Protein and Amino Acid Requirements of the Close-up Dry Cow

Robert J. Van Saun[1] and Charles J. Sniffen[2]

[1]Pennsylvania State University, Department of Veterinary & Biomedical Sciences, 115 Henning Building, University Park, PA,16802 [2]Fencrest, LLC, Holderness.
Email: rjv10@psu.edu

■ Take Home Messages

▸ Metabolic adaptations of maternal metabolism are essential to maintain nutrient availability in support of fetal development and lactation. Mobilization of body protein may be an underpinning issue leading to metabolic derangements and immune dysfunction and a greater risk of postpartum disease and impaired reproduction.

▸ Any process resulting in increased inflammation or excessive mobilization of body fat can lead to altered amino acid prioritization away from constituent protein metabolism resulting in exacerbation of protein mobilization and greater risk for disease, lost production or impaired reproduction.

▸ Close-up dry diet formulation needs to address meeting the rumen microbial population needs relative to energy and protein sources, which depends upon dietary forages and starch content, and the cow's additional needs to meet her amino acid requirements.

▸ Feeding higher amounts of metabolizable protein (MP) in dry diets may help to ensure adequate intake in the face of variable dry matter intake within a group. A concentration of 90–100 g MP/kg dry matter in the close-up diet is recommended to achieve at least 1100 g MP per day for a greater proportion of cows in a group.

▸ Research studies defining specific amino acid requirements in support of late pregnancy are limited and further research is required. A current body of research is suggesting methionine supplementation in late pregnancy may support improved health and immune status.

▪ Introduction

Dry cow nutrition and management more than 20 years ago was characterized as "management by neglect", a result of the lack of understanding we had on how nutritional management influenced all aspects of postpartum health, production and reproduction (Van Saun, 1991). Since the first description in the 1970's of "fat cow syndrome" and related energy-balance concerns, most dry cow research focused on energy metabolism and intake. Although higher protein supplementation to dry cows was suggested, subsequent research was equivocal in showing improved productive responses (Bell et al., 2000). Recent research is becoming more interested in transition cow protein needs based on documented body protein mobilization in support of early lactation (Grummer and Ordway, 2011). Anecdotal observations from the field would suggest dietary protein content, defined as metabolizable protein (MP), and possibly amino acid supply are having positive impacts on cow performance, but mainly from reproductive and metabolic health perspectives. The objective of this presentation is to address our current understanding of close-up dry cow protein requirements, provide perspective on transition protein metabolism and amino acid needs, and define practical feeding recommendations.

▪ Dry Cow Protein Requirement

Fetal growth from time of conception to birth can be described by an exponential growth curve with more than 70% of growth occurring in the last 60–70 days of pregnancy. This places the greatest nutritional burden of pregnancy on the close-up dry cow just weeks before parturition when there is potential for highly variable feed intake depending upon grouping strategies and feeding management.

Defining the Protein Requirement

The National Research Council (NRC) dairy and beef cattle publications over the past 60 years have defined and improved upon models to predict energy and protein requirements (Table 1) in support of pregnancy (NRC, 2001), though minimal differences are seen between reports due to a lack of data characterizing fetal protein requirements. Early NRC requirements were based on a 1950's extension publication and a 1956 study describing fetal growth in Danish Red cattle. The work of Bell et al. (1995) described growth characteristics for the modern day Holstein fetus and was incorporated into the most recent NRC model, though this model still did not totally account for all amino acid needs of the close-up dairy cow as it did not address mammary growth. The unknown factor in defining pregnancy protein needs is the amino acids needed to maintain labile protein reserves and their role in production, health and reproduction. All pregnancy requirement models are based on

research end points of milk yield or composition and do not address potential loss of body protein to support fetal requirements.

Table 1. Comparison of crude (CP) and metabolizable (MP) protein requirement models for a 650 kg mature cow at 270 days pregnant with a 45 kg birth weight calf.

	NRC 1989	NRC 2001	NRC 2001 Modified	CNCPS[a]/0.33
Maintenance				
Urinary, g/d	105	105	105	105
Scurf, g/d	15	15	15	15
MFN[b], g/d	410	338	338	338
Conceptus, g/d	212	355	355	480
Mammary, g/d	0	0	120 – 200	120-200
Total MP, g/d	742	813	933 – 1013	1058-1138
Crude Protein[c]	1060 g/d 8.2 – 9.6 %	1160 g/d 8.9 – 10.5 %	1332-1447 g/d 11.1 – 13.1 %	1511-1625 12.5 – 13.7%

[a] Cornell Net Carbohydrate and Protein System, ver. 3.0 with modification changing MP efficiency from 0.5 to 0.33
[b] Metabolic fecal nitrogen
[c] Assumed dry matter intake between 11 and 13 kg/d

Modeling Metabolizable Protein Requirement

Modeling gestational MP requirement is complicated as evidenced by model variation depicted in Figure 1. A proportion of the differences among these models is due to assumed efficiency of converting net protein (i.e., retained within the fetus) to MP (i.e., absorbed amino acids). Models prior to 1995 used an efficiency of 50%, whereas Bell (1995) summarized data suggesting efficiency was lower at 33%. This lower efficiency increases pregnancy MP requirement by 150%. Other challenges in predicting gestational protein requirements result from the dynamic metabolic functions of amino acids in supporting placental and uterine growth as well as the significant role amino acids play in fetal energy metabolism, none of which contribute to fetal protein retention, which is the measured end point. Another consideration is whether or not experimental diets were properly formulated to meet or exceed cow requirements to maintain a stable labile "reserve" protein pool in the cow. This is an underlying assumption of NRC models; maternal skeletal muscle is not used in support of pregnancy. McNeil et al. (1997) showed lamb birth weights were not different from ewes fed energy adequate diets with either 12% or 15% CP diets. Body compositional analysis, however, showed ewes fed the 12% CP diet (NRC requirement) had significant skeletal muscle protein loss accounting for the lack of difference in birth weights. Ewes fed the 15% CP diet had significant skeletal muscle accretion suggesting these ewes may be better positioned metabolically to adapt to negative energy balance and mobilize amino acids to support lactation. Could this situation account for the

greater rate of metabolic disease experienced by cows delivering twins in that the cow would mobilize her body protein to support the additional fetal mass with twins? Cows in the Bell et al. (1995) study consumed 10–12 kg dry matter of a total mixed ration (TMR) containing 13% and 14% (after 250 days gestation) CP. No measure of maternal protein status was determined in this study. So does the lower MP efficiency observed by Bell account for mobilization of maternal body protein?

For demonstration purposes we used the original Cornell Net Carbohydrate and Protein System (CNCPS, version 3.0) mechanistic model to predict gestational protein requirement, which accounted for an amino acid energy contribution, to predict MP requirement using an efficiency factor of 0.33 rather than the original 0.5 factor. From Figure 1 it can be seen this model greatly increases MP needs throughout gestation compared to other models. Additionally MP required to support mammary development (120-200 g MP/day) would need to be added to this model (Bell, 1995). More importantly this model shows MP needs before the 190 day cutoff used by NRC based on data extrapolation limitations. This exercise is hypothetical, but intriguing relative to potential implications for gestational MP requirements as well as explaining possible roles for amino acid nutritive status relative to health (i.e., immunologic and metabolic), productive, and reproductive outcomes during transition. This hypothetical model could potentially explain the observed positive cow responses in the field when additional protein is fed in close-up dry cow diets. Whether the response is due to higher protein requirement or meeting a specific amino acid need remains to be determined.
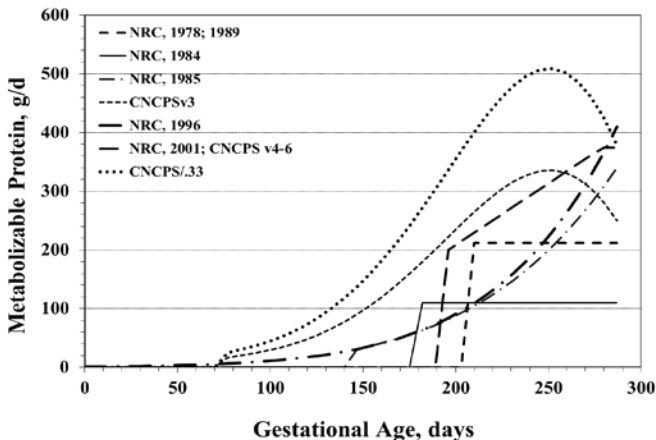

Figure 1. Different models predicting metabolizable protein (MP) requirement in support of pregnancy (45 kg birth weight) in Holstein cows (From Van Saun and Sniffen, 2014).

▪ Pregnancy Protein Metabolism

Much emphasis has been placed on energy metabolism and markers of energy balance as underpinning metabolic disturbances of transition and risk for disease. Although elevated concentrations of either nonesterified fatty acids (NEFA) or β-hydroxybutyrate (BHB) are highly associated with disease risk, their presence is not an absolute determinant. A population of cows can perform without evidence of disease with elevated concentrations of NEFA suggesting some other factor or protective element. As our understanding of transition metabolism sheds more light on its complicated nature, a more integrated perspective on transition metabolism is needed and central to this is the supply and prioritization of amino acid metabolism as it relates to cow response to diet and management. Although the body of published literature does not strongly suggest improved cow performance with greater prepartum dietary protein, there is much interest and anecdotal observations suggesting benefits from feeding diets delivering greater MP (>1100 g/day) than models would suggest is necessary to meet the cow's amino acid requirements. This observed response may be due to an underestimation of the MP requirement, providing an essential amino acid or acids, accounting for intake variability within a group allowing for adequate MP intake for cows with lower intake, or some combination of these factors.

Most studies evaluating prepartum protein nutrition essentially looked at milk yield or composition as metrics for a measured response (Bell et al., 2000). Most observations and research would suggest the primary benefit of prepartum protein feeding comes from disease prevention and improved reproductive performance. Curtis et al. (1985) reported higher prepartum protein diets decreased incidence of ketosis. Van Saun (1993) also reported lower clinical ketosis prevalence for mature Holstein cows fed 1350 g MP/day compared to cows fed 1100 g/d. In this study, all cows maintained a higher body condition score (mean 3.9 at calving), thus were more predisposed to ketosis problems. Using 3-methylhistidine as a marker of skeletal muscle degradation, van der Drift et al. (2012) showed muscle mobilization occurring prepartum through 4 weeks postpartum for dry cows fed a diet composed of grass silage and corn silage containing approximately 12.6% crude protein. Cows having higher 3-methylhistidine concentrations generally had lower BHB concentrations, suggesting a protective effect. Cows with extreme hyperketonemia had excessive muscle and fat mobilization, which could be detrimental to health and reproduction. Philips et al. (2003) showed supplementing methionine prepartum may mitigate body protein mobilization, possibly suggesting a higher amino acid requirement.

Mobilized protein from skeletal muscle and involuting uterine tissue provides a primary source of amino acids to the mammary gland to support milk protein synthesis. Lower milk protein content may reflect inadequate dietary MP

supply and repartitioning of amino acids to support the immune response or gluconeogenesis. In reviewing lactation performance across many herds, cows with low milk true protein (<2.7%) on first or second test day had lower first service and overall conception risks. Cows consuming more MP prepartum (>1350 g/d) had improved reproductive performance, and ovulation time was not influenced by negative energy balance nadir. In contrast, cows consuming lower prepartum MP intake (1100 g/d) followed by a postpartum diet high in RDP had their first ovulation time highly correlated with negative energy balance nadir (Van Saun, 1993). Availability of amino acids may be a critical factor in early follicular development and ultimately conception risk.

Unfortunately there is no single simple blood parameter that reflects protein status such as NEFA or BHB relative to energy status. Blood albumin concentration has been used as a proxy for protein status. Albumin concentration reflects dietary amino acid supply and metabolic responses repartitioning available amino acids. Increasing dietary protein in early lactation increased albumin concentration. Albumin is synthesized in the liver and is considered a negative acute phase protein meaning its rate of synthesis is decreased during an acute phase response to inflammatory cytokines (Bertoni et al., 2008). Albumin concentration pre- and postpartum was associated with greater risk for postpartum disease. Blood albumin concentration ≥35 g/L was found in primarily healthy fresh cows compared to lower concentrations being predominately associated with fresh cows having one or more disease events. Lower albumin concentration may reflect inadequate dietary MP supply, liver dysfunction, an active inflammatory response, or some combination, and may provide a marker of transition cow health status (Overton and Burhans, 2013).

It is our assessment that amino acids play a critical role in "stabilizing" metabolism of carbohydrates and lipids during transition as well as supplying substrate for tissue protein synthesis, gluconeogenesis, and other metabolic mediators. All cows experience a period of negative protein balance in early lactation that seems somewhat independent of prepartum protein feeding. If dietary protein is sufficiently deficient prepartum, however, tissue protein mobilization may occur and the reservoir of labile protein to be utilized in early lactation may be compromised resulting in greater risk for impaired health, productive efficiency, and reproductive performance (Ji and Dann, 2013).

Role of Inflammation on Protein Metabolism

A growing body of research is recognizing an association between the activated inflammatory response mediated by pro-inflammatory cytokines interleukin (IL)-1, IL-6, and Tumor Necrosis Factor (TNF)-α and altered metabolism leading to greater disease risk, poor production, and impaired reproduction (Bertoni et al., 2008; Bradford, 2015). Pro-inflammatory

cytokines can be released from adipose tissue during mobilization as well as from any stress response. Hepatic activation by these cytokines initiates the acute phase protein response resulting in up-regulated synthesis of positive acute phase proteins (+APP; i.e., ceruloplasmin, haptoglobin, serum amyloid-A, C-reactive protein, complement components) as well as enzymes and other physiologic mediators. Both IL-1 and TNF-α have profound metabolic effects promoting an increased basal metabolic rate to produce fever in concert with reducing appetite. Reduced appetite in the transition cow is a recognized lynchpin to metabolic disease susceptibility. Mobilized skeletal muscle provides amino acids to support gluconeogenesis in maintaining the higher basal metabolic rate. This response is in an effort to promote the immune response in responding to some pathogen or stressor, but is quite costly nutritionally to the animal.

Mobilization of skeletal muscle will further exacerbate negative protein balance in early lactation and may account for the predilection for more than one disease process once one has been established (Ji and Dann, 2013). In addition to mobilization of skeletal muscle, constitutive proteins synthesized by the liver, such as albumin, retinol binding protein, apoproteins, and transferrin (e.g., negative acute phase proteins, -APP) are not synthesized, most likely to further provide amino acids to support the acute phase protein response (Bertoni et al., 2008). Reduction of these constitutive proteins may adversely affect mineral and vitamin metabolism through the loss of transport proteins. Additionally, loss of apoproteins would reduce the liver's ability to synthesize very low density lipoproteins and potentially increase fatty infiltration in the face of elevated NEFA concentrations. An activated immune response is necessary during transition to deal with uterine clearance and protection from potential mastitis pathogens, but excessive stimulation of this response through environmental, social, or dietary factors will predispose to poor transition cow performance.

▪ Amino Acid Requirements and Supply

As our understanding of nutrient requirements increases there is a natural evolution of defining "protein" requirements from nitrogen-based crude protein (i.e., N x 6.25) to MP to finally defining specific amino acids, which are the actual substrates needed by the cow. Poultry and swine nutritionists have made this progression in protein requirements where they formulate for specific amino acids to achieve the "ideal protein" in the diet. At this point in dairy cattle requirements we have not defined specific amino acid requirements.

A body of research is focusing on supplemental methionine, an essential amino acid, as a critical amino acid for transition cows. The work of the Illinois team has placed emphasis on the requirement for methionine in the close-up ration. Their results have demonstrated that there is a need relative to lipid

mobilization and immune function, which has resulted in production responses (Osorio et al., 2014). The question one needs to ask is: are there essential amino acids required beyond methionine, such as the branch chain amino acids, histidine, arginine and proline (Phillips et. al., 2003)? There is evidence from work done that lysine may be essential as well. Summarized studies using milk protein yield or percent as an endpoint suggest requirements of 25 g/d and 75 g/d for methionine and lysine, respectively (French, 2012). Further, should we think beyond this to consider the gluconeogenic amino acids as well? Bell (1995) has shown that there could be a significant requirement for gluconeogenic amino acids to meet the mammary, placental and fetal requirements for energy during the late gestation period. Additionally, Bell (1995) points out there is a significant increase in the requirement for hepatic protein synthesis, which in the last 2 weeks before birth is accelerated. The acceleration is driven by increased mammary requirements, increased demand for liver size, and the high requirements of nutrient fluxes through the liver to deal with increased metabolic requirements. This becomes particularly critical as we reduce the energy provided during both early and the close-up period, which will reduce the supply of propionate but also reduces microbial yield that provides essential amino acids as well as the non-essential amino acids. Larsen and Kristensen (2012) looked at amino acid net fluxes using arterial-venous differences coupled with blood flow, prepartum and postpartum using glucogenic and ketogenic diets. They demonstrated net negative hepatic fluxes prepartum of non-essential amino acids, lending credence to the importance of adequate non-essential AA as well as essential AA. Recent work by Penn State has shown histidine to be limiting in diets where rumen microbial growth accounts for the majority of MP needs (Lee et al., 2012). Most amino acid work has focused on milk yield or composition and during early to mid-lactation. Whether or not the dry dairy cow fits into the models predicting amino acid flow remains to be seen. Some of the current study analyses would suggest amino acid content of prepartum and postpartum diets are not independent of each other and one cannot make up for prepartum deficiencies with a better balanced postpartum diet. A recent study infusing casein into early lactation cows has shown the critical importance of MP, especially essential amino acids, in supporting milk yield and composition as well as immune function (Larsen et al., 2014). The protein effect was over and above any energy deficiency.

▪ Meeting the "Protein" Requirements

Dry cow protein nutrition has been misunderstood and is still a somewhat unknown area of investigation. Controlled studies in this area have many times been confounded by the method of balancing to meet the pregnant cow protein requirement. The NRC recommendations for protein supply were based on research that unfortunately was limited and experimental rations were often formulated inappropriately providing wrong conclusions. Further,

the recommendations did not recognize the importance of the mammary requirement and protein reserves. The CNCPS system now recognizes the importance of both; however, it does not recognize the importance of labile protein reserves relative to immune function as well as the need in the early postpartum period when cows can mobilize 800 to 1000 g/day (Bell et al., 2000). This puts greater emphasis on the maintenance of labile protein reserves in the last 60 to 80 days of gestation. This is a period in late lactation and during the dry period when lower energy rations are being fed, reducing microbial protein output and MP balance can easily become negative, especially with hay-crop silage based diets. Field observations would suggest there is a need to exceed the NRC (2001) recommendations for protein and meet and not exceed the ME requirements. Coupling this with variation in dry matter intake (DMI) within a group of cows being fed a balanced ration, dictates that there be an adequate concentration of MP in the rations being fed during this time in order to ensure that all cows will be able to maintain the protein reserves that were replenished in mid-lactation. Additionally recent work has suggested that protein quality may be important as well. This would suggest it is important to pay attention to source as well as amount of MP.

With current understanding of dairy cow feeding, we need to consider the close-up dry cow diet formulation process in two stages: 1. feeding the rumen to generate microbial mass, a significant contributor to MP, and 2. feeding the cow over and above what nutrients are not provided by rumen outflow. This approach is no different than how we formulate lactating cow diets. The only issue here is whether or not the dry cow rumen dynamics fit the predictive model between dietary fermentable energy and microbial growth. Microbial growth is dictated by availability of fermentable carbohydrate and with the growing application of low energy diets does this suggest lesser microbial growth and greater need for bypass protein sources to meet the cow's amino acid needs (Kokkonen, 2014)? Rumen fiber fermentation is dependent upon availability of rumen degradable protein, thus a minimum dietary protein content of 11-12% is needed to ensure microbial fiber degradation, which is above what NRC requirements would suggest for the dry cow diet protein content (Dorshorst and Grummer, 2002).

Accurately Defining the Cow

The definition of dry cow requirements is based on carefully defining first the dry cow group that is to be fed. We often get into the mode of using one set of numbers. This is inappropriate. The impetus of defining the animal correctly is to ensure the diet will provide sufficient nutrients to all individuals within the group. The question is whether the description should be the average for the group or the upper level? For example, if average calf birth weight is 42 kg, what happens to those cows delivering a 45 or 48 kg calf? Expected birth weight can significantly influence nutritional requirements. The biggest

challenge occurs in those mixed groups of springing heifers and mature cows. Obviously some animals will be overfed, but we want to minimize the underfeeding variation.

Ensuring Adequate Nutrient Intake

One of the primary challenges of dry cow group management is formulating the diet for an appropriate intake level. Even if one provides a balanced diet for a defined average intake for a given feeding group, 50% of the animals in the group consume less than the average intake. French (2012) presented summarized prepartum intake data from Phillips et al. (2003) for multiparous Holstein cows. In this analysis the average DMI was 12.3 ± 2.5 kg/d for the last 21 days precalving with 15% of the cows consuming less than 10 kg/d (1 standard deviation below group average) and being in a state of negative nutrient balance. A recommendation from this analysis was to formulate the close-up dry diet to 1300 g or 1400 g MP as a safety factor to ensure adequate numbers of cows, 83% or 95%, respectively, consume a desired 1,080 g MP from the diet.

In another multiparous cow dataset, 21 day prepartum DMI was 13.5 ± 2.6 kg/d (Van Saun, 1993). In this study, prepartum diets differed in MP content (1100 vs. 1350 g/d) but DMI was not different across treatments. The cows consuming the higher MP diet had less metabolic disease and improved reproductive performance compared to the lower MP diet. These results would seemingly support the concept promoted by French, though a higher MP requirement is not out of consideration in explaining such responses. Clearly, large variation (higher standard deviation) of DMI within a group will result in more cows, and especially heifers in mixed groups, having lower intake and potentially experiencing a negative MP balance. These two datasets would suggest formulating a close-up diet to contain between 90 and 100 g MP/kg dry matter, which would provide at least 1000-1100 g MP for those lower intake cows within the group.

▪ Conclusions

Observational performance on farms would suggest protein content and source in the close-up dry cow diet is a critical factor in ensuring cows transition smoothly into lactation and have good health with unimpaired productive and reproductive performance. We still have gaps in our understanding of amino acid metabolism and requirements in late pregnancy and how this may be influenced by diet composition, namely carbohydrate fractions. Improved descriptions of close-up dry cows relative to expected calf birth weight, body weight and condition score as well as accounting for parity differences can improve our dietary formulations for an optimum MP requirement. Adjusting dietary MP content to account for variability in group

feed intake is a critical factor in ensuring a greater majority of the individuals within the group will consume a minimum of 1,000 g MP/day. We recommend formulating the close-up dry cow diet to contain 90-100 g MP/kg to meet MP needs of the greater proportion of the group.

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