Is Protein the Missing Piece of the Transition Metabolism Puzzle?

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Introduction

For a cow to transition from late pregnancy into lactation successfully, she needs to exquisitely coordinate metabolism in multiple tissues to ultimately provide sufficient glucose to support productive needs.[1] Daily requirements for glucose, amino acids, fatty acids and calcium for an early lactation cow (4 days postpartum; 30 kg milk, 4.7% fat and 4.2% protein) are 2.7, 2.0, 4.5 and 6.8 times greater, respectively, than those needed for pregnancy.[2] These differences represent changes in nutrient requirements over a period of only 1-to-2 weeks and occur during a period of lowest dry matter intake; highlighting the tremendous metabolic alterations necessary to adequately support lactation. The inability of the cow to metabolically adapt to this transition is the underpinning of most postpartum diseases. The late gestation diet has been shown to play a critical role in modulating a cow's predisposition to periparturient health disorders, though the role of protein remains elusive. The objective of the presentation is to review protein metabolism during transition and its influence on metabolic response relative to cow health, performance and reproduction. Other non-nutritional factors affecting the cow's ability to consume sufficient nutrients also contributes, and may contribute more significantly, to postpartum disease susceptibility. Nuances of how management and environment alter stability of these metabolic changes remains to be highlighted.

Protein Metabolism in Transition

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 NEFA or BHB have been highly associated with disease risk, their presence is not a direct 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 (Table 1). Although the body of published literature does not strongly suggest improved cow performance with higher prepartum dietary protein, there is much interest and anecdotal observations suggesting benefits from feeding diets delivering greater metabolizable protein (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.[3] Most observations and research would suggest the primary benefit of prepartum protein feeding comes from disease prevention and improved reproductive performance.[4,5] Curtis first reported higher prepartum protein diets decreased incidence of ketosis.[6] Cows fed a higher protein prepartum diet, independent of energy content, had lower serum NEFA concentration, NEFA to cholesterol ratio, and fatty liver score.[7] Van Saun also reported lower clinical ketosis prevalence for mature Holstein cows fed 1300 g MP/day compared to cows fed 1100 g/d.[8] In this study all cows maintained a higher body condition score (mean 3.87 BCS at calving), thus were more predisposed to ketosis problems. Using 3-methylhistidine (3-MH) as a marker of skeletal muscle degradation, van der Drift and colleagues 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.[9] Cows having higher 3-MH concentrations generally had lower BHB concentrations, suggesting a protective effect. Cows with extreme hyperketonemia had excessive muscle and fat mobilization. Amount of muscle mobilization was highly variable among cows in this study, though supplementing methionine may mitigate body protein mobilization.[10]

Table 1. Comparison of fetal and maternal protein sources and metabolic adaptations during transition and association with deficiency disease risks.[a,b]

Protein	Fetal	Maternal
Supply	Placental active transport of amino acids from maternal blood supply (70-80% of maternal circulating amino acids)	Microbial and dietary bypass proteins provide the metabolic source of amino acids to support body functions; mobilization of labile proteins (blood proteins, skeletal muscle) can make up for dietary deficiencies
Metabolism	Amino acids deposited into fetal tissues and placenta to support growth, oxidized for energy in fetus, amino acid exchange between fetus and placenta	Amino acids used to support body protein turnover and new protein synthesis; Up-regulation of many metabolic regulatory enzymes in support of gluconeogenesis, lipid metabolism, milk protein synthesis; Amino acids serve as intermediates in TCA cycle facilitating CHO and lipid metabolism; Amino acids may be redirected toward acute phase protein synthesis in face of inflammatory response
Disease Risks	Reduced birth weight, Inability to thermoregulate, weak calf syndrome, FPT?	Subclinical and clinical ketosis; Varying degrees of hepatic lipidosis; Reduced blood transport proteins for minerals and vitamins; Impaired immune response with increased infectious disease susceptibility, RFM, Udder edema?

[a]Abbreviations: FPT = failure of passive transfer; RFM = retained fetal membranes
[b]Adapted from Van Saun R, Sniffen CJ. Vet Clinics NA: Food Anim Pract 2014; 30:689-719.

Feeding additional rumen undegraded protein (RUP) prepartum showed improved insulin status in mature dairy cows relative to BCS and BCS score change.[11] Additionally cows fed a balanced protein diet postpartum compared to a high rumen degraded protein (RDP) diet had higher insulin sensitivity across body condition scores.[12] Insulin not only is an important regulator of glucose homeostasis, but also influences reproductive performance. Cows consuming more MP prepartum (>1300 g/d) had improved reproductive performance[8] and ovulation time was not influenced by negative energy balance nadir.[13] 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.[8]

Using production data from 55,000 lactations it was found milk protein and milk fat-to-protein ratio in early lactation were associated with reproductive performance.[14,15] Cows with low milk protein on first or second test day had lower first service and overall conception risks. Mobilized protein from skeletal muscle and involuting uterine tissue provide a primary source of amino acids to the mammary gland to support milk protein synthesis.[16] Lower milk protein content may reflect inadequate dietary MP supply and repartitioning of amino acids to support the immune response or gluconeogenesis.

Blood albumin concentration reflects dietary amino acid supply and metabolic responses repartitioning available amino acids. Increasing dietary protein in early lactation increased albumin concentration.[17] 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.[18] Albumin concentration pre- and postpartum was associated with greater risk for postpartum disease.[19] Blood albumin concentration of 3.5 g/dL or greater was found in primarily healthy cows compared to lower concentrations being predominately associated with cows having one or more disease events.[19] Cows experiencing endometritis postpartum had lower prepartum albumin concentration.[20] Lower prepartum albumin concentrations were observed in pasture-fed cows consuming a high (31.8%) nonfiber carbohydrate (NFC) compared to low (13.2%) NFC diet.[21] 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.[22]

Role of Inflammation in Metabolic Regulation

A growing body of research is recognizing an association between the activated inflammatory response mediated by proinflammatory 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.[18,23-28] Proinflammatory cytokines can be released from adipose tissue during mobilization as well as from any stress response.[24] 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 (BMR) 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 BMR. 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.[29]

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.[30] 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. 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 (VLDL) 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.

Gestational Protein Requirement

Modeling gestational protein requirements is much more complicated as evidenced by model variation depicted in recent NRC publications. A proportion of the differences among these models is due to assumed efficiency of converting MP (i.e., absorbed amino acids) to net protein (i.e., retained within the fetus). Models prior to 1995 used an efficiency of 50%;[31] whereas Bell summarized data suggesting this efficiency was much lower at 33%.[2] This difference in 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 that is the measured end point.[2] 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 and colleagues showed lamb birth weights not be different from ewes fed energy adequate diets with either 12% or 15% crude protein (CP) diets.[32] Body compositional analysis; however, showed ewes fed the 12% CP diet had significant skeletal muscle protein loss accounting for the indifference 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. Cows in the Bell et al., study consumed 10-12 kg dry matter of a total mixed ration (TMR) containing 13% and 14% (after 250 days gestation) CP diets.[33] No measure of maternal protein status was determined in this study. The CNCPS system now recognizes the importance of mammary growth and protein reserves; however, it does not recognize the importance to 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. 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.

ENSURING ADEQUATE PROTEIN 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, statistics tell us 50% of the animals in the group consume less than the average intake. French presented summarized prepartum intake data from Phillips et al.[10] for multiparous Holstein cows.[34] In this analysis the average DM intake 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 83% or 95%, respectively, of the cows consume a desired 1,080 g MP from the diet.

In another multiparous cow dataset, 21 day prepartum intake was 13.5 ± 2.6 kg/d.[8] 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 negative protein balance.

Summary

Protein nutrition of the dry cow has been misunderstood and is still a controversial area of investigation. The controlled studies in this area have many times been confounded by the method of balancing to meet the protein requirement of the pregnant cow. The NRC recommendations for protein supply were based on research that unfortunately was limited and the experimental rations were formulated incorrectly providing wrong conclusions. Further the recommendations did not recognize the importance of the mammary requirement and the protein reserves. Field observations would suggest that there is a need to exceed the NRC 2001 recommendations for protein and meet and not exceed the ME requirements. Couple this with variation in 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 that it is important to pay attention to the source of MP as well as the amount of MP. All cows experience a period of negative protein balance in early lactation that seems somewhat independent of prepartum protein feeding. However, if dietary protein is sufficiently deficient prepartum 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.

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