CSIRO PUBLISHING

Animal Production Science, 2013, **53**, 1000–1023
http://dx.doi.org/10.1071/AN12293

Review

Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking

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Abstract. The transition period is defined as the 6–8 weeks encompassing late pregnancy and early lactation, involving coordinated changes across multiple tissues and an enormous increase in nutrient requirements. Failure to transition successfully can result in reduced DM intake, milk production, delayed oestrus, failure to conceive and increased incidence of metabolic and infectious diseases, many of which are inter-related. Modern technologies have enabled the measurement of transcriptional changes in genes involved in multiple biochemical pathways across the transition period, enabling a better understanding of the implications of management and nutritional changes on cow health and productivity. Most recent research efforts have focussed on the association between pre-calving energy intake and postpartum health and productivity, with a general recognition that the positive relationship between pre-calving energy intake (and relevant circulating metabolites) and postpartum health and productivity is, for the most part, not causative (i.e. responses are very likely to reflect the same metabolic perturbation, but one is not necessarily the cause of the other). This effect is consistent in both grazing systems and in systems where cows are fed total mixed ration in confinement. These results require a paradigm shift in the extension message to farmers. Because of the focus on energy nutrition, there has been only limited recent research on the requirements of cows for protein, with recommendations based largely on predicted requirements rather than measured responses. That said, metabolisable protein is unlikely to be a limiting nutrient for late-gestation dairy cows grazing up to 50% of their diet as high-protein forages, but could potentially be limiting prepartum mammary development in animals on lower-protein diets, such as total mixed rations formulated for dry cows. The physiological role of fatty acids, in addition to the role of fat as an energy source, is an emerging and important research area, with increasing evidence, at least *in vitro*, that specific fatty acids regulate metabolic processes. Knowledge gaps and future research areas that should be prioritised are identified and discussed.

Received 21 August 2012, accepted 23 April 2013, published online 30 May 2013

Background

The 6–8 week period of transition between late pregnancy and early lactation poses an enormous metabolic challenge to the high-yielding dairy cow. Failure to adequately meet this challenge through a series of complex homeorhetic adaptations can result in a range of inter-related metabolic diseases, suboptimal production and high rates of culling in early lactation (Curtis *et al*. 1985; Goff and Horst 1997*b*; Godden *et al*. 2003). The major metabolic hallmarks characterising the transition period in healthy and functionally compromised cows have been described in numerous reviews (e.g. Bell 1995; Goff and Horst 1997*b*; Drackley 1999; Drackley *et al*. 2001). Recently, this literature has been extended to describe some of the molecular mechanisms underpinning metabolic adaptations in the peripartal cow (Loor 2010). Many other reviews have summarised the diverse array of nutritional management strategies proposed to minimise metabolic dysfunction and the risk of disease in the periparturient period (Grummer 1995; Horst *et al*. 1997; Overton and Waldron 2004).

Despite decades of intensive metabolic research and the testing of numerous nutritional strategies to maintain DM intake (DMI) and control the mobilisation of body stores of fat, calcium, and, to a lesser extent, protein in early lactation, the incidence of metabolic disease and impaired performance remain high in both intensively managed confinement and more extensive pasture-based systems. Overcoming these limitations will require a significant shift in the dry-cow feeding paradigm, with a recognition that the postpartum disease conditions have their onset before calving. The purpose of the present review is to integrate recent research findings with previous knowledge of transition-cow metabolism and nutrition, and to offer some new insights into key elements of successful management of the transition cow.

Adapting to lactation onset – physiological changes and molecular regulation

Changes in nutrient requirements

The dramatic increases in nutrient requirements following parturition have been quantified in terms of mammary demands for synthesis of milk lactose, fat and protein versus the lesser prepartum needs of the conceptus for glucose, fatty

acids (FA) and amino acids (Bell 1995). Changes in calcium and other mineral requirements have been estimated similarly (Goff and Horst 1997*b*). Briefly, it was estimated that in a Holstein cow producing 30 kg milk at 4 days postpartum, the mammary requirements for glucose, FA and amino acids are, respectively, 2.7, 4.5 and 2.0 times those of the gravid uterus during late pregnancy, and the estimated mammary requirement for energy is 3.0 times that of the uterus (Bell 1995). Similarly, Goff and Horst (1997*b*) estimated that the mammary requirement for calcium to produce 10 kg colostrum on the day of parturition is more than double that for fetal growth in late gestation. Additional nutrient requirements of non-mammary tissues also undergoing peripartal hypertrophy, such as the liver and gut, will add to the challenge.

Since average values for DMI increase by only 30–50% between late pregnancy and Week 1 of lactation (Bertics *et al*. 1992; Roche *et al*. 2005; Douglas *et al*. 2006), much of the abruptly increased requirements for specific nutrients and energy must be met by increased hepatic gluconeogenesis and the mobilisation of body stores of fat, protein and calcium. Indeed, Reynolds *et al*. (2003) and White *et al*. (2012) confirmed a major increase in hepatic gluconeogenesis in cows between 1 week prepartum and 1 week postpartum.

The massive mobilisation of body fat is achieved by a combination of increased lipolysis and decreased rates of lipogenesis and FA re-esterification in adipose tissue, leading to the net release of non-esterified FA (NEFA) and glycerol into the bloodstream (Bell 1995; Drackley *et al*. 2001; Roche *et al*. 2009). The homeorhetic changes required to achieve this tissue mobilisation appear to be primarily under genetic control (McNamara and Hillers 1986), with nutrition during the first 5 weeks of lactation having very little effect (Roche *et al*. 2006, 2009; McCarthy *et al*. 2007). Plasma NEFA are efficiently used for synthesis of mammary triacylglycerol (TAG) and can be oxidised in most non-mammary tissues. In addition, glycerol is a readily assimilated precursor for hepatic gluconeogenesis.

Mechanisms of a more moderate mobilisation of amino acids from, so-called, labile tissue protein reserves are less well understood. However, evidence points to suppression of protein synthesis and, possibly, increased proteolysis in skeletal muscle (Bell *et al*. 2000). We previously speculated that in the immediate postpartum period, there may be some diversion of amino acids from splanchnic tissue synthesis and secretion of export proteins to support much of the suddenly increased hepatic requirement for glucogenic substrate for at least a few days after calving (Bell *et al*. 2000). However, recent studies of substrates used for hepatic gluconeogenesis in the early postpartum period do not support this speculation (Larsen and Kristensen 2012).

The onset of lactation places such a large demand on mechanisms of calcium homeostasis that most cows develop some degree of hypocalcemia at calving. In some cases, concentrations of plasma calcium become too low to support nerve and muscle function, resulting in parturient paresis or milk fever (Goff and Horst 1997*b*). Adaptations to increase the blood supply of calcium very soon after calving include increased intestinal active transport, increased resorption of bone stores and decreased urinary excretion of calcium (Horst *et al*. 2005).

Homeorhetic and homeostatic regulation of physiological changes associated with the transition from pregnancy to lactation

The concept of homeorhesis was applied to metabolic regulation by Bauman and Currie (1980) to account for the initiation and coordination of chronic metabolic changes in multiple body tissues that are necessary to support a dominant physiological state. In addition to its chronic nature and simultaneous influence on multiple tissues with apparently unrelated functions, a key feature of homeorhesis is its mediation through altered responses to homeostatic effectors, such as insulin and adrenergic agents. Bell and Bauman (1996) contended that the metabolic transition from late pregnancy to early lactation offers the clearest examples of all three of these features of homeorhesis.

The chronic and diverse nature of metabolic adaptations during the transition period is readily apparent from many detailed sequential observations of whole-body and tissue carbohydrate, lipid and protein metabolism. Examples of altered responses to homeostatic signals in adipose tissue include reduced responsiveness to insulin, leading to almost total suppression of lipogenesis and an opposite effect on lipolysis (McNamara 1991; Vernon 1996), and an enhanced lipolytic sensitivity and responsiveness to adrenergic agents (McNamara 1991; Bell 1995). Responses to homeostatic signals in other key tissues, such as liver and skeletal muscle, have not been studied as systematically. However, there is evidence that the effects of insulin in suppressing hepatic gluconeogenesis and promoting the synthesis of IGF-1 are attenuated during early lactation (Boisclair *et al*. 2006).

The concept of homeorhesis implies the agency of endocrine or neuroendocrine factors capable of responding to physiological and environmental changes and of simultaneously influencing disparate metabolic functions in multiple tissues. Such a role has been convincingly demonstrated for growth hormone (GH) in lactating dairy cows, on the basis of detailed observations of metabolic, physiological and molecular responses to treatment of cows with exogenous GH and to changes in endogenous GH secretion induced by altered physiological and metabolic states (Etherton and Bauman 1998; Bauman 2000). These include most of the previously described metabolic adaptations that underpin increased fat mobilisation and hepatic gluconeogenesis during the transition state (Roche *et al*. 2009), which, most notably, are associated with a marked and sustained increase in concentrations of circulating GH (Block *et al*. 2001; Roche *et al*. 2005; Roche 2007).

The likely positive effects of GH on the mobilisation of body fat stores and increased gluconeogenesis in transition cows are associated with attenuation of its usually potent stimulation of hepatic synthesis of IGF-1 and its binding proteins (Boisclair *et al*. 2006; Lucy *et al*. 2009). This effect has been attributed to a 50% decrease in hepatic GH receptor abundance at parturition, which gradually increases during the first 14 (Radcliff *et al*. 2003) to 50 days (Lucy *et al*. 2009; Grala *et al*. 2011) postpartum and appears to be directly influenced by prevailing insulin concentrations, which, in turn, are determined by energy balance (EBAL) and/or dietary carbohydrate type (e.g. starch vs cellulose; Rhoads *et al*. 2004; Grala *et al*. 2011). Periparturient suppression of the trophic influences of IGF-1 on skeletal muscle

and other tissues is consistent with net release of amino acids and reduced glucose utilisation by peripheral tissues in support of mammary and hepatic metabolic demands.

Decreased EBAL and insulin concentrations during the transition period are also likely to be responsible for marked decreases in adipose TAG synthesis and blood concentrations of the hormone leptin, soon after calving (Block *et al*. 2001; Leury *et al*. 2003). In ruminants, as in rodents, this anorexigenic peptide appears to act principally on the hypothalamus to regulate DMI and peripheral metabolism, indicating that the prolonged depression in leptin concentrations during early lactation may assist the concomitant recovery of DMI and EBAL (Boisclair *et al*. 2006).

The known physiological actions and peripartal blood profiles of several other hormones, including oestradiol-17β, progesterone, placental lactogen, prolactin and cortisol, are consistent with homeorhetic influences on dairy cattle during late pregnancy and early lactation. However, convincing evidence of a causative role in homeorhetic processes is yet to be provided.

Molecular regulation of homeorhesis

Most research evidence, although derived mainly from rodent work, indicates that changes in expression of mRNA exert a major influence on physiological function. Many of the homeorhetic changes discussed previously correspond with changes in transcript abundance (i.e. abundance of mRNA) for key genes, indicating that molecular changes underpin the physiological perturbations associated with the transition from pregnancy to lactation (Loor 2010). With advancing technology enabling more targeted molecular assays in multiple tissues, knowledge of the mechanisms underpinning peripartal homeorhetic changes and, arguably, more importantly, the effect of environmental factors on peripartal metabolism continues to increase. Information published up to early 2010 on changes in expression of hepatic genes associated with lipid, carbohydrate and nitrogen metabolism in transition dairy cattle was reviewed recently (Loor 2010) and is included in Table 1. In general, changes in mRNA expression correspond with changes reported in biochemical studies. For instance,

- the peripartal decrease in GH receptor expression in liver coincides with low circulating concentrations of IGF-1 and correspondingly high concentrations of GH (Lucy *et al*. 2009; Grala *et al*. 2011),
- the increase in the ability of the liver to use alanine for glucose synthesis after calving (Overton *et al*. 1998; Reynolds *et al*. 2003) is consistent with an increase in mRNA expression for the enzymes pyruvate carboxylase (PC) and phosphenolpyruvate carboxykinase (PEPCK1: Greenfield *et al*. 2000; Hartwell *et al*. 2001; van Dorland *et al*. 2009; White *et al*. 2012),
- increased peroxisomal β-oxidation (Grum *et al*. 1996) in liver immediately postpartum is consistent with the increased hepatic expression of acyl-CoA oxidase (Loor *et al*. 2005), the rate-limiting enzyme in this pathway,
- increased blood NEFA and the decreased body fat mass immediately postpartum are consistent with the decrease in expression of several lipogenic enzymes in adipose tissue (Sumner-Thomson *et al*. 2011; Ji *et al*. 2012) and corresponds with lower enzymatic rates (McNamara *et al*. 1995), and
- copious synthesis of milk fat, protein and lactose postpartum is consistent with the marked increase in nutrient utilisation (Bell 1995), enzyme activity (Mellenberger *et al*. 1973) and expression of key enzymes in mammary tissue (Bionaz and Loor 2008; Bionaz and Loor 2011; Bionaz *et al*. 2012)

In the case of homeorhesis, tissue responses are coordinated by a complex network of proteins that 'share' information arising from cues (e.g. hormones and metabolites) from within the organ or from the external milieu (e.g. the blood). These networks have evolved so that tissues can accurately respond to external signals and either maintain homeostasis or provide priority to certain physiological functions (e.g. milk production); hence, regulation of mRNA transcription plays a pivotal role in coordinating physiological function during the transition period (Fig. 1).

The proteins responsible for the regulation and accessing of genetic information (i.e. transcription regulators) have evolved to function together in regulatory modules or multi-protein complexes that possess many different activities. An example of such regulation is the involvement of peroxisome proliferator activated receptors (PPAR) in the coordination of liver and skeletal-muscle adaptations to fasting and under-nutrition. In rodents, the PPAR (α, γ and δ) are activated by saturated and unsaturated long-chain FA (LCFA), essentially as a function of the intracellular concentration; for example, fasting and under-nutrition enhance influx of LCFA to liver and increase the availability of LCFA that bind to and activate PPAR (Fig. 1). These nuclear receptors represent one of the best-studied models of metabolic control at the molecular level. The pathways that can be affected by nuclear receptors encompass not only FA oxidation and ketogenesis but also gluconeogenesis and ureagenesis (Mandard *et al*. 2004). Evidence for the existence of a functional PPAR-α network in ruminant liver has been obtained in neonatal calves, in which injection of a specific pharmaceutical ligand led to up-regulation of several PPAR-α target genes (Litherland *et al*. 2010). The net result of an increase in mRNA expression is, typically, an increase in abundance of the functional protein, which, given that availability of substrate is not limiting, should lead to an overall increase in flux through the pathway (e.g. FA oxidation, gluconeogenesis and ureagenesis; Fig. 1).

Summary

The transition from pregnancy to lactation is a significant metabolic challenge, with almost instantaneous, several-fold increases in the requirement of cows for energy, protein and minerals. A successful transition involves the initiation and coordination of changes in multiple organs that facilitate the provision of these nutrients to the cow and, more specifically, to the mammary gland, often at the considerable expense of other tissues; this coordination of metabolic priorities is termed homeorhesis and there is increasing evidence that peripartal changes in the transcript abundance of genes involved in key metabolic pathways underpin this orchestration of changes in seemingly unrelated tissues. An increasing knowledge of metabolic pathway integration and more targeted functional laboratory assays are providing scientists with a greater

Table 1. Evaluation of the pattern of change of key metabolic pathways in liver, adipose and mammary tissue of dairy cattle from late pregnancy to early postpartum

Adapted from the summary by Loor (2010) and expanded with relevant data from Sumner-Thomson *et al*. (2011), Bionaz *et al*. (2012) and Ji *et al*. (2012)

Pathway	Biological process	Time postpartum	
		First week	Second to fifth week
	Liver		
Ureagenesis	Arginine biosynthesis	No change to decrease	Modest increase
Glucose metabolism	Glycolysis and TCA[A] cycle	Modest increase	No change to increase
Growth-hormone signalling	IGF-1 binding/transport	Decrease	No change to decrease
Gluconeogenesis[B]	Glucose synthesis	No change to significant increase	No change to significant increase
Lipoprotein metabolism	Synthesis of lipoprotein	Decrease	Decrease
Cholesterol metabolism	Synthesis and transport	No change to decrease	Modest increase
Fatty acid transport	Cellular uptake	Modest increase	Modest increase
Fatty acid oxidation	Mitochondrial and peroxisomal degradation of long-chain fatty acids	Modest increase	Modest increase
Fatty acid esterification	Long-chain fatty acid transfer into triacylglycerol	Increase	Increase
Ketogenesis	Synthesis of ketone bodies	Decrease	Increase
Lipid-droplet formation	Desaturation and cytosolic lipid storage	Modest increase	No change to modest increase
	Adipose[C]		
Lipid and carbohydrate metabolism[D,E]	Lipogenesis and adipogenesis; transcriptional regulation; glucose uptake	Marked decrease	Sustained decrease
Lipolysis[D,E]	Hormone-stimulated and basal lipolysis	Modest increase	Sustained modest increase
Insulin signalling[E]	IRS-1 phosphorylation	Decrease	Not assessed
Insulin-signalling pathway[E]	Gene expression	Decrease	Modest to moderate increase
Fatty acid transport and nutrient use[E]	Long-chain fatty acid uptake, transport, and lactate utilisation	Modest decrease	Modest to moderate increase
	Mammary[F]		
Lipid metabolism	Fatty acid synthesis, triacylglycerol synthesis, cholesterol and sphingolipid synthesis, desaturation	Marked increase	Marked increase
Carbohydrate metabolism	Lactose synthesis	Marked increase	Marked increase
Energy metabolism	Oxidative phosphorylation and Krebs cycle	Increase	Increase
Amino acid metabolism	His, Val, Leu, and Ile metabolism	Increase	Increase

[A]Tricarboxylic acid cycle.
[B]From White *et al*. (2012).
[C]Data encompass analyses of subcutaneous adipose biopsies harvested 30 or 14 days before calving and at 7, 14, and 21 days postpartum.
[D]From Sumner-Thomson *et al*. (2011) using microarrays.
[E]From Ji *et al*. (2012) using *in vitro* phosphorylation assay and quantitative reverse-transcription (RT) polymerase chain reaction (PCR).
[F]From Bionaz and Loor (2008, 2011) and Bionaz *et al*. (2012).

opportunity to explore the effects of environment on these important metabolic changes. These data will improve the understanding of management and nutrition on the success or failure of a cow's transition and help provide recommendations to farmers on ways that optimise this important and potentially costly period of the cow's lactation.

DM intake

Profile of change in periparturient DM intake

The central nervous system undertakes the homeostatic role of sensing nutrient intake and body reserves, integrating the information and regulating energy intake and/or energy expenditure (Roche *et al*. 2008). Information regarding metabolic state can be transmitted to the intake control centres of the brain by a diverse array of signals, such as stimulation of the vagus nerve or metabolic 'feedback' factors derived from the pituitary gland, adipose tissue, stomach or abomasum, intestine, pancreas and muscle (Woods *et al*. 1998). These signals act directly on the neurons located in the arcuate nucleus of the medio-basal hypothalamus, a key site for integration of hunger (orexigenic) and satiety (anorexigenic) responses in the brain.

In most situations, the 'drive to eat' increases with energy requirements (Woods *et al*. 1998, 2000; Roche *et al*. 2008). A major exception to this rule appears to be the prepartum dairy cow, whose DMI is reported to decline during the weeks preceding parturition (Coppock *et al*. 1972; Lodge *et al*. 1975; Bertics *et al*. 1992), despite increasing energy requirements for fetal growth and lactogenesis (Bell 1995). However, this decline is not universal. For example, Coppock *et al*. (1972) reported no decline in DMI before calving, until dietary starch concentration exceeded 25% of the ration DM. This is consistent with the

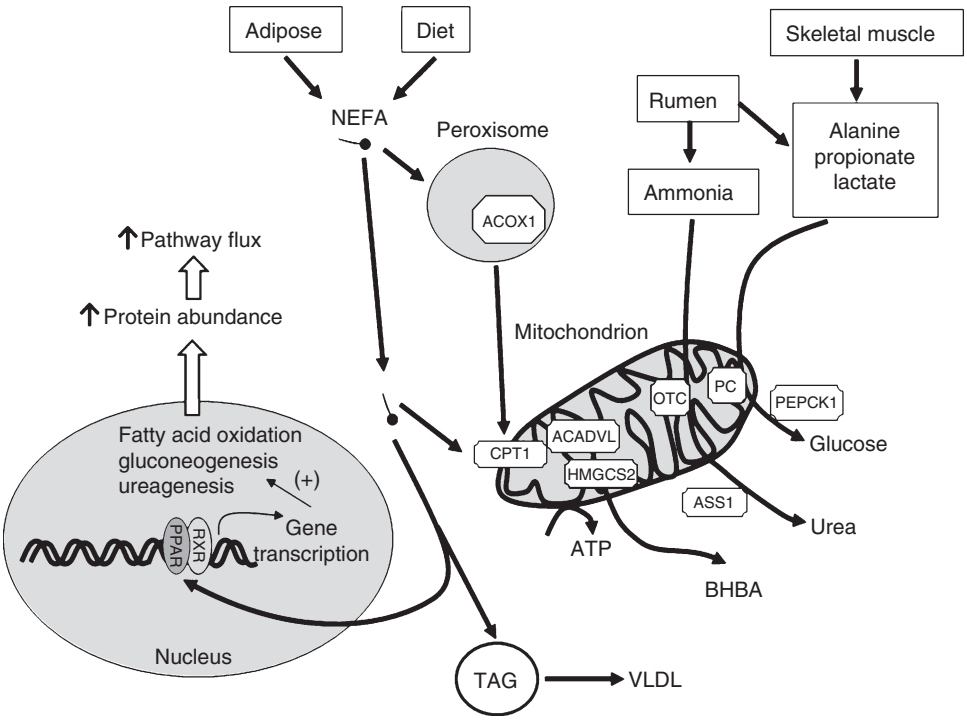

Fig. 1. Schematic model of nutrient metabolism in liver, and the underlying molecular adaptations that allow for synthesis of adenosine triphosphate (ATP), ketone bodies, urea, triacylglycerol (TAG), very low-density lipoproteins (VLDL) and glucose. Gene symbols for key enzymes in mitochondrion and peroxisomes are in upper case. Incoming non-esterified fatty acids (NEFA) can serve as activators of peroxisome proliferator-activated receptor-α (PPAR-α), thus helping coordinate metabolic adaptations to dietary fatty acids or negative energy balance. This scheme underscores the central role of liver in coordinating adaptations, not only through diet (i.e. nutrient provision) but also in peripheral tissues during catabolic states (e.g. adipose and muscle during negative energy balance). ACADVL, acyl-CoA dehydrogenase, very long chain; ACOX1, acyl-CoA oxidase 1, palmitoyl; ASS1, argininosuccinate synthase 1; BHBA, β-hydroxybutyrate; CPT1, 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial); HMGCS2, carnitine palmitoyltransferase 1A (liver); OTC, ornithine carbamoyltransferase; PC, pyruvate carboxylase; PEPCK1, phosphoenolpyruvate carboxykinase 1 (soluble); and RXR, retinoid X receptor, α.

decline in DMI in sheep intravenously infused with oestrogen when offered starch-containing pellets, but not when offered hay (Forbes 1972, 2007). The effect of oestrogen on selection against starch-containing feeds appears to be moderated by body condition score (BCS; Weston 1996), with the decline in DMI being greater in higher-BCS animals. Consistent with the effect of a dietary forage : concentrate ratio on the peripartum decline in DMI, Roche (2006) reported no decline in DMI in the weeks preceding calving in dairy cows fed a 50 : 50 mixture of fresh pasture and pasture hay, irrespective of whether the cows were fed to energy requirements or restricted to 80% of energy requirements. These results are consistent with those of Douglas *et al*. (2006) and Janovick and Drackley (2010), wherein cows fed either a high fibre ration (i.e. straw) or offered a restricted allowance of standard total mixed ration (TMR) to reduce energy intake prepartum did not decline in DMI in the weeks pre-calving, but cows fed DM and/or energy in excess of their requirements displayed the universally accepted decline in DMI.

Recent research from von Keyserlingk's group at the University of British Columbia has added further to the understanding of factors regulating the peripartum decline in DMI. Seminal work associating behavioural and DMI changes before calving with post-calving diseases (Huzzey *et al*. 2007; Goldhawk *et al*. 2009) has highlighted that cows that succumb to either ketosis or metritis after calving have a lower DMI pre-calving; however, there was no evidence of a change in feeding behaviour or DMI until the onset of parturition in those that remained healthy post-calving. Considering data on disease prevalence, the 'average' herd profile of DMI change during the weeks before calving would indicate a decline in DMI; however, the data imply that this is associated with an unidentified, non-evident malaise prepartum rather than a natural decline associated with impending parturition. Consistent with the putative influence of this non-evident prepartum malaise on postpartum health, Burke *et al*. (2010*a*) reported that subclinical uterine inflammation 28–42 days post-calving was negatively associated with blood albumin concentrations 2 weeks pre-calving, indicating that a prepartum metabolic disturbance could be contributing to early lactation disease. Thus, Huzzey *et al*. (2007) and Burke *et al*. (2010*a*) identified changes to animal behaviour and physiology before evident pathology. The low blood albumin was suggested by Burke *et al*. (2010*a*) to reflect liver dysfunction in this situation.

Collectively, these data indicated that the peripartum decline in DMI is a result of interactions among management, nutrition

and pregnancy-related hormonal changes and, although common, is not an immutable parturition-related phenomenon. Starch-based feeds have been reported to contribute to the decline in DMI, which, furthermore, has been associated with poor health post-calving. Additional research is required to determine the dietary and management factors that may be contributing to the liver dysfunction that is putatively associated with the failure of cows to transition successfully, and whether mitigation strategies will result in improvements to health and productivity during early lactation.

The importance of DM intake and energy balance before calving

Epidemiological and metabolic research undertaken over recent decades has been interpreted to support the original recommendation to 'prime' or 'steam up' the cow several weeks before calving (Boutflour 1928), on the basis of a positive association between pre-calving plasma NEFA and the incidence of metabolic disease (Dyk *et al*. 1995), and a negative association between liver TAG accumulation and prepartum energy intake (Bertics *et al*. 1992). However, closer examination of the latter two reports revealed inconsistencies in the derived conclusions; for example, there was no effect of prepartum DMI on liver TAG content at 4 weeks postpartum, but cows in the high pre-calving DMI group had greater plasma NEFA and almost double plasma β-hydroxybutyrate (BHBA) concentrations of the low pre-calving DMI group at 2 weeks postpartum (Bertics *et al*. 1992). These results are consistent with the results of more recent US (Douglas *et al*. 2006) and NZ (Roche *et al*. 2005) research reporting a positive linear relationship between early postpartum liveweight loss and pre-calving DMI (i.e. postpartum liveweight loss increased with pre-calving DMI). Also, research studies in Europe (Agenas *et al*. 2003; Holtenius *et al*. 2003), the USA (Dann *et al*. 2006; Douglas *et al*. 2006) and New Zealand (Roche *et al*. 2005; Roche 2007) provided no evidence that a pre-calving increase in plasma NEFA negatively affects postpartum metabolism or disease incidence. In fact, cows that were feed-restricted for several weeks before calving had increased capacity for hepatic gluconeogenesis and β-oxidation, reduced hepatic TAG accumulation and a reduced risk of milk fever and ketosis (Holtenius *et al*. 2003; Roche *et al*. 2005; Loor *et al*. 2006; Roche 2007; Janovick *et al*. 2011). Thus, contrary to accepted dogma, high energy intakes pre-calving may increase the risk of metabolic diseases in early lactation.

The collective literature points to a deficiency in the epidemiological research model, from which associations are often extended to reflect cause and effect before the underlying physiology is properly considered. It is now apparent that both postpartum disease and metabolic indicators of negative EBAL (NEBAL) reflect a failure of the cow to transition successfully through calving, but are, in fact, indirectly linked rather than inter-dependent, as is generally concluded. This assertion is supported by animal-behaviour data from Huzzey *et al*. (2007), Goldhawk *et al*. (2009) and Proudfoot *et al*. (2010), who reported changes in feeding behaviour and DMI several weeks pre-calving in cows with endometritis, ketosis, and lameness post-calving, although there was no evidence of either metabolic or infectious diseases when DMI declined. New Zealand data also indicated an association between plasma concentrations of liver-derived acute-phase proteins up to 2 weeks prepartum and the risk of subclinical endometritis 6 weeks post-calving (Burke *et al*. 2010*a*).

Summary

Although epidemiological studies have pointed to a positive effect of increased DMI prepartum on subsequent health and productivity, experiments to test this hypothesis do not support such an effect, with increasing evidence that a slight NEBAL pre-calving may improve post-calving EBAL, metabolic health indices and milk production. In addition, these effects appear to be independent of diet (pasture or TMR), dairy system (grazing or confinement), milk-production potential of the cow, or cow BCS. Future research needs to focus on the influence of feeding level, feed ingredients and their interaction with BCS and timing relative to calving on peripartum liver function and indicators of systemic and mucosal immune function. These factors are very likely to be associated with overall transition-cow success and, in particular, early lactation EBAL, cow health and milk production. Increased availability and reduced costs of biochemical, immunological and molecular assays will enable a greater understanding of factors influencing the cow's ability to transition through calving successfully and facilitate the provision of appropriate nutritional and management advice to farmers, tailored to their farming system.

Transition-cow ration

Is carbohydrate type important?

The drain of nutrients by the conceptus and mammary gland places considerable nutrient demand, above maintenance, on the cow during the final weeks pre-calving. Additional factors, however, have resulted in the belief and recommendation that the provision of non-fibre carbohydrates (NFC) as an energy source will improve the transition success of the dairy cow. Assumed benefits are believed to result from the following four factors:

- the ability of NFC to stimulate ruminal papillae development,
- acclimatisation of the rumen microorganisms to the high NFC ration offered post-calving,
- provision of a more readily available supply of nutrients, such as glucose, for the conceptus to prevent maternal loss of energy reserves, and
- the insulin-stimulating effect of dietary NFC, suppressing lipolysis and reducing the influx of FA into the liver, thereby reducing fatty liver and ketosis.

Papillae development

Because of the results of one study (Dirksen *et al*. 1985), providing feeds high in NFC pre-calving is often recommended to enhance papillae development. However, such a recommendation is an over-extrapolation of those results, wherein papillae development was improved in cows fed a high-grain diet compared with those fed a poor-quality forage (straw). In comparison, Andersen *et al*. (1999) compared cows fed a more typical forage-based ration for non-lactating dairy cows (pasture silage) with cows supplemented with ~4 kg DM of

barley in the morning. Macroscopic and histological examinations of the rumen epithelia indicated no differences between the two diets. This result is consistent with the predominant influence of ruminal butyrate production on rumen papillae development and maintenance (Sakata and Tamate 1979) and the fact that supplementation with cereal concentrates has little effect on ruminal butyrate production in cows fed fresh pasture or pasture silage (Dalley *et al*. 2001).

Rumen adaptation

When highly fermentable carbohydrates are introduced into the diet, the production of volatile FA increases, reducing rumen pH. This change in pH exerts selective pressure on the microbial population and needs to be undertaken gradually, thereby allowing a stable fermentation pattern to continue. Of the reasons cited earlier for a greater proportion of NFC in the pre-calving diet, this reason is, arguably, the most valid. However, Hernandez-Urdaneta *et al*. (1976) disputed the need to feed concentrates pre-calving for ruminal adaptation because cows in their study could be changed from prepartum diets of nearly all forage to high-concentrate diets (60% concentrates) postpartum without any adverse effects on feed intake, rumen fermentation or milk production. In contrast, Weimer *et al*. (2010) noted that although rumen pH and volatile FA patterns returned to normal within 24 h in cows subjected to an exchange of ruminal contents, changes to the microbial population took up to 61 days. This is consistent with the low rumen pH following concentrate feeding 28 days postpartum reported by Andersen *et al*. (1999) in cows that were supplemented with barley pre-calving. Considering there was no difference in ruminal epithelium histology, this difference could be a result of different populations of ruminal microorganisms and associated differences in the efficiency of degradation of NFC. This may mean that gradual adaptation to the post-calving diet is worthwhile, particularly if it is substantially different in NFC content from the pre-calving ration.

Further research on the length of time required for ruminal transition between feeds offered to dairy cows is needed to minimise digestive disturbances and maximise transition success.

Glucogenic precursors to spare maternal reserves

As the primary sources of energy used for conceptus metabolism are glucose, lactate and amino acids (see review by Bell 1995), the provision of gluconeogenic precursors could be expected to reduce the catabolism of labile protein stores and reduce the need for mobilisation of FA from adipose tissue for β-oxidation in peripheral tissues. In support of this, several studies (see review by Overton and Waldron 2004) reported increased DMI, reduced NEFA and BHBA, and increased postpartum milk production in cows supplemented with NFC sources pre-calving. However, as reported by Overton and Waldron (2004), the majority of these studies were confounded by energy intake as well as carbohydrate source. Smith *et al*. (2008) in TMR-fed cows and Roche *et al*. (2010) in pasture-fed cows evaluated isoenergetic diets that differed in their carbohydrate composition; Smith *et al*. (2008) compared 34% and 40% NFC and Roche *et al*. (2010) compared 13% and 32% NFC in the prepartum ration. Post-calving milk yield and net energy secreted, milk composition and BCS change were not affected by pre-calving carbohydrate type in either TMR- or pasture-fed cows, refuting the hypothesised benefits of pre-calving dietary NFC inclusion. However, Roche *et al*. (2010) did highlight treatment differences in metabolic parameters, with lower concentrations of blood albumin pre-calving and during the colostrum period in cows on the high-NFC diet. Blood albumin, a negative acute-phase protein, is an indicator of liver health, declining in situations of liver dysfunction. These data imply a potentially negative effect of NFC on peripartum metabolism in pasture-based systems, and this requires further investigation.

In apparent support of the lack of a benefit from changing pre-calving dietary carbohydrate composition, results from a recent study indicated that providing a cow with NFC instead of structural carbohydrate, under otherwise isoenergetic conditions, will not ‘spare’ maternal tissue stores (Mandok *et al*. 2012). Results, in fact, indicated a less efficient conversion of energy to liveweight gain from high-starch feeds than from feeds high in neutral detergent fibre in late-gestation dairy cows. It was hypothesised that the high NFC feeds would result in increased ruminal lactate and propionate production, increased hepatic expression of PEPCK1 and, therefore, gluconeogenesis, with the resultant glucose (and potentially lactate) being utilised by the conceptus for energy, while the acetate production from structural carbohydrate would increase *de novo* lipogenesis. Mechanisms aside, the data do not support a use of NFC in preference to structural carbohydrates as a means of sparing maternal tissues in late gestation.

To increase insulin secretion and reduce BCS mobilisation

A final reason for increasing the NFC : structural carbohydrate ratio of the pre-calving diet is the belief that the resulting increase in ruminal propionate would stimulate insulin, thereby increasing the uptake of NEFA by adipose tissue (Lee and Hossner 2002) and suppressing lipolysis (Lafontan *et al*. 2009). Such an effect would, in theory, prevent fatty liver and associated disorders. The effect of carbohydrate type during the transition period in both TMR-fed (Smith *et al*. 2008) and pasture-fed (Roche *et al*. 2010) dairy cows consuming isoenergetic diets reported either no effect of carbohydrate type (Smith *et al*. 2008) on plasma glucose, NEFA or BHBA concentrations or increased NEFA in the grazing cows consuming a higher concentration of NFC (Roche *et al*. 2010), although BHBA and glucose were unaffected. In addition, the aforementioned lower efficiency of converting energy from high-starch feeds to liveweight gain in late-gestation dairy cows than that of converting energy from feeds high in neutral detergent fibre (Mandok *et al*. 2012) do not support a metabolic reason for changing the NFC : structural carbohydrate ratio of the transition-cow ration.

The importance of dietary fat

Over the past two decades, there has been substantial interest in the effect of lipid supplementation during the periparturient period for improving liver metabolism and health (Grum *et al*. 1996; Douglas *et al*. 2004; Selberg *et al*. 2004, 2005; Petit *et al*. 2007; Andersen *et al*. 2008; Ballou *et al*. 2009;

Hayirli *et al*. 2011). The degree of lipid supplementation has been mostly within the range of traditional recommendations (e.g. 3–4% of supplemental lipid to not exceed ~6% of DM as total lipid).

The primary aim in the studies undertaken during the 1990s was to provide additional energy in the form of fat to help thin cows replenish adipose stores during the dry period (Grum *et al*. 1996); however, evidence from rodent studies at that time also sparked interest in the potential of LCFA to manipulate liver lipid metabolism about the time of parturition and prevent TAG accumulation (i.e. enhance LCFA oxidation; Drackley 1999). More recent research has explored the effects of saturated, trans-, and poly-unsaturated (PU) FA-rich sources on hepatic metabolism (Selberg *et al*. 2004, 2005; Andersen *et al*. 2008) and reproduction (Santos *et al*. 2008).

Published studies of metabolism of specific FA by ruminant liver tissue or hepatocytes are scant. Two *in vitro* experiments from University of Wisconsin used monolayer cultures of calf hepatocytes (Mashek *et al*. 2002; Mashek and Grummer 2003) to evaluate the effects of palmitic acid (16:0) alone or in combination with stearic acid (18:0), oleic (18:1), linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3; EPA), or docosahexaenoic (22:6n-3; DHA) acid on various aspects of lipid metabolism. In the first study, incubation of 1 mM palmitate plus 1 mM 18 : 1 or 20:5n-3 resulted in greater total oxidation (CO_2 and acid-soluble products) of ^{14}C-palmitic acid than did incubation of 1 mM palmitate alone (Mashek *et al*. 2002). Interestingly, the incubation containing 18 : 1 also increased the use of ^{14}C-palmitic acid for cellular TAG synthesis, but 20:5n-3 did not cause an effect. Incubations of palmitate plus 22:6n-3 or 18:3n-3 did not affect total oxidation of ^{14}C-palmitic acid or its esterification to TAG; however, 18:2n-6 decreased total oxidation and the use for TAG synthesis (Mashek *et al*. 2002). The positive effect of 18 : 1 on the use of palmitic acid for cellular TAG synthesis could have been related with data from the same group, demonstrating greater expression of the enzyme microsomal triglyceride transfer protein and Apoprotein B (ApoB) (Pires *et al*. 2006), both of which are essential for synthesis of very low-density lipoproteins (VLDL).

The second study from this group confirmed the response of 18 : 1 only in terms of enhancing the use of palmitic acid for esterification but did not confirm the effect of 20:5n-3 on acid-soluble product formation from palmitic acid (Mashek and Grummer 2003). Furthermore, there was an increase in cellular TAG with incubations of 18:3n-3 and 22:6n-3. Contrary to the first study, stearic acid (18:0) was included as a treatment and data demonstrated an increase in BHBA concentrations and oxidation of ^{14}C-palmitic acid to ketone bodies during incubations of 1 mM 16 : 0 plus 1 mM 18 : 0 (Mashek and Grummer 2003).

Additional analyses from this second study included rate of gluconeogenesis and concentration of cellular glycogen. Linolenic acid plus palmitic acid resulted in the highest rates of gluconeogenesis from [^{14}C]-propionic acid and greatest amounts of intracellular glycogen, along with reduced TAG production. An interesting finding was that 22:6n-3 plus palmitic acid or both plus 20:5n-3 increased cellular TAG content and incorporation of ^{14}C-palmitic acid into cellular TAG. In addition, 22:6n-3 plus palmitic acid decreased metabolism of ^{14}C-propionic acid to glucose in the medium or to cellular glycogen.

Although the resulting data are thought provoking, a criticism of the studies reported could be the use of supra-physiological concentrations of each FA (peak total NEFA concentration is rarely greater than 1.5 mM; Drackley 1999). In addition, isolated hepatocytes incubated for 48 h are sufficiently far removed from the regulatory responsiveness and functioning in cow's liver tissue (e.g. diminished basal gluconeogenic activity; Donkin and Armentano 1993) to make them inappropriate for addressing the biological effects of purified FA. Given the limitations in obtaining and maintaining bovine hepatocytes in culture, use of liver tissue collected via biopsy for *in vitro* studies is a feasible alternative that has not been thoroughly explored.

Intravenous infusions of TAG emulsions from oils with different FA profiles have also been used to study the potential effects of specific FA on liver lipid and carbohydrate (e.g. gluconeogenesis) metabolism (Mashek *et al*. 2005). Comparisons of tallow (containing 43% 18 : 1 and 26% 16:0), linseed (containing 51% 18:3n-3, 21% 18:1, and 6% 16:0) and fish oil (containing 32% EPA + DHA, 15% 18:1, and 20% 16:0) emulsions infused over 4 days into fasted, non-lactating, non-pregnant cows implied that different lipid sources can influence development of fatty liver. Among these oils, linseed resulted in a tendency ($P = 0.10$) for lower liver TAG compared with tallow and fish oil. Plasma NEFA were also lower with linseed oil, compared with tallow, indicating the supplemental 18:3n-3 might have had an effect on adipose metabolism (Mashek *et al*. 2005). Considering the primary FA in pasture is also 18:3n-3, these results have implications for systems in which temperate forages are offered as part of the total ration and, in particular, grazing systems; the effects of dietary FA require further investigation.

In summary, there is compelling evidence that, in addition to providing energy to the transition cow, specific FA could alter lipid and carbohydrate metabolism in the bovine liver to different extents. A greater knowledge of these effects could be used to positively influence liver metabolism peripartum, thereby reducing liver TAG accumulation and enhancing gluconeogenesis and other important aspects of liver function.

Can the liver be primed to the onset of parturition by feeding fat?

The concept of 'priming' liver metabolism ahead of calving so that the level of desired LCFA (e.g. polyunsaturated FA (PUFA) vs saturated) is enriched in liver and adipose tissue (Rukkwamsuk *et al*. 1998; Drackley 1999; Friggens *et al*. 2004; Douglas *et al*. 2006; Andersen *et al*. 2008; Ballou *et al*. 2009) merits consideration. However, to test such a hypothesis thoroughly, there is need to design dose–response studies, including both saturated and unsaturated LCFA sources and FA combinations representative of the late-prepartum and early postpartum periods. With such an approach, the degree of LCFA enrichment of the liver phospholipid pool (Douglas *et al*. 2006) or adipose stores could be assessed and how this affects metabolic pathways and, in particular, the molecular targets that regulate liver function. At the core of these studies is the

hypothesis that the intracellular concentration of a desired LCFA can be enriched and FA oxidation increased at the expense of esterification. The potential effect of LCFA supplementation could likely go beyond liver and adipose tissue metabolism *per se*, because work with rodents (Mishra *et al*. 2004; Li *et al*. 2005) has provided evidence that the very-long chain PUFA (e.g. EPA, DHA) can reduce production of pro-inflammatory cytokines through effects on gene expression. Inflammation, for example, has been reported to result in fatty liver in rats (Dickerson and Karwoski 2002) and there is increasing evidence of similar events occurring in the liver of periparturient cows (Loor *et al*. 2005, 2006; Bradford and Farney 2010).

Are there potential practical approaches to target nuclear receptors in cow tissues?

In non-ruminants, the direct metabolic effects of LCFA appear linked to their ability to bind and activate nuclear receptors (e.g. PPAR, hepatocyte nuclear factor-4-α: HNF4A), with subsequent increases or decreases in expression of sets of genes involved not only in metabolism, but in various cellular functions (e.g. cell proliferation, signal transduction, apoptosis: Desvergne *et al*. 2006). Among PPAR, PPAR-α is highly expressed in liver of both rodents and dairy cattle, and its activation in rodents increases the expression of all the key enzymes involved in cholesterol synthesis and FA oxidation; PPAR-α can also directly enhance the synthesis of some apolipoproteins (e.g. Apo-AI and Apo-AII: Puigserver 2005; Barish *et al*. 2006) and gluconeogenic genes. Unlike classical endocrine receptors that bind to high-affinity glandular hormones, PPAR bind to lower-affinity ligands generated from dietary fat or intracellular metabolism and turn on feed-forward metabolic cascades to regulate lipid homeostasis via the transcription of genes involved in lipid metabolism, storage and transport (Barish *et al*. 2006; Desvergne *et al*. 2006).

Studies reviewed here and elsewhere (Loor 2010) have indicated that PPAR have biological relevance in ruminants and might represent novel biological targets to optimise liver and adipose adaptations to the onset of parturition. Bovine PPAR also respond to pharmaceutical agonists and LCFA (Thering *et al*. 2009; Bionaz *et al*. 2012). Several recent studies have evaluated different types of lipid and their capacity to reduce liver lipid deposition, but results have been inconsistent. For example, Kulick *et al*. (2006) used a dry-cow model in which water, tallow or linseed oil was infused into the abomasum. Contrary to the results of Mashek *et al*. (2005), tallow (high in 16 : 0 and c9–18:1) was more beneficial than linseed oil in reducing liver TAG accumulation during feed restriction. In another recent study (Andersen *et al*. 2008), saturated fat (16:0 and 18 : 0 mainly) prepartum was more efficacious than flaxseeds (high in 18:3n-3) in preventing TAG accumulation at 2 weeks post-calving. This appeared to be coupled with numerically greater palmitate oxidation to CO_2 and ketone bodies. Although it is challenging to compare across studies, results appear to be consistent with *in vitro* results, indicating that longer-term incubation with 16 : 0 (Mashek and Grummer 2003) enhanced ketogenesis to a greater extent than did PUFA. The large increase in the use of palm kernel extract and palm oil as a supplement for dairy cows, with their high concentrations of saturated FA, requires investigation, particularly in their effect on periparturient liver metabolism.

Protein nutrition

Despite numerous studies on the level and quality of protein nutrition before and soon after parturition on subsequent health and production, current recommendations are based mostly on predicted requirements rather than measured responses in terms of utilisation of metabolisable protein and individual amino acids. Bell *et al*. (2000) reviewed various aspects of protein metabolism, requirements of tissues for metabolisable protein, and the potential contributions of catabolised tissue protein to overall amino acid supply during the periparturient period and early lactation. They suggested that the dynamics of protein requirements and supply are complex, with wide variations in the demand for amino acids as gluconeogenic substrates during the early postpartum period further complicating predictions of requirements for metabolisable protein and specific amino acids.

The NRC (2001) indicated a metabolisable protein requirement for their example Holstein cow and heifer during late pregnancy of ~900 g/day. However, this estimation did not include an increment for synthesis of mammary tissue, which Bell *et al*. (2000) approximated at ~120 g/day, resulting in an overall predicted requirement of between 1000 and 1100 g/day. This is unlikely to be an issue in grazing systems where at least 50% of the dry-cow diet is provided by high-protein forages (Roche *et al*. 2010). However, where fresh forage is not a major component of the prepartum ration, as in a typical dry-cow TMR during the late prepartum period, this predicted requirement cannot be met without supplemental protein sources and, in particular, ruminally undegradable sources. Consistent with this assessment, modestly positive responses have been reported by increasing level of protein feeding during the prepartum period (reviewed by Bell *et al*. 2000).

There are also indications that excessive supply of crude protein (CP) may be detrimental to performance (Putnam *et al*. 1999; Hartwell *et al*. 2001). For example, Garcia-Bojalil *et al*. (1998*a*, 1998*b*) fed high-protein diets (~21% CP) containing either low (11.1%) or high (15.7%) concentrations of rumen-degradable protein (RDP). They reported decreased milk yields and delays in both follicular development and luteal function in cows fed the ration containing 15.7% RDP. These effects may be attributed to the apparently impaired capacity of the liver to detoxify ammonia to urea as TAG accumulation increases during the immediate peripartum period (Strang *et al*. 1998; Zhu *et al*. 2000). These results have implications for grazing systems, in particular, where cows receive RDP well in excess of requirements in early lactation (Roche *et al*. 2010). However, reducing dietary CP in the transition period in grazing dairy cows through provision of low-protein supplements in isoenergetic diets (Roche *et al*. 2010) did not affect milk production, although it did reduce the duration of postpartum anoestrus (Burke *et al*. 2010*b*).

Two aspects of protein metabolism in transition cows that require clarification are the contribution of hepatic amino acid catabolism to support increased rates of gluconeogenesis after calving and the magnitude, tissue source and dietary response of

endogenous protein mobilisation in the periparturient period. *In vitro* studies have indicated an increased hepatic capacity for glucose synthesis from alanine and, possibly, other glucogenic amino acids in newly calved cows (Overton *et al*. 1998). However, several *in vivo* studies of splanchnic metabolism in transition cows have reported only modest, if any, contributions of alanine and other amino acids to the marked postpartum increase in hepatic gluconeogenesis (Reynolds *et al*. 2003; Doepel *et al*. 2009; Larsen and Kristensen 2009, 2012). Furthermore, neither feeding a glucogenic diet (Larsen and Kristensen 2012) nor supplementation of dietary protein (Hartwell *et al*. 2001) had a sparing effect on apparent gluconeogenesis from amino acids in the early postpartum period. Rather, there is evidence that increased hepatic uptake of lactate is the most important contributor to increased gluconeogenesis soon after calving, and that splanchnic tissue adaptations may actually partition amino acid fluxes to favour mammary supply over hepatic uptake (Doepel *et al*. 2009; Dalbach *et al*. 2011).

Factorial estimation of metabolisable protein and amino acid requirements versus dietary supply indicates a net deficit during the several weeks after calving (Bell *et al*. 2000), the magnitude of which is consistent with serial measurement of changes in whole-body protein content (Komaragiri and Erdman 1997; Komaragiri *et al*. 1998) and nitrogen balance during the transition period (Maltz and Silanikove 1996; Plaizier *et al*. 2000*a*). The importance of mobilisation of amino acids from skeletal muscle has been inferred from increases in urinary excretion or circulating levels of 3-methylhistidine (Plaizier *et al*. 2000*b*; Phillips *et al*. 2003; Chibisa *et al*. 2008) and increased expression of mRNA for proteolytic enzymes in muscle soon after calving (Chibisa *et al*. 2008). The importance of protein mobilisation from peripheral tissues is also consistent with the significant discrepancy between the milk secretion and the splanchnic release of essential amino acids in early postpartum cows (Dalbach *et al*. 2011). However, the techniques employed are, at best, only semiquantitative and not totally specific to skeletal muscle. Future studies must seek to better define the magnitude, pattern and responsiveness to diet and other factors of peripartal muscle amino acid metabolism as well as the likely smaller contributions of uterine involution and other tissues in transition cows.

Summary

Knowledge of the effects of nutrition on the successful transition of a cow through calving has greatly increased in the past decade. Epidemiological evidence of a positive association between pre-calving energy intake and post-calving health and productivity has been superseded by metabolic and molecular evidence of a potential negative relationship between these variables in well managed dairy systems. In addition, recent research has indicated a lack of importance of carbohydrate-type pre-calving on post-calving health and productivity, although indications of reduced liver function when grazing cows are provided with high NFC supplements pre-calving requires further investigation. Protein nutrition of the transition cow has been less intensively studied, with current recommendations still being based on calculated requirements rather than in terms of directly measured responses. In addition, because of the large amount of protein in the diets of grazing animals, a deficiency of protein has not been considered likely; however, possible negative effects of surplus RDP in an animal with reduced hepatic function requires further investigation. Finally, recognition of the functional role of individual FA in addition to fat as an energy source has led to very interesting findings on the effect of FA on liver function. However, *in vivo* research is lacking; the positive (and negative) effects of individual FA *in vitro* as well as the increased use of high-fat by-products during the transition period indicate a need for future research effort in this area.

Periparturient health problems

The transition period, although short, is when the majority of metabolic and infectious diseases occur during the dairy production cycle. The majority of cases of milk fever, ketosis, fatty liver, mastitis and uterine infections occur during this period, with much of the effort during the transition period aimed at prevention of these diseases.

There is a well reported reduction in aspects of immune function through the calving period, with reduced numbers and effectiveness of immune cells in blood (Nonnecke *et al*. 2003). Although this is often described as peripartum immune suppression, there is also a heightened peripartum inflammatory response in many tissues (Bradford and Farney 2010), with increased concentrations of positive acute-phase proteins indicating a heightened immune system; for this reason, the peripartum period should not be regarded as a period of immune suppression, but instead as one of immune dysfunction.

Metabolic diseases are complex disorders that occur when the cow's ability to adjust to a major physiological change (e.g. calving) is compromised. They have been a persistent problem for farmers for centuries, with milk fever first documented in 1793 in Germany (Schultz 1971) and ketosis reported in the USA as early as 1849 (Udall 1943). Most metabolic disorders stem from nutritional inadequacy or failure to prime metabolic processes for the change from pregnancy demands to lactational demands. Although this is true for the classical metabolic disorders, there is increasing evidence of a link between nutritional and management factors that contribute to immune dysfunction and non-classical metabolic disorders (e.g. milk fever that occurs before the drain on calcium following the first milking).

These diseases are costly; conservative estimates from New Zealand have indicated that the failure of cows to transition successfully through calving costs their dairy industry in excess of AU$1 billion/year in lost productivity and premature wastage of cows (Roche 2012). A focus of any transition-cow management program should be to ensure that peripartum disease (i.e. both clinical and subclinical) is minimised, thereby maximising the likelihood of an early return to oestrus and a successful pregnancy outcome, increasing DMI and milk production, while minimising the early lactation NEBAL.

Peripartum immune dysfunction

The immune system is impaired during the transition period due to physical, hormonal and metabolic stresses associated with gestation, parturition and the onset of lactation (Kehrli *et al*. 1989; Cai *et al*. 1994; Mallard *et al*. 1997). Numerous studies have reported decreases in immune-cell concentrations in blood

(Park *et al*. 1992), reduced gene expression for immune components (Madsen *et al*. 2002) and impaired chemotactic and phagocytic capabilities about the time of parturition when compared with mid–late lactation (Shuster *et al*. 1996; Mehrzad *et al*. 2001). The state of NEBAL is often characterised by impaired neutrophil function (e.g. Kehrli *et al*. 1989; Burvenich *et al*. 2007), including trafficking, phagocytosis and killing capacity (Burvenich *et al*. 2007). Lymphocyte numbers decrease about the time of parturition as a function of reduced proliferation (e.g. Kehrli *et al*. 1989).

There is some *in vitro* evidence indicating that a marked elevation in blood NEFA and BHBA concentrations is one of the causative factors of immune suppression. For instance, NEFA at concentrations typical of the early postpartum period (i.e. 0.5–2 mM) led to substantial necrosis and a decrease in the viability of neutrophils (Scalia *et al*. 2006), although oxidative burst activity was increased. Ketone body concentrations similar to those evident about the time of parturition also impaired the phagocytic and bactericidal capacity of neutrophils, effectively reducing udder defence mechanisms against mastitis pathogens (Suriyasathaporn *et al*. 2000). Although this effect of NEBAL is well accepted, seminal research using mastectomised cows compared with intact cows has indicated that the event of parturition and associated metabolic changes contribute to the immune dysfunction (Nonnecke *et al*. 2003), but that the lactation-induced NEBAL post-calving sustains the suppression of immune-cell function. In addition, the postpartum NEBAL, although a result of failing to coordinate energy intake with energy output at that time-point, may have its origins at an earlier time (Huzzey *et al*. 2007; Goldhawk *et al*. 2009), as previously discussed, and may not be corrected through dietary changes post-calving (Roche *et al*. 2005, 2009; Roche 2007).

Data from non-ruminant studies have indicated that both saturated and polyunsaturated LCFA can alter immune functions through activation of diverse intracellular signalling cascades (e.g. activation of Toll-like receptors and PPAR, inhibition of NF_KB: Sordillo *et al*. 2009; Contreras and Sordillo 2011). A recent study provided evidence that saturated FA (16:0 and 18:0) and EPA were more potent than other unsaturated LCFA in up-regulating the expression of PPAR-α and several inflammation-related genes (Bionaz *et al*. 2012), indicating that enhanced availability of certain LCFA might alter immune-cell pathways. From a practical standpoint, however, there are insufficient *in vivo* data on optimal levels of LCFA to potentially enhance immune cell function of dairy cows during the peripartum period.

Immune dysfunction exacerbates susceptibility to pathogenic infections during the transition period

In addition to the sharp decrease in the number of circulating polymorphonuclear (PMN) neutrophils (Park *et al*. 1992; Detilleux *et al*. 1995), the decrease in mammary T-lymphocytes (Park *et al*. 1992) from late pregnancy through calving impairs the cow's ability to fight pathogens during the early postpartum period. The PMN neutrophils constitute the primary defence of the innate immune system against invading microorganisms; thus, impairment of PMN-neutrophil function during the transition period exacerbates susceptibility to mastitis and metritis. Consistent with this premise, an increase in the incidence of these diseases has been reported in cows with impaired PMN-neutrophil function before and after parturition (Cai *et al*. 1994). Respiratory burst activity of PMN neutrophils is one of the mechanisms used by these cells to fight invading pathogens and data have indicated that the severity of clinical mastitis is greater in cows with a low respiratory burst activity (Heyneman *et al*. 1990).

From a mechanistic standpoint, the marked change in blood steroid concentrations (e.g. cortisol, progesterone and oestradiol) about the time of parturition contributes to impaired PMN-neutrophil function because it decreases the expression of genes and membrane proteins associated with apoptosis (e.g. FADD, Mcl-1, and TRAF6), protein translation (ribosomal protein S15), normal respiratory metabolism (Cytochrome *b*) and the adhesion receptor L-selectin (Weber *et al*. 2001; Madsen *et al*. 2002; Burton *et al*. 2005). Thus, hormonal changes at the time of parturition affect PMN-neutrophil function at a molecular level. However, the periparturient hormonal changes are short-lived and are unlikely to explain all of the alterations in PMN-neutrophil function throughout the transition period.

The decrease in circulating blood glucose with the onset of lactation is another possible factor associated with the impaired immune function after calving. Newsholme *et al*. (1986) reported high activities of hexokinase and glucose-6-phosphate dehydrogenase and low activities of phosphorylase in activated murine macrophages, indicating that glucose is a more important fuel than glycogen and that the pentose phosphate pathway is of high importance within the cell. Most important, glucose is the primary, if not only, fuel source used by macrophages for phagocytosis (Newsholme *et al*. 1986). It follows then that enhancing glucose availability to immune cells might be a practical means of lessening the severity of immune dysfunction around calving. However, in a recent study, Graugnard *et al*. (2012) reported that over-feeding a moderate-energy diet during the dry period reduced PMN-neutrophil phagocytosis after calving, despite those cows having a greater blood glucose concentration pre- and postpartum.

Practical approaches to alleviate immune dysfunction

There is strong evidence that supplementation of the diet with antioxidants (e.g. Vitamin E and/or α-tocopherol, selenium, Vitamin A or retinol and/or β-carotene and Vitamin C) is a practical way of enhancing immune-cell function and decreasing inflammation (Sordillo *et al*. 2009). It was also reported recently that supplementation with rumen-protected omega-3 PUFA plus Vitamin E from –21 through +21 days relative to parturition resulted in lower postpartum blood concentrations of NEFA, BHBA, bilirubin, and a greater concentration of α-tocopherol, indicating an overall improvement in liver function and attenuated inflammatory response postpartum (Trevisi *et al*. 2011). Although ingested PUFA are substantially hydrogenated in the rumen, there is evidence that dietary supplementation with omega-3-rich oils during the peripartal period can enhance the concentration of EPA and DHA in liver phospholipids and adipose tissue TAG (Ballou *et al*. 2009); these can, in turn, serve as a storage

source of these FA and potentially alter molecular pathways regulated by PPAR (Schmitt *et al*. 2011). Fresh forages, such as perennial ryegrass, may, therefore, be beneficial in the peripartum period, being high in both omega-3-rich oils and Vitamin E, and having a fast rumen-passage rate, ensuring minimal biohydrogenation of the desirable fat. These advantages must be weighed against the high dietary RDP and dietary potassium, both of which may have negative consequences for the health of dairy cows.

Metabolic diseases

Although the prevalence of metabolic diseases tends to be low in lower-yielding pasture-based herds (McDougall 2001), many farmers face annual problems that are both costly and frustrating. Clinical cases are only part of the problem, with many more cows reportedly suffering subclinical problems when clinical cases are evident. For example, New Zealand research has indicated that for every 'downer' cow, at least two more cows are clinically hypocalcaemic and 16 have subclinical hypocalcaemia (Roche 2012).

The majority of metabolic diseases occur during the transition period and, although genetic factors influence the risk of these diseases through effects on traits, such as susceptibility to milk fever (Lean *et al*. 2006; Roche and Berry 2006) and the rate of BCS loss (Roche *et al*. 2006; McCarthy *et al*. 2007), farm management in the weeks before and after calving has a major effect on the risk of disease.

The two most common metabolic diseases in pasture-based systems are milk fever and ketosis, although it is likely that 'fatty liver syndrome' and left displaced abomasum become more common with greater supplement use and higher milk production per cow. All of these diseases have secondary effects, with the occurrence of one disease increasing the risk of another (Fig. 2). In addition, they predispose cows to infectious diseases (Curtis *et al*. 1985), particularly of the udder and uterus, and reduce milk production and fertility. The principal causes of, and strategies to avoid, these metabolic diseases will be reviewed here.

Milk fever

Milk fever is best described by its technical name, *parturient hypocalcaemia*, which means lowered blood calcium about the time of calving. Approximately 90% of milk fever occurs in the 24 h after calving (Roche and Berry 2006). On average, only 2% of cows are diagnosed with milk fever in pasture-based systems (i.e. 'downer cows'; McDougall 2001). However, laboratory analyses have indicated that double this number have less than the clinical threshold of calcium circulating in blood (<1.4 mM) and 33% of cows are subclinically affected (i.e. <2.0 mM; low DMI, reduced milk production; Roche 2012).

Clinical milk fever is reported to reduce lactation yield by 14%, while cows suffering subclinical hypocalcaemia produce 7% less milk (Block 1984). There is also evidence that milk fever increases the risk of ketosis and uterine infections (Curtis *et al*. 1985) and that 5% of downer cows do not recover (Schultz 1971). The average cost of milk fever is estimated to be AU$7000/100-cow herd (Roche 2012).

Although a cow has substantial stores of calcium in her skeleton (~6 kg) and consumes a considerable amount of calcium in food (i.e. a cow eating 10 kg of pasture and pasture silage has 40–80 g calcium in her gastrointestinal tract), blood calcium is under very strict hormonal control; a cow only absorbs from food and resorbs from bones what she requires. This can create an issue at the onset of lactation, when a cow's requirement for calcium in blood can increase by more than 400% in a day (Goff and Horst 1997*b*). This requires a rapid increase in the absorption of calcium from the intestines and in the resorption of calcium from bone. Anything that interferes with these processes will increase the risk of milk fever.

Many factors affect the cow's ability to maintain blood calcium during this period.

(1) *Genetics*. Jersey cows are 2.5–5 times more likely to experience milk fever than are Holstein–Friesian cows (Lean *et al*. 2006; Roche and Berry 2006). This effect of breed is well published, with Channel Island breeds having less ability to absorb calcium and secreting more calcium in milk (Goff *et al*. 1995). Within breed, Holstein–Friesian cows of North American genetic origin have lower blood calcium and magnesium concentrations than do Holstein–Friesian cows of New Zealand origin (Roche *et al*. 2001), even when secreting a similar milk energy output, indicating a greater risk of milk fever.

(2) *Body condition score*. Cows that are excessively fat (BCS of >6.0) or excessively thin (BCS of <3.0) at calving are at an increased risk of milk fever (Roche and Berry 2006).

(3) *Weather*. Milk fever is more likely to occur during wet days and nights, probably because of lower DMI and increased stress. In addition, the greater the difference between minimum and maximum temperature (i.e. nights with frost), the greater the risk of milk fever (Roche and Berry 2006).

(4) *Diet*. Many dietary factors can contribute to the risk of milk fever, including the following:

- Magnesium intake is, arguably, the single greatest dietary factor determining the risk of milk fever (Lean *et al*. 2006; Roche and Berry 2006). Magnesium is essential for the efficient absorption and resorption of calcium; therefore, cows that have low blood magnesium about the time of calving are more likely to get milk fever. In an analysis of 30 years of data from DairyNZ No. 2 Dairy, milk fever prevalence dropped from more than 10% to less than 5% following the introduction of pre-calving magnesium supplementation in the late 1970s–early 1980s (Roche and Berry 2006). In addition, Roche *et al*. (2002) reported that supplementation with magnesium sulfate and magnesium chloride was more effective than supplementation with magnesium oxide in maintaining periparturient eucalcaemia, despite a lack of effect of differences in dietary potassium and dietary cation–anion difference (DCAD). However, use of these supplements is not practical on all farms.
- Cows absorb only as much calcium as they require and it takes several days for a cow to alter the proportion of calcium she absorbs from her diet (Braithwaite and Riazuddin 1971). When the cow calves and her

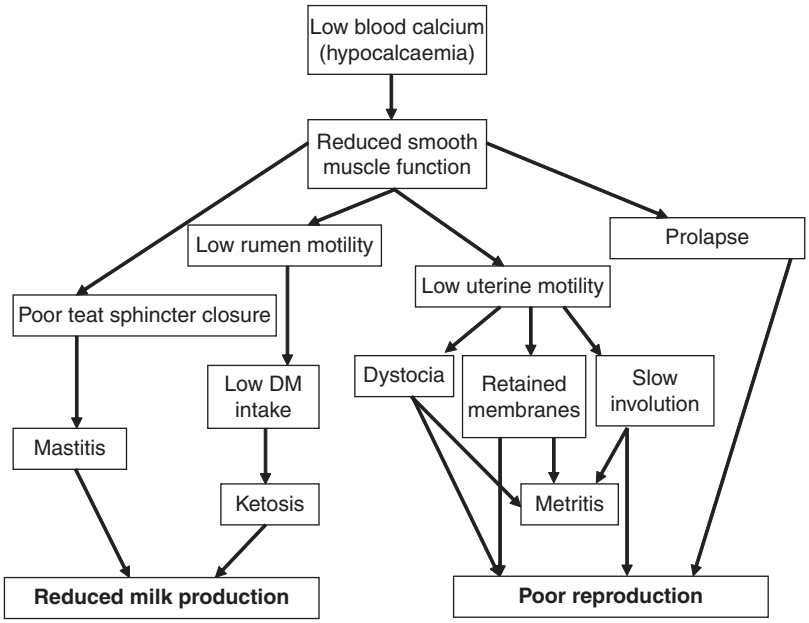

Fig. 2. One metabolic disease (e.g. hypocalcaemia) can increase the risk of other metabolic and infectious diseases and reduce milk production, health and reproduction (adapted from Curtis *et al*. 1985).

requirements for calcium increase rapidly, she cannot increase the proportion of dietary calcium absorbed sufficiently or quickly enough and milk fever occurs.

Because of this relationship between dietary calcium and calcium absorption, traditional recommendations have been to feed a low calcium diet pre-calving. In theory, this will stimulate the cow to absorb a higher proportion of calcium from her diet, such that, when she calves and is fed a high calcium diet, she will absorb enough calcium to prevent milk fever. This strategy has been demonstrated to be effective on farm (Wiggers *et al*. 1975). However, it is very difficult to reduce dietary calcium low enough during the dry period, to stimulate calcium absorption post-calving sufficiently to prevent milk fever.

Oetzel (1991) and Lean *et al*. (2006) noted that very low and very high concentrations of dietary calcium prepartum prevented milk fever, with the greatest risk of milk fever occurring between 0.5% and 2.0% DM calcium. These reports have led some to suggest that milk fever can be prevented by supplementing cows with calcium pre-calving. However, the data presented indicated that dietary calcium would need to be greater than 2.0% DM to be effective in preventing milk fever; on average, a dry cow eating 9–12 kg DM of a pasture and pasture silage mix would have to consume a further 300–400 g ground limestone each day for dietary calcium to be sufficiently high to reduce the risk of milk fever. In pasture-based systems, this would rarely be practical.

In contrast to dry cows, the provision of supplementary calcium to colostrum cows, along with magnesium supplementation, will aid in the prevention of milk fever (Roche *et al*. 2002).

- Dietary potassium also contributes to the prevalence of milk fever, but, at least in pasture-based systems, it does not appear to be as important as many claim. Research undertaken primarily in the USA has suggested that potassium is the primary nutritional factor contributing to milk fever through its effect on DCAD (Goff and Horst 1997*a*). Because of this, high-potassium forages should be minimised in the weeks before calving (NRC 2001). If this contraindication were appropriate for pasture-fed cows, 100% of cows would get milk fever due to the high potassium content of temperate pastures. In contrast, incidence of milk fever in pasture-based herds is low (McDougall 2001) and New Zealand data indicated no difference in blood calcium about the time of calving when cows were fed pastures varying from 3.3% to 4.2% DM potassium, which is the natural range evident in productive temperate pastures (Roche *et al*. 2002). This does not mean that potassium is unimportant. Potassium interferes with the absorption of magnesium in the rumen and, as magnesium is important for calcium absorption, thereby increases the risk of milk fever. However, it is secondary in importance to magnesium supplementation.
- Feeds that are high in phosphorus increase the risk of milk fever by interfering with the renal activation of Vitamin D (Kichura *et al*. 1982; Reinhardt *et al*. 1988). Therefore, feeds that are high in phosphorus (e.g. palm kernel extract, distillers grains) should be used with caution in the weeks before calving, particularly in herds prone to milk fever.
- The DCAD is calculated from the amount of potassium, sodium, chlorine and sulfur in the diet. The proportion of these minerals in the diet influences the alkalinity of blood (blood pH; Stewart 1983) and blood pH affects calcium absorption from the intestine and bone calcium homeostasis (Van Mosel *et al*. 1994; Roche *et al*. 2007). Blood pH drops when DCAD is less than +150 meq/kg DM and calcium absorption from the small intestine increases (Roche *et al*. 2003, 2007). Such a low DCAD is generally not achievable

when pasture is a significant part of the ration. Lowering the DCAD through removal of potassium from the ration where practically possible, however, facilitates the use of practical concentrations of anionic salts and will improve magnesium absorption, thereby reducing the risk of milk fever in some circumstances.

- New Zealand data (Roche 2007; Roche *et al*. 2005) have indicated that the more feed cows are offered in the 2 weeks before calving, the lower the concentration of calcium in blood at calving. The reason for this is unclear; it may be because well fed cows are receiving more calcium in their diet, reducing the proportion of calcium absorbed, or because they are consuming more potassium pre-calving. Alternatively it may be because well fed cows have lower DM intakes after calving (Roche *et al*. 2005) and, therefore, reduce their intake of calcium at the time when it is most needed.

Ketosis

Ketone bodies (acetone, acetoacetate, BHBA) are intermediates in the breakdown of NEFA that can accumulate in blood when large amounts of body fat are mobilised and there is insufficient carbohydrate to facilitate β-oxidation. The clinical state of this disease is referred to as ketosis.

There are three types of ketosis (Oetzel 2007).

- *Type I ketosis* is a result of an unexpected drop in DM intake, particularly in high-producing cows (spontaneous ketosis). This can be caused by not allocating feed properly or because adverse weather events prevent cows from eating allocated feed (e.g. heavy rain, snow). Because the underfeeding is spontaneous, the cow continues to secrete large amounts of energy in milk and must mobilise BCS to meet her energy demands. This type of ketosis can be prevented by ensuring cows are adequately fed or by ensuring any feed restriction is imposed gradually (e.g. over a week). Reducing milking frequency may also reduce the risk (Kay *et al*. 2013).
- *Type II ketosis* occurs to a degree in all cows 3–4 weeks after calving (Roche 2012), but the clinical condition generally occurs in over-conditioned cows: the risk of Type II ketosis doubles when calving BCS increases from 5.5 to 6.0 (Gillund *et al*. 2001). Prevention should be through feeding management in late lactation and during the dry period. Mature cows should be fed to achieve a calving BCS of 5.0 at calving (10-point scale; Roche *et al.* 2009).
- *Silage ketosis*. In addition to the two main types of ketosis, cows can also get ketosis from consuming poor-quality silage. Silage that has undergone a secondary fermentation will increase blood BHBA and the risk of ketosis. Such silage should not be fed to transition dairy cows.

Ketosis has long been recognised as an important disorder of energy metabolism during early lactation (Schultz 1968) and estimates of clinical ketosis incidence over time have consistently ranged from 4% to 16% of cows (Schultz 1968; Baird 1982; Merck 2011). Although there are no published estimates of incidence of clinical ketosis in grazing dairy cows, using blood BHBA concentrations as a reference point, Roche (2012) reported that, on average, 8% of cows in research studies had >2.0 mM BHBA in blood in the weeks post-calving; this is the reported reference concentration indicative of clinical ketosis in North American systems (Duffield *et al*. 1998).

Two important evolutions in how ketosis is assessed and its implications considered have occurred over the past 15 years:

- the major focus has shifted from clinical ketosis to the implications of subclinical ketosis in dairy cows, and
- the importance of timing of ketosis during early lactation has been evaluated.

In a dataset involving 1010 cows from 25 farms (mostly tiestall and component-fed) in Ontario, Duffield *et al*. (1998) reported that ~30% of control cows sampled during Weeks 1 and 2 of lactation had subclinical ketosis, as defined by circulating BHBA >1.2 mM, and Duffield *et al*. (1999) concluded that these cows had a greater risk of subsequent left displaced abomasum (odds ratio = 2.60) and metritis (odds ratio = 3.35).

Consistent with the findings of Duffield *et al*. (1998, 1999), Ospina *et al*. (2010*a*) determined that cows fed a TMR in the north-eastern United States with serum BHBA >1.0 mM from Day 5 to Day 15 of lactation had a greater risk for the development of left displaced abomasum, clinical ketosis or metritis (risk ratios ranging from 4.4 to 6.9). In the same dataset, cows with serum BHBA >1.0 mM had 393 kg less projected milk for the lactation and 13% lower risk of pregnancy during the 70 days following the voluntary waiting period (Ospina *et al*. 2010*b*). In addition, McArt *et al*. (2012*a*) determined that 43% of cows (within farm, prevalence ranging from 26% to 56%) in four large freestall, TMR-fed herds had at least one circulating BHBA concentration between 1.2 and 2.9 mM when sampled six times between 3 and 16 days in milk, indicating that the prevalence of subclinical ketosis was high and also highly variable across farms.

In comparison, Roche (2012) reported that only 12% of cows in a multi-year research database derived from pasture-based diets had blood BHBA concentrations >1.2 mM and that this prevalence was recorded 4 weeks post-calving, with <2% of cows presenting with this BHBA concentration during Weeks 1 and 2 post-calving (Fig. 3). The reason for the difference in prevalence between TMR-based and pasture-based systems is not clear, but may reflect differences in milk production and the

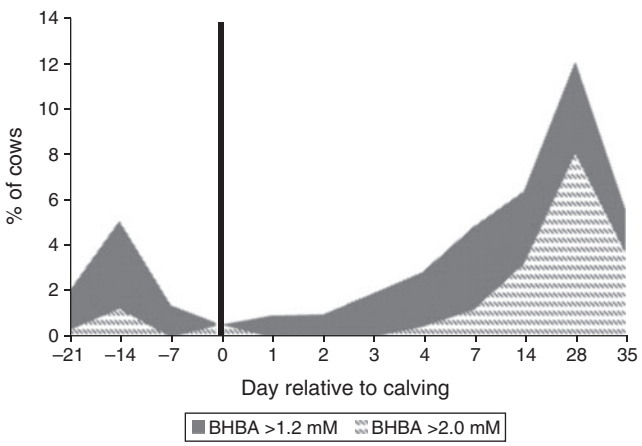

Fig. 3. Concentration of β-hydroxybutyrate (BHBA) through time in blood of pasture-based transition dairy cows. Data are derived from a research database (Roche 2007; Roche *et al*. 2005, 2010) and reported by Roche (2012).

consequent level of metabolic stress the cow is subjected to during the transition period. Nevertheless, it is consistent with the low overall incidence of metabolic disorders reported for pasture-based systems (McDougall 2001).

The second evolution in the consideration of ketosis relates to timing of incidence during early lactation. Traditionally, ketosis has been thought to have primary onset between 10 days and 8 weeks post-calving (Schultz 1968; Baird 1982). In contrast, McArt *et al*. (2012*b*) reported that peak prevalence of subclinical ketosis occurred at 5 days in milk when 29% of cows sampled across four large dairy herds had elevated circulating BHBA concentrations. Furthermore, associations of subclinical ketosis with disease outcomes or removal from the herd were much stronger if the onset of subclinical ketosis occurred from 3 to 7 days in milk than they were from 8 days in milk or later. However, these data from TMR-fed herds are not consistent with those of Roche (2012) for pasture-based systems, where peak BHBA concentrations occurred at 28 days postpartum (Fig. 3). Similarly, Ingvartsen (2006) reported a peak in plasma BHBA concentrations between 4 and 6 weeks post-calving, but with increases from Week 1. He also noted, however, that post-calving peak BHBA was affected by pre-calving energy intake, with cows on low-energy diets pre-calving having lower plasma BHBA post-calving being consistent with New Zealand observations that over-feeding in the pre-calving period increases the risk of high blood BHBA post-calving (Roche 2007). Janovick *et al*. (2011) reported a similar effect of prepartum level of feeding in TMR-fed cows, highlighting the consistency of this effect of prepartum nutrition on cow health and well-being across nutritional systems and cow genetics. Consistent with these results, Roche *et al*. (2005) also reported a negative relationship between the level of feeding pre-calving and EBAL post-calving, such that a high feeding level pre-calving was associated with greater NEBAL post-calving. Douglas *et al*. (2006), Janovick and Drackley (2010) and Janovick *et al*. (2011) all reported similar findings in TMR-fed cows. Consistent with these findings, molecular changes in liver have indicated decreased functionality (i.e. β-oxidation, gluconeogenesis, ureagenesis) post-calving in cows overfed pre-calving (Loor *et al*. 2006).

The relevance of reference ranges for BHBA derived in systems where cows are fed high-NFC diets needs to be considered for the Australian and New Zealand dairy industries. For example, Roche *et al*. (2010) reported that cows fed pasture and pasture silage (NFC = 18%) had more than double the circulating concentrations of BHBA than did similar cows fed an equivalent amount of metabolisable energy as pasture, pasture silage and 35% DM of the diet as a maize- and barley-based concentrate (NFC = 40%), despite no treatment differences in the trajectory of BCS change or circulating NEFA concentrations; also, blood albumin concentrations were lower in the high-NFC treatment, potentially indicating liver dysfunction. These data indicate a lack of relevance of reference ranges for BHBA derived from systems feeding high-NFC diets for pasture-based systems, where plasma BHBA might be a function of ruminal butyrate production and a lack of NFC to provide oxaloacetate for the oxidation of acetyl-CoA in the tricarboxcylic acid cycle, rather than an indicator of a metabolic disorder *per se*. Defining relevant reference ranges for BHBA for pasture-based systems should be a research priority.

Fatty liver

Fatty liver disease or hepatic lipidosis is a metabolic disorder that can frequently occur during the transition period. Fatty liver is associated with reduced health status, lower productivity and impaired reproductive performance (Bobe *et al*. 2004). 'Fat cow syndrome', commonly related to fatty liver disease (Morrow 1976), is a major problem in cows that are over-conditioned at parturition and results in dramatic decreases in DMI, a greater incidence of downer cows, and negative health effects that may be irreversible. Feeding of diets with greater starch content to increase energy density during the dry period can lead to fat cow syndrome, thus indirectly increasing the risk of fatty liver disease and other associated diseases postpartum. Fatty liver can also occur in cows that are not over-conditioned, with reports of up to 50% of dairy cows affected during early lactation (Jorritsma *et al*. 2001). However, there are very little data on the incidence of fatty liver in pasture-based dairy cows.

As outlined in previous sections, the rapid increase in milk yield, concomitant with physiological changes during the early postpartum period (Bell 1995), is often characterised by reduced DMI resulting in NEBAL and increased blood NEFA. The NEFA are taken up by the liver at ~26% of whole-body NEFA flux (Drackley *et al*. 2001) and can be

(1) oxidised to obtain energy by mitochondria, peroxisomes or microsomes (Drackley 1999),
(2) partly oxidised to ketone bodies, or
(3) re-esterified to TAG. The TAG are stored in the cytosol of hepatocytes or assembled with Apolipoprotein-B100 (ApoB100), cholesterol and phospholipids to synthesise VLDL (Shelness *et al*. 1999).

Excessive TAG accumulation in liver occurs when the hepatic uptake of NEFA exceeds their oxidation and secretion by the liver and it occurs primarily at some point during the first 4 weeks postpartum (Bobe *et al*. 2004). From a biochemical standpoint, the bovine liver can esterify NEFA at a rate *in vitro* similar to that in simple-stomached species, such as rat, pig and chicken, but the rates of TAG export in VLDL from ruminant liver is markedly lower than those of other species (Pullen *et al*. 1990). That feature is partly responsible for the consistent increase in liver TAG during the first few weeks postpartum that has been reported in studies over the past 10 years when cows have been overfed moderate-energy diets during the dry period (e.g. Dann *et al*. 2006; Janovick *et al*. 2011; Ji *et al*. 2012).

Early *in vitro* experiments with isolated bovine hepatocytes have indicated that accumulation of TAG in liver often decreased gluconeogenesis and ureagenesis (Cadorniga-Valino *et al*. 1997; Strang *et al*. 1998). Large-scale gene expression (Loor *et al*. 2007) and protein expression (Kuhla *et al*. 2009) of ketotic liver and liver from non-ketotic cows with lipid infiltration (Loor *et al*. 2006) postpartum has confirmed that hepatic lipidosis affects a large number of metabolic pathways. For instance, irrespective of the stage of lactation, a consistent response evident in cows with moderate to severe fatty liver due to under-nutrition (Loor *et al*. 2007; Kuhla *et al*. 2009; Akbar *et al*. 2012) is the reduction in

expression of genes and proteins associated with cholesterol synthesis, ATP production, endogenous carnitine synthesis and FA desaturation. In contrast, fatty liver is associated with an up-regulation of markers of inflammation and oxidative stress (Loor *et al*. 2007; Akbar *et al*. 2012). A recent study also revealed that after calving there is up-regulation of genes involved in endogenous carnitine synthesis and carnitine uptake (Schlegel *et al*. 2012). These molecular adaptations are in agreement with the positive effect of supplementing 50 g/day carnitine during the transition period in enhancing FA oxidation, which would account in part for the lower liver TAG (Carlson *et al*. 2007).

A practical approach to minimise the risk of cows developing fatty liver postpartum is to minimise excessive deposition of body fat during the dry period (e.g. control energy intake prepartum: Douglas *et al*. 2006; Janovick and Drackley 2010; Ji *et al*. 2012), control BCS (Hayirli *et al*. 2002; Roche *et al*. 2009), and/or enhance insulin sensitivity (e.g. use of insulin-sensitising agents) such that the rate of FA release from adipose tissue and blood NEFA concentrations postpartum are maintained within a non-pathological range (Smith *et al*. 2007, 2009). Although BCS prepartum provides a practical evaluation of the degree of body fatness, recent evidence has indicated that overfeeding energy can lead to a marked increase in visceral adipose tissue mass without appreciable differences in BCS or bodyweight (Nikkhah *et al*. 2008), underscoring the importance of nutritional management to avoid diseases associated with over-conditioning.

'Starter drenches' in early lactation

Propylene glycol is a glucogenic precursor that has been used for many years as an oral drench in the treatment of ketosis. Available studies consistently demonstrate decreased concentrations of NEFA in plasma, usually accompanied by decreased concentrations of BHBA in plasma in response to propylene glycol administered as an oral drench (Christensen *et al*. 1997; Pickett *et al*. 2003; Chagas *et al*. 2007). However, incorporation of propylene glycol into the TMR did not affect concentrations of NEFA and BHBA in plasma (Christensen *et al*. 1997). Stokes and Goff (2001) reported that administration of an oral drench of propylene glycol for 2 days beginning at parturition decreased concentrations of NEFA in plasma and increased milk yield during early lactation. Similarly, Chagas *et al*. (2007) reported lower blood NEFA concentrations during the first 5 weeks postpartum in grazing cows supplemented with propylene glycol for 16 weeks post-calving, but reported no effect on milk production. Subsequent experiments in which propylene glycol was administered as a drench beginning at parturition for either 2 days (Visser *et al*. 2003) or 3 days (Lenkaitis *et al*. 2003), or as a part of a combination drench administered for 3 days beginning at parturition (Visser *et al*. 2002), reported no productive response to propylene glycol drench. Chagas *et al*. (2007) did report reproductive benefits from propylene glycol, however, with an increase in the pulsatile release of luteinising hormone and an earlier return to oestrus in supplemented cows. McArt *et al*. (2012*a*) also reported reduced incidence of displaced abomasa and a reduction in mortality, when cows diagnosed with subclinical ketosis were treated with propylene glycol. Overall, research supports modest but inconsistent effects on metabolic variables from bolus administration of propylene glycol; therefore, the use of propylene glycol in 'at risk' cows, if they can be easily identified, could improve health and productivity. However, the lack of consistent production responses across experiments does not support the routine administration of propylene glycol.

Propionate supplements consisting of propionate complexed to calcium or trace minerals potentially could be used to supply substrate for hepatic gluconeogenesis. Published responses to peripartal supplementation with propionate supplements have been mixed. Mandebvu *et al*. (2003) reported that feeding ~110 g/day of a propionate supplement on a commercial dairy farm did not affect milk yield, but transiently decreased plasma NEFA concentrations and urine ketone score. Stokes and Goff (2001) reported that drenching cows with 0.68 kg of calcium propionate twice during the early postpartal period did not affect milk yield in early lactation, or concentrations of NEFA and BHBA in plasma. Part of the reason for the lack of measured responses to propionate supplements could be the amount of propionate provided relative to the amount produced in the rumen. Overall, existing research does not support the use of propionate supplements either through the TMR or via bolus.

Monensin is a common rumen modifier provided either through feed premix or in controlled-release capsule (CRC) form. Monensin acts by interrupting transmembrane movement and intracellular equilibrium of ions in certain classes of bacteria and protozoa that inhabit the gastrointestinal tract. This provides a competitive advantage for certain microbes at the expense of others, altering the rumen microbiota as a result (McGuffey *et al*. 2001). In modifying the rumen microbial populations, rumensin can enhance the glucose status of dairy cows through increased production of propionate. Consistent with such an effect, administration of monensin by CRC during the transition period and early lactation decreased the incidence of subclinical ketosis (>1.2 mM) in dairy cows by 50% (Duffield *et al*. 1998). Over-conditioned cows (BCS >4.0 at 21 days before expected calving, on a 5-point scale) supplemented with the monensin CRC produced significantly more milk than did unsupplemented controls during early lactation (Duffield *et al*. 1999). In a subsequent experiment, cows administered the monensin CRC had decreased circulating concentrations of NEFA during the week immediately preceding calving, but concentrations were not affected during the first week post-calving (Duffield *et al*. 2003). In contrast, cows fed 300 mg/day of monensin from 28 days before calving until calving did not have altered concentrations of NEFA or glucose during the prepartum period, but had lower circulating NEFA concentrations during the first week post-calving and tended to have increased DMI during early lactation (Vallimont *et al*. 2001). More recently, Mullins *et al*. (2012) reported that monensin supplementation by daily top-dress on TMR (400 mg/day) from 21 days before expected calving date until 21 days after calving decreased both mean and peak concentrations of BHBA and liver TAG accumulation.

Duffield *et al*. (2008*a*, 2008*b*, 2008*c*) conducted a meta-analysis of the effects of dietary supplementation or ruminal administration of monensin on health and production of dairy

cows. Across 59 studies, they determined that monensin supplementation reduced circulating BHBA and NEFA concentrations (Duffield *et al*. 2008*a*). Across 77 studies, they determined that monensin supplementation decreased DMI slightly but increased both milk yield and milk production efficiency (Duffield *et al*. 2008*b*). Finally, across 16 studies, monensin supplementation decreased risk for both ketosis and left displaced abomasum; administration by CRC resulted in greater reductions in retained placenta and metritis than did administration in the diet (Duffield *et al*. 2008*c*). In comparison, Waghorn *et al*. (2007) reviewed the research undertaken in pasture-based systems in Australia and New Zealand and, again, reported variable results. Australian studies (Lowe *et al*. 1991) reported a 30 g/cow.day increase in milk protein but these results were not achieved in subsequent trials on 18 dairy farms in Australia (Lean *et al*. 1994; Beckett *et al*. 1998), wherein monensin increased milk yield but did not increase milk protein yield. Waghorn *et al*. (2007) quoted unpublished experiments in New Zealand and Australia that reported an average increase of 40 g of fat and protein per cow per day across the dataset, but only an increase of 23 g in those studies undertaken in New Zealand.

The reason for the inconsistency in milk-production responses to monensin is not clear, but it may reflect a negative effect on DMI (Duffield *et al*. 2008*b*), or an interaction with diet quality. Waugh *et al*. (2005) reported an increased effect of monensin as dietary metabolisable energy declined. These data, and published evidence of greater responses when diet digestibility declines (McGuffey *et al*. 2001; Duffield *et al*. 2008*b*), suggest that milk production responses to monensin may be highest when pasture quality is low, while the lowest response is likely when pasture quality is highest. Further research is required to determine cow responses under different dietary conditions.

Summary

The transition period, although short, is when the majority of metabolic and infectious diseases occur, meaning that significant gains in animal health and productivity could be achieved through understanding the reasons for failure to transition successfully. Immunological and metabolic dysfunction appears to be the primary reason for the high disease incidence and, although some of this appears to be an inevitable function of the parturition event, there is considerable evidence that management and nutritional practices pre- and post-calving contribute. Understanding the molecular and metabolic changes associated with the level of feeding and feed ingredients on important biochemical processes will help validate the advice being provided to farmers and should, therefore, improve cow longevity, reproductive and productive efficiency, and animal welfare.

Conclusions and considerations for further research

Nutrition and management of the transition cow was highlighted by Boutflour (1928) as a way of improving milk production. During the past 20 years, in particular, knowledge in biochemical and molecular processes associated with a successful transition has dramatically increased. This increased knowledge has resulted in a paradigm shift in thinking about the most appropriate way in which to manage the transition cow. The cow's greater requirement for energy and protein as she transitions from pregnant to lactating (Bell 1995) highlighted the need to consider energy and protein nutrition. Subsequent work demonstrated that energy intake and feed composition have important effects on the molecular regulation of biochemical pathways (Loor *et al*. 2006; Ji *et al*. 2012), particularly in liver and adipose tissue, the tissues that have been subjected to greatest scrutiny, but also conceivably in muscle, mammary and reproductive tissues. The importance of pre-calving nutrition in postpartum health has been highlighted in studies of animal behaviour (Huzzey *et al*. 2007; Goldhawk *et al*. 2009), the epidemiology of periparturient diseases (Dyk *et al*. 1995) and force-feeding (Bertics *et al*. 1992). However, the paradigm shift in thinking is that the relationship between pre-calving energy intake and relevant circulating metabolites, and postpartum health and productivity is, for the most part, not causative (i.e. responses very likely reflect the same metabolic perturbation, but one is not necessarily the cause of the other). Consistent with this hypothesis, attempts to increase energy intake pre-calving have not produced positive animal production or reproduction responses; in fact, metabolic and molecular indices have indicated potential benefits to a controlled restriction during the weeks immediately preceding calving. Further research is needed in this area, as there are likely interactions among feed composition, cow BCS and postpartum nutrition factors (e.g. level of feeding, feed composition).

Because of the focus on prepartum energy intake, there has been limited research on the effects of dietary protein level, protein type, essential amino acids (e.g. methionine), or individual FA on the success of the cow's transition through calving. Considering the potential negative implications of excess RDP and the effects of FA on important biochemical processes, research is required in this area. As with all ruminant nutrition studies, due attention must be paid to what is leaving the rumen in addition to what the cow is consuming.

Most importantly, future studies investigating the requirements of the transition cow must involve multiple research disciplines (i.e. from applied nutrition and management to basic physiology). Improved laboratory assays will facilitate a greater understanding of how nutrition and management influence immunological and physiological functions during this important period. These technologies are not intended to replace detailed physiological studies. However, physiological studies that involve arterio-venous flux across important organs or *in vitro* assays are difficult, time-consuming and expensive, cannot be undertaken across large numbers of animals, and are, sometimes, of questionable *in vivo* relevance. In comparison, tissue biopsies are relatively easy to undertake and modern technologies (e.g. high-throughput molecular and immunological assays), coupled with bioinformatics platforms, facilitate the assessment of many biochemical pathways through time in multiple animals. Despite the increasing availability of these technologies, every endeavour must be made to associate transcription and protein changes with tissue metabolic output and whole-animal production-level data, to ensure appropriate interpretation of results and robust advice to industry.

Acknowledgements

The authors acknowledge the generous financial support of New Zealand dairy farmers through DairyNZ Inc. (Grant #AN1202) and the Ministry for Business, Innovation, and Employment, New Zealand (Grant #DRCX1201).

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