Empirical evidence suggests that prolonged underfeeding of protein to late-pregnant dry cows can have modest negative carry-over effects on milk volume and/or protein yield during early lactation, and may also cause increased incidence of metabolic diseases associated with fatty liver. However, assessment of requirements is hampered by lack of information on relationships between dietary intake of crude protein \((\text{N} \times 6.25)\) and metabolizable protein supply during late pregnancy, and by incomplete understanding of the quantitative metabolism of amino acids in maternal and conceptus tissues. Inability of the postparturient cow to consume sufficient protein to meet mammary and extra-mammary amino acid requirements, including a significant demand for hepatic gluconeogenesis, necessitates a substantial, albeit transient, mobilization of tissue protein during the first 2 weeks of lactation. Ultimately, much of this mobilized protein appears to be derived from peripheral tissues, especially skeletal muscle and, to a lesser extent, skin, through suppression of tissue protein synthesis, and possibly increased proteolysis. In the shorter term, soon after calving, it is likely that amino acids required for hepatic glucose synthesis are diverted from high rates of synthesis of splanchnic tissue and export proteins, including serum albumin. The prevailing endocrine milieu of the periparturient cow, including major reductions in plasma levels of insulin and insulin-like growth factor-I, together with insulin resistance in peripheral tissues, must permissively facilitate, if not actively promote, net mobilization of amino acids from these tissues.

Periparturient dairy cow: Protein requirements: Tissue protein mobilization

The notion that protein nutrition of dry dairy cows during late pregnancy influences postpartum health and lactation performance is pervasive, but studies designed to test this hypothesis have yielded conflicting results. Part of the problem is due to uncertainty about the relationship between dietary protein intake and metabolizable protein supply in pregnant dry cows. The metabolizable protein requirement of the pregnant dry cow is also not well defined, because of imprecisions in the factorial estimation of amino acid requirements for maintenance, conceptus growth and mamogenesis. As discussed later (p. 121), present estimates for maintenance and conceptus growth, in particular, suffer from a lack of quantitative information on the metabolic fates and efficiencies of utilization of absorbed amino acids. However, an additional factor, which is seldom formally considered in the factorial approach, is the possible need to supply amino acids for deposition in or export from maternal non-uterine tissues, including the splanchnic organs, skeletal muscle and other structural tissues, which might constitute a labile protein reserve that is drawn on during early lactation.

The present paper will briefly summarize the literature on lactation performance responses to protein feeding during the dry period, before discussing the bases for factorial estimation of the changing amino acid requirements during the periparturient period. Questions about maternal protein reserves will then be addressed, including their anatomical location, functional significance, and nutritional and endocrine regulation.

Responses during early lactation to protein feeding of dry cows

Milk yield and composition

As summarized in Table 1, effects of level of dietary protein fed to late-pregnant dry cows on postpartum lactation performance have been variable, with a majority of studies...
finding little response in milk yield, protein concentration or protein yield. Closer examination of studies in which significant positive responses were observed reveals several associated factors that may influence the response, including relative level of prepartum dietary protein, duration of supplementation and level of protein in the lactation diet.

In four of the six comparisons in which a positive milk yield response to prepartum supplementation of dietary protein was observed, the negative control diet contained less than 120 g crude protein (N×6.25; CP)/kg DM; Chew et al. (1984; Hook et al. 1989; Van Saun et al. 1993). This factor is also true of a study on dairy goats in which milk yield during early lactation responded positively to an increase in prepartum dietary protein (Sahlu et al. 1997). This factor may also be noteworthy that increased prepartum feeding of a diet containing 150 v. low rumen-undegradable protein (RUP)/kg CP to cows during early lactation may mask the impact of moderate protein insufficiency on prepartum diet protein requirements.

Two preliminary reports of negative effects of prepartum protein supplementation on subsequent milk yield (Crawley & Kilmer, 1995; Greenfield et al. 1998) are difficult to interpret without more detail. However, considering an earlier report that prepartum feeding of a diet containing 150 v. 85 g CP/kg DM was associated with markedly increased incidence of periparturient health disorders (Julien et al. 1976) and the apparent lack of productive benefit from high v. moderate protein levels in prepartum diets, there is little incentive to excessively increase the protein concentration of prepartum diets.

The expression of protein requirements in terms of dietary CP is a major flaw in the earlier analysis of postpartum responses to protein feeding during late pregnancy. In pregnant dry cows, as in lactating cows, the relationship between CP intake and metabolizable protein supply is quite variable and highly dependent on the quality and level of dietary protein, as well as the availability of fermentable energy for rumen microbial protein synthesis. Examples are shown in Table 2, based on studies in which different levels of dietary CP were fed to dry cows, and metabolizable protein supply was estimated using the Cornell Net Carbohydrate Protein system (Sniffen et al. 1992). Although all modern feeding systems for ruminants are based on dynamic predictions of amino acid availability, the tendency to express dry cow requirements in terms of dietary CP is pervasive, at least in North America, and quite misleading. The additional problem of factorial estimation

<table>
<thead>
<tr>
<th>Source</th>
<th>Prepartum dietary crude protein (N × 6.25; CP; g/kg DM)</th>
<th>Milk response</th>
<th>Yield (kg)</th>
<th>Protein(g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chew et al. (1984)</td>
<td>96 v. 120</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Hook et al. (1989)</td>
<td>99 v. 130</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Van Saun et al. (1993)</td>
<td>124 v. 153</td>
<td>-</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Crawley &amp; Kilmer (1995)</td>
<td>120 v. 150 150; low v. high RUP</td>
<td>↓</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Van Saun &amp; Sniffen (1995)</td>
<td>NR; low v. high RUP</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Moorby et al. (1996)</td>
<td>NR; low v. high RUP</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Huyler et al. (1997)</td>
<td>117 v. 156</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Greenfield et al. (1998)</td>
<td>120 v. 160 160; 260 v. 330 g RUP/kg CP</td>
<td>↓</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Carson et al. (1998)</td>
<td>139 v. 156</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Putnam &amp; Varga (1998)</td>
<td>106 v. 127</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Putnam et al. (1999)</td>
<td>130 v. 178</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

NR, not reported; RUP, rumen-undegradable protein; ↑, increase; ↓, decrease; −, no change.
of metabolizable protein requirements during late pregnancy is discussed below.

**Liver metabolism and health**

It has been suggested that protein supplementation of pregnant dry cows may decrease the incidence of metabolic disease during early lactation, which may or may not be related to observed effects on lactation performance. For example, Van Saun & Sniffen (1995) reported a significant reduction in postpartum incidence of ketosis in multiparous cows supplemented with rumen-undegradable protein during late pregnancy, although no effects on milk yield or composition were observed. Such an effect may be mediated through a reduction in the degree of hepatic lipid accumulation that appears to be central, if not causally related, to the aetiology of ketosis and several other metabolic diseases commonly seen in dairy cattle during early lactation (Grummer, 1993). This idea is consistent with the finding of Holtenius & Hjort (1990) that dry cows fed on diets high in energy but low in protein suffered a major increase in the severity of fatty liver at parturition compared with cows fed on high-energy high-protein or low-energy low-protein diets. A similar response was induced by Hippen et al. (1999) who fed cows on a diet very high in energy but with only 120 g CP/kg for 30 d before calving. The hepatic supply of protein, or specific amino acids, may be limiting for synthesis of apolipoprotein B, and therefore hepatic lipid disposal via synthesis and secretion of VLDL. Several studies have suggested that hepatic synthesis of apolipoprotein B is impeded in cows with fatty liver (Mazur et al. 1992; Griffat et al. 1996, 1997), and there is limited preliminary evidence that supplementation of cows with rumen-protected lysine and methionine may improve hepatic secretion of VLDL during early lactation (Durand et al. 1992). Later work by this group (Bauchart et al. 1998) has confirmed that supplementation of rumen-protected lysine and methionine can reduce liver triacylglycerol at 2 and 4 weeks postpartum, and plasma ketone levels at 2 weeks postpartum. This treatment also increased the hepatic concentration of apolipoprotein B100 at 4 weeks postpartum, consistent with the notion of improved capacity for VLDL synthesis and secretion.

**Changes in amino acid requirements during the periparturient period**

The factorial estimation of protein requirements for dairy cattle by the National Research Council (1989) accounts for maintenance protein requirements as the sum of endogenous urinary protein (2.75BW0.5, where BW is body weight), scurf protein (0.2BW0.6), and metabolic faecal protein (0.09 × indigestible DM excretion). Absorbed protein is assumed to be utilized with an efficiency of 67% for formation of endogenous urinary protein and scurf protein.

The net protein required for conceptus growth is calculated as 1.136BW0.70 and absorbed protein is considered to be used for conceptus growth with an efficiency of 50%. This information leads to an estimated metabolizable protein requirement of 742 g/d for a mature dry cow weighing 650 kg and approximately 3 weeks before calving. This estimate has several uncertainties. First, the maintenance algorithm takes no account of possible effects of pregnancy on amino acid metabolism in maternal tissues other than the gravid uterus, but with the absence of hard evidence to the contrary, there is little option but to retain the present estimate. Second, while the estimate of net protein requirement for conceptus growth is not inconsistent with our direct measurements of protein accretion in the gravid uterus of mature Holstein cows during late pregnancy (Bell et al. 1995), the assumed efficiency of utilization of absorbed protein is probably an overestimate. Measurements of uterine uptake of amino acids in relation to protein accretion by the conceptus in sheep indicate that the efficiency term is no more than 40% (Chung et al. 1998), while sparser data on cattle suggest a value as low as 30% (Bell, 1995). Use of a factor of 33%, as suggested by Bell et al. (1995), applied to the net requirement predicted by the National Research Council (1989) leads to a metabolizable protein requirement of about 320 g/d, compared with 212 g/d estimated by the National Research Council (1989) approach. Finally, the estimate obtained by the National Research Council (1989) approach does not account for mammary growth during the last few weeks of pregnancy. This factor is probably trivial before 3 weeks prepartum, but could account for about 120 g metabolizable protein/d

<table>
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<tbody>
<tr>
<td>Variable</td>
<td>Dietary CP</td>
<td>Dietary NFC</td>
</tr>
<tr>
<td>DM intake (kg/d)</td>
<td>Low 11·0 Medium 11·2 High 11·1</td>
<td>High 13·2 Medium 10·2</td>
</tr>
<tr>
<td>NFC (g/kg DM)</td>
<td>339 330 301</td>
<td>438 235</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>417 436 430</td>
<td>295 489</td>
</tr>
<tr>
<td>CP (g/kg DM)</td>
<td>106 127 145</td>
<td>132 144</td>
</tr>
<tr>
<td>CP intake (g/d)</td>
<td>1166 1422 1639</td>
<td>1690 1469</td>
</tr>
<tr>
<td>MP supply (g/d)</td>
<td>971 1119 1186</td>
<td>1226 829</td>
</tr>
<tr>
<td>MP:CP</td>
<td>0·83 0·79 0·72</td>
<td>0·73 0·56</td>
</tr>
</tbody>
</table>

NFC, non-fibre carbohydrate; NDF, neutral-detergent fibre; CP, crude protein (N × 6·25); MP, metabolizable protein estimated using Cornell Net Carbohydrate Protein system (Sniffen et al. 1992).
during the last 3 weeks of the dry period, based on measured values for mammary tissue growth in periparturient cows (Capuco et al. 1997) and mammary tissue protein concentration in late-pregnant ewes (McNeill et al. 1997), and an assumed efficiency of utilization of absorbed protein of 67%. Thus, a more realistic estimate of metabolizable protein requirements of the late-pregnant dry cow is probably approximately 1000 g/d.

**Requirements during early lactation**

According to the National Research Council (1989), factorial estimation of metabolizable protein requirements for lactation involves separate estimation of requirements for maintenance, as described earlier, and for milk synthesis, based on milk protein yield (net requirement) and a value of 70% for the efficiency of utilization of absorbed protein. Measured values for milk yield (30 kg/d) and milk protein concentration (420 g/kg) in cows weighing 650 kg and tested at 4 d postpartum yield an estimated metabolizable protein requirement of 2170 g/d. An additional 120 g/d for mammary growth probably should be included because this process continues into early lactation (Akers et al. 1981). Thus, the total requirement during the first week of lactation is at least 2300 g/d, which is 2.3 times that required before birth.

The factorial estimation of protein requirements of the late-pregnant dry cow is probably approximately 1000 g/d. Requirements during early lactation involve separate estimation of requirements for maintenance, as described earlier, and for milk synthesis, based on milk protein yield (net requirement) and a value of 70% for the efficiency of utilization of absorbed protein. Measured values for milk yield (30 kg/d) and milk protein concentration (420 g/kg) in cows weighing 650 kg and tested at 4 d postpartum yield an estimated metabolizable protein requirement of 2170 g/d. An additional 120 g/d for mammary growth probably should be included because this process continues into early lactation (Akers et al. 1981). Thus, the total requirement during the first week of lactation is at least 2300 g/d, which is 2.3 times that required before calving just a few days earlier. Once again, this conservative estimate does not account for the almost certain increase in maintenance requirements in lactating versus non-lactating animals.

**Impact of periparturient changes on protein balance**

We have used the conservative factorial approach described earlier, together with individually-measured values for metabolizable protein intake, to derive calculated protein balances for a group of eighty Holstein cows through the first 28 d of lactation (Fig. 1; Burhans & Bell, 1998). Calculated protein balance decreased to a nadir of about −600 g/d at 7 d postpartum, then steadily increased for the next 3 weeks, with zero balance apparently achieved at about 23 d postpartum. This estimate must be regarded as minimal, because it does not account for another potentially major avenue of amino acid utilization, hepatic gluconeogenesis.

Several lines of evidence point to an important, if transient, reliance on glucogenic amino acids to make up a shortfall in rumen propionate production, related to constrained voluntary DM intake during early lactation. Calculations based on generous predictions of availability of propionate, glycerol, and lactate for hepatic gluconeogenesis suggest a shortfall in glucose precursors of at least 500 g/d in a cow producing 30 kg milk and consuming 15 kg DM/d of a high-quality ration during the first week of lactation (Bell, 1995). The estimated metabolizable protein supply under these conditions was 1650 g/d, which barely accounts for the requirement for milk protein synthesis. This factor leads to the unavoidable conclusion that simply to fulfill the cow’s need to synthesize glucose, at least 500 g endogenous protein/d must be mobilized, in addition to that required for other maintenance purposes. Some of this additional requirement may be offset by reduced oxidation of glucose in peripheral tissues (Bennink et al. 1972), consistent with the characteristic insulin resistance in these tissues during early lactation in ruminants (Debras et al. 1989; Vernon et al. 1990). It is also evident that the liver’s capacity for converting alanine, and probably other glucogenic amino acids, is up regulated in dairy cows soon after calving (Fig. 2; Overton et al. 1998). The regulatory mechanism is not known, but presumably involves perception of glucose demand, because similar up-regulation can be elicited by treating wethers with phlorizin (Overton et al. 1999). Another piece of persuasive semi-quantitative evidence for the significant net conversion of tissue protein to glucose was provided by Wilson et al. (1988). These workers estimated the proportions of C in individual milk constituents derived from feed and body tissues in dairy cows, taking advantage of natural variations in $^{13}$C:12C present in C3- and C4-pathway plant species. These estimates suggested that during early lactation as much as 34% of the casein and 24% of the lactose in milk could...
have been derived from body tissue protein, with cows of high genetic merit having greater values than less superior cows. These estimates should be discounted for likely influence of nutrient turnover, because smaller but significant values for the contributions of tissue protein to milk constituents were obtained in late lactation, when cows were close to zero energy and protein balance. 

Thus, we believe that during the first 7–10 d of lactation, at least, high-yielding dairy cows may need to mobilize as much as 1000 g tissue protein/d to satisfy the mammary gland’s demand for amino acids and glucose. Clearly, such a tissue loss could not be sustained for very long, and should diminish as the cow’s voluntary consumption of a high-protein diet continues to increase. However, it raises several questions about the tissue source, mechanisms and regulation of endogenous protein mobilization that will be discussed now.

**Labile protein reserves in maternal tissues**

*Tissue source and mechanisms of protein mobilization*

Several serial slaughter studies have directly or indirectly investigated the net loss of protein from the empty body and specific tissues of dairy cows during different phases of the reproduction–lactation cycle (Belyea et al. 1978; Tyrrell & Haaland, 1983; Gibb et al. 1992; Andrew et al. 1994). Most studies showed a modest loss of protein from carcass tissues during early lactation, but sampling intervals were generally too long to allow detection of transient responses, and in some cases tissue specificity was not reported. We have examined the partitioning of CP among maternal and conceptus tissues in ewes during the last month of pregnancy, and observed that even in animals fed to predicted requirements for metabolizable protein there was a moderate net loss of carcass protein over this period (Fig. 3; McNeill et al. 1997). In animals that were fed on a protein-insufficient but energy-sufficient diet the loss of carcass protein was exacerbated and the net increase in protein accretion in visceral and mammary tissues seen in control animals was attenuated (Fig. 3).

Data on tissue protein synthesis in goats during early lactation provide additional insight into carcass tissue specificity as well as possible mechanisms of protein mobilization (Baracos et al. 1991). Absolute values for protein synthesis in hindlimb tissues were reduced by 45 % for skin, 14 % for bone marrow and 29 % for skeletal muscle in goats during the third week of lactation compared with dry goats. These reductions contributed 30, <1 and 69 % respectively to the total reduction in protein synthesis in hindlimb tissues during early lactation. Thus, it seems fairly clear that suppression of protein synthesis in skeletal muscle and skin could contribute to net mobilization of amino acids in the postparturient cow, consistent with observations of reduced muscle fibre diameter in early lactation (Reid et al. 1980). Augmentation of this response by increased myofibrillar protein degradation is suggested by the marked increase in urinary 3-methylhistidine:creatinine in parturient dairy cows (Overton et al. 1998), accompanied by a similar pattern of increase in plasma concentrations of 3-methylhistidine (Burhans et al. 1997). This response may be partly influenced by uterine involution and myometrial protein degradation in the early postpartum period, but the magnitude and sustained pattern of response suggests a major additional source of muscle protein breakdown.

Reduced hypertrophy of visceral tissues in protein-restricted ewes during late pregnancy (McNeill et al. 1997) suggests that modulation of the normal major increase in protein synthesis in liver (Bell, 1995), and presumably gut tissues, during early lactation may be an additional source for diversion of amino acids to catabolic fates. Such a response could be more immediate, albeit short-lived, than effects on carcass tissues because of the much greater rates of protein turnover in splanchic v. muscle and skin. In addition to temporarily constraining tissue growth of the visceral organs, this response might also reduce the synthesis of export proteins, including apolipoprotein B, with consequences for hepatic lipid disposal. Reduction in hepatic synthesis of albumin and other quantitatively important serum proteins is suggested by moderate reductions in their circulating concentrations in periparturient cows (Rowlands et al. 1975).

*Nutritional and endocrine regulation of tissue protein mobilization*

It is not known whether the size of the so-called labile protein reserve at parturition has an influence on mobilization of amino acids during the early postpartum period. However, it is evident that N balance and tissue accumulation of protein is directly responsive to dietary protein intake in dairy cows (Putnam & Varga, 1998) and ewes (McNeill et al. 1997) during late pregnancy. In ewes most of this additional protein was deposited in carcass tissues (Fig. 3). The degree to which postparturient mobilization of amino acids is responsive to nutrition during
early lactation also requires study. Intuitively, a response to dietary protein would be expected. It seems reasonable that glucogenic supplementation of newly-calved cows should ameliorate the apparent increase in muscle protein degradation, indicated by plasma concentrations of 3-methylhistidine. However, this hypothesis was not confirmed in cows orally supplemented with 500 g propylene glycol/d, despite other indices of metabolic benefit (Burhans et al. 1997). It remains possible that net mobilization of endogenous protein is nonetheless modulated by glucogenic substrate supply, via changes in synthesis rather than degradation of labile protein reserves.

No studies have directly addressed the endocrine or local regulation of tissue protein mobilization in the postparturient cow, and so much of the following discussion is speculative. However, the likely pattern of reduced protein synthesis, and possibly increased proteolysis in hindlimb and other tissues, is consistent with observed changes in plasma concentrations of, and tissue responses to, key endocrine regulators of tissue protein metabolism, including insulin and insulin-like growth factor (IGF)-I (Fig. 4). Despite a marked and persistent increase in plasma growth hormone, the precipitous decline in plasma concentrations of IGF-I parallels that in insulin, beginning about 2 weeks before calving. Other workers have reported reduced plasma levels of IGF-binding protein-3 and increased levels of IGF-binding protein-2, concomitant with the reduction of circulating IGF-I (Simmons et al. 1994; Segoale et al. 1997). Associated with lactation-induced insulin resistance in peripheral tissues (Vernon et al. 1990), these endocrine changes must have at least a permissive regulatory influence in suppressing protein synthesis and enhancing proteolysis. The removal of the normal positive influence of growth hormone on plasma IGF-I and IGF-binding protein-3 is consistent with similar observations during later lactation in cows that were moderately or severely underfed (McGuire et al. 1995). The possibly separate influences of protein v. energy insufficiency in mediating this disconnection of the growth hormone–IGF axis have yet to be clarified. However, it is interesting to note a recent report that inadequate supply of total amino acids or simply methionine was able to totally suppress the stimulation by growth hormone of IGF-I synthesis in cultured ovine hepatocytes (Wheelhouse et al. 2000).

Conclusions

Understanding relationships between dietary protein intake, tissue protein reserves, and performance and health of dairy cows during early lactation will be greatly assisted if diets are described in terms of metabolizable (absorbed) protein rather than CP content. There is also a need to more precisely determine the efficiency of utilization of amino acids for conceptus growth during late pregnancy in dairy cows. Net protein requirements have been established but must be combined with measurements of net uterine uptake of amino acids to derive a reliable efficiency factor.

Indirect evidence for the importance of tissue protein mobilization during early lactation is compelling, but requires confirmation by more direct experimental approaches under well-defined nutritional conditions. There is limited evidence that protein balance in maternal tissues can be improved by protein supplementation of pregnant ruminants, but it is unclear whether or when this might be of benefit during early lactation.

Finally, the regulatory mechanisms that permit the periparturient cow to simultaneously increase protein synthesis and accretion in splanchnic tissues and mobilize amino acids from peripheral tissues require detailed investigation. In addition to answering an intriguing academic question about regulation of nutrient partitioning, more fundamental
understanding of these processes may explain some of the large individual variation among cows in their ability to stay healthy and highly productive after the transition from pregnancy to lactation.

References


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