

NEGATIVE PROTEIN BALANCE: IMPLICATIONS FOR TRANSITION COWS

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INTRODUCTION

Traditionally, diets for lactating cows were formulated and fed to contain a high crude protein (CP) content to maximize milk and milk protein yields while providing a safety factor due to uncertainty of feed composition to ensure that a cow's requirements for limiting amino acids (AA) were met. This approach was viable with relatively cheap protein feeds and little concern about nitrogen (N) excretion to the environment. Today, excessive protein feeding is increasing feed costs, decreasing efficiency of N utilization, and increasing N loss to the environment. Thus, use of lower CP diets to reduce economic and environmental costs of feeding excess N is increasing (Chase et al., 2009). There appears to be an opportunity to reduce dietary CP by 0.5 to 1.5 units while maintaining metabolizable protein (MP) supply on a herd basis with minimal risk of lower milk production (Chase et al., 2009). Given the negative MP balance that occurs following parturition, it is unclear if lower protein diets can be used successfully in very early lactation.

NEGATIVE PROTEIN BALANCE

Formulating diets to meet the cow's requirement for MP in early lactation is challenging. The cow has an inability to consume sufficient protein to meet the mammary and non-mammary AA requirements. Generally negative protein balance is not considered as big of a concern as negative energy balance in early lactation. However, when cows are deficient in MP, they will breakdown muscle and other protein sources in the body. Within the first 5 to 6 wk postpartum, protein mobilization of labile protein reserves ranged from 8 to 21 kg (Komaragiri and Erdman, 1997; Komaragiri et al., 1998; Phillips et al., 2003; Chibisa et al., 2008). Cornell researchers (Bell et al., 2000; Burhans and Bell, 1998) calculated the MP balance of 80 Holstein cows during the first 28 d postpartum and found that the nadir was -600 g/d at 7 d postpartum. Cows returned to positive protein balance during wk 3 postpartum. Bell et al. (2000) suggested that during the first 7 to 10 d postpartum, high producing cows may need to mobilize up to 1000 g of tissue protein per day to satisfy the mammary gland's demand for amino acids and glucose. The liver's ability to convert glucogenic amino acids, in particular alanine, to glucose is increased tremendously immediately postpartum (Overton et al., 1998). Although protein mobilization is necessary to contribute to the insufficient supply of energy and protein, excessive mobilization increases the risk of metabolic disorders, immune dysfunction, and poor lactational and reproductive performance. Lower CP diets may increase the severity and duration of negative protein balance.

PROTEIN MOBILIZATION DURING EARLY LACTATION

Protein mobilization from endogenous tissues (e.g. skeletal muscle and visceral tissue) is an essential source to provide AA for milk protein synthesis and hepatic gluconeogenesis during early lactation of high producing cows when negative energy and protein balances generally occur (Bell et al., 2000). The breakdown of muscle protein releases 3-methylhistidine (3-MH; methylated AA that forms in actin and myosin) that can serve as a marker to characterize mobilization of muscle protein. Increasing dietary protein during late gestation increased N retention in maternal tissues of cows (Putnam and Varga, 1998). Thus, the size of maternal protein reserves may affect protein mobilization during early lactation. Vandeharr et al. (1999) found the ratio of urinary N-MH:creatinine was increased during the last 2 wk prepartum as the concentration of both energy (1.30 vs. 1.61 Mcal NE_L/kg) and protein (12.2 vs. 15.9% CP; NDS estimated 721 vs. 1172 g MP/d) in the prepartum diet was increased but not when protein alone was increased. Increased muscle protein degradation can occur when animals are fed diets that result in increased muscle protein synthesis which was likely the case when cows were fed higher energy and protein but not protein alone (Vandeharr et al., 1999). The ratio of N-NH:creatinine was higher postpartum, but was not affected by prepartum nutrition. Similarly, Doepel et al. (2002) observed that cows that received a diet with lower CP (12.5% CP, NDS estimated 1070 g MP/d) during the close-up period had plasma 3-MH peak at calving compared with wk 1 postpartum in cows fed a higher CP (17.5% CP, NDS estimated MP supply ranged 1400 to 1600 g/d). However, treatment did not affect plasma 3-MH postpartum. Other studies investigated the effect of prepartum dietary protein on postpartum protein mobilization and found similar results (Phillips et al., 2003). Therefore, it seems that the effect of prepartal plane of dietary protein on protein mobilization did not carry over to postpartum.

In a recent study (van der Drift et al., 2012), a large variation was observed among cows in the onset and duration of protein and energy mobilization during the transition period. Based on plasma 3-MH concentrations and muscle thickness profiles, protein mobilization started before calving and continued until week 4 of lactation. Interestingly, protein mobilization occurred before lipid mobilization in most cows. The authors speculated that it might be due to a prepartum AA deficiency in the absence of negative energy balance. In addition, it appears that the timing of protein mobilization is related to hyperketonemia. Cows with lower 3-MH concentrations had higher serum β -hydroxybutyrate concentrations. Greater protein mobilization to a certain extent after calving may provide AA for gluconeogenesis and limit ketone body production. However, this hypothesis needs to be confirmed with additional research. In a review of *in vivo* studies, Larsen and Kristensen (2013) estimated that glucogenic AA derived from muscle proteolysis were primarily utilized for milk protein synthesis and challenged the dogma that AA are significant contributors to liver gluconeogenesis in early lactation.

During early lactation, cows fed higher protein (20.9 vs. 17.3% CP; estimated 2348 vs. 2802 g MP/d) mobilized a similar amount of body protein as those fed lower protein, but produced numerically more milk with a significantly higher protein content (Komaragiri and Erdman (1997). It appeared that a greater MP supply was used for milk

production than sparing muscle protein reserves. In support, Doepel et al. (2009) concluded that in early lactation, metabolic priority is given to direct AA toward milk protein production rather than gluconeogenesis. Increased removal of lactate, rather than AA, contributed to the additional hepatic gluconeogenesis. Chibisa et al. (2008) supplemented cows with 600 g/d of propylene glycol from -7 to 45 d postpartum and found a tendency for a reduction in mRNA expression of genes associated with proteolysis on d 15, but no significant differences regarding body protein loss, N balance, and excretion of urinary 3-MH. The propylene glycol supplementation and resulting AA appeared to be used milk protein synthesis. Recently, Pires et al. (2012) reported that cows with low BCS (< 3.0) at parturition produced less milk protein than cows with medium (3.2 – 3.8) or high (> 4.0) BCS and had more intensive breakdown of body protein.

IMMUNE FUNCTION AND PROTEIN STATUS

High producing cows generally experience a period of immunosuppression peripartum. Several lines of evidence indicated that protein malnutrition can impair immune function and increase the risk of disease in other species. However, there is limited research regarding the potential effect of periparturient MP deficiency on immune function in dairy cows. In addition, nutritional models do not adequately account for the nutrient demands of maintaining a functional immune system. The potential protein and AA requirements for mounting an immune response could include recruitment and proliferation of immune cells, production of cytokines and antibodies, and effector molecules (e.g. nitric oxide). For example, the plasma concentration of TNF α was increased 50 to 120 folds in cows after 2 h of intravenous lipopolysaccharide (LPS) infusion compared with that of non-challenged group (Waldron et al., 2003). Moreover, an increase of somatic cells from 5×10^4 to approximately 1300×10^4 was observed in the udder of cows after an intramammary LPS challenge (Waldron et al., 2006). Klasing (1998) challenged that the amount of nutrients needed by the immune system is relatively minimal compared with requirement for daily production and growth. However, such an estimate neglected the possibility that requirements of certain nutrients (e.g. AA) are not equivalent to what is provided by feed or mobilized from endogenous tissues, which necessitates the nutrient wasting that was not taken into account in the calculation. For instance, glutamine (Gln, together with glucose) was highly preferred source of energy by immune cells, provides precursors for synthesis of cell DNA and RNA (Calder and Yaqoob, 1999) and intracellular glutathione, which is related with lymphocyte proliferation (Roth et al., 2002) and phagocytic activity and secretion of IL-1 of macrophage (Calder and Yaqoob, 1999). Requirement for Gln during an immune response is highly enriched compared with other AA. However, the concentration of Gln in both plasma and muscle of high producing cows had the largest decrease compared with other AA during early lactation (Meijer et al., 1995). Unlike most other AA that restored within 3 wk after parturition, Gln concentration was maintained at lower concentration for the first 15 wk of lactation (Meijer et al., 1995). Thus, Gln requirement for immune activation during early lactation probably added more burden to breakdown of muscle tissue. In addition, the acute phase response that is characterized as increased hepatic production of acute phase protein (APP), elevated

protein turnover rate, muscle wasting, whole body metabolic rate, and hepatic gluconeogenesis, was a more intensive proteinaceous process than activation of immune cells per se (Klasing, 1998). During an inflammatory response, plasma APP concentration can increase up to 100 fold. The acute phase response is accompanied by anorexia in most species, thus endogenous protein reserve is more readily mobilized. Furthermore, Reeds et al. (1994) reported the dis-match of AA composition of skeletal muscle compared with APP, which indicated over mobilization of body protein in order to satisfy the most demanding AA for synthesis of APP. Finally, restore of damaged tissue and labile protein reserve during the resolution period of infection or inflammation adds more to the overall protein cost. Energy and nutrient repartitioning from production serves as an important protective mechanism to fight infection when feed intake is depressed (Waldron et al., 2006).

Supply of MP appears to play a proactive role to enhance disease resistance and should be accounted for in ration formulation. A series of studies focused on periparturient relaxation of immunity to gastrointestinal nematode parasites (e.g. *Teladorsagia circumcincta*, *Nippostrongylus brasiliensis*) in ruminant (i.e. ewe) and rodent models have demonstrated that increasing dietary protein supply above requirement can improve immune responses and reduce infections (Houdijk et al., 2001ab ; Houdijk et al., 2005; Jones et al., 2011). In ewes, the reduced infections were attributed to improved immunity both locally and systemically as represented by greater abomasal globule leukocytes and IgE anti-larvae antibodies, respectively (Houdijk et al., 2005). In another ewe study (Houdijk et al., 2001a), ewes fed to maintain body protein reserve, rather than body fat reserve, during mid-pregnancy had better immunity to parasite infection during the periparturient period by shedding less fecal eggs than those that lost protein reserve during pregnancy. Increasing MP supply by 10% was proposed to be sufficient to overcome the infection (Houdijk et al., 2001b). Jones et al. (2011) used rats as an infection model and fed 6 diets with a combination of 4 levels of dietary CP and energy starting at parturition. Interestingly, increased diet protein supply, but not energy supply, significantly reduced worm burden, which may have been due to increased accumulation of mucosal mast cells and eosinophils in the small intestine. This is an excellent model demonstrated that immune integrity should be taken into account of MP budget at ration formulation. Recently, Osorio et al. (2013) reported that cows that received rumen-protected methionine from -3 to +3 wk postpartum tended to have greater whole-blood leukocyte phagocytotic activity at 21 d postpartum. Supply of MP for control and methionine supplemented groups was ~1200 g/d prepartum and the magnitude of negative protein balance was similar between treatments postpartum.

PROTEIN NUTRITION DURING THE TRANSITION PERIOD

The notion that protein nutrition of the dry cow influences lactation performance and health is widespread (Lean and Van Saun, 2013). The classic paper of Curtis et al. (1985) suggested that higher protein intake prepartum may reduce the incidence of metabolic and reproductive disorders postpartum. Many studies have focused on the CP, rumen degradable protein (RDP), or rumen undegradable protein (RUP) needs of dry cows with several studies finding little response in milk yield, milk protein content, or milk yield in the subsequent lactation (Bell et al., 2000; Lean and Van Saun, 2013). The

lack of response may be due to a poor or variable relationship between CP intake and MP that is impacted by the carbohydrate fermentability, protein fractions, and AA profile of the diet. Recommendations for MP for mature dry cows are typically in the 1000 to 1200 g/d range. Diets that are lower in fermentable carbohydrates, in particular starch, may need to be supplemented with RUP (e.g. protected soy products) to provide the appropriate amount of MP and the proper AA profile.

Several studies (Table 1) provided enough dietary and experimental information to estimate MP supply with the Nutritional Dynamic System (NDS v3) based on the CNCPS model. The median of estimated MP supply prepartum was 1078 g/d (~96% of requirement), which was similar to the estimation of MP requirements (1000 - 1100 g/d) during late pregnancy by Bell et al. (2000). The overview of postpartum performance and blood metabolites highlighted inconsistent results in response to increased prepartum MP supply. Some of the inconsistency may be due to quality of protein (i.e. fractions and AA profile) and amount of energy provided (i.e. fermentable carbohydrates). In addition, the means were generated from data collected over 6 to 17 wk postpartum, which lessened the power to detect differences during the first 2 to 3 wk when the most severe negative protein and energy balances would have occurred. Prepartum MP was positively related to milk protein yield in early lactation as long as cows were fed >75% of their MP requirement in early lactation (French, 2012). Based on CNCPS modeling of 12 published studies, French (2012) suggested the following guidelines for feeding close-up cows with a large safety margin to maximize milk protein: 1300 g/d of MP, 30 g/d of MP-methionine, and 90 g/d of MP-lysine. Providing more MP during late gestation should restore labile protein reserves, prevent protein mobilization prepartum, allow for more uncompromised protein mobilization postpartum, and improve lactational performance. Interestingly, Lean and Van Saun (2013) suggested that prepartum diets containing more protein lessened the impact of dietary protein postpartum whereas lower prepartum protein heightened the response to postpartum protein and AA. The quantity of protein mobilized during late pregnancy in response to a MP deficit seems to be regulated based on demand. In contrast, the proteolysis during early lactation is largely regulated by hormonal changes (e.g. hypoinsulinemia) and diminished muscle responsiveness to insulin. Hence, as long as the labile protein reserves were not extensively mobilized during late pregnancy, the mobilization and recovery of labile protein reserves during early lactation probably compensate for moderate differences in labile protein and minimized the residual effect of prepartum nutrition based on typical guidelines for energy and protein.

Unfortunately, there is less research on which to make protein recommendations for immediately fresh cows than close-up dry cows. Many of the studies evaluating early lactation CP or AA started during the prepartum period and continue postpartum, did not start immediately after calving, or analyzed the data from several weeks of lactation instead of focusing on the critical 2 to 3 wk postpartum. Given the negative MP balance in very early lactation, cows should be fed diets formulated to provide sufficient amounts of fermentable carbohydrates and RDP to promote microbial growth. In addition, diets should provide high quality RUP sources and include rumen-protected AA as needed to optimize the AA profile.

Table 1. Estimated peripartal MP supply and its effect on postpartum performance and metabolism from studies that differed in prepartum CP or RUP. Data were expressed as relative performance within study in comparison with a control group (diet with lower CP, RUP or lower energy and protein content).

Study	Prepartum MP, g/d	Days	Postpartum								
			DMI, kg/d	MP, g/d (balance, %)	Milk, kg/d	Protein, % or kg/d	Fat, % or kg/d	MUN, mg/dL	BW loss, kg	3-MH, nmol/mL	NEFA, mEq/L
Van Saun et al., 1993	791, 872	42	--	--	NS	↑	NS	--	NS	--	--
Putnam & Varga, 1998	916, 976, 1032	91	NS	1710 – 1850 (~75)	NS	NS	NS	--	NS	--	NS ↓ ^a
Huyler et al., 1999	834, 1028, 1282	70	NS	--	NS	NS	NS	--	NS	--	NS
Vandehaar et al., 1999	948, 1008	70	NS	--	NS ^b	--	--	--	NS	NS ^b	NS
Vandehaar et al., 1999	721, 1172	70	NS	--	NS ^b	--	--	--	NS	NS ^b	↑
Dewhurst et al., 2000	429, 814, 895	154	NS	--	NS	NS	NS	--	--	--	--
Greenfield et al., 2000	932, 976, 1090, 1306	56	↓	2300 – 2750 (~105)	NS	↓	↓	NS	↑	--	NS
Hartwell et al., 2000	1169, 1231	120	↓	2500 – 2800 (~110)	NS	↓	NS	NS	NS	--	NS
Santos et al., 2001	1222, 1365	120	--	--	NS:M ↑:P	NS:M ↑:P	NS:M ↑:P	--	--	--	NS
Doepel et al., 2002	1067, 1405	42	NS	1900 (80)	NS	NS	NS	--	NS	↓	NS
Doepel et al., 2002	1067, 1600	42	NS	1680 (75)	NS	NS	NS	--	NS	↓	NS
Park et al., 2002	1334, 1442, 1470, 1744, 1741	90	NS	3000 – 3210 (120)	Qu ^c	Q ^c	C ^c	Q ^c	Q ^c	Q ^c	--

NS: non-significant ($P > 0.10$); ↑, ↓ = change relative to control ($P < 0.10$); L = linear effect; Q = quadratic effect; C = cubic; Qu = quartic effect; M = multiparous cows; P = primiparous cows.

^a: There was a linear decrease in NEFA with increased CP when only obese cows (mean BCS = ~3.9) from each group were used.

^b: Solid-corrected milk yield and the ratio of 3-MH to creatinine were used.

^c: The milk yield is lower in cows fed 13.7%CP diet than others. Milk protein content (%) tended to gradually increased as dietary CP increase from 9.7 to 13.7% and decreased when dietary CP increased from 13.7 to 16.2%. Milk fat yield was lower in cows fed 13.7%CP diet than others. Milk urea N was generally lower with the increase of dietary CP content. The loss of BW tended to be less with increase of dietary CP from 9.7 to 13.7%, but tended to increase with dietary CP increase from 13.7 to 16.2%.

Cows fed a 20.9 vs. 17.3% CP diet during early lactation produced numerically greater milk with significantly higher protein content (Komaragiri and Erdman, 1997). Similarly, Law et al. (2009) found that cows fed higher CP (17.3%; estimated 2033 g MP/d) ate more and produced more milk and milk protein than cows fed lower protein (11.4 and 14.4% CP; estimated 1537 and 1817 g MP/d). All treatments had an estimated MP balance of ~83 to 85%. Postpartum supplementation of methionine and lysine increased milk yield of cows that were fed low RUP, but not high RUP during the close-up period (Wu et al., 1997). However, milk protein content increased with higher RUP. Socha et al. (2005) indicated that early lactation cows had improved lactational performance when intestinal supplies of lysine and methionine were increased pre- and postpartum through supplementation and that the responses depended on dietary CP, supply of MP, and intestinal digestibility of the RUP supplements. Ordway et al. (2009) found that supplementing methionine pre- and postpartum increased milk protein content but did not affect milk yield. Cows supplemented either methionine in lysine adequate diets pre- and postpartum increased milk yield, milk protein percentage, and milk fat yield (Osorio et al., 2013).

RESEARCH AT MINER INSTITUTE FOCUSED ON PROTEIN NUTRITION IN EARLY LACTATION

Multiparous Holstein cows (n = 84) were used to evaluate the effect of CP and MP in corn silage-based diets (Table 2) fed during the fresh and early lactation periods on performance and metabolism (Table 3). Treatments were 1) LL: a low CP diet (15.3% CP) for 13 wk postpartum, 2) HL: a high CP diet (H; 17.0% CP) for 3 wk postpartum and then a switch to a low CP diet until 13 wk postpartum, and 3) HM: a H diet for 3 wk postpartum and then a switch to a moderate CP diet (16.2% CP) until 13 wk postpartum. The MP supply at 19.1 kg dry matter intake was estimated (NDS v3) to be 1798, 1895, and 1999 g/d for L, M, and H, respectively. Post-study modeling indicated MP supply to be 2189, 2541, and 2538 g/d at 3 wk postpartum for LL, HL, and HM, respectively and 2599, 2650, and 2994 g/d at 13 wk postpartum for LL, HL, and HM, respectively. Treatment did not affect intake, milk yield, or content and yield of milk fat and true protein through 13 wk postpartum. Serum nonesterified fatty acids were not affected by treatment ($549 \pm 36 \mu\text{Eq/L}$; $P = 0.99$) though 3 wk postpartum. As expected, protein intake and concentrations of milk urea N and blood urea N were highest for HM. Milk N efficiency was higher for LL than HM.

Plasma concentration of 3-MH and the ratio of 3-MH:creatinine were peaked immediately after calving and gradually decreased to a relative stable level at 6 wk postpartum, but were not affected by treatments throughout the 13 wk postpartum (sampled on 1, 7, 14, 21, 28, 42, 56, 70 and 84). It appears that mobilization of body protein reserves was similar in all three groups of cows. Muscle was biopsied and mRNA expression of genes was determined through RT-qPCR. There was greater gene expression for *FBXO32* and *TRIM63* which encode E3 ubiquitin-protein ligases involved in protein degradation in skeletal muscle at 2 and 11 d postpartum in comparison with that at 62 d postpartum. These data follows a similar pattern as the plasma 3-MH data indicating the important role of muscle proteolysis in early lactation

cows. Similarly, the upregulation of mRNA expression of other components of ubiquitination pathway in early lactating cows were also reported by others (Chibisa et al., 2008; Greenwood et al., 2009). The lack of a treatment effect on the expression of *FBXO32* and *TRIM63* indicates muscle proteolysis was not modulated by additional MP supply. Genes (*TSC1*, *RHEB*, *MTOR*, *RPTOR*, *RPS6KB1*, and *EIF4EBP1*) involved in protein synthesis and the mTORC1 pathway were not differentially expressed among treatments. Genes associated with β -oxidation were upregulated for all treatments at 2 and/or 11 d postpartum compared with 62 d postpartum which underscored increased usage of fatty acids as fuel substrates in skeletal muscle immediately after parturition. Interestingly, the expression of *OXCT1*, involved in ketone body utilization, was increased in LL and decreased in HM from 2 to 62 d postpartum. There was lower expression of *GLUT4* on 2 and 11 d compared with that on 62 d postpartum, which is most likely associated with decreased glucose uptake in muscle with the initiation of lactation.

Table 2. Ingredients, chemical composition, and digestibility of treatments.

Item	Low (L)	Moderate (M)	High (H)
Ingredients, % of ration dry matter			
Conventional corn silage	20.1	20.1	20.1
Brown midrib corn silage	20.1	20.1	20.1
Haycrop silage	11.8	11.8	11.8
Corn meal	9.4	9.4	9.9
Canola meal	9.4	7.1	-
Wheat middlings	6.5	7.1	5.7
Distillers dried grains with solubles	4.7	-	-
Soybean meal	2.4	7.1	10.4
AminoPlus	-	2.4	7.1
Soybean hulls	5.6	5.6	5.6
Beet pulp	4.7	4.7	4.7
Other	5.3	4.6	4.6
Analyses			
Crude protein, %	15.3	16.2	17.0
Acid detergent fiber, %	20.9	20.3	19.5
Neutral detergent fiber (NDF), %	35.6	34.4	33.3
Acid detergent lignin, %	3.4	3.0	2.6
Starch, %	24.2	24.5	24.6
Sugar, %	4.5	4.5	4.8
Fat, %	3.9	3.5	3.3
24-h NDF digestibility, % NDF	59.8	60.7	62.1
7-h starch digestibility, % starch	74.8	74.6	73.8
Metabolizable protein supply, g/d ^a	1798	1895	1999

^a Estimated with NDS (v3) at 19.1 kg dry matter intake

Table 3. Least squares means for performance data.

Item	LL	HL	HM	SE
DMI, kg/d	26.2	26.2	26.5	0.4
CP intake, kg/d	3.9 ^b	4.0 ^b	4.4 ^a	0.1
Milk, kg/d	51.2	50.2	52.4	1.2
SCM, kg/d	47.9	47.5	49.7	0.9
Fat, %	3.51	3.58	3.58	0.09
True protein, %	2.82	2.86	2.89	0.04
MUN, mg/dL	8.3 ^b	9.0 ^b	11.8 ^a	0.2
BUN, mg/dL	8 ^c	10 ^b	12 ^a	<1
SCM/DMI	1.90	1.91	1.98	0.04
Milk N efficiency, %	39.8 ^a	38.6 ^{ab}	36.9 ^b	0.5

^{abc} $P \leq 0.05$

CONCLUSIONS

High producing cows experience negative energy and protein balances during early lactation. Traditionally, nutritional programs and management practices during the transition period have focused on minimizing the severity and duration of negative energy balance to optimize lactational performance, reproduction, and health. Now, there is a need to use an integrated nutritional approach that addresses energy metabolism, protein metabolism, mineral metabolism, rumen function, and immune function. Body protein mobilization is a necessary homeorhetic adaptation to support lactation through contributions of AA and energy. However, excessive mobilization during either late pregnancy or early lactation can increase the risk of metabolic disorders, immune dysfunction, and poor lactational and reproductive performance. The potential interactive role of nutritional management with muscle metabolism and their link with risk of ketosis warrants future research from both mechanism and quantitative perspectives.

Underfeeding protein to cows during late pregnancy may compromise labile protein reserves and impair lactational performance and health in the subsequent lactation. Current evidence suggests that close-up diets should be formulated to meet or slightly exceed requirements (e.g. 1100 to 1300 g MP/d) while meeting but not greatly exceeding energy requirement. Early lactation diets can be formulated to contain less CP than traditional fed by ~1.5 to 2.0 units as long as MP supply is maintained and diets are balanced for AA while achieve improve N efficiency with minimal effects on lactational performance and body protein mobilization. The mobilization of labile protein reserves is primarily regulated by hormonal changes and less responsive to the moderate changes in MP supply immediately postpartum. Reduced MP supply can negatively affect immune function. It is unclear if current protein feeding recommendations in regards to MP supply and AA profile optimizes immune function.

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