



The effect of reducing dietary crude protein on nitrogen utilisation, milk production, health and fertility in dairy COWS.

Report prepared for DairyCo

January 2013



Farmer messages

1. Dietary protein levels for lactating dairy cows could be reduced to perhaps as low as 14% crude protein (CP) with no or little loss in milk yield and quality so long as due regard is given to the composition of the diet with respect to level and nature of forage and concentrate inclusion. Predicting milk yield responses to low levels of dietary CP, however, is complicated by the paucity of data that exists for contemporary high-yielding dairy cows, particularly for UK feeding systems. More research, therefore, is required to better quantify production responses to low dietary CP levels, particularly during early lactation. In the meantime, caution should be exercised when formulating low CP diets for dairy cows.
2. There is some evidence that excess dietary protein may increase the risk of lameness, particularly solar ulcers. Hoof growth may benefit from supplementation with methionine but any benefit may be limited to high-yielding cows in early lactation. There is currently little evidence that altering dietary protein level or amino acid supplementation in late pregnancy or lactation will significantly improve other areas of cow health.
3. Increasing dietary protein level or quality in late pregnancy or lactation does not affect body fat mobilisation except in cows with a high body condition. These animals have a tendency to mobilise more body fat. Increasing protein quality does not have a consistent benefit on fatty liver syndrome.
4. High dietary crude protein is not of benefit to fertility and can be detrimental while low protein diets can be fed with no apparent detrimental effects on fertility, though this effect requires confirmation in the modern high-yielding cow.
5. Reducing dietary CP levels can increase the efficiency of N capture and reduce N excretion to the environment. To achieve maximal N use efficiency, however, milk yield, cow fertility and health must not be significantly compromised.
6. Dairy cows are particularly efficient in recycling urea when fed low-protein diets. The Feed into Milk (FiM) rationing system used in the UK demonstrates that the efficiency of using protein for milk protein synthesis is greater for diets which are deficient or marginal in protein than those which are adequate. There are many possible explanations for why the FiM system does not always predict milk true protein responses accurately. In particular, the system was designed to calculate requirements and supply of metabolisable protein (MP) to the cow rather than predict responses. With the current desire to lower protein contents of diets, however, consideration of likely responses becomes more important because the safety net of excess MP will be removed.

Abstract

In light of increasing global protein prices and, with the need to reduce environmental impact of contemporary systems of milk production, the current review seeks to assess the feasibility of reducing levels of dietary crude protein (CP) in dairy cow diets. At CP levels between 140 and 220g/kg DM, there is a strong positive relationship between CP concentration and dry matter intake (DMI). However, such effects are modest and reductions in DMI when dietary CP is below 160g/kg DM can be at least partially offset by either improving the digestibility (and amino acid profile) of the undegradable protein (UDP) component of the diet or by increasing fermentable ME. Level and balance of intestinally absorbable amino acids, in particular methionine and lysine, may become limiting at lower CP concentrations. In general, the amino acid composition of microbial protein is superior to that of UDP, so that dietary strategies that aim to promote microbial protein synthesis in the rumen may go some way to correcting amino acid imbalances in low CP diets. For example, reducing the level of neutral detergent fibre, while increasing the proportion of starch, can lead to improvements in N utilisation as great as that achieved by reducing dietary CP to below 150g/kg. A systematic review and meta-analysis of responses to rumen protected forms of methionine and lysine was conducted for high-yielding cows fed diets containing \leq 150g CP/kg DM. This analysis revealed a small but significant ($P=0.002$) increase in milk protein yield when cows were supplemented with these rumen protected amino acids. Variation in milk and milk protein yield responses between studies was not random but due to differences in diet composition between studies. Cows fed low CP diets can respond to supplemental methionine and lysine so long as DMI is not limiting, metabolisable protein (MP) is not grossly deficient and other amino acids such as histidine and leucine do not become rate limiting. Whereas excess dietary protein is known to impair reproduction and can contribute to lameness, there is no evidence to indicate that reducing dietary CP levels to around 140-150g CP/kg DM will have any detrimental effect on either cow fertility or health. Contemporary models that estimate MP requirements of dairy cows require refinement and validation in order to predict responses with low CP diets.

Introduction

The combined effects of an increased cost of soyabean meal and recent legislation on the storage and application of cattle manure and slurry has resulted in renewed interest to reduce protein levels in dairy cow diets. Most studies indicate that only around 25-35% of dietary protein is captured and secreted in milk, with most of the remaining N being lost in urine and faeces (eg [Broderick, 2003](#)). This not only represents a potential environmental hazard, but is costly in feed use. With respect to cost, although there is scope to use alternative less expensive vegetable sources of protein or non-protein nitrogen sources such as feed grade or slow-release urea formulations ([Sinclair et al., 2012](#)), real financial savings and reduced losses of dietary N to the environment necessitate lower protein levels in dairy cow diets. Indeed, it is recognised that the main factor influencing the excretion of N from dairy cows is protein intake, and there is a very strong and positive relationship between manure N output and dietary protein intake (r^2 of 0.9; [Yan et al., 2010](#)). Furthermore, a recent meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield, and efficiency of utilisation of dietary nitrogen for milk protein synthesis, concluded that the crude protein (CP) concentration of the diet is the most important dietary factor influencing milk nitrogen efficiency, and that reducing dietary CP is the most significant means by which to increase efficiency of dietary protein utilisation ([Huhtanen and Hristov, 2009](#)).

Studies have begun to assess the effects of feeding reduced levels of dietary CP on milk yield and composition, although there is only limited data on the effects of reduced CP on dairy cow health and fertility. Furthermore, systems developed to predict feed intake and the energy and protein requirements of dairy cows are based on a rather limited range of feeding regimens that offer conventional (typically 170 to 200g CP/kg DM) levels of dietary protein. Uncertainty, therefore, surrounds the ability of these systems to accurately predict animal requirements and to model animal performance at lower dietary CP levels. Looking forward to an era of feeding low CP diets to high-yielding dairy cows, it will be necessary to develop nutritional strategies that optimise nitrogen (N) capture in the rumen, enhance N digestion and absorption in the lower gut, and improve post-absorption N utilisation and partition towards the mammary gland. The current review seeks to address these issues and to provide some guidance towards future research endeavours and nutritional advice offered to dairy producers.

The scientific basis for current dietary protein recommendations

Like all mammals, the dairy cow must meet its protein needs ultimately from the diet. Unlike non-ruminants, however, which can only utilise amino acids from true protein, the dairy cow can utilise non-protein nitrogen as well as true protein. This is because rumen microbes can synthesise amino acids from non-protein nitrogen (eg urea) as well as true protein. Thus, the basic unit of protein nutrition in dairy cows is nitrogen, although this is normally expressed as CP, which is nitrogen times 6.25 (based on 16% average nitrogen content of proteins in plants and animals).

Historically, protein evaluation was concerned solely with matching dietary CP intake to outputs of CP in milk, faeces and urine. Milk yield and liveweight are the main determinants of feed intake and protein output, so it was easy to calculate the required concentration of dietary crude protein. Surprisingly, this ethos still persists and advisors often emphasise the CP content of a diet even though this gives no information about the fate of the various protein fractions (eg that degraded in the rumen and that which by-passes rumen degradation).

In 1980, the UK Metabolisable Protein (MP) system was launched ([ARC, 1980](#)). The MP system acknowledged that ruminants can utilise amino acids synthesised by rumen microbes as well as those directly from the diet, and that rumen microbes require nitrogen for growth. Yield of microbial protein depended on the rate and extent of protein breakdown by rumen microbes, and also on the supply of energy available for microbial growth in the rumen (ie fermentable energy). Thus, the critical measures of protein supply were rumen degradable protein (RDP), undegradable dietary protein (UDP) and fermentable metabolisable energy (FME). MP supply was calculated from microbial crude protein (MCP) and UDP supplies. Net Protein requirements were calculated from nitrogen output in milk, nitrogen accumulation during pregnancy, endogenous nitrogen losses, and nitrogen in liveweight change. Net protein requirements were converted to MP supply through an efficiency factor for each metabolic process. A revised system was published in 1993 which refined calculations of rumen nitrogen and energy supply, and improved estimation of host protein requirements ([AFRC, 1993](#)).

Experience with implementation of [AFRC \(1993\)](#) revealed several deficiencies. A consortium called 'Feed into Milk' (FiM) was established in 1997 to address these deficiencies and to develop an improved system. The outputs of FiM consisted of new equations for predicting

dry matter intake, energy and protein supply and requirements, rumen stability, milk composition, and amino acid responses (Thomas, 2004). The main features of relevance to protein evaluation were a new rumen model for calculating microbial protein yield, new estimates of MP requirements for maintenance, and a decision support system for reporting adequacy of essential amino acid supply.

The central feature of the FiM rumen model is that it defines energy supply for microbial synthesis as adenosine triphosphate (ATP) rather than FME (Thomas, 2004). This is biologically more meaningful because FME has a fixed value for a given diet, whereas ATP supply varies according to rumen degradation characteristics of feeds and rumen outflow rate. A further refinement is that rumen outflow rate is calculated separately for soluble and small feed particles, concentrates and forages; these fractions leave the rumen at different rates in the liquid and solid phases of digesta. As with AFRC (1993), MCP yield in FiM is calculated according to both energy (ATP) and protein (RDP) supplies and the lower value is taken for actual MCP yield.

An important change to protein requirements introduced by FiM was an increase in MP required for maintenance, which was significantly lower in AFRC (1993) than in other protein evaluation systems (Thomas, 2004). In AFRC (1993), endogenous nitrogen losses had been calculated at a maintenance level of feeding with no adjustment for higher levels of intake. In FiM, coefficients from NRC (2001) were adopted to recognise that faecal nitrogen losses increase with intake. A further adjustment was added to account for reabsorption of endogenous nitrogen from the hind gut, again using equations from NRC (2001).

A decision support system was incorporated into FiM to predict supply and adequacy of essential amino acids. This was based on the system adopted by INRA (Rulquin and Verité, 1993) to predict supply and responses to metabolisable lysine (Lys) and methionine (Met) which, in FiM, are compared to threshold response values. Unlike a true requirement or response system, the decision support system only gives a warning if supplies of Lys or Met are marginal or deficient; the user can then reformulate if required.

As part of the FiM project, the FiM protein model was evaluated against production data from five studies involving a total of 50 dietary treatments (Figure 1). When diets were categorised according to MP supply as a percentage of requirements into adequate (>102%), marginal (98-102%) and deficient (<98%), strong relationships ($r^2 = 0.71$ to 0.92) were observed between MP supply and yield of true protein (TP) in milk (Thomas, 2004). The slopes of the

regression lines indicated that for MP-deficient diets MP was used at maximum efficiency, and that efficiency was lower when MP was oversupplied. It was concluded that the FiM protein model performed well as a requirement-based model. This conclusion was reached also by [Agnew and Newbold \(2002\)](#) in their review of feeding standards for dairy cows, but they added the caveat that the FiM protein system should be evaluated with animals in a grazing situation.

A feature of the evaluation reported by the FiM consortium (Figure 1) is that the majority of diets categorised as deficient were associated with greater outputs of milk protein than diets categorised as marginal or adequate. This is particularly noticeable in the middle of the range of MP supply reported (1500 g/d), where MP-deficient diets produced 50% more milk TP than MP-adequate diets at the same level of MP supply. Conceptually, a diet classified as deficient in MP would be expected to result in lower milk protein output.

To examine whether this feature holds true for other datasets, data from two independent studies were used to evaluate the FiM MP model following the same methodology as the FiM consortium. In the first study, a diet designed to be MP-deficient was compared with diets supplying more MP either from fishmeal or a vegetable blend at low and high levels ([Allison and Garnsworthy, 2002](#)). In the second study, diets with low and high MP contents had low and high leucine concentrations in a factorial arrangement ([Garnsworthy et al., 2008](#)). For both studies, milk TP yield and MP supply were higher than most of the values included in the FiM evaluation. Nevertheless, strong relationships were observed for milk protein response to MP supply in diets categorised as deficient and marginal (combined), or adequate (Figure 2).

As reported in the FiM evaluation, the slope of the response, ie efficiency of MP utilisation, was greater for deficient/marginal diets (0.80) than for adequate diets (0.62). Furthermore, MP-deficient and MP-marginal diets produced more milk TP than MP-adequate diets at the same level of MP supply, thus concurring with the FiM evaluation report. A worrying conclusion could be reached, therefore, that diets which are deficient or marginal in MP do result in greater milk TP output than diets which are adequate in MP.

The most likely explanation for this anomaly is not that the FiM MP model is flawed, but that the evaluation methodology has a high likelihood of producing this anomaly. In both FiM and the current evaluation, diets were classified *a posteriori* according to MP balance calculated from observed performance, not from targets used in diet formulation. Consequently, a diet

that results in milk TP outputs higher than predicted from MP supply will be classified as deficient; a diet that results in milk TP outputs lower than predicted from MP supply will be classified as adequate. This does not mean, therefore, that formulating a diet to be deficient in MP will enhance milk protein yield; it means that responses were influenced by something other than MP supply.

Diets might influence milk TP output beyond that predicted by MP supply in several ways:

1. Efficiency of MP utilisation for milk synthesis is greater for diets that are marginal or deficient in MP supply; this was acknowledged in FiM, but a fixed efficiency of 0.68 was adopted.
2. Overall, nitrogen efficiency is increased for low protein diets by increased nitrogen recycling; FiM does not include a model for nitrogen recycling, although it does include greater reabsorption of nitrogen from the hind gut with increasing feed intake.
3. Overall, nitrogen efficiency is increased for low protein diets, also, by decreased catabolism of protein for energy ([Metcalf et al., 2008](#)) and for high protein diets by increased mobilisation of body reserves ([Garnsworthy and Jones, 1987](#)).
4. Amino acid profile of DUP can affect response to MP supply. Lys and Met responses are acknowledged in the FiM decision support systems as thresholds, but leucine enhanced responses in both [Allison and Garnsworthy \(2002\)](#) and [Garnsworthy et al. \(2008\)](#). Leucine is the major amino acid found in microbial protein so that when MCP contributes a greater proportion of MP the complete amino acid profile approaches that of the ideal. In contrast, diets that are marginal for MCP yield might enhance performance if they supply DUP with greater leucine content.
5. Diets supplying MP above requirements are likely to incur an energy cost for detoxifying excess ammonia; this cost is estimated to be 0.8 MJ per 100g surplus protein ([Twigge and van Gils, 1984](#)).
6. There are many interactions between energy and protein supplies in determining milk TP output. [Newbold \(1994\)](#) discussed the need for different optimal ratios of MP: ME at different levels of ME intake. [Metcalf et al. \(2008\)](#) reported responses to MP above optimal MP: ME ratios, but with decreasing efficiency of MP utilisation; ME supply was intended to be fixed, but responses to some treatments were confounded by changes in DMI. [Brun-Lafleur et al. \(2010\)](#) reported interactions between fixed supplies of ME and MP above and below requirements: the response of milk yield to energy supply was zero with a negative protein balance and increased with protein supply equal to or higher than requirements.

7. The FiM model to predict DMI includes a positive association between CP concentration of concentrates and DMI, although the effect is exaggerated when CP intake from concentrates exceeds 3.5kg/d.

Milk yield responses to dietary protein

The following discussion is based on strategies that seek to reduce the level of dietary CP in dairy cow rations, the consequences that this might have for milk yield and composition, and the means by which reductions in yield can be mitigated. It is recognised, however, that an alternative or perhaps complementary strategy to that of flat-rate reductions in dietary protein might be to oscillate daily CP levels thereby taking advantage of the ruminant animal's ability to salvage and recycle urea-N to the rumen and/or gastro-intestinal tract ([Reynolds and Kristensen, 2008](#); [Cole and Todd, 2008](#)). As acknowledged in the preceding section, this ability to recycle urea is particularly efficient when ruminants are fed low-protein diets. In low-producing ruminants, such as beef cattle and sheep, CP deficient forage-based diets can be supplemented with natural protein sources at intervals up to 72 hours with no adverse effect on animal performance. In some instances, N-retention has actually been increased ([Cole, 1999](#); [Archibeque et al., 2007](#)). While such a strategy may appear attractive, in terms of reducing dietary costs and also to reduce N excretion, the efficacy of such a feeding regimen in high-yielding dairy cows has not been properly investigated and, therefore, will not be considered further in this review. A further consideration pertains to recent interest in extended lactations for dairy cows. Some studies have considered calving intervals of up to two years (eg [Kolver et al., 2007](#); [Butler et al., 2010](#)). Intuitively, one might perceive greater scope to reduce dietary CP levels in such systems. However, to the best of our knowledge, no such studies have been conducted and, given that there are doubts regarding the economic and environmental viability of such systems (eg [Butler et al., 2010](#); [Wall et al., 2012](#)), the effects of dietary CP reduction in such systems will not be considered further in this review.

Crude protein and intake

There are well-established positive responses in milk yield that accompany increases in dry matter intake (DMI) of dairy cows ([Hristov et al., 2004](#)) and, given that DMI responds positively to increased dietary CP concentration ([Oldham, 1984](#); [Newbold, 1994](#)), it follows that a significant component of the milk yield response to dietary CP ([Ipharraguerre and Clark, 2005](#)) resides in improvements in voluntary intake. In turn, this arises in part through

improvements on digestibility (in particular that of fibre) in the rumen (Faverdin, 1999), but also due to direct and indirect effects on intermediary metabolism, nutrient partitioning to the mammary gland and metabolic signalling of intake regulation (Ingvarsen and Andersen, 2000; Huhtanen et al, 2008). It should be noted, however, that while such effects are quite well characterised in non-ruminants (particularly in rodents), this is not the case in ruminant species, and effects of dietary protein on intake regulation in dairy cows have not been a hot topic of investigation in recent years. Faverdin (1999) usefully identified nitrogenous nutrients as having both putative short- and long-term effects on feed-intake regulation. The former has been ascribed to the actions of ammonia (NH_4) which, when absorbed rapidly across the rumen wall at levels that exceed the liver's capacity to detoxify, can, as we have shown in cattle, act on central mechanisms to depress feed intake (Sinclair et al., 2000a). It is unlikely, however, that such levels of peripheral NH_4 would be attained with contemporary TMR diets of 18% CP or less, although blood NH_4 may come into play at higher levels of protein feeding, particularly with high effective (E)RDP:FME silages. Longer-term effects of CP on intake are equally difficult to characterise fully but are dependent on the digestibility and nature (ie grass vs grass silage vs maize silage) of the basal forage, as well as the level of concentrate supplementation. In general, intake responses to increased CP are greatest for diets of low digestibility, for maize rather than grass silages, and where high levels of concentrates are fed. Newbold (1994) proposed that intake response to MP for grass-based silages is probably due to MP supply, whereas, for maize-based silages, responses are due to the effects of both ERDP and MP supply. However, post-ruminal effects of amino acid supply on feed intake in dairy cows have been difficult to quantify, varying as it does by the proportion and source of ERDP and the degradability and amino acid content of DUP (Ipharraguerre and Clark, 2005). Furthermore, intake responses to increased MP usually have arisen when animals were deficient in ME.

In an era of reducing CP levels, and in order to minimise feed costs and N excretion to the environment, the challenge is how to mitigate effects associated with reduced DMI. Contemporary evidence for high-yielding dairy cows, however, indicates that reductions in intake are likely to be modest at CP levels at or above 140g/kg DM (eg Broderick, 2003; Colmenero and Broderick, 2006; Law et al., 2009a; Lee et al., 2011) and could be at least partially offset by either improving the digestibility of the undegradable protein component of the diet (Noftsker and St-Pierre, 2003), by increasing FME (Herrera-Saldana et al., 1990), and/or by increasing the overall ME of the diet (thereby altering the ME:MP ratio).

Physiological status (stage of lactation)

Dietary requirements to support milk production vary during lactation reflecting changes in milk yield, appetite and endocrine regulation of nutrient partitioning between tissues and the mammary gland. Scope, therefore, may exist to modify dietary protein levels at different stages during lactation in order to increase the efficiency of nitrogen utilisation and to maximise milk yields. Working with contemporary high-yielding Holstein-Friesians, [Law et al. \(2009a\)](#) investigated the effects of three different dietary CP concentrations on milk yield and nitrogen retention during either early or late lactation using a partial change-over design. The efficiency of N use for milk production (defined as kg of milk N output per kg dietary N intake) was 0.42, 0.39 and 0.35 for dietary CP concentrations of 114, 144 and 173g/kg DM respectively, during early (ie the first 150 days) lactation. The efficiency of N utilisation for milk production for the six treatment combinations for the remaining 155 days of lactation is depicted in Figure 3. Collectively, these results reveal that the efficiency of N capture for milk production is proportionately greater at lower CP concentrations. In this respect, these results agree with earlier observations ([Huhtanen and Hristov, 2009](#)). Whole lactation milk yields for cows on the lowest (ie 114g CP/kg DM) level of protein were 30% below that for cows on the highest (ie 173g CP/kg DM) level of protein. In contrast, whole-lactation milk yields were reduced by only 10% and 7% for cows on the 144/144 and 173/144 treatment groups, respectively, suggesting that there may be scope to mitigate losses associated with reduced dietary CP concentrations by manipulating diet composition (discussed later). This is important for strategies that aim to reduce dietary CP concentrations throughout lactation, as they offer the greatest potential to reduce feed costs and N excretion in the environment.

Diet composition and milk yield responses to crude protein

[Broderick \(2003\)](#) demonstrated the importance of energy source in determining N efficiency in lactating dairy cows. Increasing dietary energy, by reducing forage (thereby neutral detergent fibre (NDF)) and increasing the proportion of shelled corn (mostly starch), increased yields of total and true milk protein (Figure 4), and improved the efficiency of N use (defined earlier) from 0.25 to 0.30. In contrast, reducing dietary CP concentrations from 184 to 151g/kg DM had little effect on total milk protein yield and no effect on true milk protein yield, although efficiency of N use was improved. The proportion forage in that study was reduced from 0.75 to 0.50. It is uncertain by how much further efficiency of N use can be improved by altering concentrate carbohydrate source for levels of forage inclusion less than 0.50. However, level and balance of intestinally absorbable amino acids, in addition to

energy-yielding metabolites, are central to sustaining milk yield and composition with minimal dietary protein. It is generally accepted that, of the 10 essential amino acids (EAA), Met and Lys are normally the first two rate-limiting for growth and milk production. Diets high in NDF reduce the proportion of Met and Lys in duodenal proteins, perhaps as a consequence of reduced flow of microbial proteins (which are richer in Met and Lys) to the duodenum (Robinson, 2010). In general, the amino acid composition of MCP is superior to that of DUP as it more closely resembles the amino acid composition of milk and lean tissues (NRC, 2001). In contrast, the amino acid composition of DUP more closely reflects that of the various vegetable feedstuffs from which it is derived. Consequently, dietary strategies that aim to maximise the intestinal delivery of MCP may go some way to mitigating the effects of reduced supply of dietary CP. Such strategies are inextricably linked to energy metabolism within the rumen. Increased MCP probably accounted for much of the positive milk protein yield responses to dietary energy observed by Broderick (2003).

As the efficiency of conversion of MP and that of metabolisable amino acids into milk true protein declines with both increasing supply of MP (Metcalf et al., 2008) and with Met and Lys (Vyas and Erdman, 2009), it follows that production responses to rumen-protected forms of specific amino acids may increase at lower dietary protein levels so long as dietary energy intake and other amino acids do not become rate limiting. Most major protein sources contain less Met and Lys than does MCP, so that the proportion of these limiting amino acids in duodenal digesta decline at higher levels of dietary CP (Robinson, 2010). Sometimes, however, responses to added Met and Lys are influenced by the supply of other potentially limiting amino acids (eg histidine) and energy (eg gluconeogenic) metabolites. In a systematic review and analysis of dietary protein sources and responses to metabolisable Lys and/or Met, Robinson (2010) found that milk yield responses to Met and Met + Lys generally increased as CP level of the basal diet increased, although, as previously stated, marginal efficiencies of metabolisable Met and Lys use for milk protein yield decreased (Vyas and Erdman, 2009). Looking to the future, in an era of reduced dietary protein use, it will be important to establish precise responses to rate-limiting amino acids at low dietary CP levels. With this in mind, a comprehensive systematic review and meta-analysis of the pertinent literature relating to production responses to specific amino acids in lactating cows fed low-protein diets was conducted.

Meta-analysis of responses to rumen-protected amino acids for low CP diets

This meta-analysis used peer-reviewed publications accessible via Thompson Reuters Web of Science and NCBI PubMed. For the latter database, the following MeSH terms included

'cattle', 'metabolism', 'lactation', 'milk', 'protein', 'methionine', 'lysine'. To be eligible, only studies conducted since 1987 were considered, with lactating Holstein cows offered diets with a CP content of ≤ 150 g/kg DM during either early or mid-lactation. Analysis was also limited to those studies and experiments within studies where a combination of rumen-protected Met and Lys were incorporated into the diet, and measurements of milk yield and composition reported. This is because very few studies have considered Lys alone, and it is widely recognised that a balance of around 7.2% Lys and 2.5% Met within MP favours milk production (NRC, 2001). Only one study was included in the final analysis where these two amino acids were infused directly into the abomasum (Robinson et al., 2000). Source and level of protected Met and Lys varied between studies but this was ignored in the final analysis, based on the premise that their use and level of incorporation were predicted by the reporting authors to be sufficient to evoke a metabolic and/or production response. In a systematic analysis of the literature, however, Robinson (2010) noted that few studies made any attempt to actually measure or even predict delivery of absorbable amino acids to the duodenum. The final analysis, therefore, involved 13 peer-reviewed studies with 16 independent experiments. Responses analysed were fat-corrected milk yield (kg/day) and protein yield (kg/day). For the latter variate, it wasn't always clear whether studies reported crude or true milk protein, and so no attempt was made to adjust these values. Data were analysed using a fixed-effects model (Comprehensive Meta-Analysis software, Biostat, Englewood, NJ, USA; <http://www.meta-analysis.com>). Heterogeneity (ie variation in study outcomes between studies) was assessed using the χ^2 test on Cochran's Q statistic and by calculating I^2 (ie percentage of variation across studies that is due to heterogeneity rather than chance). As heterogeneity was present for both analysis of milk yield and milk protein yield, a random-effects meta-analysis was carried out for each. These analyses, however, agreed with that for the fixed model and so only outputs of fixed-model analyses are presented.

Results from these analyses revealed no overall response in FCM yield (Figure 5) but an overall increase ($P=0.002$), albeit small, in milk protein yield (Figure 6) when low CP diets were supplemented with rumen-protected Met + Lys. Furthermore, in the absence of publication bias (data not presented), both sets of analyses revealed significant ($P<0.001$) heterogeneity between studies indicating that biological factors rather than sampling error accounted for variation in responses between studies. Furthermore, the outcome of this meta-analysis matched the general responses of the largest study in this cohort (ie Polan et al., 1991) which was replicated across several institutes and involved, on average, 55 cows per treatment.

The greatest positive response to supplemental rumen-protected Met + Lys was observed in the study of [Robinson et al. \(1995\)](#), which was conducted at two sites with largely similar diets (based on a mixture of maize, lucerne and grass silages) offered to mid-lactation Holstein cows averaging around 31kg/day FCM. Although CP content of the diets was around 140-150g/kg DM they were calculated, based on chemical composition and measurements of intake, to meet but not exceed protein requirements for microbial growth and milk production. Under these conditions, supplementation of 19g/day ruminally protected L-Lys.HCl and 6.5g/day ruminally protected DL-Met evoked a positive response but by different mechanisms at the two sites; at one site, this was due to apparent increases in gross efficiency of utilisation of both protein and energy, while at the other site the response arose largely through improvements in DMI. In the other study that showed significant improvements to rumen-protected AA, [Christensen et al. \(1994\)](#) offered fat (ground high-oil shelled corn) to cows fed a diet containing 142g/kg DM CP and reported increases in FCM yield and milk true-protein yield relative to non-fat supplemented cows fed a diet containing 175g/kg DM CP. The feeding of ruminally protected AA and post-ruminal infusion of casein into cows fed fat-supplemented diets containing 160-170g/kg DM CP was previously found to increase milk protein yields ([Canale et al., 1990](#); [Chow et al., 1990](#); [Cant et al., 1991](#)).

In contrast, the greatest negative effect of supplemental Met + Lys in low (144g/kg DM) CP diets was found in the study of [Robinson et al. \(2000\)](#), where these two AA were directly infused into the abomasum of late-lactation cows receiving 140% of calculated intestinally-absorbable requirements. Such was the magnitude of this effect that removal of this study from the meta-analysis led to a small but significant ($P=0.05$) positive increase in overall FCM yield. The reduction in performance in this study was due to inhibitory effects on DMI. In the two studies of [Lee et al. \(2012 a and b\)](#), the CP content of Control diets was around 157g/kg DM and calculated to be adequate in MP. In contrast, the CP content of treatment diet was ≤ 140 g/kg DM and deemed to be deficient in MP. The inclusion of rumen-protected Met + Lys failed to increase FCM and milk protein yields to levels comparable to the MP-adequate Control groups in these studies but it did, in the study of [Lee et al. \(2012b\)](#), lead to small but significant improvements in milk protein yield relative to a second CP and MP deficient group, where no rumen-protected AA were added.

The variability in milk yield and compositional responses for diets of ≤ 150 g/kg DM CP highlighted by these analyses agree with the general findings of previous systematic reviews which considered a wider range of dietary protein levels ([Vyas and Erdman, 2009](#); [Patton,](#)

2010; Robinson, 2010). The general consensus is that it is possible to manipulate the proportions of Met and Lys in duodenal protein, but predicting this and production responses that may arise are difficult. There is also a consensus that responses to added Met and Lys are generally superior to either amino acid offered alone, particularly during early lactation, but that the response to one amino acid is not necessarily dependent on the addition of the other (Patton, 2010). Responses to added amino acids do, however, depend on diet composition (eg level and nature of forage) and animal factors such as stage of lactation, body composition, negative energy balance and milk yield; all of which will determine nutrient priorities and partitioning to the mammary gland. Variability in responses also exists between commercially available sources of rumen-protected Met and Lys, which is beyond the scope of the current article but is the subject of related reviews (eg Robert, 2005; Vyas and Erdman, 2009); and it is evident from Robinson et al. (2000) that excessive levels of rumen-protected amino acids may be detrimental.

Dietary Protein and Cow Health

Excess dietary protein has long been suggested as a contributory factor to lameness in dairy cattle with several possible modes of action proposed. These include high levels of ammonia emanating from excess rumen degradable protein, free ammonia in silage (Bazeley and Pinsent, 1984) or an allergic response to histamine (Chew, 1972; Nilsson, 1963). Evidence to support the relationship between dietary protein and lameness is, however, somewhat limited. One of the main studies to find an association (Manson and Leaver, 1988) reported an increase in locomotion score (higher scores indicating poorer locomotion) and number of clinical cases of lameness, particularly solar ulcers, when dietary CP was increased from 161 to 198g/kg DM. Additionally, there was a significant and negative relationship between locomotion and liveweight change, which may have subsequent effects on fertility or fatty liver syndrome (FLS). Others have also reported a negative relationship between dietary CP level and lameness (Bazeley and Pinsent, 1984). In contrast, protein was not identified as a risk factor for lameness in high producing dairy cows (Espejo and Endres, 2007), nor was a high CP diet associated with any changes in hoof characteristics of dairy calves (Momcilovic et al., 2000). Spring grass is often high in rumen degradable protein which in theory may present a particular risk to solar lesions. It is difficult, however, to differentiate the effects of protein from that of the rapid rate of ruminal fermentation of carbohydrate in grass and, as a consequence, there is no conclusive evidence to suggest a direct negative effect of protein levels in grass on lameness (Westwood et al., 2003). It is also increasingly likely that hormonal and physiological changes associated with the onset of calving and lactation are

the primary factors associated with claw horn lesions rather than dietary stress ([Tarlton et al., 2002](#)).

Notwithstanding the possible negative effects of excess protein on lameness, S-containing amino acids have a key role in the proliferation and differentiation of horn-forming epidermal cells in the bovine hoof ([Hepburn et al., 2008](#)), and, for cyst(e)ine in particular, are present at concentrations in the wall and sole horn well in excess of that found in microbial protein or DUP ([Galbraith and Scaife, 2007](#)). Given the oxidative instability of L-cysteine and the post-absorptive ability of animals to convert methionine to cysteine, most studies have focused on the effects of supplementation with Met or its analogues. Supplementation with L-Met *in vitro* has been demonstrated to increase protein synthesis in epidermal horn-forming cells ([Hepburn et al., 2008](#)), although the response *in vivo* is less certain. For example, [Laven and Livesey \(2004\)](#) reported no benefit to supplementary rumen-protected Met (115% vs 95% of requirements) on solar haemorrhages or locomotion score in dairy cattle, while there was no significant effect on the rates of growth or wear of the hoof horn ([Livesey and Laven, 2007](#)). Similarly, feeding animal protein that is high in S-containing amino acids had no beneficial effect on lameness or the development of solar lesions in the study of [Offer et al. \(1997\)](#). In contrast, others have reported an increase in hoof growth from amino acid supplementation, although this was not necessarily associated with an increase in hoof hardness ([Clark and Rakes, 1982](#)). The apparent inconsistency in response *in vitro* and *in vivo* may in part be explained by the relative requirements for S-containing amino acids (particularly Met) for milk production, with studies that have reported no benefit to supplementation generally being conducted on low-yielding cows (eg [Offer et al., 1997](#); [Laven and Livesey, 2004](#)). It could, therefore, be envisaged that beneficial effects of supplementary Met on solar lesions and lameness may become more pronounced as genetic potential for milk production advances.

The onset of lactation reduces neutrophil and lymphocyte function ([Kehrli and Shuster, 1994](#)) with glutamine, in particular, being utilised at high rates by leucocytes for nucleotide biosynthesis ([Goff, 2003](#)). It is well established that increasing the protein supply to ewes in late gestation improves their immune response and decreases the periparturient rise in faecal egg counts (eg [Donaldson et al., 2001](#)), but the beneficial effects in dairy cows is less well proven. Some (eg [Curtis et al., 1985](#); [Rode et al., 1994](#)) have reported a decreased risk of retained placenta and metabolic disease with increased dietary protein or amino acid supplementation but, in general, there is a paucity of studies that have been specifically designed to investigate the effect of protein on cow health. As a consequence, those that have are limited by the number of replicates, minimising the likelihood of detecting an effect

of treatment. The general consensus across these studies, however, is that neither pre- nor post-partum protein nutrition has a major effect on milk somatic cell count (SCC), incidence of mastitis or other disease (eg [Wu and Satter, 2000](#); [Garnsworthy and Jones, 1987](#); [Putnam et al., 1999](#)). Met has been implicated as having an important role in improving the immune system in dairy cattle, and supplying a rumen-protected Met supplement at a comparatively high level (30g/d) was shown to increase the proliferative response of peripheral T-lymphocytes in mid-lactation dairy cows, although milk SCC was not affected ([Soder and Holden, 1999](#)). Other studies that have examined Met supplementation have found no beneficial effect on SCC (eg [Piepenbrink et al., 2004](#); [Robinson et al., 2010](#)).

Tissue, body fat/protein mobilisation

An increase in dietary protein supply in early lactation is associated with a decrease in the responsiveness of adipose tissue to anti-lipolytic stimuli such as insulin, and increases in the responsiveness to lipolytic stimuli such as epinephrine ([Schor and Gagliostro, 2001](#); [Cadórniga and López Díaz, 1995](#)). It would, therefore, be anticipated that increasing tissue protein supply should be translated into a net increase in body fat mobilisation. The relationship between dietary protein concentration and liveweight change and body fat mobilisation (as represented by plasma non-esterified fatty acids (NEFA); Figures 7a and b), does not support this. Studies that have examined the influence of DUP level within the same dietary protein concentration have also failed to find a consistent relationship (Figure 8). The effects of protein supply on body tissue mobilisation are, however, confounded by dietary factors (eg amino acid profile, dietary energy concentration and source), and animal factors (eg body condition score, stage of lactation and DM intake). For example, [Jones and Garnsworthy \(1988\)](#) and [Cadórniga and López Díaz, \(1995\)](#) demonstrated a greater negative energy balance in cows that had a high body condition score when provided with diets with a high by-pass protein content, but not in cows with a low condition score. Additionally, increased dietary protein intake or DUP supply is often associated with an elevated DM intake, reducing the requirement for body tissue mobilisation.

Liver steatosis (or fatty liver; FLS), occurs during the periparturient period in dairy cows when excess body fat is mobilised and it has been estimated that approximately 45% of dairy cows in early lactation have a moderate steatosis ([Jorritsma et al., 2000](#); [Bobe et al., 2004](#)). It is known that FLS can influence the ability of the liver to metabolise excess ammonia ([Strang et al., 1998](#)), which may have further implications for fertility and gluconeogenesis, discussed elsewhere in this paper. Dairy cows have been shown to have a

particularly low rate of secretion of very low density lipoproteins (VLDL) from the liver ([Grummer, 1995](#)), and enhancing the rate of lipid transport from the liver offers one option to reduce the onset of FLS. Met serves as a methyl donor for the synthesis of apolipoprotein B100 and phosphatidyl choline in the liver. These metabolites are required for the hepatic production of VLDL, and are necessary for the synthesis of carnitine, required for the uptake of long-chain fatty acids across mitochondrial membranes ([Grummer, 1995](#)). It has, therefore, been suggested that a deficiency of Met may restrict hepatic VLDL synthesis, and surveys of dairy cows that have experienced severe liver steatosis in the periparturient period indicate a strong association with low plasma Met concentrations ([Shibano and Kawamura, 2006](#)). However, controlled studies that have investigated the response to supplementary Met have not produced a conclusive benefit ([Piepenbrink et al., 2004](#); [Bertics and Grummer, 1999](#); [Durand et al., 1992](#)). Indeed, some have reported an increase in hepatic lipid concentrations ([Preynat et al., 2010](#)), and, therefore, the practical role of Met on energy metabolism in early lactation is unclear.

An alternative source of amino acids for metabolism in early lactation is the mobilisation of body protein reserves. Mobilisation of body protein in early lactation can be large, with estimates of over 25% of body N ([Botts et al., 1979](#)) or in the region of 15kg ([Paquay et al., 1972](#)). This is equivalent to an extra daily dietary protein supply over the first 100 d of lactation of approximately 250g, or 5-10% of protein intake. The mobilised amino acids, particularly Met, may be used to enhance the rate of lipid transport from the liver and reduce the risk of FLS in early lactation, as described previously. A number of studies have therefore, been conducted with the objective to enhance body protein reserves for subsequent mobilisation in early lactation. Although some, but not all, studies have shown a benefit to milk protein output (see review of [Friggens et al., 2004](#)), there is little benefit on reducing liver triglyceride concentration or the incidence of health disorders in early lactation (eg [Huyler et al., 1999](#); [Vandehaar et al., 1999](#); [Greenfield et al., 2000](#)). Interestingly, cows that are most susceptible to the development of metabolic disorders (ie above BCS of 3.25 units) respond better to increased dietary protein supply in late gestation as evidenced by lower blood NEFA levels ([Putnam and Varga, 1998](#)), a situation similar to that found when supplying additional protein in early lactation. A confounding effect of feeding elevated protein levels in late gestation is the association with an increase in intake. This leads to an increase in BCS, which in itself depresses intake in early lactation and promotes fat mobilisation, predisposing the cow to health issues such as downer cows ([Grummer, 1995](#)) and metabolic disease ([Friggens et al., 2004](#)).

Dietary protein and fertility in lactating cows

Numerous research papers and reviews have addressed the impact of high dietary CP on fertility. While the overall tendency has been for studies to report negative associations between high dietary CP and a range of fertility parameters, many studies have found little or no effect. As well as variability in the results reported, many of these studies have been confounded by insufficient numbers of animals to accurately test effects on pregnancy rates and by failure to control the many and varied factors that interact to determine the outcome of mating. Furthermore, in some studies, the levels of dietary CP that have led to negative effects have been as high as 27.4% (Garcia Bojalil et al., 1994), well in excess of levels fed in commercial diets. One approach to dealing with these issues is meta-analysis and, in a recent comprehensive analysis on the effect of dietary CP on pregnancy rate, Lean et al. (2012) reported an overall 9% (P=0.019; 95% confidence interval 2% to 16%) reduction in risk of conception in cows fed diets containing higher or more degradable CP within a dietary CP range of 111 to 230g/kg. While this study represents a comprehensive analysis, it should be noted that some of the studies included were conducted over 30 years ago and their relevance to the modern high-yielding Holstein cow with her reduced reproductive competence may be tenuous. However, what appears clear is that high dietary CP is not good for fertility and can be detrimental.

While limited studies have directly addressed the influence of type of dietary protein on fertility parameters, a recent study by Aboozar et al., (2012) reported improvements in a range of reproductive traits in cows fed high levels of rumen undegradable protein. Earlier studies in dairy cows (Bruckental et al., 1989) and twin-suckling beef cows (Sinclair et al., 1994) indicated that increasing the level of DUP in the diet can have a beneficial effect on fertility when associated with reduced plasma urea concentrations. Such benefits await further confirmation in contemporary high-yielding dairy cows.

Reductions in dietary CP: Effects on cow fertility

While numerous studies have tested the hypothesis that high dietary CP is bad for fertility, far less emphasis has been placed on the effects of low CP diets on reproductive parameters. The results of 6 studies in which balanced groups of lactating dairy cows were fed either high or low levels of dietary CP are summarised in Table 1. Reductions in dietary protein can lead to a fall in milk yield and an associated reduction in negative energy balance, a key factor driving poor fertility. However, in the results presented here, while milk

yield was consistently reduced, low CP diets did not appear to have any major effects on reproductive performance. However, it should be noted that most of these studies were carried out some time ago and the milk yields recorded bear little comparison to the much higher yields achieved in the modern dairy cow. Thus, while these studies suggest that a reduction in dietary protein may be possible without impacting on fertility traits, it is essential that the effects of such an approach are assessed in modern high-yielding cows where metabolic demands are far in excess of animals studied previously.

Nitrogenous metabolites: urea and ammonia

Dietary CP intake can be reflected in concentrations of plasma or milk urea. Urea measurements have been used in a number of studies to assess the relationships between levels of the metabolite and fertility parameters. Plasma levels of urea in excess of 6.8 mmol/l have for many years been considered indicative of negative influences on reproduction ([Butler et al., 1996](#)). However, the numerous studies looking at the association between circulating urea levels and fertility within dairy herds have produced variable results. In the UK, studies have reported reduced conception rates in cows with elevated plasma urea levels (> 7.5 mmol/l; [Wathes et al., 2007](#)) while others have failed to find any relationship ([Cottrill et al., 2002](#); [Mann et al., 2005](#)). In a recent large study in Poland ([Sawa et al., 2011](#)), while significant relationships were reported between milk urea and a number of fertility parameters, the associated R values were low (typically 0.01 - 0.07) emphasising the tenuous nature of these associations. Furthermore, in a recent and comprehensive meta-analysis, [Lean et al. \(2012\)](#) found no influence of blood urea levels on the effects of dietary protein on conception rate.

That notwithstanding, various correlates of dietary protein, including ammonia and urea, have been shown to act at both the ovarian and uterine level to influence reproductive function ([Butler, 1998](#)). In vitro studies have established the ability of high ammonia/urea to impair oocyte development ([Sinclair et al., 2000b](#); [De Wit et al., 2001](#)) though, in parallel with the effects of feeding high protein diets, the effects are inconsistent. For example, [Ocon and Hansen 2003](#) reported negative effects of 7.5 mM but not 10 mM urea during in vitro maturation on subsequent blastocyst formation. What does appear more consistent is that the oocyte/blastocyst is more susceptible than the developing embryo as [Rhoads et al. \(2006\)](#) reported adverse effects of high urea in donor but not recipient animals following day 7 embryo transfers in lactating dairy cows. It has been suggested that the main site of action of the elevated ammonia/urea associated with the feeding of high protein diets may be the

uterus where elevated urea is associated with lowered uterine pH ([Elrod and Butler 1993](#)). However, in vitro studies would suggest that the period of reproductive susceptibility may occur before the embryo reaches the uterus, suggesting the oviductal environment may be more important. Furthermore, [Carroll et al., 1988](#) reported similar conception rates in cows fed low and high protein diets resulting in widely differing uterine urea levels. Thus, while a number of potential sites of action through which dietary protein can directly influence reproductive function have been identified, no conclusive mechanism has been established.

Benefits for the environment

With policy drives to reduce environmental impacts of livestock systems, increasing attention is being paid to greenhouse gas (GHG) emissions and excretion of nitrogen and phosphorus. Estimates vary, but dairy cow production systems in Europe contribute between 20 and 30% of GHG emissions and similar proportions of nitrogen and phosphate excretions ([Delft, 2008](#); [Garnsworthy, 2012](#)). The dairy cow is very efficient at converting foods unsuitable for human consumption (eg grass, forages, by-products) into a high-quality food product (milk). [Wilkinson \(2011\)](#) calculated that an average dairy cow yields 2.37 MJ of human-edible energy per MJ of human-edible energy intake and 1.64kg of human-edible protein per kg human-edible protein intake. Through using by-products, it is possible to feed cows profitably on diets containing no human-edible feed ([Garnsworthy and Wilkinson, 2012](#)). In terms of total nutrient efficiency, however, the cow is not so good – only 20 to 30% of nutrients consumed are converted into product; the remaining 70 to 80% are released to the environment.

Nitrogen is excreted either as organic nitrogen (40-50% of total N, mostly in faeces) or urea (50-60% of total N, mostly in urine). Although organic nitrogen is relatively stable, urea is readily converted to ammonia, which can be volatilised during storage and spreading of manure. Atmospheric ammonia can damage nitrogen-sensitive habitats when deposited in rain ([Misselbrook and Smith, 2002](#)). If manure nitrogen applied to soil is not taken up by plants, it can contribute to nitrate pollution of groundwater and to emissions of nitrous oxide, which is a potent GHG. The environmental impact of cattle manure depends mainly on methods for storage and handling ([Misselbrook and Smith, 2002](#)), but improving the nitrogen use efficiency (NUE) of milk production will reduce the overall impact of any dairy system.

Nitrogen use efficiency is defined as nitrogen output in milk per unit of nitrogen intake. In practice, NUE varies from 0.16 to 0.37g milk N/100g feed N ([Jonker et al., 2002](#)). Nitrogen

use efficiency is positively related to milk yield per cow because maintenance represents a lower proportion of total nitrogen requirements at higher yields. Milk yield per cow is governed by genetics and nutrient intake but, at the herd level, is affected also by fertility and health which influence the number of animals per unit of milk yield ([Garnsworthy, 2004](#)). After milk yield, the main factor influencing NUE is the supply of protein relative to requirements, as discussed earlier in this article. Excess protein is catabolised as an energy source, and the nitrogen released is excreted in urine. In a survey of 372 dairy farms by [Jonker et al. \(2002\)](#), cows in protein balance had an NUE of 0.30, and NUE changed by 0.05 per 100g N/d above or below requirements; at all levels of supply, however, NUE was highly variable among farms.

There is a direct relationship between total nitrogen excretion and dietary nitrogen intake because during digestion and metabolism some nitrogen losses are inevitable. Two areas where diet composition can affect nitrogen excretion are efficiency of microbial protein synthesis (EMPS) and amino acid composition of DUP. Microbial nitrogen capture varied between 14 and 33g microbial N/kg OM truly degraded in the rumen in a review of 320 in vivo observations ([Archimède et al., 1997](#)). Type of carbohydrate in the diet had a large influence on EMPS with the highest values being for high-starch diets. During rumen fermentation of high-starch diets, supply of fermentable energy will be better synchronised with ammonia release, thus improving microbial nitrogen capture ([Sinclair et al., 1993](#)). More generally, the ratio of RDN to MCP calculated from FME ([Thomas, 2004](#)) should be as close to 1.0 as possible in order to minimise excretion of surplus RDN. The amino acid composition of DUP affects nitrogen excretion through supply of essential amino acids, as described earlier in this article. Deficiencies of Lys, Met and leucine reduce milk protein synthesis resulting in a surplus of non-essential amino acids that will be catabolised.

Conclusions

Care is required when using contemporary dietary formulation programs to ensure that milk yield responses match predictions for cows fed low (ie ≤ 150 g/kg DM) CP diets. Dietary strategies that aim to optimise microbial protein synthesis may go some way to mitigating expected reductions in intake and milk yield when feeding low CP diets. Consideration should also be given to the nature and level of fermentable and non-fermentable energy sources in such diets. FCM and protein yield responses to the first-limiting essential amino acids Met and Lys will depend on diet composition and MP supply. Responses may be greater in early lactation but there is currently insufficient data to evaluate this for cows fed low CP diets. Finally, no detrimental effects of feeding low CP diets on either cow health or fertility are anticipated but this requires confirmation in contemporary high-yielding cows.

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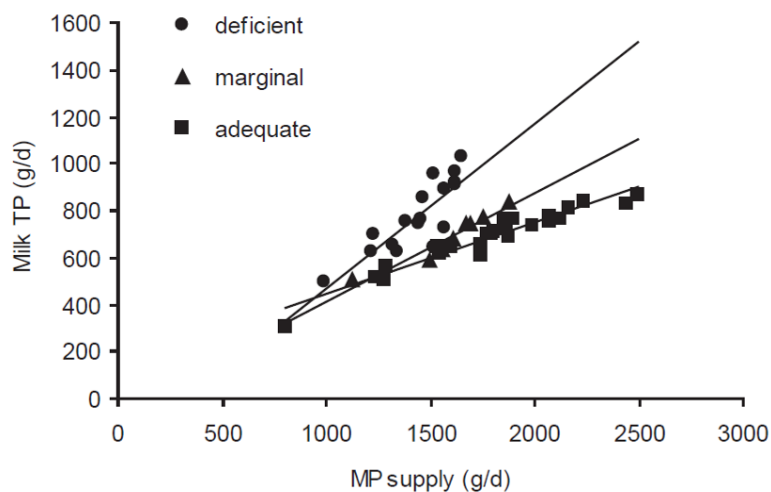


Figure 1. FiM evaluation of metabolisable protein (MP) supply against milk true protein (TP) output for diets categorised as deficient, marginal or adequate according to MP balance (from [Thomas, 2004](#)).

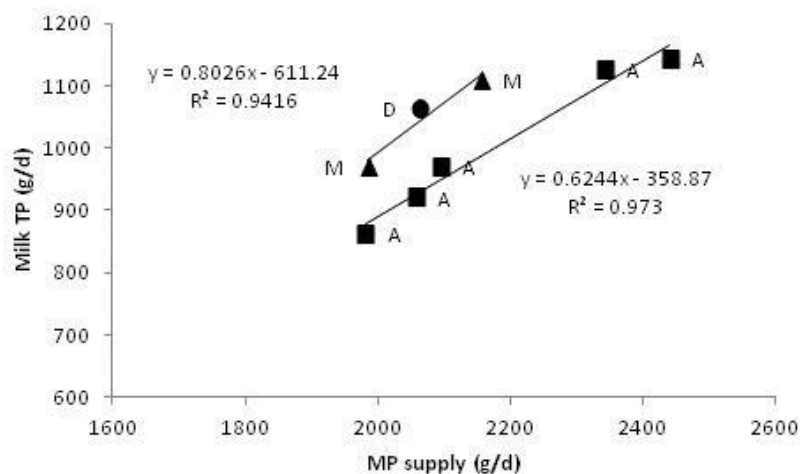


Figure 2. Evaluation of metabolisable protein (MP) supply against milk true protein (TP) output for diets categorised as deficient (D), marginal (M) or adequate (A) according to MP balance (data from [Alison and Garnsworthy, 2002](#); [Garnsworthy et al., 2008](#)).

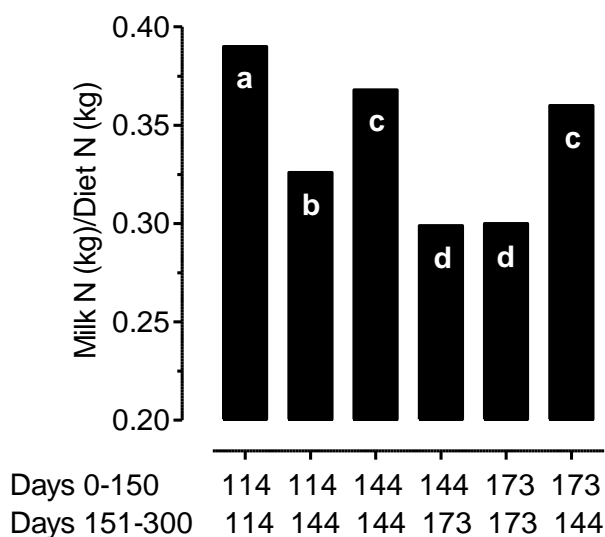


Figure 3. The effect of altering dietary protein concentrations (from 114 to 173g/kg DM) during early (Days 0-150) and late (Days 151-305) lactation on the efficiency of nitrogen (N) use (kg milk N output per kg dietary N intake) between Days 151-305 of lactation. Bars with different superscripts differ at $P < 0.001$ (data from [Law et al., 2009a](#)).

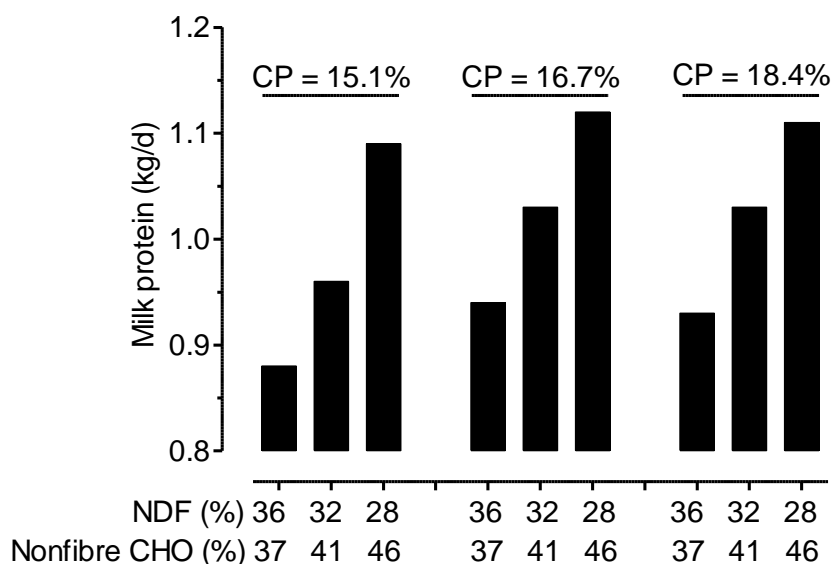


Figure 4. Effect of dietary crude protein (CP; % DM) and neutral detergent fibre (NDF; % DM) on milk protein yield (data derived from [Broderick, 2003](#)). Non fibre carbohydrates (CHO; % DM) would have consisted mainly of starch and a small amount of sugars. Diets contained high levels of maize grain.

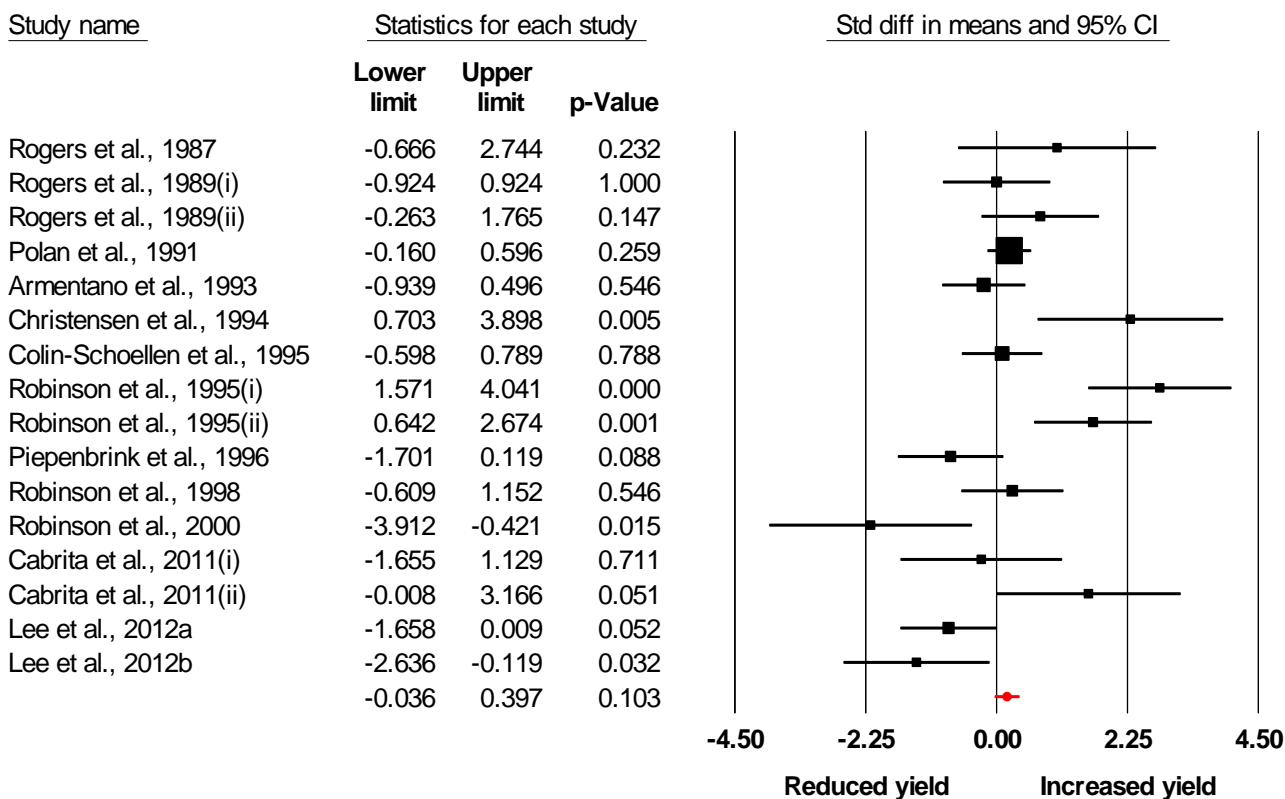


Figure 5. Effect of rumen-protected methionine plus lysine on fat-corrected milk yield (kg/day) for diets of $\leq 15\%$ crude protein. A meta-analysis of 13 studies, involving 16 experiments, where cows were fed either a 'Control' diet or a diet of similar composition but with added rumen-protected methionine and lysine. Heterogeneity (Q-statistic, χ^2) between studies = 62.1 (15 df), $P < 0.001$ ($I^2 = 75.8\%$). Mean values (■) proportional in size to each study's weight. Overall treatment effect: $Z = 1.107$ ($P = 0.103$).

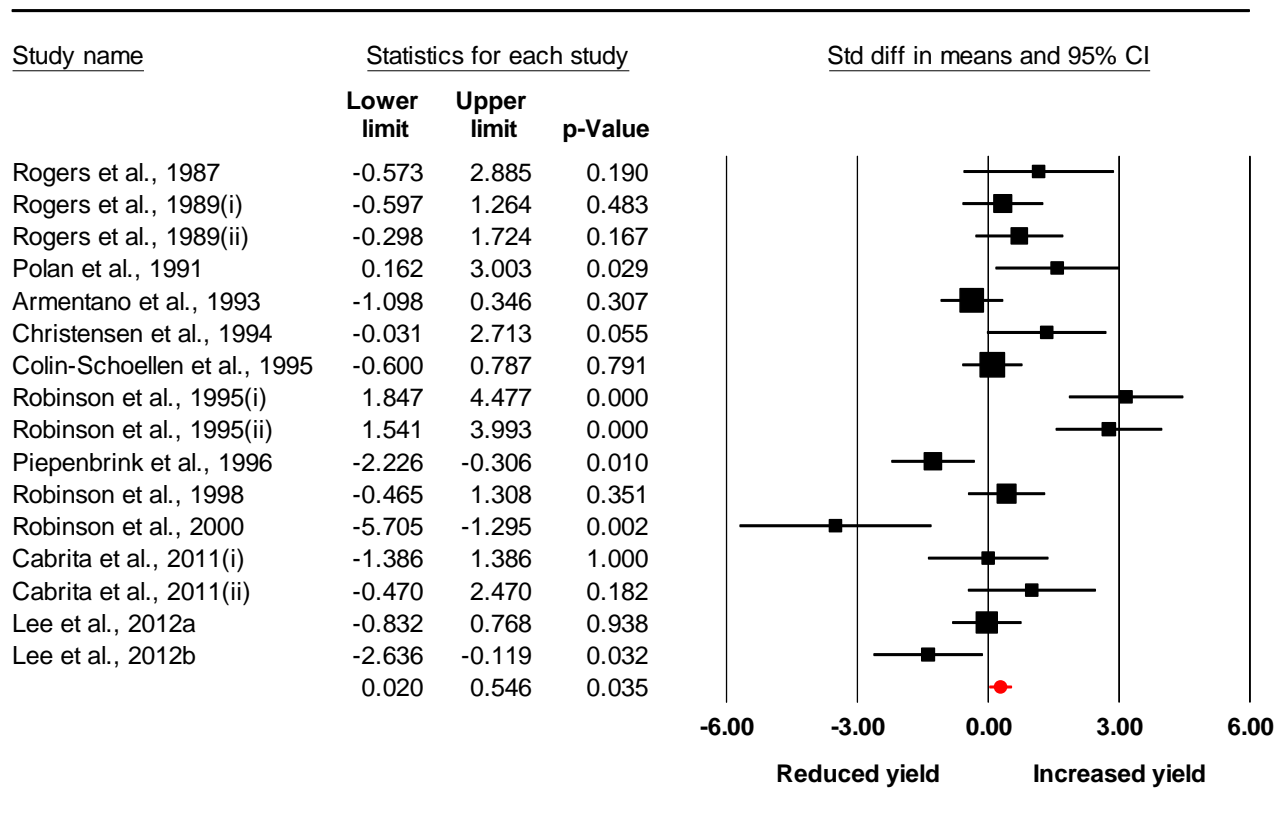


Figure 6. Effect of rumen-protected methionine plus lysine on milk protein yield (kg/d) for diets of $\leq 15\%$ crude protein. A meta-analysis of 13 studies, involving 16 experiments, where cows were fed either a ‘Control’ diet or a diet of similar composition but with added rumen-protected methionine and lysine. Heterogeneity (Q-statistic, χ^2) between studies = 63.5 (15 df), $P < 0.001$ ($I^2 = 76.4\%$). Mean values (■) proportional in size to each study’s weight. Overall treatment effect: $Z = 3.141$ ($P = 0.002$).

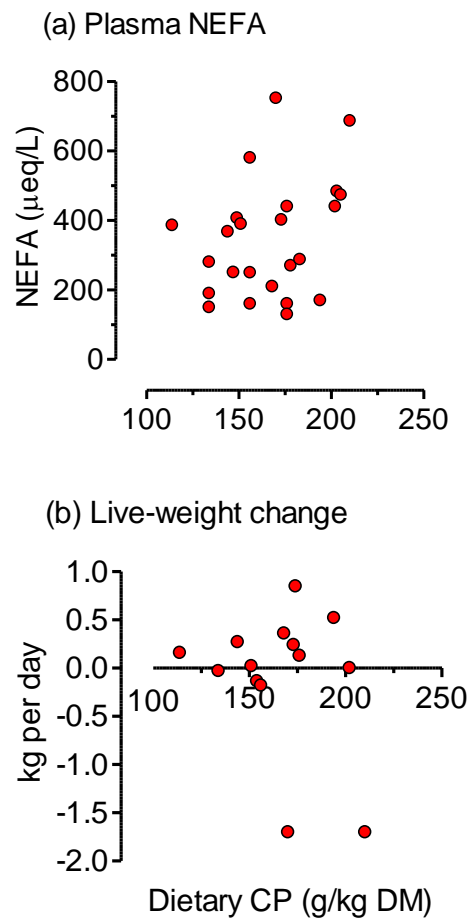


Figure 7. Relationship between dietary crude protein (CP) concentration and plasma non-esterified fatty acids (a) and liveweight change (b) in early lactation dairy cows (Data from [Law et al., 2009a](#); [Kokkonen et al., 2002](#); [Davidson et al., 2003](#); [Hongerholt and Muller 1998](#); [Nachtoml et al., 1991](#); [Wu and Satter 2000](#); [Schei et al., 2005](#); [Bach et al., 2000](#)). R^2 for NEFA = 0.10 and LWG = 0.07.

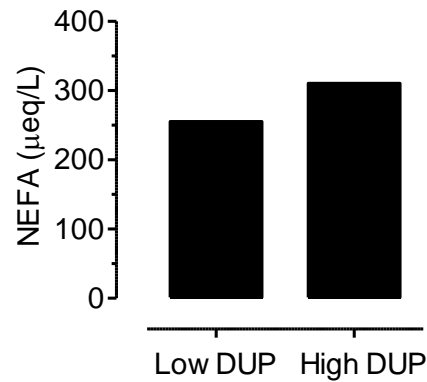


Figure 8. Relationship between dietary crude protein (CP) degradability and plasma non-esterified fatty acids (NEFA) in early lactation dairy cows. DUP = digestible, undegradable protein. (Data from [Davidson et al., 2003](#); [Hongerholt and Muller 1998](#); [Bach et al., 2000](#); [Schor and Gagliostro, 2001](#); [Westwood et al., 2000](#) and [Garci-Boljalil et al., 1998](#); [Jones and Garnsworthy, 1988](#); [Cadorniga and López Díaz, 1995](#)). Data:- Geometric means; P = 0.158.

Table 1. The effects of feeding low crude protein (CP) (<15%) on fertility traits in lactating dairy cows in which balanced groups of cows were fed diets containing either high (16.9 – 20.0%) or low (12.7 – 14.5%) levels of CP. (1 Jordan & Swanson 1979; 2 Edwards et al., 1980; 3 Howard et al., 1987; 4 Carroll et al., 1988; 5 Barton et al., 1996; 6 Law et al., 2009b). PUN = plasma urea nitrogen; CR = conception rate; PR = pregnancy rate.

Study	n	CP %	Milk Yield (kg/day)	PUN (mg/dl)	Days to Ovulation	Days to Oestrus	Days to AI	Services per	CR to 1st AI	Cumulative PR by 90 - 120	Days open
1	15	19.3	(>30)	-	16	27	-	2.47	-	-	106
	15	12.7		-	18	36	-	1.47	-	-	69
2	6	16.9	23.2	-	-	-	-	2.7	-	-	139
	6	13.1	19.9	-	-	-	-	2.3	-	-	123
3	71	19.5	26.4	26	-	38.2	-	1.47	-	84.8	79.9
	75	14.5	25.9	15	-	40.5	-	1.55	-	86.5	80.4
4	27	20	26.3	-	22	27	59	1.8	56	93	82
	28	13	25.3	-	17	24	54	1.5	64	96	72
5	32	20	25.4	21	25.8	39.5	59.9	1.75	43.7	87.5	-
	32	13	25	8.6	23.2	41.4	62.5	1.7	40.6	75	-
6	30	17.3	35.4	-	30.9	-	-	2.69	27.6	62.1	-
	30	14.4	31.8	-	33.2	-	-	2.32	29.7	66.7	-
Mean (weighted for study size)	High CP	19.2	27.7	24.4	24.9	35.2	59.5	1.9	42.0	82.5	86.6
	n =	181	166	103	104	145	59	181	89	160	119
	Low CP	13.8	26.5	13.1	23.7	37.2	58.5	1.7	44.2	82.3	79.2
	n =	186	171	107	105	150	60	186	90	165	124



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