates of undegradability of various feeds (National Research Council, 1989). The standard deviations (S.D.) and coefficients of variation (C.V.) show there is variation in UIP values of a given feed. Also, many feeds have limited numbers of determination. Blood meal has only two determinations, but has a low S.D. and C.V., meaning the UIP values are most likely useful. Whole soybeans also have only two determinations, but have large S.D and C.V. UIP values from feeds that have a large S.D. and C.V. with few determinations should be used with caution. Other feeds such as fish meal and soybean meal have large numbers of determination, so the UIP values of these feeds should be acceptable.

Feed protein that escapes rumen degradation supplies AA of non-microbial origin, similar to AA supplied by infusion and rumen protected AA. Quantity of AA available post ruminally can be readily estimated when they are rumen protected or infused. When AA are supplied from feed protein that escapes rumen degradation, quantity of AA available post ruminally depends on the degradability of the protein and the AA content of the protein that escapes rumen degradation.

The UIP and DIP requirements are not known for growing dairy heifers. National

Table 10. Ruminal Undegradability of Protein in Selected Feeds

Feed	Number of Determinations	Mean	S.D.	C.V.
Alfalfa, dehydrated	8	.59	.17	29
Alfalfa hay	12	.28	.07	25
Alfalfa silage	6	.23	.08	36
Barley	16	.27	.10	37
Beet pulp	4	.45	.14	30
Blood meal	2	.82	.01	1
Brewers dried grains	9	.49	.13	27
Casein	3	.19	.06	32
Clover, red	3	.31	.04	12
Clover-grass	2	.54	.11	21
Clover-grass silage	7	.28	.06	22
Corn	11	.52	.18	34
Corn, dry rolled	6	.60	.07	12
Corn gluten feed dry	2	.22	.11	51
Corn gluten meal	3	.55	.08	14
Corn silage	3	.31	.06	20
Cottonseed meal	21	.43	.11	25
Distillers dried grains with solubles	4	.47	.18	39
Fish Meal	26	.60	.16	26
Grass	4	.40	.10	26
Grass silage	20	.29	.06	20
Linseed meal	5	.35	.10	27
Meat and bone meal	5	.49	.18	37
Oats	4	.17	.03	15
Peanut meal	8	.25	.11	45
Rapeseed meal	10	.28	.09	31
Sorghum grain	2	.54	.02	4
Sorghum grain, dry rolled	2	.64	.08	12
Sorghum grain, steam flaked	2	.47	.07	15
Soybean meal	39	.35	.12	33
Soybeans	2	.26	.11	40
Wheat	4	.22	.06	27
Wheat middlings	3	.21	.02	11

Source: National Research Council, 1989

Research Council recommendations for a large breed growing female dairy heifer are in Table 11. Using book values for UIP content of feeds, these calculated requirements for young heifers are not easy to achieve. They could be, however, if UIP values were higher for a given feedstuff in young calves than in older cows or heifers.

Numerous studies have been conducted to evaluate UIP and milk production in lactating dairy cows (Aharoni. et al., 1993; Christensen et al., 1993; Erasmus et al., 1994; Grummer et al., 1994; Holter et al., 1993; Keery and Amos, 1993; Palmquist et al., 1993; Sklan and Tinsky, 1993; Tomlinson et al., 1994; Van Saun et al., 1993). Fewer studies relate UIP and growth of dairy heifers (Maiga et al., 1994; Reddy et al., 1993; Tomlinson, 1990; Zerbini et al., 1985). Performance (daily gain, body composition, wither height) measurements are good indicators of a response to UIP, but they are not the only method of evaluating response to high UIP diets. Feed efficiency and nitrogen retention are valuable methods because they relate the efficiency with which the animal utilizes nutrients. The ultimate value of a feed for dairy heifers should be determined by the gain it promotes per unit of intake. Even though high UIP feeds are costly, they can be economical if improved feed efficiency offsets the cost.

Titgemeyer et al. (1989) compared various protein sources fed in combination with a basal diet of corn silage, wheat straw, ground corn, urea, and casein. Six Simmental cannulated steers (336 kg BW) were fed one of 13 diets (12 treatment diets and a control

Table 11. UIP and DIP recommendations for large breed growing dairy heifers.

Body Weight	Gain	UIP	DIP
(kg)	(g)	(g)	(g)
100	600	317	57
150	600	283	150
200	600	254	239
300	600	209	413
400	600	182	592
500	600	175	785
600	600	193	1007

Source: National Research Council, 1989

diet) over eight periods, such that each steer received the control diet at least once, and the control diet was fed once each period. Corn starch, the protein supplement in the control diet, was replaced with soybean meal, corn gluten meal, blood meal, or fish meal in the treatment diets. Three levels of each protein supplement were fed to achieve a low, medium, and high protein supplement. As expected, animals fed soybean meal had the largest increase in rumen ammonia - N over the control, while the blood meal treatment had no significant increase over the control. Total AA flow (above the control) to the duodenum (g/g of supplemental N) was highest for the corn gluten meal and blood meal treatments (4.87 and 5.044 respectively), lowest for the soybean meal treatment (.656), and intermediate for the fish meal treatment (2.11). Rumen degradabilities and small intestine N absorption were also calculated (Table 12).

The results of Titgemeyer et al. (1989) indicate that as UIP and small intestine N absorption increase, AA flow increases and ammonia N decreases. This suggests that N-retention and feed efficiency would also be improved. Keery and Amos (1993) reported similar results when four cannulated Holstein steers were supplemented soybean meal, heat treated soybean meal, menhaden fish meal, or a combination of fish meal, corn gluten meal, and heat treated soybean meal. Rumen ammonia - N levels were highest for the soybean meal treatment (12.5 mg/dl) and lowest for the combination protein supplement (8.7 mg/dl) and heat treated soybean meal (6.8 mg/dl). For unknown reasons, fish meal maintained high rumen ammonia - N levels. Apparent organic matter digestibility in the

Table 12. Rumen degradability and small intestine N absorption of soybean meal, corn gluten meal, blood meal, and fish meal.

Feed	Rumen Undegradability	Small Intestine N Absorption
	(%)	(% of total N)
soybean meal	21	13
corn gluten meal	86	69
blood meal	92	68
fish meal	68	50

(Titgemeyer et al., 1989)

reticulo-rumen was highest for the soybean meal group, but whole tract digestibilities were not different, implying that the treatments with high UIP levels had a higher percentage of organic matter digested in the lower gut.

The results of Keery and Amos (1993) and Titgemeyer et al. (1989) suggest that increasing UIP levels in growing ruminant diets can be beneficial. Post ruminal digestion and AA flow appear to improve with high UIP diets. Also, high UIP diets tend to decrease rumen ammonia - N levels, possibly indicating increased N-retention. The bottom line, however, is performance response (daily gain, feed efficiency) to UIP, which Keery and Amos and Titgemeyer et al. did not measure. In addition, they did not evaluate UIP responses under normal housing and rearing conditions. They worked with cannulated animals in individual stalls.

Mantysaari et al. (1989b) found no benefit to supplementing UIP under group housing conditions to heifers that were not cannulated. They fed fish meal, meat and bone meal, or a mixture of UIP sources (meat and bone meal, meat meal, poultry meal, blood meal, feather meal) to 112 Holstein heifers ranging in age from 210 - 524 d. Diets were soybean meal and corn silage based, and ranged in UIP from 27.6% for the control (no UIP supplement) to 34.2% for the mixture of UIP sources. Dry matter intakes, ADG, and feed efficiency were not different ($p>.05$) between the treatments. A possible explanation for the lack of response to UIP could be the narrow range of UIP levels in the treatments,

and the lack of a soluble CP source. Increasing UIP levels above 40% for the treatments would have provided a wider range of UIP levels. By not supplying a soluble CP source such as alfalfa or urea, rumen ammonia levels might have been lower than needed for maximum microbial growth in the high UIP diets.

Thonney et al. (1986) supplemented growing Holstein steers with cottonseed meal or a cottonseed meal : fish meal combination. Diets were isonitrogenous and corn grain (84% of DM) and chopped alfalfa-grass hay (11% of DM) based. Average daily gains from 95 to 544 kg BW were not significantly different between the fish meal group and the cottonseed meal group. However, feed efficiency was better for the fish meal treatment (5.73 vs 6.56 kg DMI / kg BW gain, $p<.08$). In this study, improved feed efficiency of the fish meal treatment could partially be due to the high Lys and Met content of fish meal (Clark et al., 1987).

Zerbini et al. (1985) fed ruminating Holstein bull calves a basal diet (11.6% CP) or one of four treatment diets (15.5% CP) supplemented with soybean meal, corn gluten meal, cottonseed meal, or fish meal. All diets contained orchardgrass hay, ground corn, soybean meal, and minerals. Dry matter intakes were similar between all treatments, but ADG was highest for fish meal treatment. Fish meal and corn gluten meal treatments yielded the lowest rumen ammonia N levels (2.1 and 3.5 mg/dl, respectively), which is not surprising considering these treatment had the highest UIP levels. Total VFA production

was highest for the soybean meal treatment and lowest for the fish meal treatment, most likely due to the differences in degradability. The diet low in UIP (soybean meal) should have stimulated more microbial growth than the high UIP diet (corn gluten meal), resulting in more microbial products (VFA's). In accordance, serum N levels were lowest for the fish meal treatment. In this study, fish meal was a superior protein source for growing Holstein bull calves. This could be due in part to the balanced AA profile of fish meal protein.

Swartz et al. (1991) reported a response in feed efficiency when supplementing UIP. Holstein heifers (38) and intact males (22) were fed one of three diets (33,37, and 46% UIP) from 1-13 weeks of age (period 1) and from 14-25 weeks of age (period 2). The UIP source was blood meal. Average daily gain, wither height, and hip height were not different for any treatment for either period. However, period 2 feed efficiency was improved with UIP supplementation. UIP supplementation in period 1 did not improve feed efficiency. A possible explanation for the lack of response during period 1 could be immature status of the rumen at a young age. Possible reasons for the response in period 2 could be the wide range of UIP levels among treatments or the urea added to the UIP treatments, which prevented a deficit of soluble CP in the high UIP diets.

Tomlinson (1990) fed 4 levels of UIP (31, 43, 50, 55% of CP) at 100% TDN requirements (NRC, 1978) for .6 kg ADG. Thirty-two Holstein heifers between 213 and

231 kg were used in a 50 d trial. Diets were corn silage and straw based, with combinations of soybean meal, blood meal, and urea as protein supplement. Diets were isonitrogenous and isocaloric. As UIP increased, BW gain increased, DMI decreased, and feed efficiency improved. In a second study by Tomlinson (1990), Jersey heifers were fed three levels of UIP (30, 40, and 50% CP) at three levels of non-structural carbohydrate (NSC) (17, 22, and 26% of DM). Feed efficiency was improved as UIP% increased at all three NSC levels. A third study by Tomlinson (1990) evaluated 2 levels of UIP (30 and 40% CP) and two levels of TDN (95% and 115% NRC) fed to Holstein heifers. Again, an improvement in feed efficiency was reported with the addition of UIP, with the best feed efficiency occurring with the low TDN / high UIP treatment. Although Tomlinson's work showed consistent improvement in feed efficiency with high UIP diets, these trials were of short duration (50 - 105 d). In addition, Tomlinson utilized blood meal as a UIP source. Blood meal is deficient in Met, which has been implicated as a limiting AA for growth (Richardson and Hatfield, 1978). Individual AA deficiencies can be avoided by feeding a UIP source containing a combination of protein sources. A combination that provides a balanced AA profile to the animal decreases the possibility of an AA being limiting. Tomlinson improved feed efficiency by replacing soybean meal with blood meal, but efficiency improvements might have been greater with a combination protein supplement.

RUMEN UNDEGRADABLE PROTEIN FEED SOURCES

Fish Meal

Fish meals are produced from either surplus fish or fish unacceptable for human consumption, or scraps of waste material from filleting, canning and other fish processing industries. Fish meal processing involves cooking, pressing, drying, and grinding of the fish and fish scraps. Cooking coagulates the protein, freeing water and fat from the tissues, and sterilizes the raw material. After cooking, the fish are pressed to remove some of the water and fat, resulting in a pressed meal and a liquid. The pressed meal is dried to 90% DM, ground and packed. The liquid portion is centrifuged to remove the fat. The liquid less fat is known as stickwater. The stickwater is evaporated to a thick syrup consisting of 30 - 50% solids (mostly proteins), which is added to the pressed meal to form the whole fish meal. These processing steps are similar for all fish with the exception of fish with a low fat content (whitefish), which do not need pressing (Hussein and Jordan, 1991).

Fish meal has a high CP content (60.4 - 72% DM) and a moderate amount of fat (3.4 - 11.3% DM). Despite the CP variation, AA content is similar (Barlow and Windsor, 1983). Variation in fat and protein content depends upon many factors. CP

content of fish meal is lower when processed from spoiled fish or from fish scraps instead of whole fish. Amount of stickwater added also effects CP levels. Temperature and time in drying the pressed fish to pressed meal can alter the Cys, His, Lys, and Trp content (Johnson and Savage, 1987). Type of fish also influences nutrient content. Whitefish (cod, haddock) have a low fat content, and anchovy, capelin, and menhaden fish have a high fat content (Hussein and Jordan, 1991).

Fish meal is a protein source high in UIP. NRC (1989) reported that fish meal has a mean UIP content of 60%, but well preserved fish meal is 78% UIP and stale fish meal is only 48% UIP, demonstrating the importance of processing fish quickly after catching to avoid spoilage. Hassein et al. (1991) concluded that fish meal should contain 60% CP, 50-60% UIP, and a maximum of 12% fat. Fish meal that is very dark brown in color with a scorched smell is most likely heat damaged and should be avoided.

Corn Gluten Meal

The main purpose of wet corn milling is to produce corn starch, which is used to produce high fructose sweeteners, corn syrup, and ethanol. By-products of this process include corn germ meal, condensed fermented corn extractives, corn gluten feed, and corn gluten meal. These by-products arise from the primary components of a corn kernel:

starch, gluten, hull, water, and germ. Starch, the most abundant portion of the kernel, is found at the top, on the sides, and in the middle of the kernel. Gluten contains most of the protein in corn, and the hull is the fine skin on the outside of the kernel. Germ is the source of corn oil, and is found at the bottom of the center of the kernel.. Corn germ meal is ground corn germ with most of the solubles and oil removed. Condensed fermented corn extractives, or corn steep liquor, is a high protein and energy ingredient consisting of the soluble portions of the corn kernel. Corn gluten feed is what remains after extraction of starch, gluten, and germ (Weigel, 1991).

The Association of American Feed Control Officials (1993) defines corn gluten meal as "The dried residue from corn after removal of the larger part of the starch and germ, and the separation of the bran by the process employed in the wet milling manufacture of corn starch or syrup, or by enzymatic treatment of the endosperm. It may contain fermented corn extractives and (or) corn germ meal." Essentially, corn gluten meal is separated and purified gluten (Weigel, 1991).

Corn gluten meal is high in CP (67% DM basis) and high in UIP (55% CP) (NRC, 1989), and a good source of Met and Cys (Weigel, 1991). However, it is a poor source of Lys. For this reason, corn gluten meal does not complement a corn based diet, because Lys is also low in corn silage, high moisture corn, and shelled corn. Corn gluten meal is valuable to the poultry industry because it has a high xanthophyll content, which when fed

to poultry, causes a yellow color in the skin and shanks.

Blood Meal

Blood meal is produced from clean fresh animal blood, exclusive of all extraneous material such as hair, stomach belchings and urine except in traces that would normally occur under good manufacturing procedures (American Feed Manufacturers Association, 1993). Due to large cattle and hog industries, most of the blood meal in the United States is of porcine or bovine origin. Blood meal is valuable because of it's high CP (87.2%) and high UIP (82%) content (NRC, 1989). The high UIP content results from heating during processing. Blood meal is an excellent source of Lys, but a poor source of Met and Ile (Ensminger et al., 1990). In an *in vivo* study, Loerch et al. (1983) reported blood meal to have 4.57% acid detergent insoluble nitrogen (% N) and an apparent digestibility of 80.4%. The UIP, CP, and AA content vary little between cattle and hog blood meal, although cattle blood meal can contain more hair (Harlan, 1994). Hair contributes to the CP content, but is unavailable to the animal.

Blood meal is processed by one of three methods: flash dried, ring dried, or spray dried. The greatest single factor influencing blood meal quality is the processing method (American Feed Manufacturers Association, 1993). Approximately 80% of the blood

meal in the United States is processed by the flash dried method (Harlan, 1994). Processing begins with the collection of blood off the killing floor in the slaughterhouse, where it is placed in a holding tank. Next, the blood is injected with 60 p.s.i. steam to coagulate the protein, which is centrifuged to remove approximately 50% of the moisture. A partially dried, "jelly like" mixture is left. This mixture is dried in a forced hot air dryer at 175-185° F until the moisture drops to 10%. If drying is too hot or for too long, moisture will drop below 10%, which is undesirable. When moisture falls below 7%, Lys content appears to drop significantly . Moisture above 10% is undesirable because of storage and spoilage problems, therefore processors aim for 9-11% moisture. After exiting the drier, the dried blood is ground to the desired particle size and stored. Temperature after grinding is about 100° F, and the product can be used immediately upon cooling (Harlan, 1994).

The ring dried processing method is identical to the flash dried method except for the drying procedure. Instead of a forced hot air dryer, a drum with heated steam coils (analogous to a cement kiln) is used to dry the blood. This method is not common, utilized mainly by older, smaller processors. Flash dried blood meal is a uniform, reddish brown color, while ring dried blood meal is a deep red-black to black color (American Feed Manufacturers Association, 1993).

Spray dried processing is different from flash and ring drying in that high

temperatures are not needed for drying. The product is lower in UIP and more digestible than ring or flash dried blood meal because the heating process is less severe. Spray dried blood meal is finer and higher in Lys content. The high cost (twice that of ring or flash dried blood meal) and low UIP value of spray dried blood meal make it impractical for use in the dairy industry. It is, however, considered a valuable feed for young pigs (Harlan, 1994).

Evidence available today concerning heifer growth and feeding management supports some general conclusions. First, prepubertal growth rates in excess of 1.0 kg/d for large breeds will likely impair mammary development and 1st lactation milk yield. Slower gains (.8 kg/d) enable breeding at 340-385 kg at 13-14 mo of age, resulting in a 24 mo age at first calving. Research has shown 25 mo is the optimum calving age for profit per day of herdlife. Thus it appears rapid gains before puberty are unnecessary and harmful. Second, high UIP diets have improved feed efficiency in some studies but not all. Currently, high UIP diets can not be recommended for dairy heifers due to the cost of high UIP protein supplements. Diets balanced in protein and energy using forages plus soybean meal/shelled corn are adequate. Potentially, UIP sources can improve feed efficiency enough to overcome feed costs, but currently that is not certain.

Effect of undegradable intake protein and energy on growth and feed efficiency of growing Holstein heifers.

Abstract

Thirty-two Holstein heifers were used to determine the effect of energy and undegraded intake protein (UIP) on growth and feed efficiency from 6 mo of age until calving. Treatment diets were randomly assigned to one of four groups of eight Holstein heifers between 138 and 250 d of age (average of 180). Treatments were in a 2x2 factorial arrangement, consisting of two levels of energy (supporting .6 or .9 kg ADG) and two levels of UIP (30 or 50% of CP). Heifers were fed treatment diets in a confinement facility until 385 d of age, constituting phase I of the trial. Isonitrogenous diets based on corn silage, alfalfa silage, ground orchardgrass hay, shelled corn, soybean meal, blood meal, and minerals were fed as a TMR. High UIP was achieved by substitution of blood meal for soybean meal. Phase II began at the end of phase I and continued until calving. During phase II, heifers were housed together and fed a common diet to achieve .7 kg ADG. DMI (kg/d) for phase I was 4.46, 5.42, 7.38, and 5.95 for Low Energy:Low UIP, Low Energy:High UIP, High Energy:Low UIP, and High Energy:High UIP. ADG (kg) was .62, .74, 1.01, and .96. Feed efficiencies were 8.12, 7.58, 7.44, and 6.43 kg DMI/kg

BW gain, and 4.85, 4.65, 4.98, and 4.27 kg TDN intake/kg BW gain. Dry matter intake and ADG increased with high energy diets, DM efficiency improved with high energy and high UIP diets, and TDN efficiency improved with high UIP diets. Phase II ADG was .53, .43, .33, and .50 kg/d. Overall ADG (Phase I and II) was not affected by energy or UIP. Results indicate that high UIP and high energy diets improve feed efficiency, and rate of gain before breeding does not influence calving weight or age.

Introduction

The most recent NRC (National Research Council, 1989) provides UIP and DIP recommendations for growing dairy heifers. Below 200 kg BW, NRC recommends UIP levels >50% of CP. Research trials supporting these recommendations are few. Although NRC UIP recommendations appear to be extreme and difficult to achieve, research has shown UIP levels of 40-50% of CP can improve growth and feed efficiency of dairy heifers and steers. Tomlinson (1990) reported improved feed efficiency as UIP increased from 31 to 55% of CP, using blood meal as a source of UIP. Similarly, Thonney et al. (1986) reported improved feed efficiency, but not ADG when cottonseed meal was replaced with fish meal in the diet of growing Holstein steers. Zerbini et al. (1985) found improved ADG but no differences in DMI when fish meal replaced soybean meal in diets of 9 wk old Holstein bull calves. Swartz et al. (1991) reported high UIP diets (37.9-46.4 UIP, % of CP) supplemented with blood meal resulted in improved feed efficiency from 14-25 wk of age but not from 1-13 wk of age when compared to a low UIP (29.7-32.9 % of CP) soybean meal supplemented diet. Other researchers (Heinrichs et al., 1993; Heinrichs and Garman, 1992; Mantysaari et al., 1989b) found no response in ADG or feed efficiency with high UIP diets. Response to UIP is inconsistent, and appears to vary between protein sources.

High energy diets increase heifer growth rates (Kertz et al., 1987; Peri et al.,

1993), which favors early breeding. Heifers bred early (<14 mo) enter the lactating herd sooner (<23 mo). However, high growth rates (>1.0 kg/d) can result in overfattening and possibly impaired mammary development (Foldager and Sejrsen, 1982; Harrison et al., 1983; Sejrsen et al., 1982; Swanson, 1960;). Mammary development appears to be most sensitive to growth rate during the prepubertal period (Sejrsen et al., 1982). Therefore, high energy diets, fed prepubertal, could significantly impair mammary development. Benefits associated with high prepubertal growth rates (early breeding, possible early calving) may be less significant than the detrimental effects on mammary development.

The first objective of this experiment was to determine the response of Holstein heifers to widely divergent levels of UIP and energy from 6-14 mo of age. The second objective was to evaluate the effect of growth before breeding (6-14 mo of age) on performance from breeding until calving.

Materials and Methods

Thirty-two Holstein heifers were used to study effects of energy and UIP on growth and feed efficiency from 6 mo of age until calving. Prior to treatment assignment, preweaned calves were housed in calf hutches, then moved to loose housing pens in groups of 4-8 after weaning. Heifers were offered mixed grass hay, calf starter, corn and alfalfa silage in sufficient quantities to promote gains of .60-.80 kg/d. Two wk prior to start of the trial, heifers were placed in groups of eight in the research facility, with groups balanced by age and BW. After a two wk adjustment period, treatment diets were randomly assigned to groups. Ages at the beginning of the trial were between 138 and 250, with mean of 181 d (Table 1). The first 210 d of data comprised phase I of the trial. Mean initial BW (kg) were 127, 140, 151, and 130, and mean initial ages were 176, 188, 179, and 177 d. Treatments were in a 2x2 factorial arrangement, consisting of two levels of energy (supporting .59 or .90 kg ADG) and two levels of UIP (30 or 50% of CP) as described in Table 2. Due to limited numbers of heifers of similar ages, only two treatments could be conducted at one time. Therefore, for phase I, the Low Energy:Low UIP and High Energy:High UIP treatments were conducted from June 1991 through March 1992, and the High Energy:Low UIP and Low Energy:High UIP treatments from June 1992 through March 1993. Phase II began at the end of phase I, and continued until calving. During phase II, heifers were housed in a common pasture, and received a corn

silage/soybean meal-based ration formulated to achieve .7 kg ADG. Thirty-two heifers completed phase I, and 26 completed phase II. Four heifers from Low Energy:High UIP left the herd for reasons unrelated to treatments (aborted during third trimester of gestation, laminitis, pneumonia, septicemia/toxemia resulting from a thoracic abscess), as did one heifer from High Energy:Low UIP (leukosis) and one from High Energy:High UIP (broken leg). Although 50% of Low Energy:High UIP heifers died or were culled, there does not appear to be any relation to dietary treatment.

During phase I, heifers were housed in the research facility, a confinement facility of counter-slope design, and fed a TMR through a Pinpointer 4000B computerized feeder (UIS, Inc. Cookeville, TN), which recorded daily as-fed intakes of TMR (Quigley et al., 1986). The counter-slope facility was open to the south and contained four 3.6 m x 9.1 m pens. One computer feeder was on the south side of each pen. The facility enabled recording of individual intakes of heifers group housed . Heifers had 24 h access to the feeder, but only one animal could use the feeder at any one time. During phase II, heifers were housed together with other heifers at the Virginia Tech Dairy Center in a pasture and offered a soybean meal/corn silage based diet formulated to achieve .7 kg ADG. Heifers were bred beginning at 14 mo, or at the beginning of phase II.

Body weights and wither heights were measured weekly during phase I, and monthly during phase II. Body weights were measured on three consecutive d at the

beginning and end of phase I, and averaged to achieve an accurate starting and ending weight. A livestock scale (Adrian J. Paul, Inc., Duncan, OK) was used to determine BW. Feed efficiencies and intakes for phase I were calculated using weekly averages of daily as-fed intakes and calculated nutrient content of diets. Average daily gain was calculated for each wk during phase I. For phase II, ADG was calculated using weight after calving and weight at end of phase I. Average daily gain for the first 2 mo of phase II was calculated using weight at end of phase I, and weight 2 mo later.

Diets were fed as a TMR, and contained corn silage, alfalfa haylage, chopped orchardgrass hay (mean particle length=2 cm) , ground shelled corn, a 2:1 mineral, and a protein supplement (Table 3). Protein supplement was either blood meal or soybean meal (44% CP). Blood meal was of swine origin, and processed by Smithfield Packing, Suffolk, VA.. Diets were sampled weekly to monitor TMR nutrient content, but weekly ingredient analysis was used to calculate nutrient content of diets. Percent UIP was estimated using NRC values (National Research Council, 1989), and solubility was estimated using values of Stallings et al. (1985). Crude protein, TDN, DM, and ADF values from NRC (1989) were used for blood meal and soybean meal. Forages were analyzed at the Virginia Tech Forage Testing Lab. TDN of forages and shelled corn was calculated as follows:

corn silage: TDN = 80.4 - .4810 x ADF;

alfalfa silage: TDN = 93.79 - .90 x ADF;

grass hay: TDN = 100.32 - .653 x ADF;

shelled corn: TDN = 89.8 - .768 x ADF.

(Stallings, 1994)

Data were analyzed using the GLM procedure of SAS (1993). The dependent variables for phase I (intakes, diet nutrient content, ADG, BW, wither height and wither height index) were analyzed using the model:

$Y_{ijkl} = \mu + U_i + T_j + (UT)_{ij} + H_{(ij)k} + W_l + (UW)_{il} + (TW)_{jl} + (UTW)_{ijl} + E_{(ijkl)}$, where:

Y_{ijkl} = dependent variable of heifer k in UIP i and energy j for wk l

μ = population mean;

U_i = fixed effect of UIP level i, i = 1 to 2;

T_j = fixed effect of energy level j, j = 1 to 2;

$(UT)_{ij}$ = fixed effect of interaction of UIP i and energy j;

$H_{(ij)k}$ = random effect of heifer k in UIP i and energy j;

W_l = fixed effect of wk l, l = 1 to 31;

$(UW)_{il}$ = fixed effect of interaction between UIP i and wk l;

$(TW)_{jl}$ = fixed effect of interaction between energy j and wk l;

$(UTW)_{ijl}$ = fixed effect interaction between UIP i, energy j, and wk l;

$E_{(ijkl)}$ = random residual.

Effects of protein, energy, and protein*energy interaction were tested using heifer as the error term.

Feed efficiencies for phase I were calculated using total intake/total BW gain. Feed efficiencies for phase I, phase II ADG, post confinement ADG, and overall ADG were analyzed using the model:

$Y_{ijk} = \mu + U_i + T_j + (UT)_{ij} + E_{(ij)k}$, where:

Y_{ij} = dependent variable of UIP i and energy j;

μ = population mean;

U_i = fixed effect of UIP level i, i = 1 to 2;

T_j = fixed effect of energy level j, j = 1 to 2;

$(UT)_{ij}$ = fixed effect of interaction of UIP i and energy j;

$E_{(ij)k}$ = random residual.

The effects of protein, energy, and protein*energy interaction were tested by the residual. All significant differences were for $p<.05$.

Results and Discussion

Treatment diets differed in DM, TDN, ADF, soluble CP, and UIP as expected (Table 4). High energy diets contained more silage and less orchardgrass hay than low energy diets, resulting in lower DM. TDN averaged 66.7% for high energy diets, and 60.5% TDN for low energy diets. ADF was highest for the Low Energy:Low UIP diet, reflecting the high proportion of orchardgrass hay in the diet. Crude protein levels were intended to be similar, but were higher in the high UIP diets. The differences in CP were small, however, and reasonable under practical situations. Soluble CP in the low UIP diets was low (24.6% of CP) compared to the high UIP diets (41.5% of CP), possibly indicating a reduced amount of ammonia available to rumen microorganisms, resulting in decreased microbial growth (Nocek and Russel, 1988).

Diet composition is in Table 3. To increase UIP content of the high UIP diets, blood meal partially or fully replaced soybean meal. High energy diets contained shelled corn (High Energy:Low UIP) or a high proportion of corn silage (High Energy:High UIP) to increase energy content. All diets contained low quality ground orchardgrass hay.

Intakes (Table 5) of DM, CP, and TDN increased with high energy compared to low energy diets. Intakes of DM, CP, and TDN in this trial tended to differ from NRC recommendations. High energy heifers were compared to NRC (1989) recommendations

for 250 kg large breed heifers gaining .8 kg/d, and low energy heifers were compared to 200 kg large breed heifers gaining .6 kg/d. Low Energy:Low UIP and High Energy:High UIP treatments were similar to NRC recommendations for DM, CP and TDN intakes. However, Low Energy:High UIP heifers exceeded NRC by 1.03 (DM), .14 (CP), and .37 (TDN) kg/d, and High Energy:Low UIP exceeded NRC by 1.39 (DM), .28 (CP), and 1.01 (TDN) kg/d. Limited exercise, ad libitum feeding, and boredom might have contributed to higher intakes of DM. Low intakes of the Low Energy:Low UIP heifers might be attributed to high dietary ADF content.

High UIP diets resulted in increased intakes of DM, CP, and TDN at low energy, but decreased intakes at high energy, resulting in a significant interaction of energy and protein. Therefore, it appears that high UIP diets decrease intakes of DM, CP, and TDN at 67% TDN, but increase intakes at 60% TDN. At moderate energy levels (64% TDN), Tomlinson (1990) reported DMI decreased as UIP increased from 31 to 55% of CP, which was not true for the low energy treatments in this trial, but was for the high energy treatments. In contrast, Mantysaari et al. (1989b) and Zerbini et al. (1985) reported no change in DMI with increased UIP at high levels of energy (75% TDN). Perhaps diets in excess of 75% TDN result in excessive energy intake. Baumgardt (1970) postulated that DMI is controlled metabolically by energy balance, implying that DMI may be reduced at high energy levels as the animal attempts to metabolically seek energy balance. Therefore, energy and not UIP might control intake at high energy levels (>75% TDN). Mantysaari

et al. (1989a) reported similar DM intakes to those in this trial (5.66 kg at 210 kg BW, 6.76 kg at 263 kg BW). Calculated TDN intakes, using 75% TDN, were higher for Mantysaari et al. heifers (4.25 kg at 210 kg BW, 5.07 kg at 263 kg BW) than heifers in this trial, supporting the conclusion that energy and not UIP might have limited intake. Swartz et al. (1991) found DMI was highest at medium levels of UIP (37% of CP) and lowest at high levels of UIP (46% of CP) when diets contained 68% TDN. However, intake of diets containing 78% TDN was not influenced by UIP level. Therefore, it appears that UIP decreases DMI at moderate energy levels (64-68% TDN), but not at low (60% TDN) or high (75-78% TDN) energy levels.

UIP intakes increased with high energy or high UIP diets, but there was no energy*UIP interaction. UIP intakes increased with high UIP diets, regardless of energy level. High UIP heifers exceeded NRC (1989) recommendations for UIP intake by .13 kg for Low Energy:High UIP and .17 for High Energy:High UIP. However, low UIP heifers had UIP intakes similar to NRC estimates. Intakes of soluble CP were lower for high UIP heifers, due to the low protein solubility of the high UIP diets. The High Energy:Low UIP heifers had twice the intake of soluble CP of the other three treatments. However, it does not appear that soluble CP intake limited DMI, because the low energy:low UIP heifers had higher soluble CP intakes than heifers on Low Energy:High UIP or High Energy:High UIP treatment.

Phase I BW, ADG, wither heights, and wither height indexes are in Table 6. Body weights differed with respect to energy but not UIP level. Accordingly, ADG increased with energy level, but not UIP level. To achieve recommended breeding weight of 340-385 kg at 13 mo (Etgen et al., 1987; Nebel, 1993; Perkins, 1993), heifers must gain .8 kg/d. Therefore, the Low Energy:Low UIP treatment had inadequate gains (.62 kg/d) and the high energy treatments had more than adequate gains (1.0 kg/d). Body weights and ADG appear to be directly related to DMI, regardless of UIP level or energy level. As DMI increased, BW and ADG also increased. In contrast, Tomlinson (1990) found as UIP increased from 31 to 55% of CP, ADG and BW increased, and DMI decreased. Tomlinson's trial was only 50 d, therefore the response could have changed over time. Wither heights were not affected by UIP level, but were increased with energy level. This is supported by Kertz et al. (1987), who found wither height increased with high energy diets compared to low energy diets. Tomlinson (1990) reported high UIP diets did not improve wither height. Wither height index (WHI), defined as kg BW/cm WH, was larger for high energy treatments, but was not affected by UIP. Although body condition was not measured in any other manner, the WHI data imply that high energy heifers had more condition than low energy heifers.

Apparent feed efficiencies for phase I are in Table 7. DM and CP efficiencies were improved with high energy diets, and DM and TDN efficiencies were improved with high UIP diets. No significant interactions occurred. Although gains were not improved with

UIP, feed efficiency was. This was the most significant advantage to the high UIP diets in this trial. Tomlinson (1990), Zerbini et al. (1985), and Swartz et al. (1991) also found high UIP diets improved feed efficiency. In younger calves (<13 wk), Swartz et al. (1988) and Heinrichs and Garman (1992) reported feed efficiency did not improve with increasing UIP. An explanation for the lack of response in calves <13 wk old is difficult. Possibly young calves do not have the microbial population and rumen function of older heifers, which would reduce microbial protein synthesis. This could cause more protein to bypass the rumen, resulting in more protein available in the small intestine for absorption, thus increasing UIP content of the diet. In addition, rate of passage could be higher in young calves, which also could increase UIP. Therefore, high dietary UIP levels might limit substrate available for microbial protein synthesis in young calves.

Phase II began when heifers left the confinement facility and were moved to pasture. The first 2-mo of this period were analyzed separately to determine performance after leaving confinement. During this 2-mo period, heifers of all treatments except Low Energy:Low UIP lost weight (Table 8). Weight losses during this transition period can be attributed to increased exercise of heifers when first moved to pasture, which reduced energy available for growth. This emphasizes the importance of breeding heifers reared in a confinement facility prior to being moved to pasture. Weight losses likely were detrimental to normal reproductive function (Canfield and Butler, 1990; Villa-Godoy, 1990). Services per conception and age at conception are in Table 9. Average age at

conception was high at 552 d (18.4 mo.), but services per conception averaged only 1.68. Breeding started after heifers left the confinement facility, or at 14 mo of age. Heifers apparently did not cycle or did not display visible signs of heat for one to two mo after leaving confinement. Only four of the 32 heifers conceived prior to 15 mo of age.

Low Energy:Low UIP heifers had the lowest ADG for phase I (.62 kg/d), but the highest ADG for phase II (.53 kg) compared to the other treatments. Conversely, High Energy:Low UIP treatment had the highest ADG for phase I (1.01 kg/d), but the lowest for phase II (.33 kg/d). Phase II ADG had a significant energy*UIP interaction. Apparently, heifers that gained poorly during phase I experienced compensatory growth during phase II. Park et al. (1987) reported compensatory growth occurred when heifers were limited in nutrient intake (15% below NRC), then offered a diet exceeding NRC (1978) requirements by 40%. During the compensatory period, heifers had higher and more efficient (kg DM/kg gain) BW gains than heifers fed 100% NRC throughout the trial. Although diets in this study did not differ as drastically in nutrient content from phase I to phase II as they did in the Park et al. study, it is probable that heifers on low energy diets experienced compensatory growth during phase II. As a result, overall ADG was not different between treatments, suggesting high prepubertal growth rates do not carry over to calving weights. In addition, calving ages were not different between treatments (Table 10).

Feed costs per kilogram ration DM and per kilogram BW gain during phase I are in Appendix Table 5. Low Energy:High UIP was the most expensive ration per kilogram DM ($.1214) and per kilogram BW gain ($.8892). Low Energy:Low UIP was the least expensive ration per kilogram DM ($.1108), while High Energy:High UIP ration was the least expensive ration per kilogram BW gain ($.7475). The high UIP diets tended to be more expensive due to the high cost of blood meal (Appendix Table 5). Economic evaluation was not a goal of this study, therefore no attempt was made to control feed costs. Economics will change, however, based on available forages and current feed costs.

Heifers receiving high energy diets supporting .9 kg ADG from 6-14 mo of age had increased ADG, BW, and intakes of DM, CP, and TDN. In addition, feed efficiencies (DM and CP) were improved with high energy diets. Heifers receiving high UIP diets (50% of CP) from 6-14 mo of age had improved feed efficiencies (DM and TDN) compared to heifers receiving low UIP diets (30% of CP). Intakes of DM, CP, and TDN were decreased with high UIP diets at high energy levels (68% TDN), but not at low energy levels (60% TDN). From 14 mo of age until calving when heifers were fed a common diet, heifers on low energy diets from 6-14 mo of age experienced compensatory growth, resulting in no treatment differences in ADG from 6 mo of age until calving. Dietary treatment from 6-14 mo of age did not influence calving weight, calving age, or lifetime ADG.

It appears that high UIP diets improve feed efficiency of Holstein heifers, although the mode of action is not clear. Reduced feed intake accounts for differences in feed efficiency, as ADG were not increased with high UIP diets. High UIP diets were low in protein degradability and solubility, resulting in decreased nitrogen availability to rumen microbes. Nocek and Russel (1988) reported low nitrogen availability resulted in decreased DMI in lactating cows. Decreased DMI of heifers receiving high UIP diets in this study may be related to decreased microbial growth resulting from low substrate (nitrogen) availability. It is also not clear why blood meal improved feed efficiency. Blood meal is a good source of Lys, but is deficient in Met. Therefore, a Met deficiency should have limited growth of the high UIP heifers. However, a combination of protein sources high in UIP from different origins would favor a balanced profile of essential amino acids. Such a combination could potentially improve growth and feed efficiency compared to blood meal.

Table 1. Ages at beginning and end of phases I and II, and housing, diets and heifer numbers during phase I and II of Blood Meal Trial.

Phase[1]	Treatment[2]	n[3]	Age at Start (days)	Age at End (days)	housing
I	Low En:Low UIP	8	176	385	counterslope confinement facility
	Low En:High UIP	8	180	431	
	High En:Low UIP	8	189	439	
	High En:High UIP	8	177	386	
II	Low En:Low UIP	8	385	841[4]	common pasture
	Low En:High UIP	4	431	848[4]	
	High En:Low UIP	7	439	807[4]	
	High En:High UIP	7	386	832[4]	

[1] Phase I - Period when treatment diets fed.
Phase II - Period when common diet fed (corn silage and soybean meal based, formulated to achieve .7 kg average daily gain).

[2] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.
Low UIP = 25-35% of CP, High UIP = 45-50% of CP
Low Energy = energy to support .590 kg daily BW gain (NRC, 1989).
High Energy = energy to support .908 kg daily BW gain (NRC,1989).

[3] Six heifers were lost during phase II for reasons unrelated to treatments, therefore they were omitted from analysis.

[4] calving age

Table 2. Blood Meal Trial phase I treatments.

	UIP[1]	ENERGY[2]	PROTEIN SOURCE[3]
Low En : Low UIP	LOW	LOW	SBM
Low En : High UIP	HIGH	LOW	BM
High En : Low UIP	LOW	HIGH	SBM
High En : High UIP	HIGH	HIGH	BM

[1] Low UIP = 25-35% of CP
High UIP = 45-50% of CP

[2] Low Energy = energy to support .590 kg daily BW gain (NRC, 1989).
High Energy = energy to support .908 kg daily BW gain (NRC,1989).

[3] BM = blood meal
SBM = soybean meal

Table 3. Blood meal phase I diets at 225 kg BW (% DM basis).

	Low En:Low UIP[1]	Low En:High UIP	High En:Low UIP	High En:High UIP
corn silage		33	28	61
alfalfa haylage	21		36	
orchardgrass hay	73	54	20	32
dry shelled corn			12	
soybean meal	5	7	3	
blood meal		5		6
mineral	1	1	1	1

[1] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

Table 4. Nutrient composition of treatment[1] diets in Blood Meal Trial phase I.

		LOW EN LOW UIP		LOW EN HIGH UIP		HIGH EN LOW UIP		HIGH EN HIGH UIP	
item	units	mean	SD[2]	mean	SD	mean	SD	mean	SD
DM	%	70.49	5.98	63.64	2.01	55.55	2.46	52.80	2.89
CP	% of DM	13.25	0.86	14.16	0.76	13.75	0.42	13.79	0.45
TDN	% of DM	59.68	0.64	61.39	1.10	67.12	2.14	66.34	1.13
ADF	% of DM	36.23	1.04	30.91	1.68	27.30	2.74	24.46	0.07
SOL CP	% of CP	37.44	4.23	24.60	5.17	45.63	1.88	24.56	1.08
UIP	% of CP	34.18	1.62	49.98	1.52	26.58	1.42	51.94	1.94
n		30		30		30		30	

[1] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

[2] Standard deviation

Table 5. Blood Meal Trial phase I least square means for weekly intakes of dry matter, crude protein, total digestible nutrients, acid detergent fiber, soluble crude protein, and undegradable intake protein.

	LOW EN LOW UIP[2]	LOW EN HIGH UIP	HIGH EN LOW UIP	HIGH EN HIGH UIP
DM[1]	4.46	5.42	7.38	5.95
CP[1]	0.62	0.77	1.01	0.82
TDN[1]	2.78	3.32	4.94	3.95
ADF[1]	1.69	1.69	2.04	1.46
SOL CP[1]	0.23	0.19	0.46	0.20
UIP[1]	0.21	0.38	0.27	0.43
n	240	240	240	240

[1] kg/d

[2] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

Table 6. Blood Meal Trial phase I least square means and standard errors for weekly measures of body weight, average daily gain, wither height, and wither height index.

Item	LOW EN LOW UIP[5]	LOW EN HIGH UIP	HIGH EN LOW UIP	HIGH EN HIGH UIP	SE	UIP[6] p value	EN[7] p value	UIP *EN[8] p value
DMI[2]	4.46	5.42	7.38	5.95	0.04	0.22	<.01	<.01
BW[1]	189.60	221.08	267.16	233.75	0.61	0.93	<.01	<.01
ADG[2]	0.62	0.74	1.01	0.96	0.03	0.50	<.01	0.08
WH[3]	104.42	107.21	112.94	109.17	0.09	0.74	<.01	0.04
WHI[4]	1.79	2.04	2.34	2.11	<.01	0.89	<.01	<.01
n	240	240	240	240				

[1] kg
[2] kg/d
[3] cm
[4] kg BW/cm WH
[5] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.
[6] UIP effect (tested by heifer(energy*UIP))
[7] energy effect (tested by heifer(energy*UIP))
[8] energy*UIP interaction (tested by heifer(energy*UIP))

Table 7. Blood Meal Trial phase I least square means and standard errors of apparent feed efficiencies.

Item	LOW EN LOW UIP[2]	LOW EN HIGH UIP	HIGH EN LOW UIP	HIGH EN HIGH UIP	SE	UIP[3] p value	EN[4] p value	UIP *EN[5] p value
DM[1]	8.12	7.58	7.44	6.43	0.31	0.02	0.01	0.45
CP[1]	1.08	1.08	1.02	0.89	0.04	0.11	0.01	0.11
TDN[1]	4.85	4.65	4.98	4.27	0.20	0.03	0.53	0.21
n	8	8	8	8				

[1] kg intake/kg BW gain

[2] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

[3] UIP effect (tested by residual)

[4] energy effect (tested by residual)

[5] energy*UIP interaction (tested by residual)

Table 8. Average daily gains for Phase I, Phase II, post confinement, and overall periods of Blood Meal Trial.

Item	Low En Low UIP[1]		Low En High UIP		High En Low UIP		High En High UIP	
	ADG[6]	SE	ADG[6]	SE	ADG[6]	SE	ADG[6]	SE
Phase I[2b]	0.62	0.03	0.74	0.03	1.01	0.03	0.96	0.03
Phase II[3c]	0.53	0.04	0.43	0.05	0.33	0.04	0.50	0.04
post confinement[c]	0.10	0.09	-0.51	0.09	-0.57	0.09	-0.37	0.09
overall[5]	0.56	0.31	0.56	0.04	0.59	0.03	0.65	0.03

[1] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

[2] 6-14 months of age (n=960)

[3] 14 months of age until calving (n=26)

[4] 1st 2 months of Phase II (n=32)

[5] 6 months of age until calving (n=26)

[6] kg/d

[a] significant UIP effect ($p<.05$)

[b] significant energy effect ($p<.05$)

[c] significant energy*UIP interaction ($p<.05$)

Table 9. Services per conception and age at conception for heifers in Blood Meal Trial.

Treatment[1]	Services per Conception	standard deviation	Age at Conception (days)	standard deviation	n
Low Energy:Low UIP	1.13	.35	543	63	8
Low Energy:High UIP	1.33	.74	588	65	4
High Energy:Low UIP	1.63	.52	518	70	7
High Energy:High UIP	2.63	1.77	560	77	7

[1] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

Table 10. Least square means for calving weight (kg) and calving age (days) for Blood Meal Trial.

	Low En Low UIP[1]		Low En High UIP		High En Low UIP		High En High UIP	
item	mean	SE	mean	SE	mean	SE	mean	SE
Calving weight[2,3]	501	23	515	33	523	25	556	25
Calving Age[2]	841	23	849	32	808	24	832	24
n	8		4		7		7	

[1] Low En:Low UIP = low energy, low UIP treatment.
Low En:High UIP=low energy, high UIP treatment.
High En:Low UIP=high energy, low UIP treatment.
High En:High UIP=high energy, high UIP treatment.

[2] no significant energy, UIP, or UIP*energy interaction effect

[3] weight after calving

Effect of energy and quantity and quality of undegradable intake protein on growth and feed efficiency of growing Holstein heifers.

Abstract

Thirty two Holstein heifers were used to determine the effect of energy, undegraded intake protein (UIP), and protein source on growth and feed efficiency. Treatment diets were randomly assigned to one of four groups of eight Holstein heifers between 130 and 248 d of age (average of 199). Two treatments were high UIP:low energy, with one treatment containing a combination protein supplement of fish meal, corn gluten meal, and blood meal, and the other treatment containing blood meal as the protein source. Remaining two treatments were low UIP:high energy and low UIP:low energy. High and low energy diets provided energy to support .908 and .590 kg ADG, respectively. High and low UIP diets provided 30% or 50% UIP as a % of CP. The trial was 300 d. Heifers were housed in a confinement facility and fed an isonitrogenous TMR based on corn silage, alfalfa haylage, ground orchardgrass hay, ground shelled corn, minerals, and protein supplement (blood meal, combination protein supplement, or soybean meal). DMI for combination protein supplement, blood meal, low energy, and high energy heifers was 6.06, 6.91, 6.69, and 7.70 kg/d. ADG's were .74, .96, .78, and

.81 kg/d. Feed efficiencies were 8.79, 9.95, 9.52, and 8.62 kg DMI/kg BW gain. Wither height, DMI, and BW gain increased with high energy, but were not affected by UIP source or level . Overall DM efficiency was not affected by energy or UIP. Heifers receiving combination protein supplement had improved DM and CP efficiencies from 13.5-16.5 mo. of age compared to blood meal or soybean meal supplemented heifers. Plasma urea-N was not different between treatments. Results indicate high energy diets are beneficial to growth and feed efficiency from 6-16 mo of age, but high UIP diets may not be.

Introduction

The most recent NRC (National Research Council, 1989) provides UIP and DIP requirements for growing dairy heifers. Research evaluating the effects of UIP on growth and feed efficiency is inconclusive. Some researchers have reported improved feed efficiency with diets ranging from 40-50% of CP as UIP (Swartz et al., 1991; Thonney et al., 1986; Tomlinson, 1990; Zerbini et al., 1985). Other researchers (Heinrichs et al., 1993; Heinrichs and Garman, 1992; Mantysaari et al., 1989b) found no response in feed efficiency with high UIP diets.

The inconsistent response to UIP might partially be explained by UIP source. Use of a single UIP source can result in an amino acid (AA) deficiency. Amino acids absorbed in the small intestine originate from protein of microbial and dietary origin. Microbial protein is an excellent source of AA (Chandler, 1989; Mantysaari, 1989a), and is relatively constant in AA content (Purser and Buechler, 1966). However, most supplemental protein sources are deficient in one or more AA. Therefore deficiencies in the AA profile of the bypass fraction will result in an AA deficiency, unless microbial protein meets the AA requirements of the animal. For example, blood meal is deficient in Met, implying diets with blood meal as the only UIP source are theoretically deficient in Met. A combination of protein sources high in UIP from different origins would favor a balanced profile of essential AA.

Although UIP may be important, energy content must also be considered when evaluating responses to UIP. At moderate energy levels (64% TDN), Tomlinson (1990) reported increasing UIP from 31 to 55% of CP improved feed efficiency and decreased DMI of 225 kg Holstein heifers. In contrast, Mantysaari et al. (1989b) and Zerbini et al. (1985) reported no change in DMI with increased UIP at high levels of energy (75% TDN). It is possible that diets in excess of 75% TDN result in excessive energy intake. Baumgardt (1970) postulated that DMI is controlled metabolically by energy balance, implying that excessive energy intake limits DMI. Therefore, energy and not UIP might control intake at high energy levels (>75% TDN). Swartz et al. (1991) found that feed efficiency was improved and DMI was decreased with high UIP (46% of CP) compared to low UIP diets (29.7% UIP), when dietary TDN was 67.7-68.7% of DM. However, feed efficiency and intake of diets containing 77.7-78.5% TDN was not influenced by UIP level. It appears that UIP decreased DMI and improved feed efficiency at moderate energy levels (64-68% TDN), but not at high (75-78% TDN) energy levels.

Regardless of UIP level, high energy diets result in increased heifer growth rates (Kertz et al., 1987; Peri et al., 1993), which enable early breeding. Heifers bred early (<14 mo) enter the lactating herd sooner (<23 mo). However, high growth rates (>1.0 kg/d) result in overfattening and possibly impaired mammary development (Foldager and Sejrsen, 1982; Harrison et al., 1983; Sejrsen et al., 1982; Swanson, 1960;). Mammary development appears to be most sensitive to growth rate during the prepubertal period

(Sejrsen et al., 1982). Benefits associated with high prepubertal growth rates (early breeding, early calving) may be less significant than detrimental effects on mammary development.

The first objective of this experiment was to determine the response of Holstein heifers to widely divergent levels of UIP and energy from 6.5-16.5 mo of age. The second objective was to determine the effect of providing a UIP source with a balanced profile of essential AA on growth and feed efficiency of Holstein heifers.

Materials and Methods

Thirty-two Holstein heifers were used to study effects of energy and UIP on growth and feed efficiency from 6-16 mo of age. Prior to treatment assignment, preweaned calves were housed in calf hutches, then moved to loose housing pens in groups of 4-8 after weaning. Heifers were offered mixed grass hay, calf starter, corn silage and alfalfa silage in sufficient quantities to promote gains of .60-.80 kg/d. Two wk prior to start of the trial, heifers were placed in the research facility in groups of eight, with groups balanced by age and BW. After a two wk acclimation period, treatment diets were randomly assigned to groups. Treatments were: low energy:high UIP with a combination of corn gluten meal, fish meal, and blood meal as a protein supplement (CPS); low energy:high UIP with blood meal as a protein supplement (BM); low energy:low UIP with soybean meal as a protein supplement (Low Energy); and high energy:high UIP with soybean meal as a protein supplement (High Energy) (Table 11). Mean initial BW (kg) were 156, 162, 158, and 149.

Due to limited number of heifers of similar ages, only two treatments could be conducted at a time. The BM and Low Energy treatments were conducted from March through December, 1993. The CPS and High Energy treatments started in September, 1993 and ended in May, 1994. Ages at the beginning of the trial were between 130 and 248 d, however, BM and Low Energy heifers received treatment diets for 42 d before data

collection started (Table 12). This was due to an unavoidable age variation among heifers in treatments. Ages of heifers in the BM and Low Energy treatment varied by 38 and 45 d, but ages varied by 110 and 118 d in the High Energy and CPS heifers. Prior experience with heifers in the research facility indicated that heifers <130 d of age did not adapt well to the facilities. Therefore, for the youngest heifer in the second set of treatments to not begin treatment <130 d of age, the average age at the beginning of the trial for the CPS and High Energy heifers was increased to 200 d. Low Energy and BM had already been started at an average of 157 d of age. Therefore, the first 42 d of data for BM and Low Energy were not used in analysis.

Heifers were housed in a counter-slope design confinement facility, and fed a TMR through a Pinpointer 4000B computerized feeder (UIS, Inc. Cookeville, TN), which recorded daily as fed intakes (Quigley et al., 1986). The counter-slope facility was open to the south, and contained four 3.6 m x 9.1 m pens. One computer feeder was on the south side of each pen. The facility enabled recording of individual intakes of group housed heifers. Heifers had 24 h access to the feeder, but only one animal could use the feeder at any one time.

Body weights and wither heights were measured weekly throughout the trial, and on three consecutive d at the beginning and end of the trial to achieve an accurate estimation of starting and ending BW and wither height. A livestock scale (Adrian J. Paul,

Inc., Duncan, OK) was used to determine BW. Feed efficiencies and intakes were calculated using weekly averages of daily as fed intakes and calculated nutrient content of diets. Feed efficiencies were calculated overall and for seven six wk periods. Average daily gain was calculated for each wk.

Diets were fed as a TMR and contained corn silage, alfalfa haylage, ground orchardgrass hay (mean particle length=2 cm), ground shelled corn, a 2:1 mineral, and a protein supplement. Protein supplement was either blood meal, soybean meal (44% CP), or a combination protein supplement (CPS premix) containing corn gluten meal (60% CP), blood meal and fish meal (Appendix Table 1). CPS premix was mixed in a horizontal mixer before addition to the TMR. Blood meal was of swine origin, and processed by Smithfield Packing, Suffolk, VA.. Corn gluten meal was processed by Corn Products, Inc., Winston Salem, NC. Sea-Lac™ ruminant grade fish meal (Zapata Protein (USA) Inc., Hammond, LA) was used. Diets were sampled weekly to monitor TMR nutrient content, but weekly ingredient analysis was used to calculate nutrient content of diets. Percent UIP was estimated using NRC values (National Research Council, 1989), and solubility was estimated using values of Stallings et al. (1985). Values of NRC (1989) for CP, TDN, DM, and ADF were used for blood meal, fish meal, corn gluten meal, and soybean meal. Forages were analyzed at the Virginia Tech Forage Testing Lab. TDN of forages and shelled corn was calculated as follows:

corn silage: TDN = 80.4 - .4810 x ADF;

alfalfa silage: TDN = 93.79 - .90 x ADF;

grass hay: TDN = 100.32 - .653 x ADF;

shelled corn: TDN = 89.8 - .768 x ADF.

(Stallings, 1994)

CPS premix was developed to reflect expected AA needs of growing heifers. Amino acid content of protein sources (blood meal, corn gluten meal, fish meal, and soybean meal) was compared to AA content of muscle tissue to estimate protein quality. Chandler (1989) and Schingoethe (1991) used milk as a base of comparison for AA needs of lactating dairy cows. However, tissue is an appropriate base of comparison for growing animals. Estimated AA composition of protein sources, microbial protein and tissue are in Appendix Table 3. Microbial protein is similar to tissue in AA content and overall the best source of AA, but is deficient in Arg, His, Lys, and Met as compared to muscle tissue. Blood meal is an excellent source of Lys, but a poor source of Met. Fish meal is not severely deficient in any one AA, but is adequate only in Leu and Met compared to tissue. Corn gluten meal is a poor source of Trp and Lys, but a good source of Met. Soybean meal has a balanced AA profile compared to tissue, but the high degradability of soybean meal (65% of CP, NRC, 1989) limits it's use as a rumen undegradable AA source. Rumen microorganisms degrade the majority of AA in soybean meal to synthesize microbial protein.

With the knowledge that an individual protein source will not meet the estimated AA needs of growing dairy heifers, a blend was formulated to closely match the AA profile of tissue. However, rumen undegradable feed proteins are not the only source of AA to the small intestine. The contribution of AA from microbial protein should be taken into account. In the high UIP (50% of CP) diets, approximately 50% of the CP should bypass rumen degradation. Therefore, it was assumed that 50% of the AA absorbed in the small intestine were of microbial origin, and 50% were dietary AA. Estimated bacterial protein production was calculated to validate this assumption. Bacterial crude protein (BCP), bacterial true protein (BTP), and digestible bacterial true protein (DBTP) were calculated using equations of NRC (1985) as follows:

BCP = (-31.86 + 26.12 x TDN) x 6.25;

BTP = BCP x .8;

DBTP = BTP x .8;

where TDN = kg/d TDN intake.

Similar to Chandler (1989) and Schingoethe (1991), an index was developed to evaluate protein sources. The index accounts for the calculated percentage deficit of each individual AA in the protein source compared to tissue. The AA content of each protein source or protein source combination evaluated was averaged with microbial protein AA content before comparison to tissue. The percentage deficit for each AA was calculated

as:

AA deficiency (g/100 g AA) =

tissue AA content - (AA content of protein source + AA content of microbial protein)/2.

Percent deficit =

100 x (AA deficiency, g/100g)/(g/100 of AA in tissue).

Amino Acid values of Polan (1992) were used in calculations. A percent deficit of 0 was used for AA that were more abundant in feed protein than in tissue. Percent deficit was calculated for each essential AA, and two deficiency index's were calculated to compare protein source combinations as follows:

Index A = average percent deficiency of all ten essential AA.

Index B = average percent deficiency of essential AA with a percent deficiency >0.

Schedule for plasma urea-N, urea space, and loineye measurements are in Table 12. Ages were consistent for all treatments across all measurement d. Blood samples for plasma urea-N analysis were taken once every four hours over a 24 h period. Ten ml

blood was obtained by jugular venipuncture and immediately transferred to a 10 ml collection tube containing 100 ul sodium heparin (286 IU/ .1 ml), and placed on ice. Blood was centrifuged at 5,000 RPM for 10 min, and plasma removed and frozen (-30^0 C) for later analysis. Urea-N was determined using the phenol-hypochlorite reaction as described by Weatherburn (1967). Longissimus muscle area (loineye) was determined using an Aloka 210 ultrasound machine with a 3 MHz probe (Corometrics Medical Systems, Inc. Wallingford, CT). Loineye area was estimated between the 12th and 13th ribs. The ultrasound image was recorded on a VCR, and sketched on computer using Animorph Version 1.40 (1991 Woods Hole Educational Ass.) to estimate loineye area in cm^2.

Empty body composition was estimated by urea space (Preston and Kock, 1973). Heifers were fasted 12 h prior to estimation. Heifers were weighed, and an initial blood sample drawn for baseline value through a 16 gauge needle. Immediately after the initial sample, a 20% urea solution dissolved in .9% saline was injected through the same needle. Dosage of urea solution was 130 mg urea/kg BW. Post infusion sample was obtained 12 min after completion of urea dosing. Urea space (US) was estimated as follows:

$$\text{Urea space} = \frac{\text{(volume urea solution infused x concentration of solution)}}{\text{(post injection urea-N minus baseline urea N)}}$$

Volume infused was .65 ml/kg BW and concentration of solution was 93.4 mg urea-N/ml

solution. Urea space as a percent of BW was calculated as follows:

$$\text{urea space as a percent of BW (US\%BW)} = \frac{\text{urea space} \times 10}{\text{BW (kg)}}$$

Percent empty body water (%EBW), percent empty body protein (%EBP), and percent empty body fat (%EBF) were estimated using the equations of Hammond et al. (1990):

$$\%EBW = 83.5 - .16\ US\%BW - .032\ BW$$

$$\%EBP = 16.6 - .009\ US\%BW + .005\ BW$$

$$\%EBF = -5.9 + .14\ US\%BW + .030\ BW$$

Carcass protein and carcass fat were estimated using equations of Swartz et al. (1991):

$$\text{Protein (kg)} = 3.03 + .10BW - .02US\%BW$$

$$\text{Fat (kg)} = -2.5 + .07BW - .006US\%BW$$

Data were analyzed using the GLM procedure of SAS (1993). Feed efficiency, intake, ADG, BW, wither height, wither height index, body composition, and loineye were analyzed using the model:

$Y_{ijk} = \mu + T_i + H_{(i)j} + P_k + (TP)_{ik} + E_{(ijk)}$ where:

Y_{ijk} = dependent variable of heifer j in treatment i for period k;

μ = population mean;

T_i = fixed effect of treatment i, i = 1 to 4;

$H_{(ij)}$ = random effect of heifer k in treatment i and period k;

P_k = fixed effect of period k, k = 1 to 7 (feed efficiency),

1 to 42 (intake, BW, ADG, WH, WH index),

1 to 3 (loineye and body composition estimates);

$(TP)_{ik}$ = fixed effect of interaction between treatment i and period k;

$E_{(ijk)}$ = random residual.

Effects of treatment were tested by heifer(treatment). All observations for each variable were used for analysis, with the exception of feed efficiencies, which were calculated using total intake/total BW gain for seven 6 wk periods.

Diet nutrient content was analyzed using the model:

$Y_{ij} = \mu + T_i + W_j + E_{(ij)}$ where:

Y_{ij} = dependent variable for diet of treatment i for wk j;

μ = population mean;

T_i = fixed effect of treatment i, i = 1 to 4;

W_j = fixed effect of wk j, j = 1 to 42;

$E_{(ij)}$ = random residual.

Loineye change was analyzed using the model:

$Y_{ij} = \mu + T_i + E_{(i)j}$ where:

Y_{ij} = dependent variable of loineye change for heifer j on treatment i;

μ = population mean;

T_i = fixed effect of treatment i, i = 1 to 4;

$E_{(i)j}$ = random residual.

Loineye change was calculated as the difference between loineye measurements 2 and 1, 2 and 3, and 1 and 3.

Plasm urea-N was analyzed using the model:

$Y_{ijkl} = \mu + T_i + H_{(i)j} + D_k + (TD)_{ik} + (HD_{(i)})_{jk} + P_l + (TP)_{il} + (DP)_{kl} + (TDP)_{ikl} + E_{(ijkl)}$ where:

Y_{ijkl} = dependent variable of heifer j in treatment i for d k and period l;

μ = population mean;

T_i = fixed effect of treatment i, i = 1 to 4;

$H_{(i)j}$ = random effect of heifer j in treatment i;

D_k = fixed effect of day k, k = 1 to 5;

$(TD)_{ik}$ = fixed effect of interaction between treatment i and day k;

$(HD)_{(i)jk}$ = fixed effect of interaction between heifer j and day k;

P_l = fixed effect of period l, l = 1 to 6;

$(TP)_{il}$ = fixed effect of interaction between treatment i and period l;

$(DP)_{kl}$ = fixed effect of interaction between day k and period l;

$(TDP)_{ikl}$ = fixed effect of interaction between treatment i, day k and period l;

$E_{(ijkl)}$ = random residual.

Treatment was tested by heifer(treatment). Day and day*treatment interaction were tested by day*heifer(treatment). Periods in the model refer to six 4 hour periods during one d.

Significant differences were for $p<.05$. Trends were discussed for $p<.10$. Non orthogonal contrasts (Appendix Table 8) evaluated effects of UIP level, UIP source, and energy level. Due to confounding with energy, comparisons of UIP level did not consider high energy treatment. Energy level comparisons did not consider CPS or blood meal treatments to avoid UIP confounding.

Results and Discussion

Least square means for treatment diets are reported in Table 13. Crude protein levels were not different between any treatments, but DM% was highest for CPS, due to the large proportion of orchardgrass hay (54.5%) in the diet. Treatment diets differed in UIP and soluble CP as expected, with the exception of the two high UIP diets. The BM treatment had higher UIP and soluble CP content than CPS. More CPS premix had to be fed in CPS than blood meal in BM to approach UIP levels of BM. Therefore, more CP was supplied from the protein supplement in CPS than BM treatment. For this reason, more low protein orchardgrass hay and less high protein alfalfa haylage were fed in CPS diet, resulting in differences in soluble CP. However, soluble CP was higher in CPS diet (28.5 % of CP) than the high energy:high UIP (24.56% of CP) and low energy:high UIP (24.60% of CP) diets in the first trial in this thesis (Blood Meal Trial). Percent TDN differed between UIP source and UIP level, but differences were small and acceptable under practical conditions. Dry matter differences were attributable to varying proportions of dry orchardgrass hay (90% DM) and wetter silages.

All treatment diets contained corn silage, alfalfa haylage, orchardgrass hay, dry shelled corn, and minerals (Table 14). High Energy treatment had more shelled corn than other treatments to increase energy content of the diet. Low UIP treatments (High Energy and Low Energy) contained soybean meal, and the two high UIP diets (BM and

CPS) contained blood meal or CPS premix. Blood meal is higher in UIP and CP than CPS premix, but lower in energy (Appendix Table 2). For this reason, BM diet contained more shelled corn and corn silage than CPS diet. Chopped orchardgrass hay was the most abundant forage in all diets.

Calculated deficiency indexes (Table 15) were used to evaluate protein sources, with the lowest index indicating the protein most similar in AA content to tissue. Based on these two deficiency indexes, microbial protein is the best source of AA. Similarly, Chandler (1989) and Schingoethe (1991) developed AA indexes using milk protein as a base of comparison, and found microbial protein was a better source of AA than any feed protein. Fish meal had the best deficiency indexes of any feed source, but a supplement containing large amounts of fish meal was avoided due to the fatty acid content of fish meal. Spain et al. (1994) suggested that polyunsaturated fatty acids (PUFA) in fish meal may alter post ruminal lipid metabolism. Several studies reported lower milk fat yields when fish meal was fed (Mattos and Palmquist, 1974; Spain et al., 1990; Wohlt et al., 1991). Although Zerbini et al. (1985) had success feeding fish meal to dairy bull calves, fish meal was limited in this study to avoid possible alteration of post ruminal lipid metabolism. Therefore, various mixtures containing different proportions of fish meal, corn gluten meal, and blood meal were evaluated, limiting fish meal to one third of the total mixture. The mixture with the lowest deficiency index was an as fed mixture of 41.5% corn gluten meal, 21% blood meal, and 37.5% fish meal, with each feed

contributing one third of the total UIP (Appendix Table 1). The combination protein supplement (CPS premix) had the lowest deficiency index A (8.5) and B (14.1) compared to other feed sources. Deficiency index B of CPS (14.1) was better than that of microbial protein (16.8).

Bacterial protein production estimates are in Table 16. They validate the assumption that 50% of the amino acids absorbed in the small intestine of heifers receiving the high UIP diets were of dietary origin. The ratio BCP / (CP intake) was used to estimate the percentage of amino acids of microbial origin. The CPS, Blood Meal, and Low Energy treatments had a BCP / (CP intake) ratio of .47, implying that approximately 50% of the amino acids available post ruminally were of dietary origin, and 50% were of microbial origin. The High Energy treatment had a higher ratio (.59), reflecting higher energy intakes. Even though the equations of NRC (1985) do not account for protein degradability, they do give a reasonable estimate of bacterial protein production.

Intakes are reported in Table 17, and contrasts are reported in Appendix Table 9. Intakes of DM, CP, TDN, and UIP were greater for all treatments compared to NRC (1989). The CPS, BM, and Low Energy heifers were compared to NRC recommendations for 250 kg large breed heifers gaining .6 kg/d (5.31 kg DM, .637 kg CP, 3.48 kg TDN, and .229 kg UIP), and High Energy heifers were compared to 300 kg large breed heifers gaining .8 kg/d (7.06 kg DM, .848 kg CP, 4.52 kg TDN, and .236 kg

UIP). Limited exercise, ad libitum feeding, and boredom in the confinement facility might have contributed to higher intakes than NRC estimates.

TDN intakes were greater for high energy heifers, and there was a trend for higher DM and CP intakes. By design, high UIP heifers had higher UIP intakes and lower soluble CP intakes as compared to low UIP heifers. BM heifers had higher intakes of UIP and soluble CP than CPS heifers, due to DMI differences. Contrary to Tomlinson (1990) and Swartz (1991) and similar to Zerbini et al. (1985) and Mantysaari et al. (1989b), DM intake was not influenced by UIP level. There were also no differences or trends in DMI between BM and CPS heifers.

Least square means and contrasts for overall apparent feed efficiencies are in Table 18 and Appendix Table 10. There were no significant differences or trends in overall DM, CP, or TDN efficiencies between treatments or among contrasts. This is unexplainable, as other researchers (Tomlinson, 1990; Zerbini et al., 1985; Swartz et al., 1991) and the first trial in this thesis have shown an increase in feed efficiency with high UIP diets. Overall DM efficiencies for all treatments averaged 9.2 kg DM intake/kg BW gain, with heifers averaging 275 kg BW. Tomlinson (1990) achieved improved feed efficiencies (5.66 kg DM intake/kg BW gain) at 225 kg BW with corn silage/barley straw based diets. Perhaps lower CP levels (11.8 - 12.7% CP) or higher energy levels (63.6-64.4% TDN) contributed to the superior feed efficiencies reported by Tomlinson. The results are difficult to

explain, however, because blood meal was used to achieve high UIP levels. Blood meal is deficient in Met, so Tomlinson heifers should not have achieved superior feed efficiencies, based on a methionine deficiency. From 7 - 17 mo of age, Mantysaari et al. (1989b) reported mean DM efficiencies similar to this trial (8.4), using soybean meal, fish meal, meat and bone meal, or one of two commercial high UIP mixes.

Table 19 and Appendix Tables 11-13 report least square means and contrasts for apparent feed efficiencies for six 7 wk periods of the trial. No significant differences were evident for periods one, two, three, four, and five, but there were differences for periods six and seven (the last 12 wk of the trial). High UIP treatments had improved TDN and DM efficiencies, and a trend for improved CP efficiencies for period six. Heifers fed High Energy diet had improved CP and TDN efficiencies for period seven. The largest differences were evident between the BM heifers and the CPS heifers. CPS heifers had improved CP, TDN, and DM efficiencies compared to BM heifers for periods six, and a trend for improved TDN efficiency for period seven. Heifers averaged 410 d of age at the beginning of period six, and 492 d of age at the end of period seven. Therefore, it appears that CPS premix supplementation improved feed efficiencies from approximately 13.5 mo. of age until 16.5 mo. of age. Swartz et al. (1991) reported a response at different ages. From 1-12 wk of age, high UIP diets (46% UIP, as a % of CP) did not improve feed efficiency. However, from 12-25 wk of age, the same group of heifers improved feed efficiency when fed a high UIP (38% UIP, as a % of CP) diet. Even though Swartz et al.

animals were younger compared to the heifers in this trial, they displayed a similar delayed response to UIP.

Least square means and contrasts for ADG, BW, wither height, and wither height index are reported in Table 20 and Appendix Table 14. All heifers had adequate BW gains to achieve breeding by 13-14 mo (Nebel, 1992; Perkins, 1992) and were similar to gains reported by Tomlinson (1990), but lower than .94 kg/d reported by Mantysaari. ADG increased with high energy, but was not affected by UIP level or source. Kertz et al. (1987) reported high energy diets increase ADG. Mantysaari et al. (1989b) and Swartz et al. (1991) reported no change in ADG with increased UIP. Body weight, wither height, and wither height index was not affected by UIP level. There was a trend for greater wither heights with high energy diets, but no differences in wither height index or BW were apparent. Performance traits measured in this trial (ADG, BW, WH) show no effect of UIP level and no difference between UIP sources. In summary, high energy promoted growth in stature and BW.

Zerbini et al.(1985) reported Holstein bull calves that received a fish meal supplemented diet had lower serum urea-N levels (8.5 mg/dl) than bull calves fed a soybean meal supplemented diet higher in soluble and degradable protein (10.9 mg/dl). They postulated that higher serum urea-N levels were related to higher NH_3 losses from the rumen of heifers receiving highly degradable diets. Excess protein in the rumen is

converted to ammonia, then eventually to urea, which will travel through the blood. Therefore, CPS and BM heifers should have had lower plasma urea-N levels than High Energy or Low Energy heifers. However, there were no overall differences with respect to UIP level or UIP source (Table 21). Plasma urea-N differed on certain d with respect to UIP source and level (Table 22; and Appendix Table 15), but no pattern was evident. Heifers had 24 h access to feed, so feeding time was inconsequential. No diurnal pattern of plasma urea-N was evident.

The unexpected similarity in plasma urea-N levels with respect to UIP level is partially explained by the procedure used to determine plasma urea-N (Weatherburn, 1967). The procedure requires 10 µl of plasma diluted with 7 ml of reagents. Ten µl is a small quantity, therefore accuracy of plasma transfer by pipette is critical. A small error could result in a significant change in volume of plasma transferred. Compounding this problem was the large quantity of fibrin observed in the plasma samples. Fibrin results from blood clotting, when the blood protein fibrinogen is converted to fibrin to form clots (Lehninger, 1993). Sodium heparin (100 µl, 286 IU/.1 ml) was added to blood collection tubes to eliminate clotting in the test tubes. However, large quantities of fibrin were visible in the samples. The reason for this is unclear. An attempt was made to centrifuge samples (7,000 RPM, 15 min) before urea analysis to force fibrin to the bottom of the test tube, thus avoiding fibrin in the aliquot transferred by pipette. However, it was observed that fibrin was still present in the samples. A small piece of fibrin could easily change the

volume transferred via pipette by 20-30%. In hindsight, serum would have been more appropriate than plasma. Plasma is identical to serum except for the presence of fibrin (Graaff et al., 1986). Therefore, urea-N values should be identical between serum and plasma. Using serum, however, would eliminate pipette transfer errors due to fibrin. Therefore, plasm urea-N values reported in this study should be interpreted with caution.

Loineye measurements were obtained on approximately d 30, 100, and 240 of the trial (Table 12). Least square means are reported in Table 23, and contrasts in Appendix Tables 16 and 17. High energy heifers had a larger loineye area on d 30 and 100, but not d 240. On d 240, high UIP heifers had smaller loineye areas (34.1 cm^2) than low UIP heifers (38.1 cm^2), and CPS heifers (32.5 cm^2) had smaller loineye areas than BM heifers (35.73 cm^2). Total change in loin eye area from d 33 to d 240 was reduced with high UIP diets, but not affected by energy level. Loineye area results are unexplainable because BW and ADG were not different. The data suggest that high UIP diets are detrimental to loineye growth. However, reliability of ultrasound to estimate loineye area is questionable (Waldner, 1992). In addition, loineye growth may not be indicative of overall muscle growth in dairy heifers. It does not appear that ultrasonic loineye area estimation is a valid method of evaluating muscle growth in growing dairy heifers.

Least square means for urea space estimates of body composition are presented in Tables 24-27, and contrasts in Appendix Tables 18-24. Overall %EBW was lower and

%EBF was higher for BM heifers compared to CPS heifers. There were no differences with respect to energy or UIP level. As expected, overall %EBP was not different. There was an unexplainable difference in %EBP with respect to UIP level at 310 d of age. Carcass protein and carcass fat overall and measured at 235, 310. and 440 d of age were not different. The only difference in body composition using urea space estimation was with respect to UIP source. The CPS premix appeared to promote leaner gains than blood meal. Swartz et al. (1991) stated that urea space did not improve estimation of body composition for dairy heifers, although other researchers (Preston and Kock, 1973; Hammond et al. 1990) reported accurate results using dairy and beef steers. The major fault with urea space equations is that they apply only to the small number of animals in the data set. Visual differences in body condition were apparent between Low Energy and High Energy heifers, but urea space estimates did not indicate this. Without differential slaughter data, it is difficult to make conclusions from urea space estimation of body composition in this trial.

Feed costs were determined using Feedstuffs (1994) price estimates. Appendix Table 7 contains feed costs per kg ration DM and per kg BW gain. Low Energy ration was the least expensive per kg ration DM ($.1131). CPS and Blood Meal rations were the most expensive per kg ration DM ($.1185 and $.1178), due to the high cost of blood meal, corn gluten meal, and fish meal (Appendix Table 4). Low Energy and High Energy diets were the least expensive per kg BW gain ($.9341 and $.9304). Economic evaluation

was not a goal of this study, therefore no attempt was made to control feed costs. Economics will change, however, based on available forages and current feed costs.

The results of this trial question the validity of supplementing growing dairy heifers with UIP. The only trait measured that responded to UIP was feed efficiency from 13.5-16.5 mo of age. Three factors may have contributed to the lack of response. First, CP levels were probably too high (14%) to elicit a large response to UIP. Intakes of CP exceeded NRC recommendations, thus creating an excess of CP. Tomlinson (1990) reported increasing UIP from 31 to 55% of CP improved feed efficiency with 12% CP diets. Second, previous response to UIP in growing dairy animals has occurred in diets containing 64-70% TDN (Swartz et al., 1991; Tomlinson, 1990; Zerbini et al., 1985; Blood Meal Trial in this thesis). UIP diets in this trial averaged 60.7% TDN. Third, this trial was designed to test three effects (energy, UIP source, and UIP level) with only four treatments. Eight treatments are needed to accurately test three treatments in a factorial design. Therefore, statistical differences were difficult to detect in this study. The contrasts were non-orthogonal, which made Bonferroni F values necessary. Bonferroni F's are larger than normal F's, thus reducing the possibility of detecting statistical differences. In hindsight, this trial should have been conducted with three high UIP (50% of CP) treatments and one low UIP (30% of CP) treatment, utilizing one of three different UIP sources or soybean meal as the protein supplement. Energy level should have been higher (64-68% TDN), and CP levels should have been lower (12-13%), with all diets

isonitrogenous and isocaloric.

However, ignoring the faults of this study, it appears that high energy diets (sufficient to support .908 kg BW gain/d) increased BW gains of Holstein heifers from 6 - 16.5 mo of age. CPS premix improved feed efficiency from 13.5-16.5 mo of age, but did not improve feed efficiency from 6-16.5 mo of age. High UIP (50% of CP) did not improve ADG or feed efficiency from 6-16.5 mo of age. Urea space and ultrasonic loineye estimation do not appear to be accurate techniques of estimating body composition.

Table 11. Combination Protein Supplement Trial treatments.

	UIP[1]	ENERGY[2]	PROTEIN SOURCE[3]
CPS	HIGH	LOW	FM, CGM, BM
BLOOD MEAL	HIGH	LOW	BM
LOW ENERGY	LOW	LOW	SBM
HIGH ENERGY	LOW	HIGH	SBM

[1] Low UIP = 25-35% of CP
High UIP = 45-50% of CP

[2] Low Energy = energy to support .590 kg BW gain (NRC, 1989)
High Energy = energy to support .908 kg BW gain (NRC, 1989)

[3] FM = fish meal
CGM = corn gluten meal
BM = blood meal
SBM = soybean meal

Table 12. Average heifer age for each treatment throughout Combination Protein Supplement Trial.

Item		TREATMENT[1] CPS	HIGH ENERGY	BLOOD MEAL	LOW ENERGY
		(AGE IN DAYS)			
DIETS STARTED		201	200	157	155
TRIAL STARTED		201	200	199	197
PLASMA UREA-N SAMPLING[2]	1	223	222	222	220
	2	279	278	273	271
	3	336	335	341	339
	4	392	391	389	387
	5	462	461	460	458
UREA SPACE[3]	1	241	240	232	230
	2	310	309	312	310
	3	440	439	442	440
LOINEYE MEASUREMENT.[3]	1	234	233	234	232
	2	306	305	309	307
	3	439	438	439	437
TRIAL END		494	493	492	490

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] Sample day 1-5.

[3] Measurement 1, 2, and 3.

Table 13. Nutrient composition of treatment[1] diets in Combination Protein Supplement Trial.

		CPS		BLOOD MEAL		LOW ENERGY		HIGH ENERGY	
item	units	mean	SD[2]	mean	SD	mean	SD	mean	SD
DM	%	70.28	2.42	66.06	8.37	65.95	7.51	62.74	1.94
CP	% of DM	14.37	0.47	14.35	0.74	14.37	0.71	14.32	0.51
TDN	% of DM	60.90	0.85	60.47	0.81	61.08	1.23	67.76	0.95
ADF	% of DM	32.67	1.22	31.87	1.17	33.22	1.80	25.86	1.57
SOL CP	% of CP	28.52	0.78	30.48	1.15	40.15	4.68	33.41	2.01
UIP	% of CP	45.02	0.82	49.50	1.47	28.99	2.05	31.01	1.14
n		42		42		42		42	

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] Standard deviation

Table 14. Mean weekly ration composition (% of DM) from Combination Protein Supplement Trial.

	CPS[1]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY
corn silage	18.0	19.7	15.5	30.7
alfalfa haylage	17.3	20.2	32.0	18.1
orchardgrass hay	54.5	47.3	32.4	25.9
dry shelled corn	2.1	6.9	4.8	14.1
soybean meal			14.6	10.5
blood meal		5.3		
CPS	7.3			
mineral	.7	.7	.7	.6
n	42	42	42	42

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 15. Percent deficiency indexes[1] for protein sources in Combination Protein Supplement Trial, based on deficiency of individual amino acids[2] compared to tissue, considering contribution of microbial protein[3].

Protein Source	Index A[4]	Index B[5]
Blood meal	10.2	34.1
Fish Meal	8.8	14.7
Corn gluten meal	14.5	24.2
Combination protein supplement premix[6]	8.5	14.1
Soybean meal	8.8	17.5
Microbial Protein	6.6	16.8
Tissue	0	0

[1] Estimated using amino acid data of Polan (1992)

[2] amino acid deficiency (g/100 g amino acids)=tissue amino acid content - (amino acid content of protein source+amino acid content of microbial protein)/2]. Percent deficit=100* [amino acid deficiency, g/100 g amino acids)/(g/100 g of amino acid in tissue)].

[3] microbial protein estimated to contribute 50% of amino acids

[4] Index A=average percent deficiency of all ten essential amino acids

[5] Index B=average percent deficiency of essential amino acids with a percent deficiency>0.

[6] Combination protein supplement premix; amino acid content calculated from blood meal, fish meal, and corn gluten meal estimates.

Table 16. Estimated bacterial protein production of heifers in Combination Protein Supplement Trial, using equations and assumptions of NRC(1985).

Item	CPS[1]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY
BCP[2] (g/d)	403	482	467	653
CP intake (g/d)	860	980	950	1100
BTP[3] (g/d)	323	385	374	522
DBTP[4] (g/d)	258	308	299	418
BCP/CP intake	.47	.49	.49	.59

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[2] Bacterial Crude Protein, calculated as (-31.86 + 26.12*TDN)*6.25; where TDN=kg/d TDN intake
[3] Bacterial True Protein = BCP*.8
[4] Digestible Bacterial True Protein = BTP*.8

Table 17. Least square means for weekly intakes of dry matter, crude protein, total digestible nutrients, acid detergent fiber, undegradable intake protein, and soluble crude protein for Combination Protein Supplement Trial.

Item	units	CPS[1]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY
DM	kg/d	6.06	6.91	6.69	7.70
CP	kg/d	0.86	0.98	0.95	1.10
TDN	kg/d	3.69	4.17	4.08	5.22
ADF	kg/d	1.99	2.21	2.24	1.98
SOL CP	kg/d	0.25	0.30	0.39	0.37
UIP	kg/d	0.39	0.49	0.28	0.34
n		336	336	336	336

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 18. Feed efficiency least square means for treatments and periods of Combination Protein Supplement Trial.

Item		n	DM EFF[1]	CP EFF[2]	TDN EFF[3]
TREATMENT[4]	CPS	56	8.79	1.26	5.34
	BLOOD MEAL	56	9.95	1.41	6.01
	LOW ENERGY	56	9.52	1.35	5.81
	HIGH ENERGY	56	8.62	1.23	5.84
	Standard Error		0.32	0.05	0.24
PERIOD[5]	1	32	5.52	0.86	3.53
	2	32	7.73	1.11	4.86
	3	32	8.07	1.16	5.00
	4	32	8.50	1.23	5.24
	5	32	8.30	1.16	5.15
	6	32	14.27	1.96	8.88
	7	32	12.16	1.70	7.58
	Standard Error		0.49	0.07	0.32

[1]DM EFF = kg dry matter intake / kg body weight gain. TMT F=2.85, p=.0554 (error=heifer(TMT))
[2]CP EFF = kg crude protein intake / kg body weight gain. TMT F=2.53, p=.0771 (error=heifer(TMT))
[3]TDN EFF = kg TDN intake / kg body weight gain. TMT F=1.44, p=.2520 (error=heifer(TMT))
[4] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] Seven six week periods, across all treatments

Table 19. Feed efficiencies for seven six week periods of Combination Protein Supplement Trial.

Item	Period[2]	CPS[1]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY	SE
kg DMI/ kg body weight gain	1	5.76	4.87	4.38	7.07	1.03
	2	9.62	6.68	6.77	7.86	1.03
	3	9.66	7.61	6.85	8.17	1.03
	4	8.00	10.99	8.41	6.59	1.03
	5	6.90	9.96	9.08	7.26	1.03
	6	10.64	16.32	16.68	13.43	1.03
	7	10.94	13.24	14.50	9.95	1.03
kg CP intake/ kg body weight gain	1	0.88	0.78	0.69	1.08	0.14
	2	1.38	0.94	0.96	1.14	0.14
	3	1.41	1.08	0.99	1.18	0.14
	4	1.16	1.58	1.22	0.95	0.14
	5	0.96	1.41	1.27	1.00	0.14
	6	1.50	2.21	2.26	1.87	0.14
	7	1.52	1.87	2.04	1.38	0.14
kg TDN intake/ kg body weight gain	1	3.59	3.00	2.72	4.85	0.64
	2	5.85	4.06	4.23	5.30	0.64
	3	5.82	4.55	4.20	5.44	0.64
	4	4.81	6.67	5.11	4.38	0.64
	5	4.24	5.95	5.46	4.94	0.64
	6	6.50	9.81	10.12	9.10	0.64
	7	6.60	8.04	8.78	6.88	0.64

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[2] Eights measurements per treatment per period.

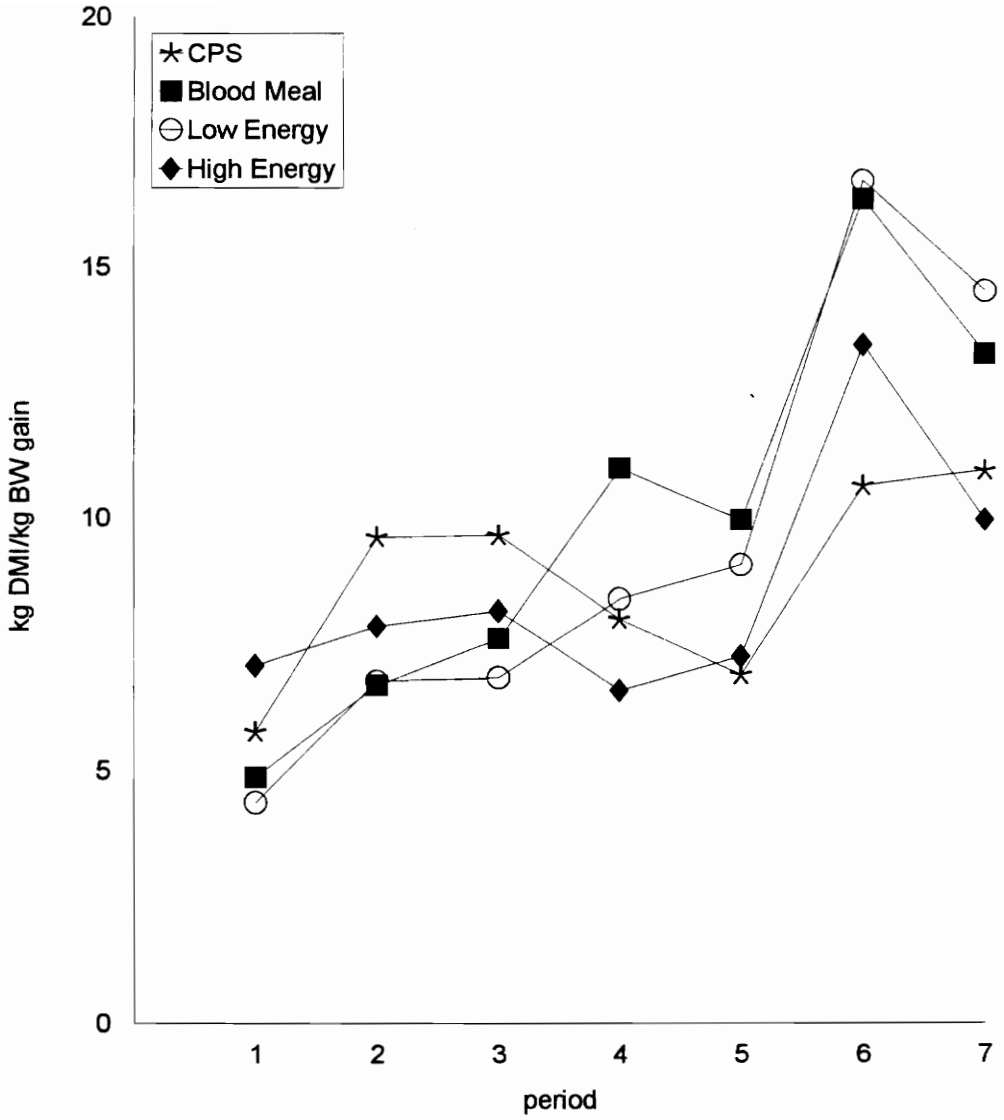


Figure 1. Dry matter efficiency for Combination Protein Supplement Trial heifers for 7 six wk periods.

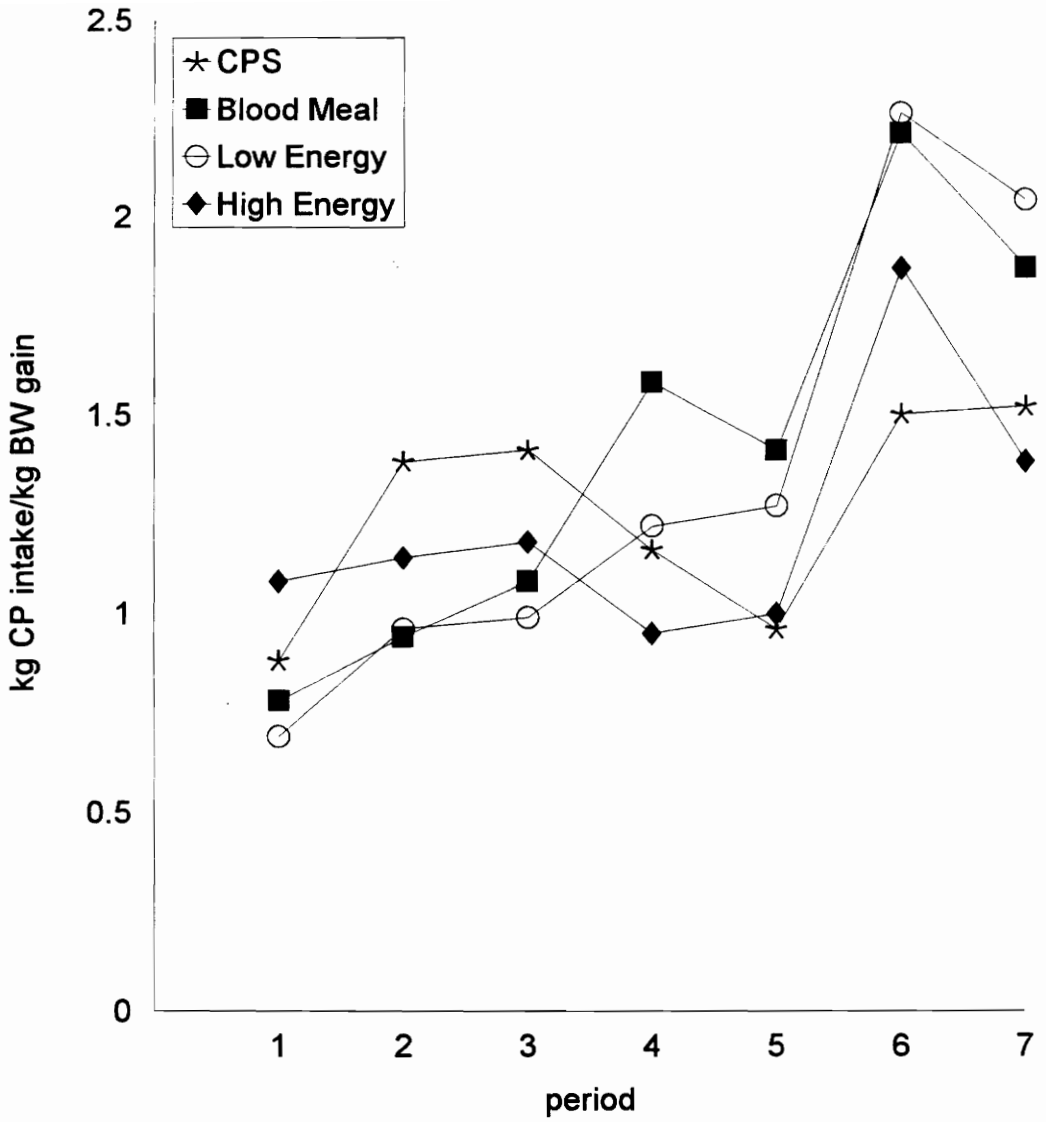


Figure 2. Crude Protein efficiency for Combination Protein Supplement Trial heifers for 7 six wk periods.

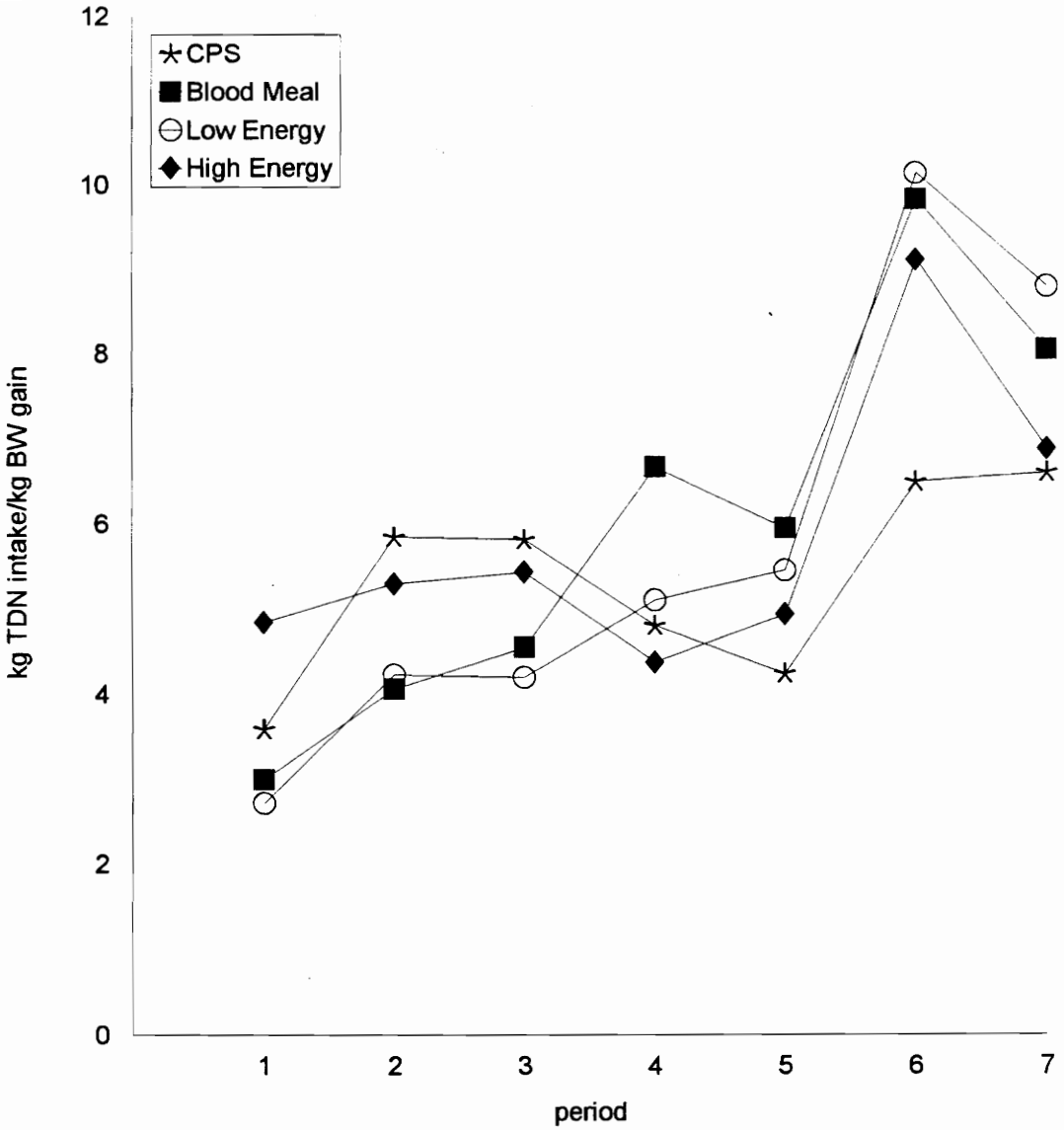


Figure 3. TDN efficiency for Combination Protein Supplement Trial heifers for 7 six wk periods.

Table 20. Least square means and standard errors for DMI, ADG, BW, WH, and WHI from Combination Protein Supplement Trial.

	CPS[5]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY	SE	TMT F[6]	p
DMI[1]	6.06	6.91	6.69	7.70	0.30	4.96	<.01
ADG[1]	0.74	0.78	0.81	0.96	0.03	14.29	<.01
BW[2]	255.00	279.00	276.00	297.00	10.63	2.67	0.07
WH[3]	114.20	116.20	114.80	119.50	1.33	3.21	0.04
WHI[4]	2.19	2.37	2.37	2.44	0.07	2.37	0.09
n	336	336	336	336			

[1]kg/d; DMI=dry matter intake, ADG=average daily gain
[2]body weight, kg
[3]wither height, cm
[4]wither height index, kg BW/cm WH
[5] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[6] tested by heifer (TMT)

Table 21. Plasma urea nitrogen least square means (mg urea-N/dl plasma) and standard errors for day 1-5 and hour 1-6 of Combination Protein Supplement Trial.

Item		LS means	Standard error
DAY[1]	1 (220 days of age)	11.70	0.21
	2 (275 days of age)	9.81	0.21
	3 (335 days of age)	10.05	0.22
	4 (390 days of age)	9.85	0.21
	5 (460 days of age)	10.43	0.21
HOUR[2]	1	10.26	0.15
	2	10.50	0.15
	3	10.01	0.15
	4	10.66	0.15
	5	10.51	0.15
	6	10.25	0.15
TREATMENT[3]	CPS	10.80	0.32
	BLOOD MEAL	9.98	0.32
	LOW ENERGY	10.36	0.32
	HIGH ENERGY	10.33	0.32

[1] F for DAY = 13.63, p<.0001. error term: heifer*day(TMT)
[2] F for HOUR = 2.46, p=.0316. error term: residual.
HOUR = one of six samples per day, taken every four hours.
[3] F for TMT = 1.13, p=.3527. error term: heifer(TMT).
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 22. Plasma urea nitrogen least square treatment means (mg urea-N/dl plasma) and standard errors for day 1-5 of Combination Protein Supplement Trial.

DAY[1]	CPS[2]	SE	BLOOD MEAL	SE	LOW ENERGY	SE	HIGH ENERGY	SE
1	10.78	0.30	11.47	0.30	15.20	0.31	9.39	0.30
2	10.88	0.30	9.31	0.30	9.26	0.30	9.79	0.30
3	9.47	0.30	9.94	0.31	11.15	0.32	9.70	0.30
4	10.76	0.30	10.19	0.30	7.13	0.30	11.32	0.30
5	12.11	0.30	8.99	0.30	9.17	0.30	11.47	0.30

[1] DAY 1=220 days of age; DAY 2=275 days of age; DAY 3=335 days of age; DAY 4=390 days of age; DAY 5=460 days of age.
[2] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 23. Least square means and standard errors of loineye area measurements for Combination Protein Supplement Trial.

Item	CPS[7]	BLOOD MEAL	LOW ENERGY	HIGH ENERGY	SE
LOINEYE 1[1]	20.38	21.28	18.71	24.96	1.07
LOINEYE 2[2]	24.24	24.84	23.47	32.27	1.07
LOINEYE 3[3]	32.46	35.73	38.05	41.04	1.07
PER 1 CHANGE[4]	3.85	3.56	4.77	7.31	1.09
PER 2 CHANGE[5]	8.23	10.88	14.57	8.77	1.71
TOT CHANGE[6]	12.07	14.44	19.34	16.08	1.65

[1] Loineye area (cm^2) 1 (231 days of age, 30 days into treatment)
[2] Loineye area (cm^2) 2 (306 days of age, 100 days into treatment)
[3] Loineye area (cm^2) 3 (440 days of age, 240 days into treatment)
[4] Change in loineye area (LOINEYE 2 - LOINEYE 1)
[5] Change in loineye area (LOINEYE 3 - LOINEYE 2)
[6] Change in loineye area (LOINEYE 3 - LOINEYE 1)
[7] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 24. Combination Protein Supplement Trial least square treatment means for urea space body composition estimates utilizing equations of Hammond et al. (1990).

Item	CPS[4]		BLOOD MEAL		LOW ENERGY		HIGH ENERGY	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
%EBW[1]	65.22	1.28	60.06	1.34	65.36	1.28	66.59	1.28
%EBP[2]	17.11	0.09	16.97	0.09	17.25	0.09	17.46	0.09
%EBF[3]	10.55	1.31	15.11	1.36	10.47	1.31	9.42	1.31

[1] % empty body water. TMT F=4.82, p=.0079. (error=heifer(TMT)).
[2] % empty body protein. TMT F=5.79, p=.0033. (error=heifer(TMT)).
[3] % empty body fat. TMT F=4.76, p=.0084. (error=heifer(TMT)).
[4] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 25. Combination Protein Supplement Trial least square treatment means for urea space body composition estimates utilizing equations of Hammond et al. (1990).

Item	CPS[10]		BLOOD MEAL		LOW ENERGY		HIGH ENERGY	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
%EBW 1[1]	73.35	2.75	67.43	2.50	66.77	2.50	70.32	2.50
%EBW 2[2]	58.60	2.50	49.60	3.06	62.46	2.75	67.96	2.50
%EBW 3[3]	63.71	2.50	63.15	2.50	66.83	2.50	61.51	2.75
%EBP 1[4]	17.16	0.16	16.92	0.15	16.83	0.15	17.17	0.15
%EBP 2[5]	16.60	0.15	16.30	0.18	16.97	0.16	17.37	0.15
%EBP 3[6]	17.57	0.15	17.70	0.15	17.95	0.15	17.84	0.16
%EBF 1[7]	3.32	2.41	8.52	2.18	9.09	2.18	6.02	2.18
%EBF 2[8]	16.29	2.18	24.24	2.68	12.97	2.41	8.18	2.18
%EBF 3[9]	12.03	2.18	12.56	2.18	9.36	2.18	14.07	2.41

[1] % empty body water 1 (235 days of age).
[2] % empty body water 2 (310 days of age).
[3] % empty body water 3 (440 days of age).
[4] % empty body protein 1 (235 days of age).
[5] % empty body protein 2 (310 days of age).
[6] % empty body protein 3 (440 days of age).
[7] % empty body fat 1 (235 days of age).
[8] % empty body fat 2 (310 days of age).
[9] % empty body fat 3 (440 days of age).
[10] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 26. Combination Protein Supplement Trial least square treatment means for urea space body composition estimates utilizing equations of Swartz et al.(1991).

	CPS[3]		BLOOD MEAL		LOW ENERGY		HIGH ENERGY	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Carcass Protein[1]	24.29	1.00	25.93	1.04	26.35	1.00	28.64	1.00
Carcass Fat[2]	12.93	0.72	14.30	69.30	14.33	0.72	15.85	0.72

[1] kg, TMT F=3.22, p=.0377.
[2] kg, TMT F=2.95, p=.0499.
[3] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Table 27. Combination Protein Supplement Trial least square treatment means for urea space body composition estimates utilizing equations of Swartz et al. (1991).

Item	CPS[7]		BLOOD MEAL		LOW ENERGY		HIGH ENERGY	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
CF 1[1]	9.01	0.61	9.66	0.55	9.17	0.55	10.80	0.55
CF 2[2]	11.25	0.55	13.03	0.68	12.97	0.61	14.10	0.55
CF 3[3]	18.53	0.55	20.21	0.55	20.86	0.55	22.63	0.61
CP 1[4]	19.13	0.93	19.67	0.85	18.91	0.85	21.54	0.85
CP 2[5]	21.37	0.85	23.33	1.03	24.15	0.93	26.20	0.85
CP 3[6]	32.37	0.85	34.78	0.85	35.98	0.85	38.19	0.93

[1] carcass fat 1 (235 days of age).
[2] carcass fat 2 (310 days of age).
[3] carcass fat 3 (440 days of age).
[4] carcass protein 1 (235 days of age).
[5] carcass protein 2 (310 days of age).
[6] carcass protein 3 (440 days of age).
[7] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

Conclusions

In the Blood Meal Trial, feed efficiency improved with addition of UIP and energy. Dry matter and CP efficiency improved with high energy, and DM and TDN efficiency improved with high UIP. In the Combination Protein Supplement Trial, high energy improved CP and DM efficiency from 15.0-16.5 mo. of age, and high UIP improved DM and TDN efficiency from 13.5-15.0 mo. of age. Supplementation with CPS premix improved DM, CP, and TDN efficiency over blood meal from 13.5-15.0 mo of age. Feed efficiency was not affected by UIP or energy from 6-13.5 mo. of age, and overall (6-16.5 mo. of age) feed efficiency for the Combination Protein Supplement Trial was not improved with high UIP or high energy diets. It appears that high UIP or high energy diets have the potential to improve feed efficiency in growing Holstein heifers, although the response is not consistent.

In both trials, high energy diets increased BW gains. High energy diets increased wither height growth in the Blood Meal Trial, but only a trend for increased wither height with high energy diets was evident in the Combination Protein Supplement Trial. Average daily gain during phase II of the Blood Meal Trial was highest for the low energy, low UIP treatment and lowest for the high energy, low UIP treatment, resulting in no difference in overall ADG for the entire trial between any treatments. Excessive gains before breeding do not appear to be necessary. Apparently, heifers that gain poorly before

breeding experience a compensatory growth period between breeding and calving. Therefore, high energy diets are not needed for prebreeding age heifers in confinement, provided heifers reach breeding weight by 13-14 mo of age.

Plasma urea-N, urea space estimates of body composition, and loineye area showed little difference between treatments in the Combination Protein Supplement Trial. Estimating muscle growth by measuring loineye area, and estimating urea space do not appear to be good predictors of body composition. Although these estimates did not show differences in body composition, visual differences were apparent between the three low energy treatments and the high energy, low UIP treatment. Heifers on the high energy, low UIP treatment appeared to have more condition. Although urea space works well with beef cattle, it appears to be a poor predictor of body composition in dairy heifers, due to differences in size and body composition.

Conclusions regarding UIP and heifer growth were difficult to make due to the inconsistent response to UIP between the two trials. One reason for the discrepancy could be the statistical design of the experiments. Statistical differences in the Combination Protein Supplement Trial were difficult to detect, due to the poor design of the trial. Four treatments were not adequate to study three effects (UIP source, UIP level, energy level). An ideal design would have been a 2x2x2 factorial, but time and facilities did not permit this. A better solution would have been to drop one of the effects, and have a 2x2 design.

Differences were easier to detect in the Blood Meal Trial , due to the balanced 2x2 factorial design. Other than statistical design, diet differences between the two trials could have resulted in varying responses. Diet nutrient content was similar between both trials, with the exception of crude protein. Diets in the Blood Meal Trial averaged 13.7% CP, while diets in the Combination Protein Supplement Trial averaged 14.4%CP. The higher protein levels in the Combination Protein Supplement Trial could have resulted in excess dietary protein, decreasing the possibility of high UIP improving efficiency of protein utilization. Another reason could be the poor performance of the low energy, low UIP treatment in the Blood Meal Trial. They had poor gains and feed efficiencies compared to the low energy, low UIP treatment in the Combination Protein Supplement Trial. Thus, differences between high and low UIP, and high and low energy were easier to detect in the Blood Meal Trial. The only explanation for the poor performance of the low energy, low UIP treatment in the Blood Meal Trial was the disproportionate amount of low quality orchardgrass in the diet (73%).

It appears that improved feed efficiency of heifers fed diets containing 50% of CP as UIP is mainly a factor of decreased intake. Decreased microbial growth from lack of degradable and soluble nitrogen could be the cause of decreased intake. Both trials had similar BW gains regardless of UIP level, but DMI decreased with high UIP diets, resulting in improved feed efficiency. Therefore, dietary or management factors that influence intake also influence heifer response to UIP. Baumgardt (1970) implied that

excessive energy intake will limit DMI. Using NRC TDN recommendations as a barometer (65-67 %TDN for 200-300 kg large breed heifers), diets in excess of 70% TDN might be supplying excess energy to the heifer, thereby reducing intake. Results reported in literature (Swartz et al., 1991; Tomlinson, 1990; Zerbini et al., 1985) support the conclusion that energy levels in excess of 70% TDN metabolically regulate intake, regardless of UIP level, but TDN levels near 65% do not effect intake. Results from the Blood Meal Trial support the conclusion that feed efficiency can be improved with high UIP diets (50% of CP) by reduction of feed intake at moderate energy levels (65-70% TDN). The lack of response to UIP at lower energy levels in both trials cannot be supported by the literature. Mantysaari et al. (1989b), Swartz et al. (1991), Tomlinson (1990), and Zerbini et al. (1985) evaluated UIP at 64-78% TDN, while the low energy treatments in both trials were 60% TDN. A possible explanation is that at low energy levels, feeding the rumen microbes for maximum growth is essential. Reducing nitrogen available to rumen bacteria by increasing UIP could significantly impair microbial growth at low energy levels. However, at high energy levels, efficient use of nitrogen (recycling, minimal waste) combined with available energy could maintain microbial growth at a reasonable rate.

Although the two trials presented in this thesis do not offer conclusive evidence that UIP or energy is beneficial or unnecessary, it does warrant the need for further research. The response to UIP shown in the Blood Meal Trial occurred even though

blood meal is deficient in Met, a limiting AA. Based on the results of the Blood Meal Trial, the CPS premix, offering a balanced AA profile, should have improved feed efficiency compared to blood meal, and especially compared to a low UIP diet. A better designed experiment with lower CP levels (13%) and higher energy levels (65% TDN) might produce improvements in feed efficiency when providing a UIP source with a balanced AA profile.

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Appendix of Tables

Appendix Table 1. Composition[1] of combination protein supplement premix (CPS premix).

	% of total as fed	% of total dry matter	CP, as a % of total CP	UIP, as a % of total UIP
corn gluten meal	41.5	41	38.7	33.3
blood meal	21	21.2	25.9	33.3
fish meal	37.5	37.8	35.4	33.3

[1] calculated using NRC (1989) values

Appendix Table 2. Nutrient content of protein sources in Combination Protein Supplement Trial.

	CP[1]	TDN[1]	UIP[1]
	% DM	% DM	% CP
corn gluten meal	67.2	89	55
blood meal	87.2	66	82
fish meal, menhaden	66.7	73	60
combination protein supplement premix	71.2	78	64
soybean meal	49.9	84	35

[1] From NRC, 1989.

Appendix Table 3. Amino acid composition[1] of tissue, microbial protein, and protein supplements used in Combination Protein Supplement Trial.

Source	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TRP	VAL
Blood meal	4.8	6.2	0.9	13.4	9.1	0.9	6.7	3.5	1.1	7.9
Fish meal, menhaden	4.8	2.1	4.2	7.4	7	2.7	3.4	3.5	1.1	5.1
Corn gluten meal	2.8	1.8	3.4	14.1	1.5	2.8	5.7	3	0.4	4
CPS premix[2]	4.1	3.4	2.8	11.6	5.9	2.1	5.3	3.3	0.9	5.7
Soybean meal	6.9	2.2	5.1	6.9	5.9	1.3	4.5	3.5	1.4	4.9
Tissue	6.8	3	5.5	7.2	8.2	2.7	4.6	4.6	1.2	5.2
Microbial Protein	5.1	2	5.7	8.1	7.9	2.6	5.1	5.8	4.2	6.2

[1] Polan (1992), g/100 g amino acids

[2] Combination protein supplement premix; amino acid content calculated from blood meal, fish meal, and corn gluten meal estimates.

Appendix Table 4. Estimated[1] deficit or excess (g/100 g amino acids), and percent deficiency of individual amino acids of protein sources in Combination Protein Supplement Trial, using tissue as a base of comparison, and considering contribution of microbial protein.[2]

Protein Source[3]	Amino Acid deficit	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TRP	VAL
BM	g/100g AA[4]	-1.85	1.10	-2.20	3.55	0.25	-0.95	1.30	0.05	1.45	1.85
	%[5]	27	0	40	0	0	35	0	0	0	0
FM	g/100g AA[4]	-1.85	-0.95	-0.55	0.55	-0.80	-0.05	-0.35	0.05	1.45	0.45
	%[5]	27	32	10	0	10.	2	8	0	0	0
CGM	g/100g AA[4]	-2.85	-1.10	-0.95	3.90	-3.55	0.00	0.80	-0.20	1.10	-0.10
	%[5]	42	37	17	0	43	0	0	4	0	2
CPS[6]	g/100g AA[4]	-2.18	-0.32	-1.23	2.67	-1.37	-0.33	0.58	-0.03	1.33	0.73
	%[5]	32	0	22	0	17	12	0	1	0	0
SBM	g/100g AA[4]	-0.80	-0.90	-0.10	0.30	-1.35	-0.75	0.20	0.05	1.60	0.35
	%[5]	12	30	2	0	16	28	0	0	0	0
Microb. Protein	g/100g AA[4]	-1.70	-1.00	0.20	0.90	-0.35	-0.10	0.50	1.20	3.00	1.00
	%[5]	25	33	0	0	4	4	0	0	0	0

[1] Estimated using amino acid data of Polan (1992), g/100 g amino acids

[2] microbial protein estimated to contribute 50% of amino acids

[3] BM=blood meal, FM=fish meal, CGM = corn gluten meal, CPS=combination protein supplement premix, SBM=soybean meal.

[4] Amino acid deficiency (g/100 g amino acids)=[tissue amino acid content - (amino acid content of protein source+amino acid content of microbial protein)/2]

[5] Compared to tissue, % deficit=100* [(amino acid deficiency, g/100 g amino acids)/(g/100 g of amino acid in tissue)]

[6] Combination protein supplement premix; amino acid content calculated from blood meal, fish meal, and corn gluten meal estimates.

Appendix Table 5. Feed prices for feeds used in Blood Meal Trial and Combination Protein Supplement Trial.

	FOB price ($/ton)	freight[1] to Blacksburg, VA ($/ton)	delivered price, Blacksburg, Va ($/ton)
corn silage[2]	—	—	30
alfalfa haylage[2]	—	—	50
orchardgrass hay[2]	—	—	90
dry shelled corn[3]	98	6	104
soybean meal, 44%[4]	185	35	220
blood meal[4]	405	35	440
fish meal, menhaden[4]	340	35	375
corn gluten meal, 60%[5]	300	30	330
2:1 mineral[2]	—	—	308

[1] estimated freight, assuming 25 ton truckload
[2] estimated local price in Blacksburg, VA
[3] FOB Roanoke, VA (Virginia Agriculture Commodity Newsletter, 1994)
[4] FOB Atlanta, GA (Feedstuffs, 1994)
[5] FOB Baltimore, MD (Feedstuffs, 1994)

Appendix Table 6. Total feed costs in $/kg ration DM and $/kg BW gain for heifers in Blood Meal Trial from 6-14 months of age.

Treatment	Total Feed Costs[1]	
	$/kg ration DM[2]	$/kg BW gain[3]
Low Energy:Low UIP	.1108	.7970
Low Energy:High UIP	.1214	.8892
High Energy:Low UIP	.1128	.8242
High Energy:High UIP	.1206	.7475

[1] Calculated using feed prices in Appendix Table 5.
[2] Mean ration cost.
[3] Calculated as: (mean ration cost per kg DM * mean kg DMI)/mean kg ADG.

Appendix Table 7. Total feed costs in $/kg ration DM and $/kg BW gain for heifers in Combination Protein Supplement Trial.

Treatment[1]	Total Feed Costs[2]	
	$/kg ration DM[3]	$/kg BW gain[4]
CPS	.1185	.9704
BLOOD MEAL	.1178	1.0436
LOW ENERGY	.1131	.9341
HIGH ENERGY	.1160	.9304

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[2] Calculated using feed prices in Appendix Table 5.
[3] Mean ration cost.
[4] Calculated as: (mean ration cost per kg DM x mean kg DMI)/mean kg ADG.

Appendix Table 8. Non-orthogonal contrast sets used in analysis of Combination Protein Supplement Trial.

	Name	Contrast[1]
Contrast Set	UIP LEVEL	CPS & BLOOD MEAL vs. LOW ENERGY
	UIP SOURCE	CPS vs. BLOOD MEAL
	ENERGY	HIGH ENERGY vs. LOW ENERGY
Interaction Contrast[2] (Treatment * Period)	UIP LEVEL	CPS & BLOOD MEAL vs. LOW ENERGY (within each per.)
	UIP SOURCE	CPS vs. BLOOD MEAL (within each per.)
	ENERGY	HIGH ENERGY vs. LOW ENERGY (within each per.)

[1] CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[2] Used to test treatment differences within period for urea space body composition estimates, loineye area measurements, plasma urea-N, and feed efficiencies.

Appendix Table 9. Non-orthogonal contrasts for dry matter intake from heifers in Combination Protein Supplement Trial.

Item	CONTRAST[1]	F	significant[2]
Dry Matter Intake	UIP LEVEL	0.31	
	UIP SOURCE	3.87	
	ENERGY LEVEL	5.52	*

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.447

Appendix Table 10. Feed efficiency non-orthogonal contrasts for Combination Protein Supplement Trial.

Item	CONTRAST[1]	F	significant[2]
kg DMI / kg BW gain	UIP LEVEL	0.11	
	UIP SOURCE	4.92	
	ENERGY LEVEL	2.98	
kg TDN / kg BW gain	UIP LEVEL	0.18	
	UIP SOURCE	3.94	
	ENERGY LEVEL	0.02	
kg CP / kg BW gain	UIP LEVEL	0.04	
	UIP SOURCE	4.28	
	ENERGY LEVEL	2.53	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.447

Appendix Table 11. Non-orthogonal contrasts for DM efficiencies for seven six week periods of Combination Protein Supplement Trial.

PERIOD	CONTRAST[1]	F	significant[2]
1	UIP LEVEL	0.37	
	UIP SOURCE	0.56	
	ENERGY LEVEL	3.42	
2	UIP LEVEL	1.20	
	UIP SOURCE	4.09	
	ENERGY LEVEL	0.56	
3	UIP LEVEL	2.00	
	UIP SOURCE	2.01	
	ENERGY LEVEL	0.82	
4	UIP LEVEL	0.74	
	UIP SOURCE	4.26	
	ENERGY LEVEL	1.58	
5	UIP LEVEL	0.26	
	UIP SOURCE	4.43	
	ENERGY LEVEL	1.57	
6	UIP LEVEL	6.49	**
	UIP SOURCE	15.27	**
	ENERGY LEVEL	5.00	*
7	UIP LEVEL	3.65	
	UIP SOURCE	2.52	
	ENERGY LEVEL	9.79	**

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.577
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 5.866

Appendix Table 12. Non-orthogonal contrasts for CP efficiencies for seven six week periods of Combination Protein Supplement Trial.

PERIOD	CONTRAST[1]	F	significant[2]
1	UIP LEVEL	0.57	
	UIP SOURCE	0.23	
	ENERGY LEVEL	3.51	
2	UIP LEVEL	1.23	
	UIP SOURCE	4.60	*
	ENERGY LEVEL	0.73	
3	UIP LEVEL	2.10	
	UIP SOURCE	2.50	
	ENERGY LEVEL	0.87	
4	UIP LEVEL	0.75	
	UIP SOURCE	4.28	
	ENERGY LEVEL	1.68	
5	UIP LEVEL	0.24	
	UIP SOURCE	4.70	*
	ENERGY LEVEL	1.73	
6	UIP LEVEL	5.08	*
	UIP SOURCE	12.15	**
	ENERGY LEVEL	3.48	
7	UIP LEVEL	3.75	
	UIP SOURCE	3.06	
	ENERGY LEVEL	10.42	**

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.577
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 5.866

Appendix Table 13. Non-orthogonal contrasts for TDN efficiencies for seven six week periods of Combination Protein Supplement Trial.

PERIOD	CONTRAST[1]	F	significant[2]
1	UIP LEVEL	0.54	
	UIP SOURCE	0.43	
	ENERGY LEVEL	5.57	*
2	UIP LEVEL	0.85	
	UIP SOURCE	3.89	
	ENERGY LEVEL	1.40	
3	UIP LEVEL	1.60	
	UIP SOURCE	1.95	
	ENERGY LEVEL	1.89	
4	UIP LEVEL	0.65	
	UIP SOURCE	4.23	
	ENERGY LEVEL	0.64	
5	UIP LEVEL	0.21	
	UIP SOURCE	3.57	
	ENERGY LEVEL	0.33	
6	UIP LEVEL	6.26	**
	UIP SOURCE	13.38	**
	ENERGY LEVEL	1.26	
7	UIP LEVEL	3.47	
	UIP SOURCE	2.53	
	ENERGY LEVEL	4.37	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.577
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 5.866

Appendix Table 14. Non-orthogonal contrasts for ADG, BW, WH, and WHI of Combination Protein Supplement Trial.

Item	CONTRAST[1]	F	significant[2]
ADG	UIP LEVEL	2.48	
	UIP SOURCE	1.55	
	ENERGY LEVEL	17.47	**
BW	UIP LEVEL	0.54	
	UIP SOURCE	2.61	
	ENERGY LEVEL	1.89	
WH	UIP LEVEL	0.08	
	UIP SOURCE	1.13	
	ENERGY LEVEL	6.41	*
WHI	UIP LEVEL	1.21	
	UIP SOURCE	3.18	
	ENERGY LEVEL	0.51	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.447

Appendix Table 15. Non-orthogonal contrasts for plasma urea-N for five sampling days of Combination Protein Supplement Trial.

DAY	CONTRAST[1]	F	significant[2]
1	UIP LEVEL	118.07	**
	UIP SOURCE	2.61	
	ENERGY LEVEL	181.01	**
2	UIP LEVEL	5.02	*
	UIP SOURCE	13.43	**
	ENERGY LEVEL	1.55	
3	UIP LEVEL	14.17	**
	UIP SOURCE	1.21	
	ENERGY LEVEL	10.96	**
4	UIP LEVEL	81.09	**
	UIP SOURCE	1.81	
	ENERGY LEVEL	95.30	**
5	UIP LEVEL	13.83	**
	UIP SOURCE	52.73	**
	ENERGY LEVEL	28.73	**
Overall[3]	UIP LEVEL	.01	
	UIP SOURCE	3.43	
	ENERGY LEVEL	.04	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.577
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 5.866

[3] contrasts for 5 days combined

Appendix Table 16. Non-orthogonal interaction contrasts for loineye area measurements of heifers in Combination Protein Supplement Trial.

Item	CONTRAST[4]	F	significant[5]
LOINEYE 1[1]	UIP LEVEL	2.64	
	UIP SOURCE	0.35	
	ENERGY LEVEL	17.15	**
LOINEYE 2[2]	UIP LEVEL	0.66	
	UIP SOURCE	0.16	
	ENERGY LEVEL	33.92	**
LOINEYE 3[3]	UIP LEVEL	9.17	**
	UIP SOURCE	4.68	
	ENERGY LEVEL	3.92	

[1] Loineye area (cm^2) 1 (age: 231 days)
[2] Loineye area (cm^2) 2 (age: 306 days)
[3] Loineye area (cm^2) 3 (age: 436 days)
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210

Appendix Table 17. Non-orthogonal contrasts for change in loineye area of heifers in Combination Protein Supplement Trial.

Item	CONTRAST[4]	F	significant[5]
PER 1 CHANGE[1]	UIP LEVEL	0.63	
	UIP SOURCE	0.04	
	ENERGY LEVEL	2.70	
PER 2 CHANGE[2]	UIP LEVEL	5.74	*
	UIP SOURCE	1.20	
	ENERGY LEVEL	5.75	*
TOT CHANGE[3]	UIP LEVEL	9.08	**
	UIP SOURCE	1.03	
	ENERGY LEVEL	1.96	

[1] Change in loineye area (LOINEYE 2 - LOINEYE 1)
[2] Change in loineye area (LOINEYE 3 - LOINEYE 2)
[3] Change in loineye area (LOINEYE 3 - LOINEYE 1)
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.447

Appendix Table 18. Combination Protein Supplement Trial non-orthogonal contrasts for urea space body composition estimates utilizing equations of Hammond et al. (1990).

Item	CONTRAST[1]	F	significant[2]
%EBW	UIP LEVEL	2.95	
	UIP SOURCE	7.78	**
	ENERGY LEVEL	.47	
%EBP	UIP LEVEL	4.02	
	UIP SOURCE	1.29	
	ENERGY LEVEL	2.98	
%EBF	UIP LEVEL	2.88	
	UIP SOURCE	7.86	**
	ENERGY LEVEL	.43	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6447.

Appendix Table 19. Combination Protein Supplement Trial non-orthogonal interaction contrasts for % empty body water estimates using equations of Hammond et al (1990).

Item	CONTRAST[4]	F	significant[5]
%EBW 1[1]	UIP LEVEL	1.35	
	UIP SOURCE	2.54	
	ENERGY LEVEL	1.01	
%EBW 2[2]	UIP LEVEL	6.10	*
	UIP SOURCE	5.21	*
	ENERGY LEVEL	2.19	
%EBW 3[3]	UIP LEVEL	1.24	
	UIP SOURCE	0.03	
	ENERGY LEVEL	2.06	

[1] % Empty body water 1
[2] % Empty body water 2
[3] % Empty body water 3
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210.

Appendix Table 20. Combination Protein Supplement Trial non-orthogonal interaction contrasts for % empty body protein estimates using equations of Hammond et al. (1990).

Item	CONTRAST[4]	F	significant[5]
%EBP 1[1]	UIP LEVEL	1.27	
	UIP SOURCE	1.29	
	ENERGY LEVEL	2.73	
%EBP 2[2]	UIP LEVEL	6.87	**
	UIP SOURCE	1.73	
	ENERGY LEVEL	3.31	
%EBP 3[3]	UIP LEVEL	3.19	
	UIP SOURCE	0.40	
	ENERGY LEVEL	0.26	

[1] % Empty body protein 1
[2] % Empty body protein 2
[3] % Empty body protein 3
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210.

Appendix Table 21. Combination Protein Supplement Trial non-orthogonal interaction contrasts for % empty body fat estimates using equations of Hammond et al. (1990).

Item	CONTRAST[4]	F	significant[5]
%EBF 1[1]	UIP LEVEL	1.35	
	UIP SOURCE	2.56	
	ENERGY LEVEL	0.99	
%EBF 2[2]	UIP LEVEL	6.07	*
	UIP SOURCE	5.29	*
	ENERGY LEVEL	2.16	
%EBF 3[3]	UIP LEVEL	1.21	
	UIP SOURCE	0.03	
	ENERGY LEVEL	2.11	

[1] % Empty body fat 1
[2] % Empty body fat 2
[3] % Empty body fat 3
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210.

Appendix Table 22. Combination Protein Supplement Trial non-orthogonal contrasts for urea space body composition estimates utilizing equations of Swartz et al. (1991).

Item	CONTRAST[1]	F	significant[2]
Carcass Protein	UIP LEVEL	1.00	
	UIP SOURCE	1.28	
	ENERGY LEVEL	2.64	
Carcass Fat	UIP LEVEL	.70	
	UIP SOURCE	1.86	
	ENERGY LEVEL	2.39	

[1] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.

[2] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.937.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6447.

Appendix Table 23. Combination Protein Supplement Trial non-orthogonal interaction contrasts for carcass fat estimates using equations of Swartz et al. (1991).

Item	CONTRAST[4]	F	significant[5]
CF 1[1]	UIP LEVEL	0.06	
	UIP SOURCE	0.63	
	ENERGY LEVEL	4.32	
CF 2[2]	UIP LEVEL	1.22	
	UIP SOURCE	4.10	
	ENERGY LEVEL	1.91	
CF 3[3]	UIP LEVEL	4.77	
	UIP SOURCE	4.58	
	ENERGY LEVEL	4.63	

[1] Carcass fat 1
[2] Carcass fat 2
[3] Carcass fat 3
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210.

Appendix Table 24. Combination Protein Supplement Trial non-orthogonal interaction contrasts for carcass protein estimates using equations of Swartz et al. (1991).

Item	CONTRAST[4]	F	significant[5]
CP 1[1]	UIP LEVEL	.22	
	UIP SOURCE	.18	
	ENERGY LEVEL	4.84	*
CP 2[2]	UIP LEVEL	2.46	
	UIP SOURCE	2.15	
	ENERGY LEVEL	2.65	
CP 3[3]	UIP LEVEL	5.38	*
	UIP SOURCE	4.07	
	ENERGY LEVEL	3.11	

[1] Carcass protein 1
[2] Carcass protein 2
[3] Carcass protein 3
[4] UIP level contrast = CPS and BLOOD MEAL vs. LOW ENERGY
UIP source contrast = CPS vs. BLOOD MEAL
Energy Level contrast = HIGH ENERGY vs. LOW ENERGY
CPS = low energy, high UIP with combination protein supplement premix;
BLOOD MEAL = low energy, high UIP with blood meal;
LOW ENERGY = low energy, low UIP with soybean meal;
HIGH ENERGY = high energy, low UIP with soybean meal.
[5] * significant at ($p<.10$), Bonferroni F (Alpha = .10) = 4.792.
** significant at ($p<.05$), Bonferroni F (Alpha = .05) = 6.210.

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Abstracts:

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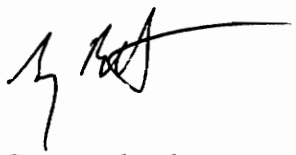

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