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# Ruminally undegraded protein and ruminally protected amino acids for dairy heifers

Brian David Garthwaite  
*University of New Hampshire, Durham*

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**RUMINALLY UNDEGRADED PROTEIN AND RUMINALLY  
PROTECTED AMINO ACIDS FOR DAIRY HEIFERS**

**BY**

**Brian D. Garthwaite**  
**B.S., Animal Sciences, University of Wisconsin River Falls, 1990**  
**M.S., University of Illinois at Urbana-Champaign, 1992**

**DISSERTATION**

**Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of**

**Doctor of Philosophy**

**in**

**Animal and Nutritional Sciences**

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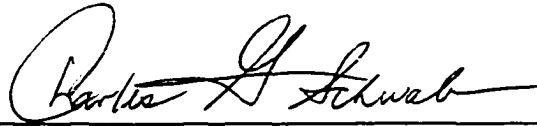
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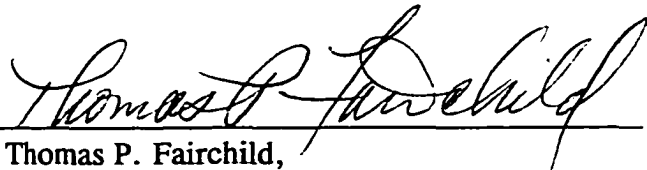
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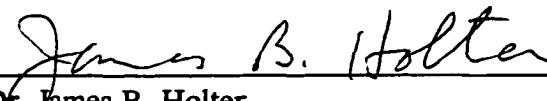
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Assistant Professor of Animal and Nutritional Sciences



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Dr. Thomas P. Fairchild,  
Professor of Animal and Nutritional Sciences



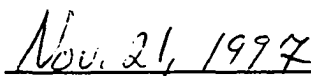
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Dr. James B. Holter,  
Professor of Animal and Nutritional Sciences, Emeritus



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Dr. Willard E. Urban,  
Professor of Biometrics/Genetics Biometrics, Emeritus



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Date

## **DEDICATION**

Family, faith, and friends gave love, encouragement, and support.

One creature provided the impetus to pursue higher education.

This dissertation is dedicated to:

**BDG Elegant Lock**

So strong an influence on the joyous, but sometimes difficult lessons of life.

She is in my heart, forever.

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As silly as it may seem, I thank all of the heifers that were part of my project. They taught me things I had never considered to be important. Despite the seemingly constant poking and prodding, the required deviations from normal behavior, and the days when my mood was dark, they always were happy to see me and I could count on them to do something to cheer me up. In exchange for what little time and attention I could give them, they gave me all theirs.

Mookie, this one's for you! Nobody but I believed in you. We had the last laugh, didn't we?

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## ABSTRACT

### RUMINALLY UNDEGRADED PROTEIN AND RUMINALLY PROTECTED AMINO ACIDS FOR DAIRY HEIFERS

by

Brian D. Garthwaite

University of New Hampshire, December, 1997

A series of growth experiments was conducted to evaluate the feeding of ruminally undegraded protein alone, or in combination with ruminally protected lysine and methionine. Eighty Holstein heifer calves were blocked by date of birth as they became available at 6 wk of age and assigned to a 2 x 2 factorial arrangement of treatments. Main effects of the 2 x 2 factorial were amount of ruminally undegraded protein in diet dry matter and whether ruminally protected lysine and methionine were supplemented to diets. Four phases of growth were evaluated: Phase 1) 6 wk to 100 kg; Phase 2) 100 to 175 kg; Phase 3) 175 to 245 kg; Phase 4) 245 to 410 kg. Feeding higher concentration of ruminally undegraded protein during Phase 1 improved average daily gain and feed efficiency, which reduced days on feed. No improvement of skeletal growth was evident by feeding more ruminally undegradable protein. Responses were limited to the young calves; no benefits were observed for heifers greater than 100 kg by feeding higher concentrations of ruminally undegraded protein or ruminally protected lysine and methionine. Results suggest that recommendations for ruminally undegraded protein fed to older heifers may be too generous. No improvements for growth were achieved by feeding ruminally protected lysine and methionine, but responses may have been masked by high intake of total absorbable protein.

## INTRODUCTION

A fundamental goal of rearing dairy replacements is to reduce the interval between birth and first parturition. Achieving that goal requires intelligent management decisions related to animal health, facilities, labor efficiency, and nutrition. The quest for young age at first calving must be tempered by the recognition that any management decision in one facet may have profound, negative effects in another. The growing dairy heifer is an investment in the future productive success of the dairy business; thus, the growing heifer no longer can be relegated to less than satisfactory care.

Past research has indicated that increasing the concentration of ruminally undegraded protein in diets of dairy heifers may improve skeletal growth, feed efficiency, and average daily gain. Results have been inconsistent either because of errors in calculation of requirements, use of protein supplements of varying quality or containing a poor complement of essential amino acids, or lack of understanding about the fundamentals of amino acid nutrition.

The objectives of the research presented in this thesis were to evaluate whether improvements of growth by dairy heifers 6 wk of age to 410 kg of body weight could be achieved by feeding higher concentrations of ruminally undegraded protein, and whether those responses could be enhanced further by supplying potentially limiting amino acids in ruminally protected form.



## CHAPTER I

### REVIEW OF LITERATURE

#### Introduction

The ultimate goal of raising dairy heifers is to supplant inferior cows culled from the lactating herd in favor of replacements having higher productive potential. The importance of that goal is illustrated by data of Smith (1993), who summarized Wisconsin Dairy Herd Improvement Association records of 11,000 Holstein herds. Herds with highest production of milk (> 11,350 kg) contained the greatest proportion of first lactation heifers (43%) and had the lowest average age at first calving; those herds also had the highest cull rate for multiparous cows. Most dairy producers raise excess heifers to maintain a static herd size, but that is necessary only for high culling rates or high age at first parturition.

Whether replacements are raised on farm, contracted to professional growers, or purchased, average costs of rearing range from \$1,150 to \$1,250 per heifer (Cady and Smith, 1996). Expenses accrued during the rearing period must be viewed as an investment toward future production because they represent 15 to 20% of total dairy farm expenditures that are not recouped until the heifer begins lactating (Webb, 1992; Karszes, 1996). The expedience to which heifers enter the lactating herd is important, but efforts to reduce costs must be counter-balanced by ensuring that adequate nutritional, health, and housing needs are provided such that future production is not compromised.

### **Target Age and Size at First Parturition**

The 24 mo age at first calving (AFC) has been a long-standing recommendation. Only recently has that guideline been amended for Holsteins to include acceptable targets of 545 to 615 kg of body weight (BW) (Keown and Everett, 1986; Heinrichs and Hargrove, 1987; Hoffman and Funk, 1992; Heinrichs, 1993; Grummer et al., 1995), 133 cm for wither height (Heinrichs and Hargrove, 1987), and body condition score of 3.5 (Grummer et al., 1995). Adoption of the recommended 24 mo AFC has been relatively slow in U. S. dairy herds; from 1960 to 1982 the mean AFC for Holsteins remained fairly constant at 27 mo (Powell, 1985; Nieuwolf et al., 1989). More recent evidence (National Animal Health Monitoring Service, 1996) indicates that AFC declined to 26 mo in 1996.

The relationship among AFC, BW, body condition score, and wither height should seem somewhat obvious, but researchers (Miller and McGilliard, 1959; Clark and Touchberry, 1962; Little and Kay, 1979; Fisher et al., 1983; Thompson et al., 1983; Bettenay, 1985; Keown and Everett, 1986; Lin et al., 1986; Gardner et al., 1988; and Moore et al., 1991) have not always considered them in a related manner.

Clark and Touchberry (1962) reported that age independently influenced yield of milk in first lactation, but BW had approximately fourfold greater influence. In contrast, Moore et al. (1991) determined that correlations for yield of milk in first lactation were more positive for age than BW. Hoffman and Funk (1992) pointed out that reports by Clark and Touchberry (1962), Fisher et al. (1983), and Moore et al. (1991) used few records of heifers <24 mo AFC; thus, negative effects of AFC <24

mo may be questioned.

Lin et al. (1986) determined that heifers of 23 mo AFC produced less milk during first parity than did heifers of 26 mo AFC, but yield per day of life at the end of first lactation was not different. A later publication (Lin et al., 1988) reported that three parity lifetime performance of those same heifers did not differ for total milk produced or total milk produced per day of herd life; however, 61-mo total milk and milk produced per day of herd life was greater for heifers of 23 mo AFC because they partially had completed the fourth lactation. Gardner et al. (1988) found similar results with younger heifers (22.2 vs. 24.6 AFC), and Gill and Allaire (1976) determined that optimal AFC was 23 mo. Efforts to reduce AFC < 23 mo have demonstrated problems with dystocia (Wickersham and Schultz, 1963; Amir et al., 1967; Bettenay, 1985), lower first lactation (Plum and Harris, 1968; Gardner et al., 1977) or lifetime production (Swanson, 1960; Little and Kay, 1979; Bettenay, 1985), or no significant detriment (Van Amburgh et al., 1994).

Data supporting an optimum BW at first parturition are sparse (Keown and Everett, 1986; Heinrichs and Hargrove, 1987; Hoffman and Funk, 1992; Lacasse et al., 1993; Grummer et al., 1995; Hoffman, 1996). Keown and Everett (1986) determined that the BW at which first lactation yield of milk began to decline for additional increase of BW was between 540 and 570 kg. A BW of  $526 \pm 66$  kg for 24 mo old Holsteins, estimated by using heart girth, was suggested by Heinrichs and Hargrove (1987). Hoffman (1996) used an empirical approach based on data (Keown and Everett, 1986; Heinrichs and Hargrove, 1987; Grummer et al., 1995) and

suggested that Holsteins should weigh 615 kg at first calving.

Despite discrepancy about recommendations for target age and weight at first calving for Holsteins, sufficient evidence indicates that minimums of 24 mo AFC and 570 to 615 kg of BW prepartum should be considered acceptable goals. Scant information exists for recommendations of wither height or body condition score, but wither height of 133 cm (Heinrichs and Hargrove, 1987) and body condition score of 3.5 (Grummer et al., 1995) seem reasonable until more data is available.

### **Peripubertal Age and Weight**

The peripubertal period will be defined as the period from 100 to 350 kg of BW. This definition is based on the biologic observation that Holstein heifers normally will experience puberty between 250 and 300 kg of BW (Wickersham and Schultz, 1963; Sejrsen et al., 1983) independent of age.

The biologically fixed BW at puberty should serve as the physiologic benchmark from which age and BW at first calving are manipulated. Perhaps some of the confusion about optimal AFC and BW can be related to the presumption that older heifers have higher BW than do younger heifers, which may or may not be true. Chronologic age should not be considered analogous to physiologic age because the former is simply the quotient of BW divided by rate of gain; thus, age at puberty can be varied by different prepubertal rates of gain.

This presents multiple scenarios where age at breeding (hence, AFC), BW at first parturition, or both may be manipulated. For example, similar rates of gain

applied prior to puberty and heifers confirmed pregnant at different ages could result in different AFC; applying similar or different rates of gain after confirmed pregnancy could result in different or similar BW at calving. Likewise, different rates of prepubertal gain and confirmed pregnancy at identical BW could result in different AFC; similar or different rates of gain after confirmed pregnancy could result in similar or different BW at calving. Other schemes can be constructed, but chronologic age at puberty can be varied only by subjecting heifers to unequal rates of gain.

#### **Nutritional Manipulation of Peripubertal Rate of Gain**

Research efforts spanning the last 40 yr (Swanson and Spann, 1954; Swanson, 1960; 1961; Gardner et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; 1983; Harrison et al., 1983; Bettenay, 1985; Dutrow et al., 1991; Daccarett et al., 1993; Van Amburgh et al., 1994) purposefully altered peripubertal rate of gain to allow for younger AFC.

Swanson (1960) fed Jersey twins either ad libitum or limited concentrate and forage to evaluate effects on production; fat-corrected milk production of ad libitum fed heifers was nearly 85% of controls. Little and Kay (1979) used mixed, British dairy breeds fed to gain 1.0 or .74 kg/d and evaluated milk production through four lactations. Milk yield for all lactations was reduced significantly for rapidly reared heifers. Gardner et al. (1977) fed Holstein heifers ad libitum hay and concentrates to support gain of 1.1 kg/d or restricted diets to support .8 kg/d; heifers were inseminated to calve at 19.7 and 26.9 mo, respectively. Milk yield was reduced significantly during first lactation, but not in later lactations. Van Amburgh et al. (1994) fed

prepubescent Holstein heifers three levels of energy and achieved AFC of 24.2, 22, and 21 mo. Milk yield was lower, but not significantly different for heifers of 22 and 21 mo AFC compared to 24.2 mo. Others (Swanson and Spann, 1954; Sejrsen et al., 1982; Bettenay, 1985) observed lower production from heifers fed for high peripubertal rate of gain. Recent experiments (Dutrow et al., 1991; Daccarett et al., 1993) evaluated the performance of heifers fed 100 or 115% of NRC (1989) requirements from 3 mo of age to calving; first lactation performance was not significantly different, but AFC was reduced significantly (24.6 versus 23.1 mo for 100 and 115% of NRC (1989) requirements, respectively).

Sufficient evidence exists to indicate that high peripubertal rate of gain may reduce first lactation yield of milk, but interpretation is difficult. Nebulous terms used to describe treatments, such as "high plane of nutrition", "high rate of feeding", or "nutrient dense diets", and the use of impractical diets and breeds of low productive capability makes difficult the application of much of the previous research to current feeding situations. Those ambiguities have led to the general recommendation that accelerated rate of gain should be avoided, but recent advances in the understanding of mammary growth and development may offer strategies to ameliorate or eliminate the negative associations of peripubertal rate of gain and production of milk.

#### **Effect of Peripubertal Rate of Gain on Mammary Development**

A preponderance of data (Little and Kay, 1979; Sejrsen et al., 1982; 1983; Harrison et al., 1983; Petitclerc et al., 1983; Capuco et al., 1988; Stelwagen and Grieve, 1990) has identified high energy consumption during the peripubertal period as

having a detrimental effect on mammary secretory development. The primary detriment is deposition of excess adipose tissue at the expense of secretory tissue in the developing gland. Harrison et al. (1983) found total mammary gland weight to be unaffected by growth rates of 570, 760, and 1180 g/d, but secretory tissue in the gland was reduced by the 1180 g/d treatment. Sejrsen et al. (1982) fed prepubertal heifers a 60:40 ratio of forage to concentrate either for restricted or ad libitum intake. Ad libitum feeding prior to puberty lowered secretory tissue by 23% compared with restricted feeding; that effect was not observed in postpubertal heifers, indicating that the effect was limited to prepuberty.

The complete mechanism by which this occurs is not totally understood, but evidence suggests that there is a specific period related to mammary tissue differentiation and hormonal control.

*Mammary Tissue Differentiation.* Tucker (1987) and Akers and Sejrsen (1996) described the growth, differentiation, and hormonal control of mammary development in the bovine. From birth to about 2 to 3 mo of age, growth of the mammary circulatory system and fat pad, into which the duct system elongates, occurs isometrically (i.e., in proportion to the body). At 2 to 3 mo of age (approximately 80 to 100 kg of BW) the mammary gland shifts to an allometric rate of growth (faster than the rest of the body), which is characterized by rapid proliferation and penetration of ductular epithelial tissue (parenchyma) into the fat pad matrix. The allometric phase continues until puberty, at which time the synchronous secretion of estrogen and progesterone signal to slow the rate of growth to an isometric rate; epithelial ducts are

stimulated to branch and elongate (Akers and Sejrsen, 1996).

*Hormonal Modulation of Mammary Growth.* Sinha and Tucker (1969)

determined that mammary gland tissue proliferated 3.5 fold that of the rest of the body during allometric growth. Sejrsen et al. (1982) compared restricted (613 g/d) versus accelerated (1218 g/d) average daily gain (ADG); allometric growth was 2.4 and 1.8-fold, respectively, indicating that high ADG attenuated mammary growth. Subsequent work (Sejrsen et al., 1983) indicated that concentration of endogenous growth hormone was significantly lower for accelerated heifers compared to controls, and that the negative influence of excess feeding on mammary secretory tissue may be associated with decreased concentration of growth hormone in blood. Their supposition was validated (Sejrsen et al., 1986) by injecting exogenous growth hormone and measuring mammary parenchyma; treated heifers had 46% more parenchyma and 15% less extra-parenchymal tissue. Sandles and Peel (1987) administered growth hormone to one member of 12 sets of twin heifers for 21 wk prepuberty. Total mammary parenchyma was increased, but milk yield was not affected by prepubertal administration of growth hormone.

The results of Sejrsen et al. (1986), Sandles and Peel (1987), and discussions (Akers and Sejrsen, 1996; Hoffman and Funk, 1992) of other hormonal modulators of mammary growth offer opportunity to ameliorate the negative affects of high energy diets on mammary growth; however, given the relative lack of research attention to heifer nutrition, future efforts may be better focused on feeding strategies to avoid over conditioning and development of fatty udders during the peripubertal period.



### **Protein Nutrition of Dairy Heifers**

The *Nutrient Requirements of Dairy Cattle* (National Research Council (NRC), 1989) estimates protein requirements for dairy heifers based on the concept of absorbable protein (AP), which is the summation of protein that must be absorbed to meet the needs for maintenance and production. The AP is supplied by microbial crude protein (MCP) synthesized in the rumen and by feed protein that escapes ruminal degradation. Together, they provide the essential amino acids (EAA) needed for synthesis of body proteins. The NRC (1989) partitions the dietary protein needed to supply AP into two fractions, degraded intake protein and undegraded intake protein; each has a specific purpose. Degraded intake protein, for which the currently accepted terminology is "ruminally degraded protein" (RDP), supplies the nitrogen needed by ruminal microorganisms for synthesis of MCP. The MCP supplies EAA for protein synthesis by the host, but not in sufficient quantities to maximize production (NRC, 1985). The undegraded intake protein, for which the currently accepted terminology is "ruminally undegraded protein" (RUP), contributes the remainder of EAA needed for maintenance and production.

#### **Contribution of Ruminally Degraded and Ruminally Undegraded Protein to Absorbable Protein**

*Ruminally Degraded Protein.* The requirement for RDP more correctly should be considered a requirement for the ruminal microorganisms, rather than the host. Microbial growth in the rumen requires RDP, which may include dietary nonprotein nitrogen, or a net ruminal influx of endogenous urea from either saliva or across the

rumen wall (NRC, 1985). The production of MCP, in grams per day, for growing cattle is described as a function of total digestible nutrients (TDN) intake (NRC, 1985), in kilograms, as follows:

$$\text{MCP} = 6.25(-31.86 + 26.12\text{TDN}).$$

As pointed out in *Nutrient Requirements of Beef Cattle* (NRC, 1996) the negative intercept is not biologically logical, especially for young cattle. For example, a weaned calf consuming less than 1.53 kg of starter feed that contains 80% TDN would have negative net synthesis of ruminal MCP. In that case, the underprediction of ruminal MCP synthesis would increase the required contribution of RUP to 100% of AP. That fallacy likely is the result from lack of available information for dairy cattle <200 kg of BW in the data base used to generate the equation for predicting synthesis of ruminal MCP (NRC, 1985). For that reason, the tabular requirements (NRC, 1989) for RUP in diets of young dairy heifers may be too low, and should be viewed with suspicion until data are available for refinement of the model.

*Ruminally Undegraded Protein.* The requirement for RUP represents the remainder of AP not supplied by MCP. When synthesis of MCP is low, the requirement for RUP is high, assuming a highly productive state. Conversely, when MCP production is high, or maximized, the contribution required of RUP is low. Considering the calculation of MCP synthesis described in the preceding section, a young dairy heifer would require most of the AP in the form of RUP. Research pertaining to that issue will be discussed in a later section.

### **Nutritive Value of Ruminally Synthesized Microbial Protein**

Ruminally synthesized MCP can supply most of the AP when diets are formulated properly. When growing ruminants with a functional rumen are fed purified diets containing only nonprotein N (NPN) as a N source, there is adequate MCP produced to support BW gain of about 65% of the level at which they gain when they are fed practical energy ingredients and protein supplements (Oltjen, 1969). The intestinal digestibility of MCP is high, fairly constant, and not influenced markedly by changes in diet composition or level of feed intake (Schwab, 1995).

The proportion of each EAA to total EAA (i.e., "balance") in MCP appears to be similar to that required for growth, because the balance of EAA in ruminally synthesized bacteria is similar to lean tissue (Table 1; from Schwab and Garthwaite, 1996). This suggests that MCP, in comparison to most feed proteins (Table 1), is a superior source of EAA for growth, and once absorbed, will be used for growth with high efficiency; however, Richardson and Hatfield (1978) demonstrated that methionine and lysine are first and second limiting, respectively, for retention of nitrogen by growing steers fed a semi-purified diet devoid of RUP.

### **Nutritive Value of Ruminally Undegraded Protein**

All feedstuffs other than NPN supplements, such as urea or ammonia salts, contain some RUP (NRC, 1989). The amount and intestinal digestibility both differ among feedstuffs, and can be highly variable within a feedstuff (Table 2; from Stern et al., 1994). Feedstuffs also vary greatly in balance of EAA and the EAA composition of most feed proteins differs from MCP (Table 1); therefore, the easiest way with

conventional feeds to change the balance of EAA in AP is to feed large amounts of high-RUP protein supplements for which the balance of EAA is most deviant from MCP.

### **Supplements of Ruminally Undegraded Protein for Dairy Heifers**

The disappointing results discussed earlier about feeding more energy to increase ADG and reduce AFC has stimulated research interest in evaluating whether improving rate of growth or feed efficiency without overconditioning is possible by feeding more RUP. Secondly, the requirements for RUP (NRC, 1989) seem impractical owing to the high level (>60 % of CP) indicated for small (<200 kg of BW) heifers and the apparent linear decline of the requirements as BW increases.

Research endeavors prior (Cummins et al., 1982; Zerbini and Polan, 1985; Amos, 1986; Thonney and Hogue, 1986; Mantysaari et al., 1989) and subsequent (Swartz et al., 1991; Herrera-Seldana et al., 1992; Steen et al., 1992; Heinrichs et al., 1993; James, 1993; Casper et al., 1994; Bethard et al., 1997; Tomlinson et al., 1997) to the release of (NRC, 1989) have evaluated the merits of feeding more RUP to growing dairy heifers.

Cummins et al. (1982) varied the content of RUP (40, 55, and 70% of CP) in diets fed to calves (62 kg of BW) by substituting combinations of corn gluten, cottonseed, or dehydrated alfalfa meals for soybean meal in diets of different physical forms (ground, chopped, or grain only). No significant differences for dry matter intake (DMI) were observed, but calves given the 55% RUP treatment, which contained cottonseed and dehydrated alfalfa meals, retained less dietary N than those

given either the 40 or 70% RUP diets. Zerbini and Polan (1985) reported improvements in ADG of bull calves supplemented with fish meal compared with calves receiving corn gluten or cottonseed meals. Amos (1986) reported two studies evaluating 30 and 70% RUP diets for 120-kg heifers. Distillers dried grains and dehydrated alfalfa meal replaced soybean meal to achieve higher RUP; ADG was increased by feeding more RUP. In contrast, Mantysaari et al. (1989) observed no significant responses to supplementing RUP from fish meal, meat and bone meal, or animal protein blend compared with soybean meal; Thonney and Hogue (1986) found similar results when comparing the feeding value of fish meal versus cottonseed meal.

Most reports published after the release of NRC (1989) were designed to test whether recommendations of RUP for heifers were valid. Swartz et al. (1991) used combinations of blood meal, soybean meal, and corn proteins in diets of 30, 34, and 38% RUP (% of CP) for 100-kg calves. Feed efficiency improved with increasing RUP as a result of lower DMI and no difference in ADG. In that study (Swartz et al., 1991), comparative slaughter showed no differences of body composition. In contrast, Steen et al. (1992) observed a slight increase of empty-body protein, measured ultrasonically, in heifers fed a 40% RUP diet containing cottonseed meal and animal protein. No significant differences were measured for skeletal dimensions compared with a low RUP control.

The interactions of RUP and source of fermentable carbohydrates were investigated by Casper et al. (1994) and reported by James (1993). The study by Casper et al. (1994) evaluated two levels of RUP (30 and 35% of CP), which was

supplied by extruded soybean meal, and two sources of fermentable carbohydrate (corn versus barley) fed to 150-kg heifers. No effects of carbohydrate were evident, but ADG increased by addition of extruded soybean meal; however, the ranges of RUP and fermentable carbohydrate were small and RUP was less than recommended (NRC, 1989).

James (1993) reported an experiment evaluating three levels each of RUP and fermentable carbohydrate in a factorial arrangement for heifers (193 kg of BW); blood meal replaced soybean meal to increase RUP and combinations of barley and corn were used to alter fermentable carbohydrate. The amount of carbohydrate seemed to have a greater influence on ADG than did level of RUP; wither height increased as RUP increased within level of fermentable carbohydrate. The apparent efficiency of use of digestible energy was improved by increasing RUP, which resulted in a tendency for higher content of body fat as estimated by using the urea space procedure.

The relationship of RUP and digestible energy content of diet DM were investigated by Bethard et al. (1997). Two combinations of TDN formulated to support ADG of 600 or 900 g/d and two levels of RUP (30 or 50% of CP) were fed to 137-kg heifers; blood meal replaced soybean meal to increase RUP. No significant effects of RUP were observed for DMI, ADG, or wither height. The opposite was true for the effects of energy concentration, suggesting that energy was more limiting for growth than was RUP. A similar experimental protocol was used to evaluate dried brewers grains as the supplement of RUP (James, 1993); TDN content was 85 and 115% of requirements for 680 g/d ADG. The ADG did not differ significantly

between low (30%) and high (40%) RUP when TDN was high, but did differ between low TDN treatments; the effect of RUP in the high TDN diet may have been masked by greater production of MCP. Feed efficiency was highest for heifers given high RUP and low TDN because DMI decreased and ADG increased for that treatment.

Tomlinson et al. (1997) fed diets containing 31, 43, 50, and 55% of total N as RUP to heifers (225 kg of BW); blood meal and urea replaced soybean meal. The ADG and apparent efficiency of use of digestible energy increased linearly with increasing RUP, but DMI decreased linearly. Post-weaned calves showed improvements of ADG and feed efficiency when animal proteins replaced soybean meal (Herrera-Seldana et al., 1992), but Heinrichs et al. (1993) found no advantage to feeding more RUP from animal sources to older heifers. Heinrichs et al. (1993) did not report a sufficiently large range of RUP (35.5 vs. 37.1 % of CP), fed low CP (12% of DM), and they restricted ADG to 700 g/d for both treatments; a response should not be expected.

The composite of results has yielded information that is contradictory, sometimes unexpected, and often difficult to interpret. The inconsistencies have been suggested to have occurred, at least in part, because no two protein supplements have: (1) identical concentrations of CP, (2) identical ratios of RUP and RDP, (3) identical intestinal digestibilities of RUP, (4) identical content of EAA, and (5) the same type or quality of RDP for synthesis of MCP (Schwab and Garthwaite, 1996). Furthermore, the convention by which RUP and RDP typically is expressed, as % of CP, is faulty and should be abandoned. By expressing RUP as % of CP, the importance of RDP

essentially is ignored. For example, two isonitrogenous diets of 18% CP and 30 and 60% of CP as RUP would contain 5.4 and 10.8% of DM as RUP (e.g.,  $18 \times \text{RUP}\% \text{ of CP} \div 100 = \text{RUP}\% \text{ of DM}$ ). Likewise, those same diets would contain 12.6 and 7.2% of DM as RDP; thus, when isonitrogenous diets are formulated to contain increasing RUP as % of CP, RDP as % of DM decreases in a reciprocal fashion. The reduced RDP content may be insufficient to support maximal synthesis of MCP and offer cause for reinterpretation of the experiments presented. Lastly, most researchers only casually considered the EAA composition of RUP and how it may have affected their results.

### **Amino Acid Nutrition of Growing Cattle**

#### **Limiting Amino Acids**

Ruminants, like all mammals, have metabolic requirements for amino acids (AA) rather than for protein per se. Of the approximate 20 standard AA found in plant and animal proteins, ten typically are considered to be essential, each for which an animal has a different requirement. When the EAA are absorbed in the correct proportion (i.e., balance) relative to the requirements, their efficiency of use for maintenance and growth is maximized. The EAA that is in shortest supply relative to requirements is defined as the first limiting EAA.

Direct evidence provided by intestinal infusion (Burriss et al., 1976; Richardson and Hatfield, 1978; Hill et al., 1980; Schwab et al., 1982; Titgemeyer and Merchen, 1990; Merchen and Titgemeyer, 1992), postprandial administration by maintaining reflex closure of the esophageal groove (Abe et al., 1997), or feeding high quality



sources of ruminally protected methionine (RPMET) or ruminally protected lysine (RPLYS) (Lusby, 1994; Klemesrud and Klopfenstein, 1994) indicates that methionine (MET) and lysine (LYS) are generally the two most limiting EAA for growing cattle.

#### **Sequence of Limiting Essential Amino Acids**

The sequence of Lys and Met limitation is determined by their relative concentrations in RUP. Methionine and Lys were first and second limiting, respectively, when ruminally synthesized MCP was the sole source of AP (Richardson and Hatfield, 1978). Titgemeyer and Merchen (1990) observed a 17% increase in N retention by abomasal infusion of Met in steers fed a diet containing small amounts of RUP for which MCP supplied nearly all of the absorbed EAA. Schwab et al. (1982) determined that Met was first limiting for steer calves consuming a diet based on cereal grains. Lusby (1993) observed a 9% increase of ADG by feeding RPMET to calves grazing pasture, and Klemesrud and Klopfenstein (1994) determined that Met was first limiting for steers consuming a diet of sorghum silage, corn cobs, urea, and meat and bone meal as the main source of RUP.

In contrast to those experiments, Lys was first limiting when steers consumed large amounts of corn, and urea provided most of the supplemental protein (Burriss et al., 1976; Hill et al., 1990). Abe et al. (1997) fed basal diets of corn, corn gluten meal, and straw to young post-weaned calves. Lysine and Met were dissolved in water and administered postruminally via nipple feeders. Sensory conditioning was used to maintain reflex closure of the esophageal groove to ensure that supplemental AA were provided postruminally. Lysine was first limiting under conditions that seemed

conducive to restricted ruminal synthesis of MCP.

Considered wholly, these studies provide clear evidence that in cases where ruminal fermentation is not hindered or sources of RUP are chosen judiciously such that no individual EAA is severely deviant from that which would be contained in MCP, the AP would be used with a good to excellent efficiency. On the other hand, restricting ruminal fermentation by limiting RDP or fermentable carbohydrate would reduce MCP synthesis and require more RUP; if the source(s) of RUP had poor or divergent content of EAA, then the potential for a limitation of one or more EAA would be likely and efficiency of use of AP would be fair to poor.

#### **Essential Amino Acid Requirements of Dairy Heifers**

The NRC (1985; 1989) has not established requirements of AA for dairy cattle. The Cornell Net Carbohydrate and Protein System (CNCPS) and associated AA submodel (O'Conner et al., 1993) is a dynamic, factorial model that can be used to predict requirements and intestinal availability of EAA for growing and lactating cattle. The EAA requirements of Holstein heifers for three levels of ADG as predicted by using the CNCPS are shown in Table 3. Of particular interest is the lack of influence of level of ADG on the "predicted" proportional requirements of most EAA, including Lys and Met; estimates of the latter are 16.3 and 5.1% of EAA, respectively. This is similar to the EAA of lean tissue (Table 1).

Unfortunately, no data have been published that have evaluated the accuracy of CNCPS to predict intestinally available EAA. Ainsle et al. (1993) used feeding trials and comparative slaughter data to evaluate CNCPS predicted AP and EAA allowable

gain; the model accounted for 87 and 73% of the variation, respectively. The NRC (1996) has incorporated many of the elements of the CNCPS, but continued research and aggressive field evaluation are needed to refine the model. Whether the CNCPS can be used to evaluate if improvements of animal performance or efficiency of utilization of N can be made related to AA nutrition is unclear because no data exist to formulate a judgement.

### **Implication to Research Conducted**

Attempts to improve or accelerate ADG of dairy heifers by feeding RUP have yielded conflicting results. Whether feeding too much or too little RUP, too little RDP, or an imbalance of EAA is the culprit is unclear. Secondly, few experiments conducted with dairy heifers extend treatments for the entire period of weaning to confirmed pregnancy. The ultimate measure of heifer performance is whether influences during the rearing period affect future production of milk. Reducing the length of the rearing period must not affect negatively first lactation performance such that lost milk income is greater than the lowered costs of rearing.

The objectives of the research conducted were to evaluate the merit of increasing RUP by using highly digestible soybean sources while providing similar content of RDP and similar content of fermentable carbohydrates in diets of dairy heifers from 6 wk of age to 410 kg of BW. Measures of feed efficiency, ADG, days on feed and to confirmed pregnancy, and skeletal growth were established as the main response criteria. Secondly, the CNCPS was used to predict intestinally available EAA; supplements of RPMET and RPLYS (Smartamine™ ML and Smartamine™ M;

Rhône-Poulenc Animal Nutrition) were given to assess whether improvements in balance of absorbable EAA could enhance further the measured responses.

**TABLE 1. A comparison of the EAA profiles of body tissue with that of ruminal bacteria and protozoa and common feeds.<sup>1</sup>**

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
	-----(% of total EAA)-----									
Lean tissue	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1
Rumen microbes										
Bacteria	10.4	4.2	11.5	15.9	16.6	5.0	10.1	11.3	2.7	12.3
Protozoa	9.3	3.6	12.7	15.8	20.6	4.2	10.7	10.5	2.8	9.7
Forages										
Alfalfa	10.9	5.2	10.9	18.4	11.1	3.8	12.2	10.6	3.4	13.5
Corn silage	6.4	5.5	10.3	27.8	7.5	4.8	12.0	10.1	1.4	14.1
Haycrop silage	8.9	5.3	11.0	18.9	10.3	3.8	13.5	10.3	3.3	14.7
Grains										
Corn	10.8	7.0	8.2	29.1	7.0	5.0	11.3	8.4	1.7	11.5
Corn gluten feed	12.0	7.9	8.5	24.6	8.2	4.6	10.1	9.6	1.6	12.8
Oats	15.6	5.4	9.5	18.1	10.0	4.3	11.5	9.2	3.2	13.3
Plant proteins										
Corn gluten meal	6.9	4.7	9.3	36.4	3.8	5.5	13.8	7.5	1.5	10.7
Corn DDG w/solubles	7.7	7.2	9.8	26.3	6.2	5.2	11.1	10.3	2.7	13.4
Cottonseed meal	25.4	6.0	7.7	13.9	9.6	3.8	12.2	7.7	2.9	10.8
Soybean meal	16.3	5.7	10.8	17.0	13.7	3.1	11.0	8.6	3.0	10.6
Animal proteins										
Blood meal	7.6	11.2	2.1	22.8	15.7	2.1	12.3	8.1	2.7	15.4
Feather meal	14.7	1.1	10.0	29.3	3.9	2.1	10.0	10.5	1.5	17.1
Fish meal (menhaden)	13.1	5.7	9.3	16.5	17.0	6.3	8.8	9.5	2.4	11.3
Meat and bone meal	20.5	5.5	7.8	16.2	14.2	3.6	9.2	9.0	1.8	12.1

<sup>1</sup>Adapted from Schwab and Garthwaite (1996).

TABLE 2. Ruminally undegraded protein (RUP) and intestinal digestion (ID) of CP of various protein supplements.<sup>1</sup>

Protein supplement	n	RUP (% of CP)	ID (% of RUP)
		Avg $\pm$ SD (range)	Avg $\pm$ SD (range)
Blood meal, batch dried	12	88 $\pm$ 6 (78-98)	63 $\pm$ 17 (29-86)
Brewers grains, dried	5	57 $\pm$ 5 (50-63)	77 $\pm$ 2 (73-79)
Corn gluten meal	2	83 $\pm$ 2 (82-85)	89 $\pm$ 4 (86-91)
Cottonseed meal, solvent	1	46	33
Distillers grains, dried	5	56 $\pm$ 8 (47-64)	81 $\pm$ 5 (72-85)
Feather meal, hydrolyzed	12	76 $\pm$ 11 (50-88)	67 $\pm$ 6 (58-75)
Fish meal, menhaden	13	65 $\pm$ 4 (59-73)	80 $\pm$ 5 (73-88)
Meat and bone meal	11	59 $\pm$ 13 (40-88)	55 $\pm$ 10 (41-70)
Soybean meal	5	25 $\pm$ 3 (22-29)	90 $\pm$ 4 (86-93)
Soybean meal, lignosulfonate	6	66 $\pm$ 8 (57-77)	88 $\pm$ 4 (82-92)
Soybean meal, expeller	3	46 $\pm$ 8 (38-53)	99 $\pm$ 1 (98-100)

<sup>1</sup>Adapted from Stern et al. (1994).

TABLE 3. Requirements of Holstein heifers for absorbed EAA at three levels of gain as determined by using the Cornell Net Carbohydrate and Protein System.<sup>1</sup>

EAA	454 g/d		682 g/d		910 g/d	
	g/d	% of EAA	g/d	% of EAA	g/d	% of EAA
Arg	16.8	16.9	21.5	17.0	29.5	17.0
His	6.3	6.3	8.0	6.3	11.1	6.4
Ile	7.6	7.6	9.6	7.6	13.1	7.5
Leu	18.0	18.0	22.7	17.9	30.9	17.8
Lys	16.3	16.3	20.7	16.3	28.5	16.3
Met	5.0	5.1	6.4	5.1	8.8	5.1
Phe	9.0	9.0	11.5	9.1	15.8	9.1
Thr	10.0	10.0	12.7	10.0	17.5	10.1
Val	10.8	10.8	13.6	10.7	18.6	10.7
Total EAA	99.8		126.7		173.8	

<sup>1</sup>Example is for a 5 mo old, 160 kg of BW Holstein heifer; EAA do not include Tryptophan.

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## CHAPTER II

### RUMINALLY UNDEGRADED PROTEIN AND RUMINALLY PROTECTED AMINO ACIDS SUPPLEMENTED TO HEIFERS 6 WEEKS OF AGE TO 175 KG OF BODY WEIGHT

#### Abstract

Eighty Holstein heifer calves were blocked by date of birth and assigned to a 2 x 2 factorial arrangement of treatments at 6 wk of age to 100 kg of body weight (Phase 1) and 100 to 175 kg of body weight (Phase 2). Diets were (% of DM) low RUP (5.8% RUP, 10.2% RDP, and 16.0% CP), low RUP plus rumen-stable Lys and Met, high RUP (8.8% RUP, 10.2% RDP, and 19.0% CP), and high RUP plus rumen-stable Lys and Met. Heifers were weighed weekly and advanced to Phase 2 at minimum of 98.5 kg of body weight. Diets were 50 and 60% of DM as forage for Phase 1 and Phase 2, respectively; soybean protein, as provided by xylose-treated soybean meal (Soy Pass<sup>®</sup>, LignoTech USA, Inc., Overland Park, KS), was the supplemental source of RUP. The Cornell Net Carbohydrate and Protein System was used to estimate Lys, Met, and total essential amino acids flowing to the small intestine. Combinations of rumen-stable supplements of Lys and Met (Smartamine<sup>™</sup> ML and Smartamine<sup>™</sup> M, Rhône-Poulenc Animal Nutrition, Atlanta, GA) were given in amounts to increase intestinal Lys and Met to 16.2 and 6.0% of total essential amino acids, respectively. Average daily gain increased (758 vs. 700 g/d), apparent DMI

efficiency increased (.34 vs .31) days on feed decreased (56.2 vs. 61.9 d), DMI decreased (2.2 vs. 2.3 kg/d), hip height decreased (.155 vs. .165 cm/d), heart girth increased (.27 vs. .24 cm/d), and serum urea N increased (10.9 vs. 9.5 mg/dl) by feeding high RUP in Phase 1. Serum urea N increased (17.0 vs. 13.8 mg/dl) and pin width increased (.06 vs. .05 cm/d) by feeding high RUP in Phase 2. Supplements of rumen-stable Lys and Met in Phase 2 diets increased frame size (1.54 vs. 1.51 units). Increased concentration of RUP in diets of Holstein calves < 100 kg of body weight improved average daily gain and some measures of skeletal growth; supplemental RUP of soybean origin provided no benefit to heifers > 100 kg or < 175 kg body weight.

(**Key words:** dairy heifers, growth, ruminally undegraded protein, lysine, methionine )

**Abbreviation key:** ADF=acid detergent fiber, ADG=average daily gain, Arg=arginine, BCS=body condition score, BW=body weight, CNCPS=Cornell Net Carbohydrate and Protein System, CP=crude protein, DM=dry matter, DMI=dry matter intake, EAA=essential amino acid, Lys=lysine, MCP=microbial crude protein, Met=methionine, NDF=neutral detergent fiber, NDIN=neutral detergent insoluble nitrogen, NSC=nonstructural carbohydrate, RDP=ruminally degraded protein, RPAA=ruminally protected amino acids, RUP=ruminally undegraded protein, TDN=total digestible nutrients, TMR=total mixed ration

### **Introduction**

Current NRC (1989) guidelines indicate that dairy heifers < 200 kg of body weight (BW) and not consuming milk or milk replacer be fed diets containing high amounts of RUP, such that 50 to 85% of absorbed protein is RUP. That recommendation is based on the observation that ruminal synthesis of microbial crude protein (MCP) in young heifers is minimal and not adequate for maximum growth



(Orskov, 1977; NRC, 1985; 1989). Research to investigate the feeding of high RUP diets to young dairy heifers has yielded inconsistent results. Some have reported increases of average daily gain (ADG) by feeding more RUP (Zerbini and Polan, 1985; Amos, 1986; Herrera-Seldana et al., 1992; James, 1993; Casper et al., 1994; Tomlinson et al., 1997), whereas others observed improvement of feed efficiency (Swartz et al., 1991; Thonney and Hogue, 1986; Bethard et al., 1997) or no response (Cummins et al., 1982; Mantysaari et al., 1989; Heinrichs and Garman, 1992; Heinrichs et al., 1993). The variable responses may be related partly to interaction of RUP and dietary energy (James, 1993; Casper et al., 1994), which should be expected because fermentable carbohydrate influences amount of MCP synthesized in the rumen (NRC, 1985); responses to RUP seem to be greater when TDN is low (James, 1993). A more likely cause may be related to the selection of the source of supplemental RUP. Sources of RUP that contain an essential amino acid (EAA) composition most deviant from MCP, or from the requirement of the animal, may not be used efficiently because one or more EAA may be more deficient in the supplemented diet compared with the control (Merchen and Titgemeyer, 1992). Moreover, all studies unintentionally decreased RDP while increasing RUP because of efforts to maintain isonitrogenous diets; thus, any advantage of more total absorbable EAA from RUP may have been offset by less amount of absorbable EAA from MCP.

Lysine (Lys) and methionine (Met) are generally the two most limiting EAA in growing cattle (Schwab and Garthwaite, 1996). The regression equations developed by Socha (1994) for predicting content of Lys and Met in duodenal digesta of lactating

cows indicate that as dietary RUP is increased, the relative content of Lys and Met in duodenal digesta decreases; that observation is supported by Merchen and Titgemeyer (1992) for growing ruminants. Efforts to improve or increase the balance or amount of Lys or Met in absorbable protein by feeding diets high in RUP may accentuate their qualitative deficiency and reduce the efficiency of use of absorbed protein. A potentially more effective method may be to supplement Lys and Met by feeding them in ruminally protected form. Lusby (1993) observed a 9% increase of ADG by feeding ruminally protected Met to beef calves grazing pasture. Klemesrud and Klopfenstein (1994) reported 65% improvement for the efficiency of use of dietary protein by supplementing ruminally protected Met to steers (234 kg of BW) fed a high roughage diet containing meat and bone meal; the efficiency of use of dietary N increased as amount of meat and bone meal increased. Whether the use of ruminally protected amino acids (RPAA) may improve growth or feed efficiency by dairy heifers fed practical diets has not been evaluated.

The objectives of this experiment were to evaluate whether increasing RUP by feeding a highly digestible soybean source and providing similar content of RDP and similar content of fermentable energy in diets of dairy calves 6 wk of age to 175 kg of BW would improve ADG, gain:feed ratio, and skeletal growth and whether increasing amounts of Lys and Met in absorbable EAA by feeding RPAA could enhance further the responses.

## **Materials and Methods**

### **Experimental Design and Treatments**

All procedures related to animal care were conducted with approval by the University of New Hampshire Institutional Animal Care and Use Committee. Eighty Holstein heifer calves were blocked by date of birth (4 heifers per block) as they became available at 6 wk of age and assigned to a 2 x 2 factorial arrangement of treatments. Main effects of the 2 x 2 factorial were amount of RUP (low versus high) and whether RPAA were supplemented; the four treatments were: 1) low RUP; 2) low RUP+RPAA; 3) high RUP; and 4) high RUP+RPAA. Treatments were applied to heifers for two, separate phases of growth (Phase 1: 6 wk to 100 kg of BW; Phase 2: 100 to 175 kg of BW), but during Phase 2 each heifer continued receiving the factorial combination initially assigned for Phase 1. The TMR for each phase are shown in Table 1; differences of diet composition between Phase 1 and Phase 2 reflect a larger inclusion of forage in diets for Phase 2 to account for expected differences in DMI for larger BW. The low RUP TMR for each phase was formulated to meet or exceed slightly the NRC (1989) recommendations to support 700 g/d ADG for heifers of BW respective to each phase (Table 2). The high RUP TMR were formulated to supply an additional 3% of DM as RUP, but TDN and RDP similar to the low RUP TMR (Table 2). The RPAA were provided as combinations of Smartamine ML™ and Smartamine M™ (Rhône-Poulenc Animal Nutrition, Atlanta, GA) and were fed in amounts to achieve intestinal concentrations of 16.2 and 6.0% of total EAA for Lys and Met, respectively as estimated by using the Cornell Net Carbohydrate and Protein System

(CNCPS) (O'Connor et al., 1993). The amounts of supplemental RPAA were calculated individually for each heifer assigned to the RPAA factor combinations, based on weekly BW, DMI, and environmental conditions; thus amounts of RPAA were specific to conditions individual to each heifer within and across treatments. The concentrations of 16.2 and 6.0% of EAA for Lys and Met, respectively, were chosen because they represent closely the required concentrations in metabolizable protein as suggested, but not confirmed, for growing heifers (Ainslie et al., 1993). One heifer mistakenly was assigned to the high RUP treatment instead of the high RUP+RPAA treatment. One heifer assigned to the high RUP+RPAA treatment died as a result of injury at the beginning of Phase 2.

### **Feeding and Management**

Heifers commenced receiving treatments in 1.2 x 2.4 x 1.2 m individual stalls in a naturally ventilated barn and treatments were fed from 18.9-L plastic buckets. Heifers were moved to a naturally ventilated freestall barn equipped with Calan Doors (American Calan, Inc., Northwood, NH) when space requirements or size of heifers were sufficient; effort was taken to move block-mates simultaneously, but that was not always possible. Heifers were trained to use the Calan Doors by assisting entry and exit for intervals not longer than 6 h between bouts of eating until evidence indicated heifers could enter and exit the Calan Doors ad libitum. The TMR (Table 1) were mixed and fed once daily between 0830 and 1000 h by using a mobile drum mixer (Data Ranger; American Calan, Inc., Northwood, NH) for ad libitum DMI; orts were collected and weighed daily at 0800 h. The feed was stirred several times daily to

discourage sorting and redistribute fines. Samples of haycrop silage were analyzed weekly for DM; TMR were adjusted accordingly. A sample of each forage was submitted monthly (NEDHIC, Ithaca, NY) for wet chemistry analyses for CP, ADF, NDF, NSC, TDN, Ca, P, K, Mg, and S; concentrate feeds were analyzed every 3 mo for the same chemical constituents. Amounts of forage in diet DM for low and high RUP TMR within phases were held constant throughout the experiment, but amounts of corn meal, soybean meal, and xylose-treated soybean meal (Soy Pass<sup>®</sup>, LignoTech USA, Inc., Overland Park, KS) were adjusted for changes in chemical composition of forages. One-half of the daily allotment of RPAA was top-dressed and covered lightly into the top 4 cm of TMR at time of feeding; the remainder of daily RPAA was given between 1600 and 1630 h.

#### **Body Measurements and Collection and Analysis of Samples**

*Body Weight and Skeletal Measures.* Heifers were weighed once weekly .5 h prior to feeding by using an electronic scale (GRI AgriTech, Inc., Billings, MT). Weekly BW were used as input into the CNCPS for determining amounts of RPAA to supplement; heifers advanced from Phase 1 to Phase 2 when BW was no less than 98.5 kg. Measurements of wither and hip height, body length (point of shoulder to pin), heart girth, external width of hips, thurls, and pins, rump length (hip to pin), and body condition score (BCS 1 = thin, 5 = obese; .25 increments) were collected weekly.

*Feeds, TMR, and Orts.* Prior to initiation of the experiment haycrop silage, and every week thereafter, haycrop silage and Orts were sampled for determination of DM, dried to 88% DM, ground to pass through a 1-mm screen, and composited across

weeks within Phases 1 and 2 and analyzed for CP, NDF, ADF, Ca, P, K, Mg, and S (NEDHIC, Ithaca, NY). The average chemical composition of concentrates and chopped, grass hay was calculated by weighted composition of samples collected and analyzed over the course of the two phases.

*Blood Sampling.* Samples of blood were collected 3.5 to 4 h post-feeding from each heifer in Phase 1 by jugular venipuncture into untreated evacuated tubes (Vacutainer<sup>®</sup>, Becton Dickinson, Rutherford, NJ) on Monday, Wednesday, and Friday of the week subsequent to 85 kg of BW. Blood was transported immediately to the laboratory, chilled overnight (4 °C), centrifuged at 3,000 x g for 30 min, and serum was composited across day within heifer. Composited serum was stored frozen (-20 °C) until analyzed for concentration of urea (Sigma kit 535-A, Sigma Chemical Corp., St. Louis, MO). Samples of blood were collected from each heifer in Phase 2 in the same manner as for Phase 1, except sampling commenced at 135 kg of BW and continued weekly until 175 kg of BW; an additional sample of composited serum was submitted to the Virginia Polytechnic Institute and State University (R. M. Akers) for analysis of concentration of growth hormone.

#### **Calculations and Statistical Analyses**

Initial measurements of skeletal growth for Phase 1 were subject to correlation analysis; all skeletal measures were significantly correlated (Table 3). A measure of frame size, considered to be unitless, was calculated as:

$$[.5(\text{withers height} + \text{hip height}) \times \text{body length} \times \text{heart girth}] \div 1,000,000.$$

That value was intended to represent total skeletal growth (Pat Hoffman, personal

communication). Initial values for skeletal measures and BW were subtracted from final measures and divided by days on feed to calculate ADG for each variable. Repeated measures for DMI were reduced to means within phase.

The ANOVA was conducted by using the GLM procedure of SAS (1989) for a randomized block design with a 2 x 2 factorial arrangement of treatments; df were partitioned among block, main effects of RUP and RPAA and their interaction, covariate, and residual. Phase 1 and Phase 2 were analyzed separately. Initial BW within each phase was used as a covariate for DMI, ADG, days on feed, and gain:feed ratio; initial values of skeletal measurements and BCS within each phase were used as covariates for those measurements. Least square means were declared significantly different at  $P < .05$ .

## **Results and Discussion**

### **Diets**

The mean chemical composition of the low and high RUP diets consumed during Phase 1 and Phase 2 are shown in Table 2; values do not include intake of RPAA. The content of NSC in the high RUP diet may have been underestimated because the process of manufacturing xylose-treated soybean meal (Soy Pass<sup>®</sup>; LignoTech USA Inc., Overland Park, KS), results in high NDF insoluble N (NDIN), which would be included in the NDF fraction of the equation for calculating NSC; however, Stern et al. (1994) reported that xylose-treated soybean meal had high intestinal digestibility of RUP ( $88 \pm 4\%$  of RUP), indicating that the process does not reduce severely its value as a source of RUP. Hall (1997) suggested a modified

equation for calculating NSC by subtracting NDIN from NDF, but feedstuffs for this experiment were not analyzed for that fraction. No significant effects of RPAA or interaction with RUP were observed for DMI (Table 4); thus, mean chemical composition is reported only for main effect of RUP. The chemical composition of consumed diets differed little from the mean chemical composition of formulated diets. The additional RUP in the high RUP diets replaced NSC. The mean content of RUP and RDP in diets was not determined; therefore, amounts of RUP and RDP consumed are not reported.

#### **DMI, Days on Feed, Gain:Feed, ADG, CP and TDN Intake**

*Phase 1.* Dry matter intake, days on feed, TDN intake, and NSC intake were reduced significantly by feeding high RUP (Table 4). The reduced DMI is consistent with other reports about feeding high RUP to young heifers (Swartz et al., 1991; Tomlinson et al., 1997); however whether the decrease is related to lower intake of NSC or higher intake RUP is unclear.

The significant decrease of TDN intake by heifers fed high RUP likely is a combination of lower DMI and the slightly lower TDN content in the high RUP diet. The significantly higher intake of CP by heifers fed high RUP was expected because total CP was 2.8 percentage units higher in that diet. The ADG increased significantly by feeding high RUP (700 versus 758 g/d; Table 4), which agrees with Casper et al. (1994), who observed increased ADG by calves fed extruded soybean meal in place of solvent soybean meal. The ADG for the low RUP diet was equal to the ADG for which diets were formulated (700 g/d). The lower DMI and higher ADG for heifers



fed high RUP resulted in significantly greater gain:feed ratio, which indicates a greater apparent efficiency of use of DM for gain. Others (Thonney and Hogue, 1986; Swartz et al., 1991; Herrera-Seldana et al., 1992; Bethard et al., 1997; Tomlinson et al., 1997) reported improved apparent feed efficiency, but sources of RUP were different from this experiment.

Supplemental RPAA had no effect on any feed intake related variables.

Whether supplemented Lys or Met provided any benefit cannot be stated definitively; the CNCPS may not predict correctly the content of Lys, Met, or total EAA in duodenal digesta, neither Lys nor Met may have been limiting, or the amount of RPAA may have been insufficient. The numerically lower, but not significantly different, concentrations of urea-N in serum of RPAA-supplemented heifers suggest that RPAA may have had transient effects on use of absorbed N. Heifers fed high RUP had significantly higher concentrations of urea-N in serum, which suggests that even though ADG and apparent feed efficiency were improved by feeding more RUP, the absorbed N may have been in excess of requirements. The CNCPS frequently predicted Arg to be the first limiting EAA for the low RUP diet, which is curious because ample evidence indicates that either Lys or Met generally is the first limiting EAA in growing cattle (Schwab and Garthwaite, 1996).

*Phase 2.* No significant differences were observed for DMI, days on feed, apparent feed efficiency, ADG, or intake of TDN during Phase 2; intake of NSC was significantly lower for heifers given high RUP, which was similar to the observation for Phase 1 (Table 5). The DMI was higher than expected, and ADG were greater

than for what was formulated (923 g/d across treatments versus 700 g/d formulated for the low RUP diet; Table 5). Intake of CP was significantly higher for heifers given high RUP compared with low RUP (831 versus 713 g/d), which explains the higher concentration of urea-N in serum for heifers fed high RUP (Table 5). Apparently, the low RUP diet provided sufficient absorbable protein because ADG was similar to that of the high RUP diet; the additional RUP that was supplied by xylose-treated soybean meal was not needed for heifers during Phase 2.

No significant effects of RPAA were observed. Concentration of growth hormone in serum was not affected by treatment; growth hormone was measured to evaluate whether improved protein or AA nutrition may potentially modulate mammary development, but no reports are available to support or refute that hypothesis. The data by Sejrsen et al. (1982; 1983; 1986) indicate clearly that secretion of endogenous growth hormone is decreased by feeding dietary energy in excess of requirements. The intake of TDN was not significantly different in this experiment; thus any effects on concentration of growth hormone may not be measurable. Growth hormone is secreted in a pulsatile fashion (Sejrsen et al., 1982); therefore, the sampling protocol used in this experiment may not have been adequate to measure mean daily concentration.

### **Skeletal Measurements**

*Phase 1.* The average daily growth of heifers during Phase 1 is shown in Table 6. Average daily gain of heart girth dimension was increased significantly by feeding high RUP. A significant interaction of RUP and RPAA was observed for heart girth. Heifers given low RUP and no RPAA had lower gain of heart girth than did heifers

fed low RUP + RPAA (.23 vs. .25 cm/d), whereas heifers offered the high RUP and no RPAA had higher gain of girth than did heifers fed high RUP + RPAA; a logical explanation is elusive. Heifers fed high RUP treatments had significantly lower gain of hip height than heifers fed the low RUP treatments, but that observation seems questionable given the highly significant correlations among skeletal measures (Table 3); a similar response should be expected for all skeletal measurements.

*Phase 2.* The average daily gain of skeletal measures during Phase 2 is shown in Table 6. The average gain of pin width was increased by feeding high RUP, but the biological relevance is unclear. Final frame size was increased by feeding RPAA, which partly can be explained by numerically higher values of hip height and body length for heifers receiving the RPAA treatments.

### **Conclusions**

Results from this experiment indicate that heifers < 100 kg of BW responded to increased concentration of RUP from xylose-treated soybean meal in diet DM; heifers < 100 kg of BW achieved acceptable ADG when fed low RUP diets. Evidence does not support feeding RUP in excess of 5.6% of DM to heifers of 100 to 175 kg of BW when diets contain 70% TDN. Whether feeding RPAA is an effective means of improving balance of absorbable EAA, and subsequently, enhanced efficiency of use of absorbed protein could not be determined in this experiment.

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TABLE 1. Average ingredient composition of diets offered during Phase 1 (6 wk to 100 kg of BW) and Phase 2 (100 to 175 kg of BW).<sup>1</sup>

Ingredient	Phase 1		Phase 2	
	Low RUP	High RUP	Low RUP	High RUP
	-----(% of DM)-----			
Chopped, grass hay <sup>2</sup>	16.0	16.0	16.0	16.0
Haycrop silage <sup>3</sup>	34.0	34.0	44.0	44.0
Shelled corn <sup>4</sup> , finely ground	38.4	31.6	30.4	23.6
Soybean meal <sup>5</sup> , solvent, 48% CP	10.0	5.8	8.2	4.0
Soybean meal <sup>6,7</sup> , xylose-treated	---	11.0	---	11.0
Minerals and vitamins <sup>8</sup>	1.6	1.6	1.4	1.4

<sup>1</sup>Percentages are averages for the duration of the experiment; forage concentrations were held constant but shelled corn and the two soybean meals were adjusted as needed to accommodate changes of chemical composition of the feeds.

<sup>2</sup>Contained [% of DM, (range)]: 8.2% (6.5 to 11.4%) CP; 43.8% (41.0 to 46.2%) ADF; 70.2% (66.6 to 76.2%) NDF.

<sup>3</sup>Contained [% of DM, (range)]: 17.2% (13.4 to 24.5%) CP; 36.8% (26.5 to 44.8%) ADF; 54.5% (34.1 to 64.4%) NDF.

<sup>4</sup>Contained [% of DM, (range)]: 9.3% (8.8 to 10.0%) CP; 3.2% (2.3 to 4.9%) ADF; 10.9% (7.1 to 12.0%) NDF.

<sup>5</sup>Contained [% of DM, (range)]: 53.4% (52.7 to 55.4%) CP; 4.6% (3.3 to 8.0%) ADF; 10.7% (5.1 to 14.5%) NDF.

<sup>6</sup>Soy Pass® (LignoTech USA, Inc., Overland Park KS); non-enzymatically browned, xylose-treated soybean meal.

<sup>7</sup>Contained (% of DM): 53.4% CP, 8.7% ADF, and 10.7% NDF.

<sup>8</sup>Contained: 15.3% Ca, 4.1% P, 1.0% Mg, .1% K, 1.3% S, 13.4% Na, 20.6% Cl, 1375 ppm Zn, 1000 ppm Mn, 315 ppm Cu, 150 ppm Fe, 18 ppm Se, .1 ppm Co, .25 ppm I, 3300 ppm Lasalocid.

**TABLE 2. Chemical composition and measures of nutritive value of offered and consumed<sup>1</sup> diets.**

Item	Phase 1				Phase 2			
	Low RUP		High RUP		Low RUP		High RUP	
	Offered	Consumed	Offered	Consumed	Offered	Consumed	Offered	Consumed
	-----(% of DM)-----							
CP	16.0	16.1	19.0	18.9	16.0	16.1	19.0	18.9
RUP <sup>2</sup>	5.8	---	8.8	---	5.6	---	8.6	---
RDP <sup>2</sup>	10.2	---	10.2	---	10.4	---	10.4	---
TDN	72.5	72.3	72.4	72.1	70.1	69.4	69.9	69.0
NDF	35.6	34.9	35.1	34.6	39.8	36.7	39.3	39.3
ADF	21.2	21.0	21.8	21.6	24.8	24.8	25.4	25.3
NSC <sup>3</sup>	38.7	39.4	36.0	33.6	34.2	34.4	31.6	28.7
Ca	.6	.6	.6	.6	.6	.7	.6	.7
P	.4	.4	.4	.4	.4	.4	.4	.4
Mg	.2	.1	.2	.2	.2	.2	.2	.2
K	.6	1.8	.6	1.9	.6	2.0	.6	2.1
S	.22	.2	.2	.2	.2	.2	.2	.2

<sup>1</sup>The chemical composition of consumed diets was calculated by dividing the difference between the quantities of offered and refused nutrients by DMI.

<sup>2</sup>Calculated from NRC (1989), except RUP for Soy Pass<sup>®</sup> was 70% of CP (Stern et al., 1994); RUP and RDP were not determined for orts.

<sup>3</sup>Non-structural carbohydrates = 100% - (% NDF + %CP + %EE + %ash).



TABLE 3. Correlation coefficients of initial skeletal measures for Holstein heifers assigned to treatments.<sup>1</sup>

Measure	Heart girth	Body length	Wither height	Hip height	Hip width	Thurl width	Pin width	Rump length
Rump length	.52	.59	.68	.73	.39	.54	.48	
Pin width	.47	.44	.44	.43	.39	.58		
Thurl width	.52	.54	.68	.68	.51			
Hip width	.36	.37	.44	.47				
Hip height	.56	.59	.92					
Wither height	.51	.56						
Body length	.53							
Heart girth								

<sup>1</sup>All variables significantly correlated.

TABLE 4. Least squares means and standard errors for DMI, days on feed, gain:feed ratio, ADG, CP, TDN, and NSC intakes, and serum urea nitrogen of Phase 1 (6 wk of age to 100 kg of BW) heifers.<sup>1,2</sup>

Item	Treatment <sup>3</sup>				SE	Effect (P) <sup>4</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	19				
DMI, kg/d	2.3	2.3	2.2	2.2	.04	*		
Days on feed	60.8	63.0	55.7	56.6	1.50	*		
Gain:feed	.32	.31	.35	.34	.01	*		
ADG, g/d	701	700	777	739	.90	*		
CP Intake, g/d	386	379	447	439	6.90	*		
TDN Intake, g/d	1765	1753	1699	1681	29.80	*		
NSC <sup>5</sup> Intake, g/d	739	710	704	663	15.30	*	*	
Serum urea N, mg/dl	9.7	9.3	12.9	11.9	.62	*		

<sup>1</sup>Initial BW was used as a covariate for DMI, days on feed, Gain:feed, ADG, CP intake, TDN intake, and NSC intake.

<sup>2</sup>Initial average BW at 6 wk of age for all heifers was 58.6 ± 7.9 kg; range was 42.7 to 78.2 kg.

<sup>3</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>4</sup>\*P ≤ .05.

<sup>5</sup>NSC = 100% - (NDF% + CP% + EE% + ash%)

TABLE 5. Least squares means and standard errors for DMI, days on feed, gain:feed ratio, ADG, CP, TDN, and NSC intakes, and concentration of urea nitrogen and growth hormone in serum of Phase 2 (100 to 175 kg of BW) heifers.<sup>1</sup>

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP +RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	18				
DMI, kg/d	4.3	4.4	4.2	4.3	.07			
Days on feed	83.4	82.1	82.6	84.6	1.7			
Gain:Feed	.21	.21	.21	.21	.01			
ADG, g/d	914	922	943	911	16.90			
CP intake, g/d	705	722	827	836	11.52	*		
TDN intake, g/d	3042	3110	2982	3026	46.88			
NSC <sup>4</sup> intake, g/d	1523	1552	1216	1234	21.72	*		
Serum Urea N mg/dl	14.1	13.4	17.3	16.6	.45	*		
Growth hormone ng/ml	3.2	2.9	1.9	3.2	.48			

<sup>1</sup>Initial BW in Phase 2 was used as a covariate for DMI, days on feed, Gain:feed, ADG, CP intake, TDN intake, and NSC intake.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>NSC = 100% -(NDF% + CP% + EE% + ash%).

TABLE 6. Least squares means and standard errors for skeletal measurements of Phase 1 (6 wk of age to 100 kg of BW) heifers.<sup>1</sup>

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	19				
Skeletal growth, cm/d								
Heart girth	.23	.25	.28	.26	.01	*		*
Wither height	.19	.18	.18	.17	.01			
Hip height	.16	.17	.16	.15	.01	*		
Body length	.24	.23	.24	.25	.01			
Hip width	.09	.09	.09	.09	.01			
Thurl width	.06	.06	.06	.06	.01			
Rump length	.06	.06	.06	.06	.01			
Pin width	.04	.05	.05	.04	.01			
Frame <sup>4</sup>								
Final frame	.92	.95	.91	.93	.01			
Body condition								
Final score	2.74	2.71	2.78	2.77	.02			

<sup>1</sup>Initial measures for skeletal growth were used as covariates.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>Frame = [.5(wither height + hip height) x body length x heart girth] ÷ 1,000,000.

**TABLE 7. Least squares means and standard errors for skeletal measurements of Phase 2 (100 to 175 kg of BW) heifers.<sup>1</sup>**

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	18				
<b>Skeletal growth, cm/d</b>								
Heart girth	.25	.26	.25	.25	.01			
Wither height	.17	.17	.18	.18	.01			
Hip height	.16	.17	.17	.18	.01			
Body length	.22	.23	.22	.23	.01			
Hip width	.08	.09	.08	.08	.01			
Thurl width	.07	.08	.07	.08	.01			
Rump length	.07	.07	.07	.06	.01			
Pin width	.05	.05	.06	.06	.01	*		
<b>Frame<sup>4</sup></b>								
Final frame	1.52	1.54	1.51	1.54	.01		*	
<b>Body condition</b>								
Final score	2.84	2.81	2.85	2.86	.02			

<sup>1</sup>Initial measures for skeletal growth in Phase 2 were used as covariates.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>Frame = [.5(wither height + hip height) x body length x heart girth] ÷ 1,000,000.

### CHAPTER III

#### RUMINALLY UNDEGRADED PROTEIN AND RUMINALLY PROTECTED AMINO ACIDS SUPPLEMENTED TO HEIFERS 175 TO 410 KG OF BODY WEIGHT

##### Abstract

Seventy-nine heifers were used during two phases of growth (175 to 245 kg of BW; Phase 3) and (245 to 410 kg of BW; Phase 4) and assigned treatments in a 2 x 2 factorial arrangement. The factorial combinations for Phase 3 were: 1) low RUP (5.0% of DM; 15% CP) no ruminally protected Lys and Met; 2) low RUP plus ruminally protected Lys and Met; 3) high RUP (8% of DM; 18% CP) no ruminally protected Lys and Met; and 4) high RUP plus ruminally protected Lys and Met. Phase 4 was restricted to include only the first 16 blocks of heifers. The factorial combinations for Phase 4 were: 1) low RUP (4.3.0% of DM; 13% CP) no ruminally protected Lys and Met; 2) low RUP plus ruminally protected Lys and Met; 3) high RUP (7.38% of DM; 16% CP) no ruminally protected Lys and Met; and 4) high RUP plus ruminally protected Lys and Met. No significant responses were observed for growth during either phase. Heifers > 175 kg of BW consuming ad libitum diets may not benefit from additional RUP.

**(Key words:** dairy heifers, growth, ruminally undegraded protein, lysine, methionine )

**Abbreviation key:** ADF=acid detergent fiber, ADG=average daily gain, BCS=body condition score, BW=body weight, CNCPS=Cornell Net Carbohydrate and Protein System,

**CP**=crude protein, **DM**=dry matter, **DMI**=dry matter intake, **EAA**=essential amino acid, **Lys**=lysine, **Met**=methionine, **NDF**=neutral detergent fiber, **NSC**=nonstructural carbohydrate, **RDP**=ruminally degraded protein, **RPAA**=ruminally protected amino acid, **RUP**=ruminally undegraded protein, **TDN**=total digestible nutrients, **TMR**=total mixed ration

### **Introduction**

Few experiments have been conducted to evaluate whether feeding higher concentrations of RUP to older heifers is beneficial ( Mantysaari et al., 1989; Heinrichs et al., 1993; Bethard et al., 1997; Tomlinson et al., 1997). No responses were observed by feeding higher concentration of RUP in diets of heifers > 220 kg of BW (Mantysaari et al., 1989; Heinrichs et al., 1993), but those researchers fed diets containing very low CP such that differences in content of RUP were negligible. Tomlinson et al. (1997) observed positive, linear responses of ADG by feeding blood meal in diets for 225 kg heifers. Bethard et al. (1997) also used blood meal to evaluate interaction of energy and RUP on performance of heifers from 3 to 14 mo of age; no significant responses to RUP were observed other than slight improvements in apparent DM efficiency. The NRC (1989) suggests that diets contain 12% CP, of which nearly 4.4% of DM should be supplied as RUP, for heifers of 250 kg BW gaining 800 g/d; the recommendation declines precipitously such that 400 kg heifers need only 2.1% of DM in the form of RUP, which likely would be supplied even by marginal quality forages. Recent research efforts (Dutrow et al., 1991; Daccarett, et al., 1993; Van Amburgh et al., 1994) attempting to improve growth of heifers during the peripubertal period have shown that a general increase of all nutrients does not negatively affect first lactation

performance. A sufficiently high average daily gain can be achieved to permit young age at first calving (21 to 22 mo; Van Amburgh et al., 1994) without over conditioning.

The merit of feeding increased concentration of RUP in diets of older heifers has not been examined sufficiently. Older heifers should have sufficient DMI to subsist solely on high quality forage, but to achieve early age at first parturition the supplies of nutrients must support high rates of gain. Improving further the EAA composition of absorbable protein by supplementing ruminally protected Lys or Met may be possible, especially when RUP is the major fraction of low CP diets.

The objectives of this experiment were to assess the validity of NRC (1989) recommendations for RUP in the diet of older heifers by further evaluating the performance of heifers described previously (Garthwaite, 1997) and assessing whether ruminally protected Lys and Met may be of any benefit for growth and feed efficiency of dairy heifers.

## **Materials and Methods**

### **Experimental Design and Treatments**

All procedures related to animal care were conducted with approval by the University of New Hampshire Institutional Animal Care and Use Committee. Seventy-nine of the heifers originally assigned to the previous experiment continued receiving the factorial combinations assigned initially. Experimental periods of 175 to 245 kg of BW (Phase 3) and 245 to 410 kg of BW (Phase 4) were evaluated in this experiment. The factorial combinations for Phase 3 were: 1) low RUP (5.0% of DM; 15% CP) no ruminally protected Lys and Met (RPAA); 2)



low RUP + RPAA; 3) high RUP (8% of DM; 18% CP) no RPAA; and 4) high RUP + RPAA. Phase 4 was restricted to include only the first 16 blocks of heifers. The factorial combinations for Phase 4 were: 1) low RUP (4.3.0% of DM; 13% CP) no RPAA; 2) low RUP + RPAA; 3) high RUP (7.38% of DM; 16% CP) no RPAA; and 4) high RUP + RPAA. The TMR for each phase are shown in Table 1; 70% of diet DM was supplied by forage. The low RUP TMR for each phase was formulated to support average daily gain (ADG) of 800 g/d (NRC, 1989); high RUP TMR were designed to supply an additional 3% of DM as RUP, but similar TDN and RDP to the low RUP TMR (Table 2). The amounts of RPAA to supplement were determined as described previously (Garthwaite, 1997).

### **Feeding and Management**

Heifers were housed among pens in a naturally ventilated barn equipped with Calan Doors (American Calan, Inc., Northwood, NH). The TMR (Table 1) were mixed and fed once daily between 0900 and 1200 h by using a mobile drum mixer (Data Ranger; American Calan, Inc., Northwood, NH); orts were collected and weighed daily at 0830 h. Samples of haycrop and corn silages were analyzed weekly, or as climatic conditions warranted, for DM; TMR were adjusted accordingly. Amounts of forage and soy hulls in diet DM for low and high RUP TMR within phases were held constant throughout the experiment, but corn meal, soybean meal, and xylose-treated soybean meal (Soy Pass<sup>®</sup>, LignoTech USA, Inc., Overland Park, KS) were adjusted for changes in chemical composition of forages. The bulky nature of the diets required modification of feeding RPAA. One-half of the daily RPAA was poured

next to the mouths of heifers while heifers were eating TMR at time of feeding and the other half was given at 1630 h in the same manner; this was done to minimize filtering of RPAA to the bottom of the mangers. That strategy worked favorably because few pellets of RPAA were observed in orts.

### **Body Measurements and Collection and Analysis of Samples**

*Body Weight and Skeletal Measures.* Heifers were weighed once weekly 1 h prior to feeding by using an electronic scale (GRI AgriTech, Inc., Billings, MT). Weekly BW were used as input into the CNCPS for determining amounts of RPAA to supplement; heifers advanced from Phase 3 to Phase 4 when BW was no less than 243.5 kg. Measures of wither and hip height, body length, heart girth, external width of hips, thurls, and pins, rump length and body condition score (BCS 1 = thin, 5 = obese; .25 increments) were collected weekly.

*Blood sampling.* Samples of blood were collected 3.5 to 4.5 h postfeeding from each heifer in each phase by jugular venipuncture into untreated evacuated tubes (Vacutainer®, Becton Dickinson, Rutherford, NJ) on Monday, Wednesday, and Friday of each week; sampling of individual heifers ceased at confirmed pregnancy. Blood was transported immediately to the laboratory, chilled overnight (4 °C), and centrifuged at 3,000 x g for 30 min. Serum was composited across day within heifer within each phase of growth. Aliquots of serum were retained for analysis of concentration of urea (Sigma kit 535-A, Sigma Chemical Corp., St. Louis, MO), and submitted to the Virginia Polytechnic Institute and State University (R. M. Akers) for analysis of concentration of growth hormone.

*Observations for Estrus and Insemination of Heifers* . Heifers were observed for 20 min three times daily for signs of estrus. Heifers were assigned to the breeding list when BW was no less than 340 kg and the intent was to inseminate at the first observed estrus after heifers attained breeding weight.

### **Calculations and Statistical Analyses**

Initial values for skeletal measures and BW and the start of each phase were subtracted from final measures and divided by days on feed to calculate ADG for each variable.

Repeated measures for DMI were reduced to means within phase. Reproductive performance was summarized, but not analyzed statistically or reported because several people inseminated heifers, some heifers were inseminated before they had reached minimum breeding weight, and some were not inseminated to observed estrus.

The ANOVA was conducted by using the GLM procedure of SAS (SAS<sup>®</sup>, 1989) for a randomized block design with a 2 x 2 factorial arrangement of treatments. Initial BW for each phase was used as a covariate for DMI, ADG, days on feed, and gain:feed ratio. Initial values for skeletal measures and BCS within each phase were used as covariates for skeletal measures and BCS. Least square means were declared significantly different at  $P < .05$ .

## **Results and Discussion**

### **Diets**

The mean chemical composition of the low and high RUP diets consumed during Phase 3 and Phase 4 is shown in Table 2; values do not include intake of RPAA. The chemical

composition of consumed diets differed little from the mean chemical composition of formulated diets, except TDN was about 1 to 2 percentage units lower in consumed diets.

#### **DMI, Days on Feed, Gain:Feed Ratio, ADG, CP and TDN Intakes**

*Phase 3.* No significant effects of RUP or RPAA were observed for DMI, days on feed, gain:feed ratio, or ADG (Table 3). The ADG were much higher than intended (1030 g/d across factor combinations vs. goal of 800 g/d); however, BCS was not unreasonably high (Table 5). According to NRC (1989), heifers in Phase 3 consumed TDN and CP well over the requirement for 800 g/d. Dry matter intake was higher than expected, and total intake of TDN and CP very likely masked any effects of RUP and RPAA. Heifers reared in confinement have shown increases for feed efficiency of 12 to 25% compared with a more open environment (Quigley et al., 1986).

Serum urea N was significantly higher for heifers given the high RUP treatment (Table 3), which reflects the higher content of total CP in that diet. No significant differences were observed for concentrations of growth hormone in serum (Table 3).

*Phase 4.* The ADG across treatments for Phase 4 (941 g/d) was closer to the target ADG, but no improvements for measured responses were observed (Table 4). The concentration of urea in serum was significantly higher for heifers fed the high RUP diet (Table 4). No significant effects of RUP or RPAA were observed for concentrations of growth hormone in serum.

### **Skeletal Measures**

*Phase 3.* The average gain of wither height was significantly higher for the low RUP diet (Table 5). Whether such a small difference has biologic meaning is difficult to rationalize, especially at an older age. The RUP X RPAA interaction for heart girth is perplexing and may be spurious more than meaningful.

*Phase 4.* No significant differences were observed for skeletal measurements of heifers in Phase 4.

### **Conclusions**

Results from this experiment indicate that NRC (1989) recommendations for RUP may be too high for heifers > 175 kg of BW; however, heifers in this experiment were allowed to consume ad libitum DMI, and with the exception of the chopped hay were fed good quality forages. In situations where forage quality or DMI is low, supplements of RUP may be warranted.

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TABLE 1. Average ingredient composition of diets offered during Phase 3 (175 to 245 kg of BW) and Phase 4 (245 to 410 kg of BW).<sup>1</sup>

Ingredient	Phase 3		Phase 4	
	Low RUP	High RUP	Low RUP	High RUP
	-----(% of DM)-----			
Chopped, grass hay <sup>2</sup>	20.0	20.0	18.8	18.8
Corn silage <sup>3</sup>	15.0	15.0	31.9	31.9
Haycrop silage <sup>4</sup>	35.0	35.0	19.4	19.4
Shelled corn <sup>5</sup> , finely ground	21.3	14.4	17.0	10.0
Soybean meal <sup>6</sup> , solvent, 48% CP	7.0	2.7	4.2	.3
Soybean meal <sup>7, 8</sup> , xylose-treated	---	11.2	---	10.9
Soybean hulls <sup>9</sup>	---	---	6.9	6.9
Minerals and Vitamins <sup>10</sup>	1.7	1.7	1.8	1.8

<sup>1</sup>Percentages are averages for the duration of the experiment; forage and soy hull concentrations were held constant, but shelled corn and the two soybean meals were adjusted as needed to accommodate changes of the feeds.

<sup>2</sup>Contained [% of DM, (range)]: 8.2% (6.5 to 11.4%) CP; 43.8% (41.0 to 46.2%) ADF; 70.2% (66.6 to 76.2%) NDF.

<sup>3</sup>Ensiled with .5% urea added: Contained [% of DM, (range)]: 11.0% (8.3 to 12.8%) CP; 26.5% (19.5 to 31.5%) ADF; 42.2% (32.8 to 50.0%) NDF.

<sup>4</sup>Contained [% of DM, (range)]: 17.2% (13.4 to 24.5%) CP; 36.8% (26.5 to 44.8%) ADF; 54.5% (34.1 to 64.4%) NDF.

<sup>5</sup>Contained [% of DM, (range)]: 9.3% (8.8 to 10.0%) CP; 3.2% (2.3 to 4.9%) ADF; 10.9% (7.1 to 12.0%) NDF.

<sup>6</sup>Contained [% of DM, (range)]: 53.4% (52.7 to 55.4%) CP; 4.6% (3.3 to 8.0%) ADF; 10.7% (5.1 to 14.5%) NDF.

<sup>7</sup>Soy Pass® (LignoTech USA, Inc., Overland Park KS); non-enzymatically browned, xylose-treated soybean meal.

<sup>8</sup>Contained: 53.4% CP, 8.7% ADF, and 10.7% NDF.

<sup>9</sup>Contained [% of DM, (range)]: 12.6% (11.0 to 17.4%) CP; 46.4% (38.9 to 49.1%) ADF; 65.19% (57.4 to 68.8%) NDF

<sup>10</sup>Contained: 15.3% Ca, 4.1% P, 1.0% Mg, 0.1% K, 1.3% S, 13.4% Na, 20.6% Cl, 1375 ppm Zn, 1000 ppm Mn, 315 ppm Cu, 150 ppm Fe, 18 ppm Se, 0.1 ppm Co, 0.25 ppm I, 3300 ppm Lasalocid.

TABLE 2. Chemical composition and measures of nutritive value of offered and consumed<sup>1</sup> diets.

Item	Phase 3				Phase 4			
	Low RUP		High RUP		Low RUP		High RUP	
	Offered	Consumed	Offered	Consumed	Offered	Consumed	Offered	Consumed
	-----(% of DM)-----							
CP	15.0	15.1	18.0	17.9	13.0	13.0	16.0	15.9
RUP <sup>2</sup>	5.0	---	8.0	---	4.3	---	7.4	---
RDP <sup>2</sup>	10.0	---	10.0	---	8.7	---	8.6	---
TDN	68.5	67.5	68.4	67.0	69.0	67.5	68.9	66.9
NDF	42.5	42.8	41.9	42.4	43.8	44.6	43.2	44.2
ADF	26.3	26.8	26.9	27.3	26.9	28.1	27.5	28.6
NSC <sup>3</sup>	32.1	31.8	29.5	26.5	33.0	32.2	30.4	27.0
Ca	.4	.7	.4	.7	.4	.7	.4	.7
P	.3	.4	.3	.4	.3	.4	.3	.4
Mg	.2	.2	.2	.2	.2	.2	.2	.2
K	.6	1.8	.6	1.8	.6	1.6	.6	1.7
S	.2	.2	.2	.2	.2	.2	.2	.2

<sup>1</sup>The chemical composition of consumed diets was calculated by dividing the difference between the quantities of offered and refused nutrients by DMI.

<sup>2</sup>Calculated from NRC (1989), except RUP for Soy Pass<sup>®</sup> was 70% of CP (Stern et al., 1994); RUP and RDP were not determined for orts.

<sup>3</sup>Non-structural carbohydrates = 100% - (% NDF + %CP + %EE + %ash).



**TABLE 3. Least squares means and standard errors for DMI, days on feed, gain:feed ratio, ADG, CP, TDN, and NSC intakes, and serum urea N and growth hormone of Phase 3 (175 to 245 kg of BW) heifers.<sup>1</sup>**

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	19				
DMI, kg/d	6.2	6.3	6.1	6.2	.1			
Days on feed	69.7	67.4	70.0	70.2	1.60			
Gain:feed	.16	.17	.17	.17	.01			
ADG, g/d	1021	1057	1010	1030	23.10			
CP Intake, g/d	947	965	1109	1119	16.62	*		
TDN Intake, g/d	4251	4296	4128	4182	67.10			
NSC <sup>4</sup> Intake, g/d	2002	2033	1617	1647	29.95	*		
Growth hormone, ng/ml	2.1	2.2	1.8	2.2	.35			
Serum urea N, mg/dl	14.4	14.1	17.9	17.4	.42	*		

<sup>1</sup>Initial Phase 3 BW was used as a covariate for DMI, days on feed, Gain:Feed, ADG, CP intake, TDN intake, and NSC intake.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>NSC = 100% - (NDF% + CP% + EE% + ash%)

**TABLE 4.** Least squares means and standard errors for DMI, days on feed, gain:feed ratio, ADG, CP, TDN, and NSC intakes, and concentration of urea N and growth hormone in serum of Phase 4 (245 to 410 kg of BW) heifers.<sup>1</sup>

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	16	16	16	15				
DMI, kg/d	8.4	8.5	8.5	8.4	.12			
Days on feed	173.6	174.8	172.7	181.0	4.31			
Gain:feed	.11	.11	.11	.11	.01			
ADG, g/d	936	945	959	923	23.71			
CP intake, g/d	1155	1174	1420	1413	14.86	*		
TDN intake, g/d	6004	6072	5961	5936	69.06			
NSC <sup>4</sup> intake, g/d	2872	2877	2383	2371	31.94	*		
Serum urea N, mg/dl	13.1	11.7	15.9	15.6	.36	*		
Growth hormone, ng/ml	1.2	1.5	1.0	1.2	.24			

<sup>1</sup>Initial BW was used as a covariate for DMI, days on feed, Gain:feed, ADG, CP intake, TDN intake, and NSC intake.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>NSC = 100% - (NDF% + CP% + EE% + ash%)

TABLE 5. Least squares means and standard errors for skeletal measurements of Phase 3 (175 to 245 kg of BW) heifers.<sup>1</sup>

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	20	20	21	18				
Skeletal growth, cm/d								
Heart girth	.21	.23	.23	.22	.01			*
Wither height	.14	.14	.13	.13	.01	*		
Hip height	.13	.14	.14	.14	.01			
Body length	.18	.16	.16	.17	.01			
Hip width	.07	.07	.07	.07	.01			
Thurl width	.06	.06	.06	.06	.01			
Rump length	.06	.07	.06	.06	.01			
Pin width	.04	.05	.05	.05	.01			
Frame <sup>4</sup>								
Final frame	2.06	2.06	2.05	2.04	.02			
Body condition								
Final score	2.89	2.84	2.90	2.85	.02			

<sup>1</sup>Initial skeletal measures for Phase 3 were used as covariates.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>Frame = [.5(wither height + hip height) x body length x heart girth] ÷ 1,000,000.

TABLE 6. Least squares means and standard errors for skeletal measurements of Phase 4 (245 to 410 kg of BW) heifers.<sup>1</sup>

Item	Treatment <sup>2</sup>				SE	Effect (P) <sup>3</sup>		
	Low RUP	Low RUP + RPAA	High RUP	High RUP + RPAA		RUP	RPAA	RUP X RPAA
n	16	16	16	15				
Skeletal growth, cm/d								
Heart girth	.16	.16	.16	.16	.01			
Wither height	.08	.08	.08	.08	.01			
Hip height	.08	.08	.08	.08	.01			
Body length	.11	.11	.11	.11	.01			
Hip width	.05	.05	.05	.05	.01			
Thurl width	.04	.04	.04	.04	.01			
Rump length	.04	.04	.04	.04	.01			
Pin width	.03	.03	.03	.03	.01			
Frame <sup>4</sup>								
Final frame	3.2	3.2	3.2	3.2	.01			
Body condition								
Final score	3.12	3.12	3.09	3.04	.04			

<sup>1</sup>Initial Phase 4 skeletal measures were used as covariates.

<sup>2</sup>RUP = ruminally undegraded protein; RPAA = ruminally protected Lys and Met.

<sup>3</sup>\*P ≤ .05.

<sup>4</sup>Frame = [.5(wither height + hip height) x body length x heart girth] ÷ 1,000,000.

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