Even though we’ve been at it for over 150 years, we keep finding out new things about the rumen, the cow, and feed that affect how animals perform. Some of this information can be useful for better formulation of rations, though it may take a while to fully understand what exactly we are working with.

Over the years, occasionally reports would come in from nutritionists that increasing protein over recommended levels gave better milk responses than predicted, or that excessive protein caused what looked like ruminal acidosis. The cows are always right, but what was going on? Was the protein in feed less available than usual or being used as an energy source? Even in the research literature, protein effects that look like what we usually think of as carbohydrate or energy effects have been reported, but often with little explanation. In this paper, we’ll explore a relationship between ruminally degradable protein (RDP) and carbohydrates that are rapidly assimilated by rumen microbes. We may be able to use this information to improve feed efficiency and manipulate the nutrient supply to better meet the animal’s needs.

Carbohydrate is fermented in the rumen to produce organic acids (acetate, propionate, butyrate, lactate, valerate), gases (carbon dioxide and methane), and the energy to drive production of microbial cells (Figure 1). Availability of carbon skeletons and energy from fermentable carbohydrate are important to allow conversion of nonprotein nitrogen to microbial protein (Dellow et al., 1988). Microbes also incorporate carbohydrates into their cells as carbohydrate or for the synthesis of other molecules. The extent to which carbohydrate ferments in the rumen, or passes out unfermented is determined by competing rates of fermentation and passage. Carbohydrate fermentation can be depressed if RDP is limiting (Heldt et al., 1999), but no other ruminal effects of RDP on carbohydrate are typically discussed. Fermentation of carbohydrates in the rumen is perceived to be the main determinant of ruminal organic acid production.

**Protein Effects on Carbohydrate Fermentations: In Vitro**

Protein can affect formation of microbial products from carbohydrates in ways that may differ from our usual thinking. Increasing the supply of amino acids and peptides increased microbial yield of ruminal microbes linearly at each amount of carbohydrate tested (Figure 2; Argyle and Baldwin, 1989). How was it that the nitrogen sources were limiting to microbial cell synthesis at the lower amounts of protein, but still able to generate increasing yield responses as more carbohydrate was supplied? The study focused on protein effects, but begs the question of what was happening with the carbohydrate as microbial yields changed.
Glycogen is a microbial product that is often left out of discussion on carbohydrate fates, but it could help to explain a protein effect. For rapidly available carbohydrates such as glucose, fructose, sucrose, starch, and fructan (a water-soluble carbohydrate in cool season grasses), but not for lactose, rumen microbial products include:

Organic Acids + Gas + Microbial Cells + Glycogen (+ other microbial products like biofilms?)

Glycogen is a carbohydrate synthesized by bacteria and protozoa to store carbohydrate they’ve sequestered but have not yet fermented; it has essentially the same structure as starch. Glycogen storage increases with the amount of rapidly available carbohydrate present (Prins and Van Hoven, 1977), but decreases as dietary protein is increased (McAllan and Smith, 1974). Through an effect on glycogen accumulation, protein has potential to alter ruminal concentrations of organic acids by changing whether the carbohydrate is fermented immediately, or is stored and fermented more slowly (Figure 3). Protein supplementation has also been shown to increase lactic acid production (Malestein et al., 1984), possibly by increasing the flux of carbohydrate through glycolysis (Counotte and Prins, 1981) (Figure 3). When not produced in excessive quantities, lactic acid is not a problem per se. It is normally transient in the rumen, and is fermented to acetate and propionate.

Figure 3. Proposed scheme describing factors affecting carbohydrate fates in the rumen. Solid lines indicate direction of reactions from substrate to product. Dashed lines designate effects on formation of products with “+” indicating an effect that increases and “−” indicating an effect that decreases product formation. The effect of RDP, amino acids and nonprotein nitrogen on glycolysis is assumed based on the negative effect of these sources of N on glycogen synthesis and positive effect on lactic acid production. (PO43− = orthophosphate, Glc-6-P = glucose-6-phosphate). (M.B. Hall, unpublished).

Figure 4A-C. Effect of amount and type of protein source on A) microbial growth (nitrogen accumulation), B) glycogen accumulation, and C) organic acid production in vitro with mixed ruminal microbes (Hall, 2012a). The LoN treatment contained ammonia and amino acids and peptides, but had 12.5% less N than the HiN treatments. The HiN treatments had added urea (HiNU) or amino acids and peptides (HiNT). Organic acids are presented as the amount of carbon in the acids.
In an experiment to investigate how protein amount and type affected the fate of a rapidly assimilated sugar (glucose), we found that offering mixed rumen microbes more urea or amino acids + peptides did result in less glycogen accumulation and in more microbial cell nitrogen (cell growth), at some time points (Figures 4A-C; Hall, 2012a). The response in cell growth depended on what time point was evaluated, with low nitrogen culture (LoN) having less growth at 2 h, but surpassing the greater nitrogen culture supplemented with urea (HiNU) at 3 hours. Organic acid production differed among the treatments in the very early time points, but not by the end of the fermentation, even though there was no difference in the rate of glucose disappearance. The organic acid response differs from results of the cow studies. It raises the question as to what other products microbes were making with the carbohydrate.

### Protein Effects on Carbohydrate Fermentations: In Cows

For this alleged protein effect on carbohydrate to matter at all on the farm, does glycogen production by rumen microbes happen in cows? Measurements made on lactating dairy cows fed grass-clover silage and various supplements showed a net ruminal synthesis of “starch” that passed to the small intestine of 0.6 + 0.3 lb/day (value differed from zero, P=0.14; Larsen et al., 2009). That “starch” is microbial glycogen that would have been produced from other dietary carbohydrates. Accordingly, it is possible for cows to have something that digests like starch be produced in the rumen and flow to their small intestine, even if there was no starch in their diets.

Research studies with lactating cows have shown protein effects on what are usually considered carbohydrate-driven responses. Take for example a study in which cows given fresh-cut grass that had been fertilized with urea to give higher or lower nitrogen (N) contents (Table 1), and were or were not drenched 4 times daily with a 50:50 mix of dextrose and cornflour (starch) in water (Carruthers and Neil, 1997). The cows gave the expected ruminal ammonia responses: cows receiving the higher N grass or no carbohydrate supplement had greater ammonia concentrations than the opposing treatments (Table 2). This response was expected because ruminal degradation of a feed with greater protein content should give rise to greater production of ammonia, whereas increased amounts of available carbohydrate like that in sugar and starch allow microbes to capture the ammonia as microbial mass. Unexpectedly, the volatile fatty acid concentrations were greater on average, and 13 to 14 millimolar greater in later hours on the high N than low N grass (P<0.001), whereas carbohydrate supplementation only increased the concentration by 5 millimolar in later hours (P<0.05) (Table 2). There is potential for increased N fertilization of grasses to increase fiber digestibility, but the difference in organic acid concentrations found in this study is beyond what would be expected.

In another study investigating RDP by carbohydrate interactions, lactating cows were offered total mixed rations with greater or lesser concentrations of RDP and with more rapidly (high moisture corn) or more slowly (ground ear corn) available carbohydrate (Aldrich et al., 1993). Rumen pH of cows fed diets with high moisture corn were lower (6.28) when fed more RDP

| Table 1. Chemical composition (% of dry matter unless stated) of high (HN) and low (LN) nitrogen grasses (Carruthers and Neal, 1997). |
|-----------------|-----------------|
| **HN**          | **LN**          |
| Dry matter (% of fresh weight) | 15.7 | 17.6 |
| Organic matter  | 90.4 | 90.8 |
| Crude protein   | 17.6 | 13.2 |
| Soluble protein N | 0.15 | 0.12 |
| Soluble non-protein N | 0.66 | 0.51 |
| Neutral detergent fiber | 46.0 | 45.7 |
| Water-soluble carbohydrate | 22.0 | 27.1 |
Table 2. Average ruminal ammonia-N, pH, and total volatile fatty acid concentrations of dairy cows offered fresh cut grass of high (HN) and low (LN) nitrogen content with (+NSC) and without (-NSC) non-structural carbohydrate supplementation (Carruthers and Neal, 1997).

<table>
<thead>
<tr>
<th></th>
<th>HN</th>
<th>LN</th>
<th>p-values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>+NSC</td>
<td>-NSC</td>
<td>+NSC</td>
</tr>
<tr>
<td>NH3-N, mmol/L</td>
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<td>17.6</td>
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<td>pH</td>
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<td>6.11</td>
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<tr>
<td>Total VFA, mmol/L</td>
<td>136</td>
<td>132</td>
<td>126</td>
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As compared to less RDP (pH = 6.28 vs. 6.39, respectively, P<0.01). Cows fed more RDP also tended to have greater ruminal concentrations of organic acids (145.8 vs 137.1 mmol/L; P<0.08). In this study, there was no difference among protein treatments in organic matter digested ruminally, a change which could have altered organic acid production. In another lactating cow study, increasing dietary RDP gave greater average ruminal lactate concentrations, and tended to increase maximal lactate concentrations (Hall, 2012b).

Why Would This Happen?

Why would protein affect carbohydrate fates in the rumen? Protein may be having an effect by changing microbial energy demands (Figures 3 and 6). When added amino acids and protein increased microbial growth (Argyle and Baldwin, 1989; Figure 2), the total energy demands of the microbes also increased to support that growth. The microbes would shut down unnecessary processes that use ATP (Adenosine TriPhosphate, a major energy transfer molecule) to shunt the energy towards growth.

Storage of glycogen or energy spilling qualify as unnecessary processes when cell growth has higher priority. Storage of carbohydrate as glycogen costs 1 ATP per hexose (Stouthamer, 1973), which is 25 to 50% of the total ATP that bacteria may derive from the fermentation of a hexose (Russell and Wallace, 1988). Glycogen storage decreases with increased concentrations of breakdown products of ATP, which signal decreased energy status of the cell (Ball and Morell, 2003) and increased cellular demand for ATP. Energy spilling is the dissipation of “excess” ATP as heat; increased protein availability decreases energy spilling (Russell, 1993).

Figure 6. Effects of degradable protein or amino acids and peptide supplementation on ATP usage related to glycogen synthesis, flux of hexose through glycolysis, cell growth, and energy spilling. Nitrogen source effects on processes are designated as “+” (increase) or “-” (decrease). The effect on glycolysis is assumed based on the negative effect of N sources on glycogen synthesis and positive effect on lactic acid production. (Glc-6-P = glucose-6-phosphate). (M.B. Hall, unpublished).

When energy is not limiting, glycogen production or energy spilling utilize available ATP. These processes are apparently responsive to factors that affect energy status of the cell. Fundamentally, if cells have immediate need for energy, hexose is fermented and not stored as glycogen, if they do not, the storage of glycogen allows them to sequester substrate internally for future usage.

By shifting ATP use to production of microbial cells from glycogen synthesis or energy spilling, the efficiency of microbial growth per unit of carbohydrate fermented should be increased. An increase in the proportion of energy derived from carbohydrate that is used for growth would explain how the yield of microbes per unit of carbohydrate increased as more protein was provided (Argyle and Baldwin, 1989). To translate this increased microbial efficiency to increased amounts of microbial protein delivered to the cow, passage from the rumen would have to be such that more microbes flowed the small intestine than died and recycled in the rumen.

Thoughts On Application

The effect of ruminally degradable protein on fates of rapidly available carbohydrate seems to be a place where synchrony between protein and carbohydrate sources may give effects we can detect in cows. It also has potential to let us refine what we are doing...
with diet formulation to improve feed efficiency and manipulate the nutrient supply to better meet the animal’s needs. However, there’s more that needs to be sorted out regarding practical application of this concept. In the meantime, some thoughts to consider:

• Don’t overfeed total protein. If it’s the rapidly available carbohydrates that are affected, you should only need rapidly available protein relative to the amount of that carbohydrate.

• Does it need to be nonprotein nitrogen or amino acids + peptides? We don’t know, yet.

• Do you want glycogen, or organic acids + microbial protein? Hypothetically, if a herd is in a position where the corn has been in the silo all winter, the starch is fermenting more rapidly, and veering toward creating ruminal problems, would feeding less degradable protein help to slow down acid production in the rumen? It looks like it should; feeding some more NDF and/or effective fiber will also gain some margin of safety. Alternatively, greater efficiency of microbial growth with appropriately increasing the degradable protein could gain more microbial protein for the cow, given that passage rates supported that harvest.

• Rate of passage will decide whether the greater glycogen or microbial yield in the rumen ferments or recycles ruminally, or passes on to the small intestine where the cow can digest it and use the released glucose and amino acids. What are the options for changing rate of passage?

• No matter what you try, pay attention to the basics of good diet formulation.

References
Hall, M. B. 2012b. Corn source and dietary protein degradability: effects on ruminal measures and proposed mechanism for degradable protein effects. J. Dairy Sci. 95 (Suppl. 2): 613 (abstract).