PROTEIN METABOLISM OF THE TRANSITION COW

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INTRODUCTION

Nutritional approaches for dairy cows during the dry period and early lactation continue to be refined. The evolution during the past 6 to 8 years toward lower energy diets for dry cows and the adoption of lower protein [albeit balanced for metabolizable protein(MP)] diets for lactating cows raises questions regarding protein and amino acid (AA) nutrition and diet formulation during the late prepartum and early postpartum periods. Furthermore, the enhanced use of blood-based biomarkers for metabolic health of transition cows in the dairy industry has led to questions regarding the potential use of specific biomarkers to assess protein status of cows during this timeframe. The objectives of this paper are to revisit and describe our broad understanding of the adaptations of protein metabolism that take place during the late prepartum and early postpartum periods, infer recommendations for protein nutrition during this timeframe, and assess the potential for use of specific biomarkers to assess cow- and herd-level protein status.

DYNAMICS OF PROTEIN METABOLISM DURING THE TRANSITION PERIOD

The generalized dynamics of protein and AA metabolism in dairy cattle is depicted in Figure 1 (Boucher, personal communication). Typically, net contributions to the metabolizable AA pool come predominantly from the intestinal absorption of MP. There is continuous synthesis and degradation of muscle protein; however, in mature animals the net contribution of muscle protein to the metabolizable AA pool approximates zero. The transition period represents a different situation in one can clearly predict a negative protein balance (Bell et al., 2000; Figure 2) that supports the concept that net mobilization of tissue AA must occur to meet overall metabolizable AA needs.

Available evidence indicates that mobilization of skeletal muscle protein occurs during early lactation. Komaragiri and Erdman (1997) utilized an isotope dilution technique to estimate body composition in Holstein cows at -2, +5, and +12 wk relative to calving and concluded that cows mobilized 21 kg of body protein along with 54 kg of body fat between wk -2 and +5. Some additional body fat was mobilized from wk 5 to 12, but there was no additional mobilization of body protein. Data from Overton et al. (1998) using urine-based markers for skeletal muscle protein degradation suggest that much of this protein mobilization may occur during the first 10 to 14 d postcalving, which would match the pattern of predicted negative protein balance illustrated in Figure 1 reasonably well.

Figure 1. Dynamics of protein and AA metabolism in the cow. Adapted from Boucher (personal communication).

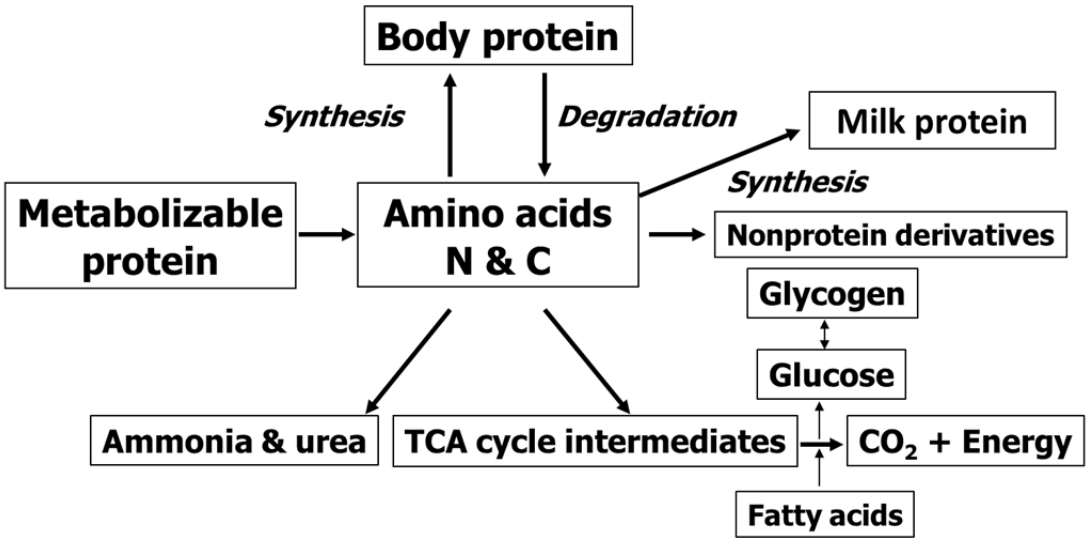
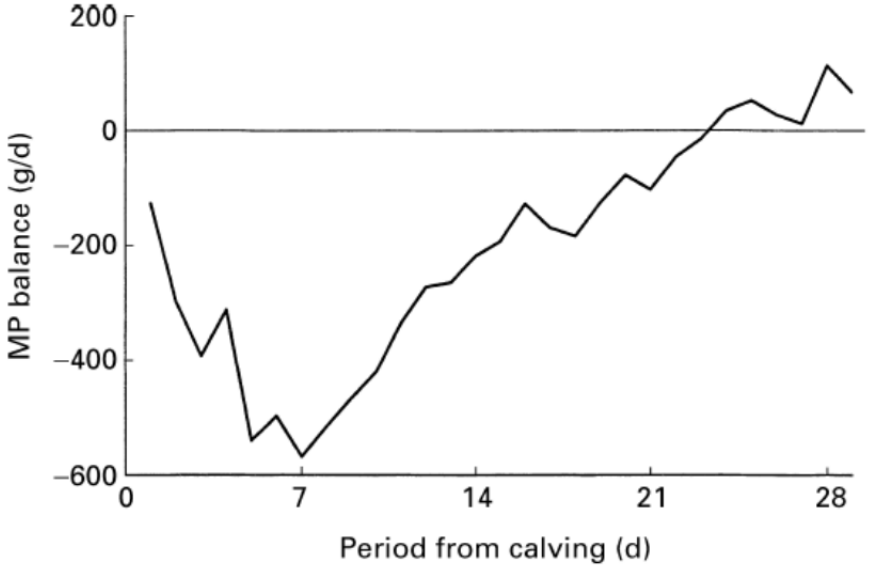


Figure 2. Calculated metabolizable protein (MP) balance in postparturient cows (n = 80) fed on a ration containing 17.8% CP and 1.7 Mcal/kg of NE_L. Individual values were calculated from daily individual measurements of crude protein intake and milk yield, and weekly measurements of milk composition. From Bell et al (2000).



Although the concept that protein mobilization occurs during the early postpartum period and likely is important for overall adaptation of transition cows to lactation is clear, less clear are the factors that affect the degree to which this protein mobilization occurs or its specific functions. Limited evidence suggests that dietary protein supplies

per se during the prepartum and postpartum periods do not affect the degree of predicted negative N balance nor mobilization of body protein. Burhans (2006) fed cows either adequate (~ 1,300 g/d predicted MP; 15.7% CP) or substantially deficient (~ 700 g/d predicted MP; 7.9% CP) amounts of MP from about 50 d prior to expected calving until calving. After calving, all cows were fed the same lactation diet. Detailed characterization of plasma metabolites and hormones was conducted at d -18 before calving during the treatment period and again at d +3 postcalving. Cows fed the higher protein diet during the dry period had higher concentrations of plasma urea N at d -18, but treatment differences were not significant at d +3. Treatment differences for all other metabolites and hormones measured in this experiment (plasma glucose, nonesterified fatty acids, B-hydroxybutyrate, total plasma protein, lactate, insulin, and somatotropin were not significant at either d – 18 or d +3 (Table 1).

A nitrogen balance study also was conducted from d +2 to 7 postcalving (Burhans, 2006). Interestingly, prepartum dietary protein treatments did not result in differences in any of the variables measured. Cows previously fed the high and low protein diets during the dry period averaged – 49 and – 63 g/d of retained N, consistent with mobilization of body protein during this timeframe. Despite the overall lack of differences in both plasma metabolites and N balance, cows fed the higher protein diet during the dry period may have had improved performance during the first week postcalving (36.4 vs. 31.8 kg/d of milk from d 2 through 7; P = 0.25) – the dataset lacked sufficient replication (n = 5) per treatment to adequately assess production outcomes.

In the study of Komaragiri and Erdman (1997), cows were fed diets formulated to contain either 16% CP (6% RUP) or 19% CP (9% RUP) beginning 2 wk precalving and continuing through 114 d postpartum. Analyzed CP percentages were slightly higher than formulated and RUP content estimated using in situ methodology were slightly different than formulated; nevertheless, these differences in dietary protein supply did not affect the amount of protein mobilized during any of the periods studied. Despite this lack of treatment on protein mobilization, cows fed the high CP diet produced 42.4 kg/d and cows fed the low CP diet produced 39.8 kg/d of milk during the 114-d postpartum period; an interaction of treatment and time existed such that cows fed low CP produced more milk for the first 6 wk of lactation, but cows fed the high CP diet produced substantially more milk than cows fed the low CP diet for the remainder of the treatment period. However, treatments were applied both pre- and postpartum, thus the effect of prepartum dietary protein is confounded with postpartum protein intake in that study.

Burhans (2006) reviewed 13 studies published between 1997 and 2002 with 21 comparisons of prepartum protein levels exclusively. For almost all the prepartum protein level comparisons there was no significant difference by treatment for either milk volume yield or milk protein yield (19 of 21 volume yield comparisons were not significant, 20 of 21 protein yield comparisons were not significant. Remarkably, despite wide variation in amount and degradability of prepartum CP, there were similarly negligible differences in postpartum intake, postpartum energy metabolites, or health reported in those studies.

Recent evidence suggests that protein mobilization may be linked to aspects of energy metabolism and metabolic health during the transition period. Van der Drift et al. (2012) characterized muscle protein catabolism using plasma 3-methylhistidine concentrations as a marker of myofibrillar protein degradation and determined that protein catabolism typically started within the final week before parturition, peaked during week 1 postpartum, and was comparatively low by week 4 postpartum. These data were supported by a reduction in longissimus muscle thickness between wk – 1 and +4. Interestingly, a relationship existed between moderate hyperketonemia (> 1.2 mM but exclusion of 3 cows from the dataset with high BHBA) and plasma concentrations of 3-methylhistidine such that cows with low plasma 3-methylhistidine had high BHBA. This would support the concept that mobilization of protein might be important for providing anapleurotic intermediates for the TCA cycle for complete oxidation of acetyl CoA rather than ketogenesis.

Anaplerosis refers to the reactions that replace TCA intermediates that are lost to cataplerosis, cataplerosis meaning the reactions which remove TCA cyle intermediate compounds (Owen et al., 2002). These two processes must be balanced, so at periods when extensive removal of TCA intermediates occurs such as during early lactation when gluconeogenesis is removing large quantities of intermediates, the supply of TCA intermediates must be replaced. Failure to replace them would limit intermediates available for utilization in other critical processes such as lipid oxidation, which if impaired could result in increased ketogensis and lipid accumulation in the liver. It may be that amino acid catabolism in the immediate postpartum is stimulated by the low insulin levels extant at that time, but has as its primary function ensuring an adequate supply of anaplerotic moieties to maintain TCA cycle function rather than functioning to provide gross amounts of substrate for gluconeogenesis, as has been postulated previously (Bell 1995). The increased 3-methylhistidine concentrations observed in peripartum dairy cattle may reflect the unique hormonal milieu of the transition cow, and seem somewhat insensitive to glucose supply. Burhans (2006) measured peripartum plasma 3-methylhistidine concentrations in a study where cows received either 0 or 500g /day of the glucogenic supplement propylene glycol, and found that 3-methylhistidine concentrations increased in the immediate peripartal period in both treated and control cows; the postpartum increase tended to be greater in cows administered propylene glycol. This suggests that gluconeogenic substrate supply, potentially depending upon the source of substrate and where it enters the TCA cycle, may alter the extent of myofibrillar protein mobilization. It seems likely that the transient increase in labile protein mobilization that occurs immediately postpartum may be a teleological anaplerotic mechanism to ensure adequacy of TCA cycle intermediates rather than a mechanism for providing gluconeogenic substrate per se.

Table 1. Least-squares means for BW, DMI, and plasma metabolite and hormone concentrations for cows fed diets with either LOW or HIGH crude protein concentration throughout the dry period. For each outcome the adjusted mean (top line) and the 95% confidence interval for the mean (bottom line: *lower bound – upper bound*) are reported.

	Day From Calving					*P*-value		
	-58	-18		+3		Diet d -18	Diet d +3	Day -18 vs. +3
Item	Covariate Period	LOW	HIGH	LOW	HIGH			
BW, kg	692	723	742	624	648	0.28	0.41	<0.01
	660 - 723	*710 - 736*	*710 - 774*	*588 - 661*	*601 - 695*			
DMI, kg/d	12.8	11.5	11.3	10.4	13.3	0.88	0.15	0.70
	11.8 - 13.8	*9.1 - 13.5*	*9.3 - 13.6*	*9.6 - 13.9*	*9.7 - 14*			
Metabolites								
Plasma urea , mg/dl	16.6	5.3	18.6	16.8	16.1	<0.01	0.75	<0.01
	15.5 - 17.7	2.7 - 7.9	16.4 - 20.8	*13.2 - 20.3*	13.7 - 18.5			
Plasma protein mg,dL	8.22	8.09	8.06	7.45	7.40	0.95	0.91	0.01
	7.83 - 8.62	*7.20 - 8.99*	*7.55 - 8.58*	*6.64 - 8.26*	*6.99 - 7.82*			
Lactate mg/dL	5.33	4.50	4.71	3.63	4.37	0.81	0.48	0.31
	4.55 - 6.11	*3.86 - 5.23*	*3.19 - 6.94*	*2.13 - 6.20*	*3.73 - 5.11*			
NEFA, µeq/L	154	172	170	703	713	0.96	0.96	<0.01
	140 - 169	*115 - 288*	*122 - 254*	*524 - 993*	*477 - 1179*			
BHBA mg/ dL	5.77	6.14	5.19	10.88	12.43	0.30	0.63	<0.01
	5.20 - 6.34	*4.65 - 8.50*	*4.40 - 6.77*	*7.6- 17.0*	*8.7 - 19.1*			
Hormones								
Insulin ng/ml	1.15	0.68	0.77	0.14	0.23	0.73	0.24	<0.01
	0.95 - 1.40	*0.41 - 1.13*	*0.48 - 1.25*	*0.08 - 0.25*	*0.12 - 0.42*			
Somatotropin ng/ml	7.90	4.89	4.50	10.47	7.02	0.76	0.16	<0.01
	6.04 - 9.76	*2.57 - 7.22*	*2.92 - 6.08*	*6.60 - 14.3*	*4.41 - 9.62*			

Table 2 Arithmetic means for measured nitrogen balance and estimated energy and glucose balances for the period from d 2 through 7 postpartum for cows fed diets with either LOW or HIGH crude protein concentration throughout the dry period.

	Treatment		Significance	
	LOW	HIGH	SED	*P* =
Nitrogen, g/d				
Intake N total	483	531	52	0.53
Fecal N output	140	152	15	0.62
Urinary N output	204	190	13	0.47
Milk N output	201	238	17	0.18
Retained N	-63	-49	36	0.70
Apparent N digestibility,%	71.0	71.5		
Total AMN Output[1], g/d	405	427	95	0.57
Urine N, % AMN output	50.4	44.4	5.8	0.10
Milk N, % AMN output	49.6	55.6	5.8	0.10
Energy Balance,[2] Mcal/d	-12.1	-16.9	2.2	0.16
Estimated Glucose Balance[3]	149	-6	114	0.36

[1] Apparent Metabolizable Nitrogen (AMN) calculated as sum of milk N output and urinary N output.

[2] Calculated from predicted dietary Nel intake and NRC (1989) equations for maintenance and milk energy.

[3] Calculated as glucose supplied – (288 + (lactose * 1.22)), where daily glucose supplied is estimated by the equation of Wieghart et al. (Wieghart et al., 1986; Bell et al., 2000) : 24*(1.89 + 1.70*DE intake), with +7.5% additional estimated for renal gluconeogenesis.

RECOMMENDATIONS FOR PROTEIN NUTRITION OF THE TRANSITION COW

Most recommendations for protein nutrition of the transition cow continue to be based largely upon predicted requirements rather than results from controlled experiments. Bell et al. (2000) reviewed various aspects of protein metabolism, requirements of tissues for metabolizable protein, and the potential contributions of catabolised tissue protein to overall amino acid supply to the cow during the periparturient period and early lactation and suggested that the dynamics of protein requirements and supply are significant during this timeframe. Furthermore, the apparently increased demand for amino acids as gluconeogenic substrates during the early postpartum period further complicates predictions of requirements for metabolizable protein and amino acids.

The NRC (2001) indicated a metabolizable protein requirement of their example Holstein cow and heifer during late pregnancy of approximately 900 g/d. However, this estimation did not include an increment for synthesis of mammary tissue, which Bell et al (2000) approximated at about 120 g/d, resulting in a total predicted requirement between 1100 and 1200 g/d. This predicted requirement cannot be met without supplemental protein sources, likely ruminally undegradable sources, fed during the late

prepartum period. Modestly positive responses have been observed by some when increasing level of protein feeding during the prepartum period (quoted by by Van Saun and Sniffen, 1996; Bell et al. 2000; Roche et al., 2013); however, more exhaustive reviews of the literature described more fully above (Burhans, 2006) suggest that increasing the amount of CP and/or RUP generally did not increase postpartum production or improve health. In most of these studies, predicted metabolizable protein supply was not provided and differences on a metabolizable protein basis may have been more modest than when on a CP or RUP basis in some of these studies. There are also indications that excessive supply of CP may be detrimental to performance (Putnam et al., 1999; Hartwell et al., 2000); we speculate that this may be attributed to the apparently impaired capacity of liver to detoxify ammonia to urea as triglyceride accumulation increases during the immediate peripartum period (Strang et al., 1998; Zhu et al., 2000).

For dairy cows during the immediate postcalving period, protein requirements and predictions of supply are complicated by both the increased demand for glucogenic amino acids (Reynolds et al., 2003) and the contributions of catabolized body proteins to the overall amino acid pool as described above. Garcia-Bojalil et al. (1998a, 1998b) fed high protein diets (~21% CP) containing either low (11.1%) or high (15.7%) concentrations of rumen-degradable protein (RDP). They measured decreased milk yield and delays in both follicular development and luteal function in cows fed the ration containing 15.7% RDP. To the authors' knowledge, the subsequent presentation in these proceedings (Dann et al., 2013) represents the first research focused on varying dietary protein supply using metabolizable protein-based approaches for formulating diets during early lactation.

POTENTIAL FOR USE OF BLOOD-BASED BIOMARKERS TO ASSESS PROTEIN STATUS IN TRANSITION COWS

Interest in using blood-based biomarkers for herd-level evaluation of transition cow opportunities continues to increase. Although limited data are available that characterize changes in major circulating proteins during the periparturient period, relatively little is known relative to their sensitivity and specificity as potential diagnostic tools. Piccione et al. (2011) measured serum protein fractions in cows during the late prepartum and early postpartum periods and determined that serum total protein concentrations decreased from the prepartum period to wk 1 postpartum, with decreased concentrations of globulins largely responsible for the decline in total protein concentrations. Concentrations of serum albumin were relatively stable during the periparturient period, but increased slightly at the sample collected at or around the time of calving. Cows with elevated inflammatory response during the postcalving period had decreased serum albumin concentrations and changes in a variety of other serum and plasma components (Bossaert et al., 2012; Trevisi et al., 2012). Consistent with this, Burke et al. (2010) reported that pasture-fed cows diagnosed with endometritis during early lactation had decreased concentrations of plasma albumin and a lower albumin:globulin ratio than cows without endometritis. Furthermore, Rezamand et al.

(2007) reported that cows with new intrammary infections postcalving had lower concentrations of plasma albumin.

Although it appears that serum protein or protein fractions may be associated with aspects of health at the cow level, the sensitivity and specificity of using them as markers of herd-level opportunities is not known. Furthermore, responses of these to dietary factors or management have not been well-characterized. Cozzi et al. (2011) measured concentrations of a variety of blood-based markers in 740 Holstein cows in 33 dairy herds. They reported significant herd variance components for albumin as well as parity and season of production effects on total protein and globulin; however detailed study of diets or management practices was not conducted in their survey. As described above (Table 1), Burhans (2006) reported that cows fed high vs. low protein diets during the entire dry period had similar total concentrations of protein in plasma. Law et al. (2009) fed increasing concentrations of dietary protein (11.4, 14.4, and 17.3% CP) beginning at calving and continuing through 150 d postpartum. Increasing protein supply increased milk yield and also plasma concentrations of total protein and albumin in plasma. Furthermore, Raggio et al. (2007) determined that supplementation of increasing amounts of metabolizable protein to lactating dairy cows increased plasma albumin concentrations but did not affect plasma total protein concentrations.

Overall, it appears that changes in plasma protein fractions, particularly albumin, may be associated with health and reproduction in cows. Furthermore, herd-level variance suggests the potential for evaluation of these as diagnostic tools; however, large datasets will be required to assess the robustness (e.g., sensitivity and specificity) of these tools for use in herd-level evaluation.

SUMMARY AND CONCLUSIONS

In summary, protein metabolism in the periparturient cow is dynamic, and mobilization of muscle protein appears to be an important adaptation to support lactation. Dietary protein supply during the prepartum and postpartum periods does not appear to modulate protein mobilization. Recommendations for metabolizable protein supply during the prepartum period continue to be based upon predicted requirements as published responses based upon increasing supply of CP and/or RUP have been modest, although in some cases assessment of the treatments on a metabolizable protein basis would have decreased the apparent difference of the treatment comparisons. Research evaluating increasing metabolizable protein supply starting at calving is nearly nonexistent. Circulating proteins, particularly albumin, appear to be associated with health outcomes at the cow level and may be modestly responsive to changes in diet; however, more comprehensive evaluation is required to determine the usefulness of this and other circulating protein fractions as herd-level diagnostic tools.

REFERENCES

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves, and lactation performance in dairy cows. Proc. Nutr. Soc. 59:119-126.

Bossaert, P., E. Trevisi, G. Opsomer, G. Bertoni, S. De Vliegher, and J. L. Leroy. 2012. The association between indicators of inflammation and liver variables during the transition period in high-yielding dairy cows: an observational study. Vet. J. 192:222-225.

Burhans, W. S. 2006. Effects of Nutritional Management on Peripartum Glucose and Energy Metabolism and Postpartum Health in Transition Dairy Cows. Ph.D. Dissertation. Cornell University, Ithaca, NY.

Burke, C. R., S. Meier, S. McDougall, C. Compton, M. Mitchell, and J. R. Roche. 2010. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J. Dairy Sci. 93:5363-5373.

Cozzi, G., L. Ravarotto, F. Gottardo, A. L. Stefani, B. Contiero, L. Moro, M. Brscic, and P. Dalvit. 2011. Short communication: Reference values for blood parameters in Holstein dairy cows: effects of parity, stage of lactation, and season of production. J. Dairy Sci. 94:3895-3901.

Garcia-Bojalil, C. M., C. R. Staples, C. A. Risco, J. D. Salvo, and W. W. Thatcher. 1998a. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: productive responses. J. Dairy Sci. 81:1374–1384.

Garcia-Bojalil, C. M., C. R. Staples, C. A. Risco, J. D. Salvo, and W. W. Thatcher. 1998b. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: reproductive responses. J. Dairy Sci. 81:1385–1395.

Hartwell, J. R., M. J. Cecava, and S. S. Donkin. 2000. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites, and milk production in transition dairy cows. J. Dairy Sci. 83:2907-2917.

Komaragiri, M.V.S., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. J. Dairy Sci. 80:929–937.

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92:1001-1012.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, DC.

Overton, T. R., J. K. Drackley, G. N. Douglas, L. S. Emmert, and J. H. Clark. 1998. Hepatic gluconeogenesis and whole-body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. J. Dairy Sci. 81(Suppl. 1):295.

Owen, O. E., S. C. Kalhan, and R. W. Hanson. 2002 The key role of anaplerosis and cataplerosis for citric acid cycle function. J. Biol. Chem. 277:30409-30412.

Piccione, G., V. Messina, A. Schembari, S. Casella, C. Giannetto, and D. Alberghina. 2011. Pattern of serum protein fractions in dairy cows during different stages of gestation and lactation. J. Dairy Res. 78:421-425.

Putnam, D. E., G. A. Varga, and H. M. Dann. 1999. Metabolic and production responses to dietary protein and exogenous somatotropin in late gestation dairy cows. J. Dairy Sci. 82:982–995.

Raggio, G., G. E. Lobley, R. Berthiaume, D. Pellerin, G. Allard, P. Dubeuil, and H. Lapierre. 2007. Effect of protein supply on hepatic synthesis of plasma and constitutive proteins in lactating dairy cows. J. Dairy Sci. 90:352-359.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201-1217.

Rezamand, P., T. A. Hoagland, K. M. Moyes, L. K. Silbart, and S. M. Andrew. 2007. Energy status, lipid-soluble vitamins, and acute phase proteins in periparturient Holstein and Jersey dairy cows with or without subclinical mastitis. J. Dairy Sci. 90:5097=5107.

Roche, J. R., A. W. Bell, T. R. Overton, and J. J. Loor. 2013. Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking. Anim. Prod. Sci. 53:1000-1023.

Strang, B. D., S. J. Bertics, R. R. Grummer, and L. E. Armentano. 1998. Effect of long-chain fatty acids on triglyceride accumulation, gluconeogenesis, and ureagenesis in bovine hepatocytes. J. Dairy Sci. 81:728-739.

Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res. Vet. Sci. 93:695-704.

Van der Drift, S.G.A., M. Houweling, J. T. Schonewille, A.G.M. Tielens, and R. Jorritsma. 2012. Protein and fat mobilization and associations with serum B-hydroxybutyrate concentrations in dairy cows. J. Dairy. Sci. 95:4911-4920.

Van Saun, R. J., and C. J. Sniffen. 1996. Nutritional management of the pregnant dairy cow to optimize health, lactation, and reproductive performance. Anim. Feed Sci. Tech. 59:13-26.

Zhu, L. H., L. E. Armentano, D. R. Bremmer, R. R. Grummer, and S. J. Bertics. 2000. Plasma concentration of urea, ammonia, glutamine around calving, and the relation of hepatic triglyceride, to plasma ammonia removal and blood acid-base balance. J. Dairy Sci. 83:734-740.