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Grass Silage: Factors Affecting Efficiency of N Utilisation in Milk Production

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The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

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- Steen, R.J.W. (1986). An evaluation of effluent from grass silage as a feed for beef cattle offered silage based diets. *Grass and Forage Science*, 41, 39-45.
- Steen, R.W.J. (1989). Recent research on protein supplementation of silage-based diets for growing and finishing beef cattle. 62nd Annual report, *Agricultural Research Institute of Northern Ireland*, pp. 12-20.
- Steen, R.W.J. (1991). Recent advances in the use of silage additives for dairy cattle. In: C.S. Mayne (ed.) *Management Issues for the Grassland Farmer in the 1990's. Occasional Symposium No. 25, British Grassland Society*, 87-101.
- Steen, R.W.J. (1996). Effects of protein supplementation of grass silage on the performance and carcass quality of beef cattle. *Journal of Agricultural Science, Cambridge*, 127, 403-412.
- Steen, R.W.J. (1998). Effect of the proportion of grass silage and concentrates in the diet on the performance of beef cattle. *Proceedings of a Research Seminar on Ruminant Production, Agricultural Research Institute of Northern Ireland*, April, pp. 49-63.
- Steen, R.W.J., D.J. Kilpatrick & M.G. Porter (2002). Effects of the proportions of high or medium digestibility grass silage and concentrates in the diet of beef cattle on liveweight gain, carcass composition and fatty acid composition of muscle. *Grass and Forage Science*, 57, 279-291.
- Stewart, T.A. (1980). Prevention of damage to grass swards from applications of silage effluent by neutralisation or dilution of effluent acids. *Grass and Forage Science*, 35, 47-54.
- Wilson, R.K. & C.P. Collins (1980). Chemical composition of silages made from different grass genera. *Irish Journal of Agricultural Research*, 19, 75-84.
- Wilson, R.K. & A.V. Flynn (1979). Effects of fertiliser N, wilting and delayed sealing on the chemical composition of grass silages made in laboratory silos. *Irish Journal of Agricultural Research*, 18, 13-23.
- Wright, D.A., J.P. Frost, D.C. Patterson & D. Kilpatrick (2000). The influence of weight of ryegrass per unit area and treatment at or after mowing on rate of drying. *Grass and Forage Science*, 82, 86-98.
- Zimmer, E. & R.J. Wilkins, (1984). Efficiency of silage systems : A comparison between unwilted and wilted silages. *Landbauforschung Volkenrode, Sanderheft*, 69, 5-12.

Grass silage: factors affecting efficiency of N utilisation in milk production

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Key points

1. Low efficiency of N utilisation for milk production in cows fed grass silage-based diets is mainly due to excessive N losses in the rumen.
2. The type and extent of *in silo* fermentation can alter the balance of absorbed nutrients.
3. There is very little experimental evidence that the capture of N in the rumen can be improved by a better synchrony between energy and N release in the rumen. Nitrogen losses in the rumen can be reduced by decreasing the ratio between rumen degradable N and fermentable energy.
4. Rapeseed meal has increased milk protein output more than isonitrogenous soybean meal supplementation, probably due to higher concentration of histidine in rapeseed protein.
5. Efficiency of N utilisation for milk production is not necessarily lower for the grass silage-based diets compared to other diets.

Keywords: grass silage, dairy cow, microbial protein synthesis, protein degradation, nutrient balance

Introduction

Grass and maize silage comprise a major proportion of conserved forages used in dairy cow rations in Central and Northern Europe. Development of early maturing varieties, lower labour requirements, improved nutrient management on dairy farms and some nutritional benefits has increased the amount of maize silage being used at the expense of grass silage. It can be expected that the use of forage maize will continue to expand into northern climates if the predicted increases in global climate warming occur. In regions that are not suitable for maize production whole-crop cereal silages represent a viable alternative or a complimentary forage for grass and legume silages.

Dairy farming is known to contribute to both atmospheric and hydrospheric pollution (Tamminga, 1992). Excessive nitrogen (N) excretion is a major environmental concern especially for intensive grass and grass silage-based systems, since the efficiency of nitrogen (N) utilisation for milk production on grass silage-based diets is lower compared to maize silage-based diets (Givens & Rulquin, 2004). Grass silage provides a substantial proportion of crude protein the (CP) in the diet of high producing dairy cows in many regions of the EU. Despite a relatively high concentration of CP in dairy cow rations containing grass silage and cereal grains, protein supplementation has consistently elicited positive production responses. Responses to supplementary protein are indicative of inefficient utilisation of N in the basal diet. Protein supplementation can alleviate certain nutritional limitations of CP in grass silage, but does result in reduced overall efficiency of N utilisation and increased N emissions. Increases in N emissions into the environment are generally a result of greater excretion of N in urine N, which is more of a concern than an increase in faecal N output due to greater exposure to evaporative losses and leaching into water supplies. Therefore other strategies to improve N utilisation of grass silage-based diets are urgently required, not only to reduce environmental N emissions, but also to reduce feed costs. The overall objective of this paper is to review research examining N metabolism of dairy cows fed grass silage-based diets, with

particular emphasis on nutritional concepts that may facilitate improved N utilisation for milk production from grass silage.

Utilisation of silage nitrogen

Microbial protein synthesis

Givens & Rulquin (2004) recently concluded that the utilisation of grass and legume CP for milk production is low compared with other forages. The poor utilisation of N from grass silage N was attributed primarily to the low efficiency of capture of rumen degradable N (RDN). However, the generally accepted conclusion that the efficiency of microbial protein synthesis (MPS) is lower for grass silage-based diets compared with other diets is largely based on indirect evidence with few direct comparisons (ARC, 1984; Givens & Rulquin, 2004). The values for the energetic efficiency of MPS (EMPS) reported in the literature are highly variable, which may, in part, reflect differences in experimental techniques and difficulties in the determination of MPS (Shingfield, 2000). It should also be noted that errors in estimating digesta flow potentially have a two-fold influence on estimates of EMPS values. Overestimating DM flow from the rumen overestimates microbial N supply, but underestimates the amount of organic matter apparently digested in the rumen. Consequently, the differences in EMPS are greater than those in total microbial N. A direct comparison of silage and barn-dried hay harvested from the grass same sward at the same time did not suggest a lower ruminal N utilisation in the rumen for silage compared to hay (Jaakkola & Huhtanen, 1993) (Table 1). Microbial N flow at the duodenum and EMPS were significantly ($P < 0.05$) higher for the silage diets compared with the hay diets, but undegraded feed protein flow was higher for the hay diets. Rumen ammonia N concentration was marginally higher in cattle fed silage-based diets (12.9 vs. 11.6 mmol/l), but the difference can be attributed to the higher CP content of the silage based diets (164 vs. 152 g/kg DM for silage and hay, respectively) rather than to an effect of forage conservation method on EMPS. Similarly, the flow of N to the duodenum has been shown to be unaffected when lucerne is conserved by ensiling compared with drying (Hristov & Broderick, 1996). Furthermore, studies have shown no significant differences in the yields of milk and milk protein between grass hay and silage harvested at the same stage of maturity (Bertilsson, 1983).

Table 1 Effects of forage conservation method and proportion of concentrate on ruminal N metabolism in growing cattle (Jaakkola & Huhtanen, 1993)

| Forage | Silage | | | Hay | | |
|--------------------------|--------|------|------|------|------|------|
| | 250 | 500 | 750 | 250 | 500 | 750 |
| Concentrate (g/kg DM) | | | | | | |
| CP (g/kg DM) | 168 | 165 | 161 | 148 | 152 | 155 |
| Rumen ammonia N (mmol/l) | 13.9 | 12.8 | 12.0 | 11.3 | 11.7 | 12.0 |
| N intake (g/d) | 178 | 181 | 174 | 161 | 165 | 173 |
| Duodenal flow (g/d) | | | | | | |
| Non-ammonia N | 142 | 152 | 150 | 132 | 146 | 150 |
| Microbial N | 77 | 89 | 85 | 64 | 76 | 79 |
| Feed N | 53 | 52 | 54 | 56 | 59 | 59 |
| N degradability | 0.71 | 0.72 | 0.68 | 0.65 | 0.65 | 0.65 |

Most of the evidence from measurements of duodenal N flow, rumen ammonia N concentration and milk production responses are not consistent with the widely held view that ensiling of grass necessarily results in reduced efficiency of N utilisation for milk production.

A low efficiency of silage N utilisation often reported in dairy cows fed grass silage-based diets can be attributed both to poor ensiling techniques and/or extensive *in silo* fermentation rather than being a result of conservation method *per se*. On both a theoretical basis (Chamberlain, 1987) and from consideration of experimental evidence (Jaakkola & Huhtanen, 1992), it is clear that the end-products of silage fermentation are not a significant source of energy for rumen microbial growth. Although the review of Dewhurst *et al.* (2000) suggested that there are no consistent effects of silage additives on EMPS, most of the evidence from within study comparisons suggests increased ruminal MPS in response to restricting *in silo* fermentation by using high levels of acid-based additives (van Vuuren *et al.*, 1995). Earlier data indicating a strong positive association between residual water soluble carbohydrate content and MPS (Jaakkola *et al.*, 1993) support the concept that MPS is related to the supply of fermentable energy for rumen microbes (Table 2). Analysis of a large data set (230 treatment means) from production trials examining the effects of silage fermentation characteristics on milk production, indicated significant inverse relationships between both silage total acid and silage ammonia-N concentrations, and the concentration and yield of milk protein (Huhtanen *et al.*, 2003).

Table 2 The effects of the rate of formic acid application on grass silage fermentation characteristics and duodenal N flow (Jaakkola *et al.*, 1993)

| | Formic acid (l/t) | | | |
|--------------------------|-------------------|-------|-------|-------|
| | 0.0 | 2.0 | 4.0 | 6.0 |
| In silage (g/kg DM) | | | | |
| WSC | 3 | 19 | 37 | 92 |
| Lactic acid | 62 | 78 | 47 | 17 |
| VFA | 63 | 18 | 13 | 10 |
| Ammonia N (g/kg total N) | 103 | 46 | 28 | 21 |
| Duodenal flow (g/d) | | | | |
| Non-ammonia N | 114.5 | 126.1 | 128.4 | 136.9 |
| Microbial N | 49.0 | 57.3 | 58.4 | 65.4 |
| Feed N | 53.4 | 56.7 | 57.9 | 59.4 |

Ruminal production of microbial N *in vitro* was lower from the soluble N in lucerne silage than from the corresponding fraction in lucerne hay (Peltekova & Broderick, 1996) suggesting that utilisation of soluble N from silage is reduced due to a shortage of available protein and peptides. Evaluation of *in vivo* data from studies in sheep and growing cattle has consistently shown an increase in MPS in response to protein supplementation (Weiss *et al.*, 2003). In contrast, measurements of nutrient flow into the omasum in cows fed a range of protein supplements (soybean meal, fishmeal, maize gluten meal, rapeseed feeds, and urea) do not support the hypothesis that MPS in dairy cows fed grass silage-based diets is limited by the supply of protein or peptides available to rumen microbes (Figure 1). Efficiency of MPS tended to decrease in response to protein supplementation, which may reflect ruminally degraded protein supplying less energy for rumen microbes than fermentable carbohydrates.

Two reasons for the lack of response in EMPS to protein supplementation in dairy cow studies could be suggested: (1) the feeding level was substantially higher and the supply of peptides and amino acids was probably more constant due to a more consistent eating pattern and (2) the silages in dairy cow studies were restrictively fermented, whereas the silages in the studies as reviewed by Weiss *et al.* (2003) were more extensively fermented. Restricting the extent of *in silo* fermentation is known to increase the proportion of peptide N in silage soluble N (Nsereko & Rooke, 1999) which can result in a more optimal supply of N fractions to rumen microbes.

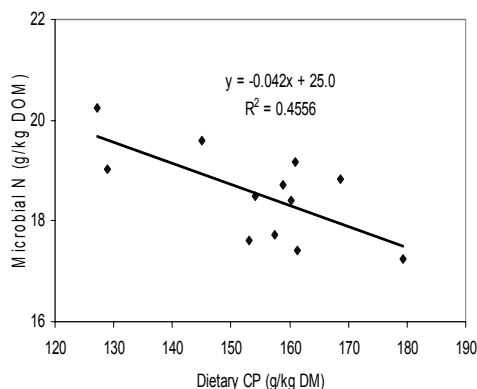


Figure 1 Relationship between dietary CP concentration and efficiency of MPS (data from Ahvenjärvi *et al.*, 1999; 2002; Korhonen *et al.*, 2002b). The values are adjusted for a random study effect.

Ruminal degradability of silage crude protein

Due to extensive proteolysis *in silo* and the high relative amount of N in the form of NPN, a large proportion of silage N is degraded to ammonia in the rumen. The values reported for the effective ruminal degradability (EPD) of silage N typically varies between 0.70 and 0.90. Factors such as forage species, maturity, wilting and the use of ensiling additives have been associated with changes in ruminal degradability of silage N. However, there is very little evidence demonstrating that variations in EPD of silage N are of any nutritional importance. Wilting has consistently reduced the EPD values of silage (Givens & Rulquin, 2004), while production experiments have shown rather small positive production responses to wilting, which were linked to increases in silage DM intake which are mainly derived from the higher DM of the wilted silages. Verbic *et al.* (1999) reported proportionally 0.19 and 0.27 higher metabolisable protein (MP) values for extensively wilted silage and hay compared with formic acid-treated silage. Such differences as determined *in situ* are in contrast with measurements of duodenal N flow and data from production studies examining the effects of forage conservation method.

The lack of progress in this area can be attributed to the inadequacies of current methods to provide reliable estimates of ruminal degradability of silage CP. Some differences in EPD estimated by the *in situ* technique may simply reflect differences in microbial contamination of undegraded residues and particle losses from the bags. Recent evidence strongly suggests that the assumptions of the Ørskov & McDonald (1979) model used to estimate effective

protein EPD are not valid. Implicit in the *in situ* method is that the rapidly degradable fraction (*a*-fraction) is instantaneously degraded or that degradation rate is infinite with no escape. Recent studies (Choi *et al.*, 2002; Volden *et al.*, 2002) have clearly demonstrated that variable proportions of dietary N can escape from the rumen in the liquid phase as non-ammonia non-microbial N. To overcome this problem, Hvelplund and Weisbjerg (2000) proposed a model in which the escape of the *a*-fraction is incorporated into the model. The second problem with the model proposed by Ørskov & McDonald (1979) is the assumption of random first-order passage kinetics of feed particles from the rumen. However passage kinetic studies based on duodenal sampling have shown mechanisms of selective retention of feed particles in the rumen (Huhtanen *et al.*, 2005).

It is clear that methodological limitations in the *in situ* technique result in an overestimation in the range of EPD values for forages. For example, accounting for the passage of amino N in the liquid phase (escape of *a*-fraction) and retention of feed particles in the rumen non-escapable pool would reduce the effects of conservation method, e.g. wilting and restricting the extent of *in silo* fermentation on EDP. Similarly, the reduction in the EPD value with advance in the maturity of ensiled grass is more likely to be related to greater microbial contamination of undegraded residues than true differences in N degradability. Possible decreases in the EPD of silage with advancing maturity are at least partly compensated for by an increase in the proportion of ADF-bound N (Rinne *et al.*, 1997) and reduced intestinal digestibility of undegraded feed protein. Before clear progress can be made in this area, methods for estimating quantitative – not just qualitative - differences in the supply of undegraded protein from forages are urgently required. The lack of a simple and reliable reference method has delayed progress in developing reliable tools for estimating ruminal CP degradability. At present, using a constant EPD value probably results in at least as good a prediction of silage MP value as utilising values obtained by the *in situ* technique.

Amino acid supply from grass silage-based diets

Theoretically N utilisation for milk production could be improved by increasing the supply of regarded as the first limiting amino acid (AA). Lysine (Lys) and methionine (Met) have long been limiting or co-limiting AA in cows fed diets based on maize silage and maize (NRC, 2001). However, Lys and Met infusions have not increased milk protein yield on grass silage-based diets. The studies conducted at the Hannah Research Institute and at MTT have demonstrated that histidine (His) is the first limiting AA for milk protein synthesis when grass silage is the basal forage in the diet. Abomasal infusion of 6.5 g His per day increased milk protein yield in cows fed grass silage and a cereal grain-based supplement, but Met and Lys AA did not elicit any further response in addition to His when infused either individually or as a mixture (Vanhatalo *et al.*, 1999). Use of feather meal to further increase the imbalance in AA supply from grass silage-based diets demonstrated a substantial milk protein yield response to abomasal His infusion (Kim *et al.* 1999, 2001a). Subsequent studies also demonstrated a positive response to His infusion in cows fed a grass-silage based diet without feather meal (Kim *et al.*, 2001b). Increases in milk protein yield to His infusions have been shown to be linear up to the levels of 6 g/d (Korhonen *et al.*, 2000). A transfer efficiency from the abomasum to milk of 0.28 was clearly below default values of MP utilisation used in current feed protein evaluation systems (e.g. 0.67; NRC, 2001).

That His is the first limiting AA for milk protein synthesis in cows fed grass silage-based diets can be attributed to the high contribution of microbial protein to total MP supply. Since the concentration of His in milk protein is markedly higher than microbial protein, whereas

Lys and Met content in milk protein is very similar to that in microbial protein. Low plasma His concentrations in cows fed grass silage and cereal grain based diets (Vanhatalo *et al.*, 1999; Korhonen *et al.*, 2000) and numerically smaller amounts of His than Met in digesta flow to the small intestine compared with Met (Korhonen *et al.*, 2000; 2002b) also support the view that His is the first limiting AA with diets based on grass silage and cereal grains. Application of the NRC (2001) AA sub-model to data from production experiments conducted at MTT (72 diets) indicated that only the His content of MP was positively associated with milk protein yield (Huhtanen, 2005).

Attempts to identify the second limiting AA for grass silage-based diets have not been particularly successful, and in some cases, even the ranking of the first limiting AA has been variable (Kim *et al.*, 2000). The AA profile of MP in cows fed grass silage-based diets is relatively well balanced, except for His, and therefore even small changes in the AA profile of CP available for absorption can change the ranking order of the next limiting AA. Even if the second limiting AA could be identified, its potential to increase milk protein output is probably rather limited. However, if protein supplements of low ruminal degradability are limited in the supply of certain AA, then substantial milk protein yield responses could be obtained by balancing AA supply as evidenced by the responses to His when feather meal is included in grass silage-based diets (Kim *et al.*, 1999; 2001a).

Protein supplementation

The CP content of concentrate supplements can be increased by replacing cereal grains or other energy rich ingredients with protein feeds. Statistical analysis of data from studies conducted mainly in Finland demonstrated positive effects of protein supplements on total DM intake (Figure 2). Increased DM intake in response to protein has been attributed to improved cell wall digestibility (Oldham, 1984). Whilst OM digestibility was improved with protein supplementation in these studies (Figure 2), the effect is too small to explain increases in DM intake. It is likely that the effects of protein supplementation are mediated partially via metabolic mechanisms, probably related to the protein or AA energy ratio. It is evident that constraints on rumen fill are not the sole factor regulating the intake of highly digestible grass silage based diets as indicated by the decrease in rumen NDF fill with increases in silage OM digestibility (Rinne *et al.*, 2002). Protein supplementation improves nutrient balance (amino acid: ME ratio), which increases milk yield, and thereby increases energy requirements which would stimulate an increase in DM intake.

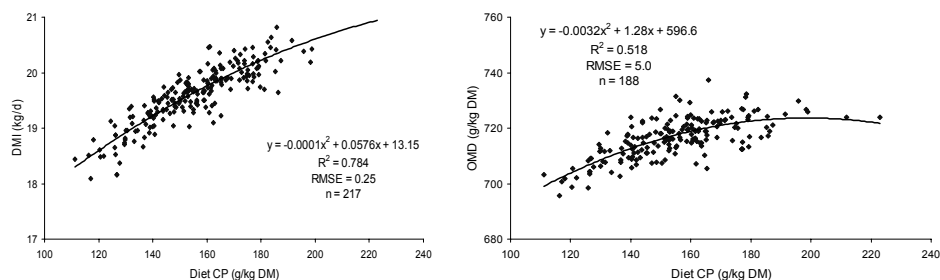


Figure 2 Effects of increasing dietary crude protein (CP) concentration on DM intake and organic matter digestibility (OMD). The values are adjusted for a random study effect.

A metabolic mechanism of intake response regulation, potentially mediated through improvements in the ratio of amino acids:energy at the tissue level, has been known for some time, and is clearly demonstrated in studies involving post-ruminal casein infusions which showed that increases in hay intake were accompanied by changes in rumen fill (Egan, 1970). Similar responses in dairy cows fed grass-red clover silages were observed by Khalili & Huhtanen (2002). Duodenal casein infusions resulted in higher DM intake, increased rumen NDF fill and total chewing time compared with infusions of the same amount of casein in to the rumen.

The effect of protein supplementation on OM digestibility is curvilinear with greater incremental responses being observed at low dietary CP concentrations. This suggests that the supply of rumen degradable protein may limit cell wall digestion of grass silage-based diets at low CP concentrations. Digestibility of digestible NDF determined by 12 d *in situ* incubations was increased when the CP content of diets fed to dairy cows was increased by replacing cereal grains with soybean meal or rapeseed expeller (Shingfield *et al.*, 2003). Whether this response was related to the stimulation of cellulolytic bacteria by an increased supply of preformed amino acids and peptides, or to differences in the intrinsic digestion properties of grain and protein supplement or to reduced starch content of the diet remains unclear.

Milk and milk protein yields are both enhanced in a quadratic manner with increased protein supplementation (Figure 3). Due to an increase in both DM intake and OM digestibility, milk yield responses can be attributed to increased energy intake as well as to enhanced AA supply. The effects of protein supplementation in an individual experiment have seldom been significant, but analysis of a large number of studies have shown that protein supplementation had a significant ($P < 0.001$) linear effect on milk protein content (0.17 g/kg per 10 g CP/kg diet DM). However, when milk protein content was corrected for the contribution from urea N, the response decreased to 0.06 indicating that proportionally 0.65 of the increase in milk protein content was due to elevated urea levels. Even though protein supplementation has produced substantial milk yield responses, these responses have consistently been associated with a reduction in the efficiency of dietary N utilisation. In this MTT data set an increase of 10 g/kg DM in dietary CP concentration reduced N efficiency by 0.014 units. In addition to less efficient N utilisation, protein supplements will often increase phosphorus emissions, since protein sources are generally rich in this mineral.

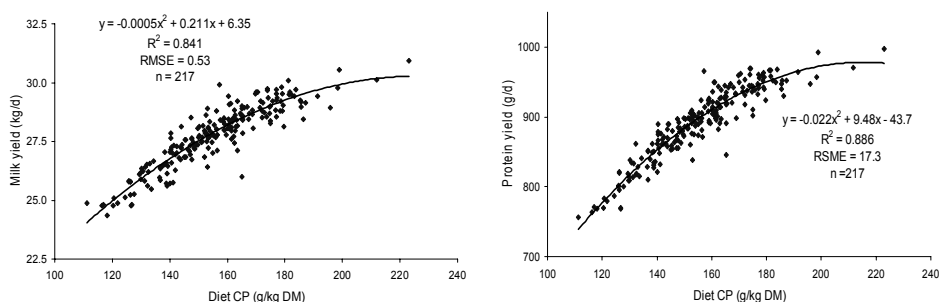


Figure 3 Effects of increasing dietary crude protein (CP) concentration on yields of milk and protein. The values are adjusted for a random study effect.

In addition to level, the source of protein supplementation is an important determinant of milk production responses, since both ruminal protein degradability and the balance between essential amino acids influence the supply of the most limiting amino acids to the mammary gland. Chamberlain *et al.* (1989) reported higher milk yield responses to fishmeal compared with soybean meal (0.15 vs. 0.12 g per 1 g increase in CP intake). However, because protein supplements of animal origin are not permitted for use in ruminant diets in the EU due to the incidence of bovine spongiform encephalopathy, the importance of high quality sources of vegetable protein for intensive milk production has further been highlighted. Indirect evidence has suggested that utilisation of rapeseed protein is at least equal to that of soybean protein (Korhonen, 2003; Lock & Shingfield, 2004). Marginal milk protein yield responses to additional protein (g milk protein per g additional CP intake) were lower (0.12) for soybean meal compared to fishmeal and rapeseed feeds (0.15 and 0.16, respectively) based on 13, 10 and 13 studies, and 33, 21 and 38 treatment means, respectively (Korhonen, 2003).

Shingfield *et al.* (2003) fed incremental amounts of either solvent extracted soybean meal or heat-moisture treated rapeseed expeller cake with a fixed amount of a cereal based concentrate (10 kg/d on a fresh weight basis) containing 120, 150, 180 or 210 g CP/kg DM and grass silage *ad libitum*. Inclusion of rapeseed elicited slightly higher milk protein output responses than soybean meal (Table 3). Feeding rapeseed expeller was associated with higher plasma concentrations of His and branched-chain amino acids (BCAA). The changes in plasma amino acids were indicative of an increased supply of both total amino acids and His which, when coupled with a lower concentration of milk urea, are indicative of more efficient utilisation of absorbed AA for diets containing rapeseed than soybean protein supplements. Other studies have shown that rapeseed containing feeds result in similar milk protein yields compared with a mixture of fishmeal and soybean meal (Dewhurst *et al.*, 1999) and higher milk protein output than a mixture of soybean meal and maize gluten meal (Khalili *et al.*, 2001; Vanhatalo *et al.*, 2003).

Table 3 Effects of inclusion of soybean meal and rapeseed expeller on feed intake and milk production of cows fed grass silage *ad libitum* and 10 kg concentrate per day (Shingfield *et al.*, 2003)

| Concentrate CP (g/kg DM) | Soybean meal | | | | Rapeseed cake | | |
|--------------------------|--------------|------|------|------|---------------|------|------|
| | 120 | 150 | 180 | 210 | 150 | 180 | 210 |
| Intake (kg DM/d) | 20.0 | 20.3 | 20.4 | 20.5 | 20.6 | 20.9 | 20.8 |
| Yield | | | | | | | |
| Milk (kg/d) | 26.2 | 26.9 | 27.0 | 28.5 | 28.2 | 29.3 | 30.1 |
| ECM (kg/d) | 29.8 | 30.3 | 30.4 | 32.1 | 31.2 | 31.6 | 32.8 |
| Fat (g/d) | 1325 | 1335 | 1342 | 1404 | 1354 | 1343 | 1410 |
| Protein (g/d) | 859 | 902 | 889 | 954 | 930 | 967 | 993 |
| Concentration | | | | | | | |
| Fat (g/kg) | 51.2 | 50.8 | 50.7 | 49.9 | 48.9 | 46.9 | 47.3 |
| Protein (g/kg) | 33.5 | 34.3 | 33.6 | 33.8 | 33.9 | 33.7 | 33.4 |
| Urea (mmol/l) | 3.30 | 4.37 | 5.19 | 5.87 | 3.90 | 4.71 | 5.24 |

Improving N utilisation of grass silage based diets

There is now increasing concern about excessive emissions of N into the environment from milk production and feeding grass silage-based diets to dairy cows has been implicated as one of the main sources for the low efficiency of N utilisation. However, it should be noted that attempts to alter N efficiency in the animal is a much less effective strategy for improving on-farm N utilisation than restricting fertiliser and concentrate N use, and increasing the efficiency of N uptake from the soil (Van Bruchem *et al.*, 1999). The greatest potential to improve N utilisation is to reduce rumen N losses, but improving the balance between absorbed nutrients (e.g. amino acid balance and glucose supply) can also result in substantial improvements in the efficiency of N utilisation. Rumen N losses in cows fed grass silage-based diets are probably higher than current estimates based on duodenal sampling. Analysis of nutrient flows at the omasum indicated that zero ruminal N balance occurs at a diet CP concentration of 130 g/kg DM (Schwab *et al.*, 2005), a level that is markedly lower than is typically fed on-farm.

Forage management and feeding

Intensive dairy farming based on grass silage, typically uses high fertiliser N inputs and harvests grass at an early stage of maturity. While these strategies both increase silage N concentration, the effects on N efficiency are markedly different. Shingfield *et al.* (2001) investigated the opportunities to improve the efficiency of N utilisation by comparing the effects of additional N derived from silage due to increased N fertilisation or from urea, wheat gluten meal and rapeseed expeller supplements. Interestingly, urea and increased N fertilisation decreased the efficiency of N utilisation more than protein supplements, particularly when compared with rapeseed expeller.

In contrast to N derived from increased N fertilisation, incremental protein from earlier harvesting is utilised at a higher efficiency for milk protein secretion. Rinne *et al.* (1999a) harvested timothy-meadow fescue swards at one week intervals, which resulted in silages with a wide range in CP content (113 to 172 g/kg DM). Even though the efficiency of N utilisation decreased from 0.333 to 0.283, as harvesting date progressed, the marginal milk protein response of 0.16 was comparable to that attained with fishmeal or rapeseed feeds. Higher utilisation of additional N from earlier harvesting compared with that derived from N fertilisation is related to increased supply of fermentable energy and higher capture of N in the rumen. Rumen N losses could also be reduced by replacing early-harvested grass silage with maize silage and whole-crop cereal silages. Because grass silage provides excess RDP, whereas maize and whole-crop silages are often deficient in degradable N, and therefore mixtures of these forages could also be used to improve the balance between RDP and fermentable energy supply.

Carbohydrate supplementation

The effects of synchronisation of energy and N release in the rumen on MPS have been intensively investigated during recent decades. However, few benefits in terms of improved efficiency of MPS from a more synchronous release of N and energy release in the rumen have been reported. Often, the effects of synchronisation and diet composition have been confounded. Sugar supplements have sometimes been more efficient than starch in promoting MPS, but these effects may be related to higher numbers of rumen protozoa when starch is fed (Chamberlain *et al.*, 1985) and increased intra-ruminal N recycling. Part of the difference

between sugar and starch supplements may be attributed to a partial escape of starch from ruminal fermentation, thereby providing less ATP for microbial growth. When the synchrony between energy and N release in the rumen of cattle fed a grass silage-based diet was manipulated by feeding sucrose either twice daily or as continuous infusion, the latter promoted a higher MPS and lower rumen ammonia N concentration than feeding sucrose twice daily (Khalili & Huhtanen, 1991). Twice-daily feeding would have been expected to provide more energy during the post feeding period of extensive proteolysis and to stimulate microbial growth. Reviewing the available literature, Chamberlain & Choung (1995) concluded that there is no convincing evidence of a close synchronisation of energy and N release in the rumen having beneficial effects on MPS. It can be concluded that the ratio between RDP and fermentable energy is much more important determinant of ruminal N losses than synchrony in the rates of N and energy release.

Protein supplementation

High producing dairy cows require an abundant supply of absorbed AA. Without accurate predictions of the absorption and utilisation of AA, an over supply of protein often occurs to ensure that the genetic potential for milk production is expressed. However, this approach reduces the efficiency of N utilisation. In cows fed grass silage-based diets the supply of RDP, which is often in excess of microbial N requirements, is further increased by protein supplementation and a major proportion of the additional N will be excreted as urea-N in the urine.

Theoretically, decreasing ruminal protein degradability by physical or chemical treatment should be an effective strategy to improve the efficiency of N utilisation for milk production. Treatments used to reduce ruminal protein degradability have often resulted in lower EPD values as determined by *in situ* incubation, but production responses to reductions in EPD are inconclusive (Santos *et al.*, 1998). The absence of a response to reduced degradability could be due to the untreated protein supplement already satisfying AA requirements. To test this hypothesis, Rinne *et al.* (1999b) fed incremental levels of solvent extracted rapeseed meal and heat-moisture treated rapeseed expeller, which differed in EPD by 0.18 units (0.82 vs. 0.64). While both protein supplements linearly increased milk protein output, the rapeseed expeller with lower EPD did not produce a greater increase in yield of protein.

Preventing a potential deficiency of the most limiting AA in the mammary gland is another reason for feeding protein in excess. It is probable that His is the first limiting AA when the supply of absorbed AA is largely a reflection of the profile in microbial protein, as often is the case for grass silage-based diets. With the limitations in the use of animal proteins for ruminants, the scope to balance the limited His supply by high quality plant proteins is rather limited. Rapeseed meal, which is one of the best sources of His amongst vegetable proteins, has the same amount of His per g protein as milk. Rumen protected lysine and methionine supplements have none or only small positive effects on N utilisation in cows fed diets based mainly on maize-silage or maize (Satter *et al.*, 1999), and it is unlikely that protected sources of His would markedly improve the efficiency of N utilisation on grass silage diets due to the relatively balanced profile of absorbed AA under these circumstances. Increasing His concentration in rapeseed protein by gene technology (Wahlroos, 2004) provides a new and interesting opportunity to supply a more balanced profile in absorbed AA in the future.

Glucose supply

Modifications to the chemical composition of herbage during ensilage can have a major impact on the balance of nutrients absorbed from the gastro-intestinal tract of ruminant animals. Diets based on restrictively-fermented grass silage containing relatively high concentrations of water soluble carbohydrates and minimal amounts of lactate are characterised by a rumen fermentation pattern rich in lipogenic volatile fatty acids (Chamberlain & Choung, 1993), associated with a relatively high EMPS (van Vuuren *et al.*, 1995). Diets based on high lactate silages are associated with a rumen fermentation pattern rich in propionate (Figure 4) and a lower EMPS compared with restrictively fermented silages. An increased supply of microbial protein at the duodenum with restrictively fermented silages is seldom associated with a corresponding increase in milk protein secretion (Huhtanen *et al.*, 2003). These general considerations of the effects of silage fermentation characteristics suggest that diets based on restrictively-fermented silages are more limited in the supply of glucose, while the available AA are more limiting in diets based on high lactate silages. For diets based on restrictively-fermented silages, the benefits of increased microbial protein may be negated by a concomitant reduction in glucose supply from ruminal propionate production. Higher AA and lower glucose concentrations in the plasma of cows fed diets based on restrictively-fermented silage compared with high lactate silage (Miettinen & Huhtanen, 1997) indicate that influencing silage fermentation characteristics has the potential to alter the balance of absorbed nutrients.

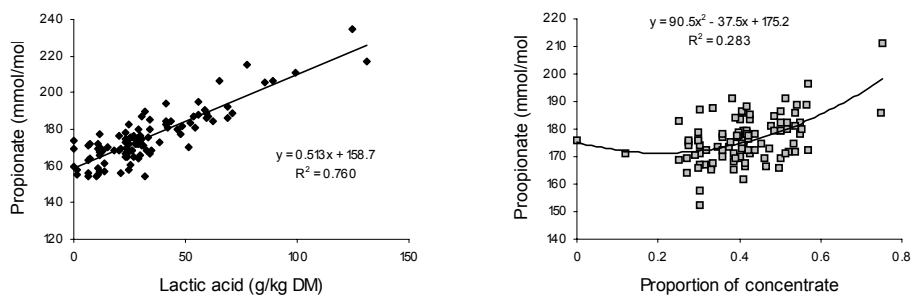


Figure 4 Effects of dietary concentration of lactic acid and proportion of concentrate on molar proportion of propionate in rumen VFA (data from Finnish studies). The values are adjusted for a random study effect.

Increasing the supply of glucose by infusing propionate into the rumen or infusing glucose post-ruminally has on average resulted in small positive production responses (Huhtanen 1998; Rigout *et al.*, 2003). Higher milk protein yield responses to infusions of His plus glucose (Huhtanen *et al.*, 2002) or casein plus glucose (Vanhatalo *et al.*, 2003a) as combined infusions rather than single infusions are consistent with the utilisation of AA for milk protein synthesis being compromised by limited glucose supply from the basal diet.

Because increasing the proportion of concentrate in the diet has only small effects on the proportion of glucogenic VFA in cattle fed grass-silage-based diets (Lock & Shingfield, 2004, Figure 4), increased starch digestion in the small intestine is an alternative strategy to enhance milk protein production by sparing glucogenic AA from catabolism in the liver. However,

Nocek & Tamminga (1991) concluded that production studies show no clear evidence that post-ruminal starch digestion increases milk production. Keady *et al.* (1996; 1999) obtained increases in the concentration of milk protein, but with no effects on total DM intake or milk production, by increasing starch in the concentrate with grass silage-based diets. In a recent study, milk protein yield tended to increase linearly to post-ruminal starch infusions (Reynolds *et al.*, 2001), but at best, only proportionally 0.175 of the gross energy in starch was recovered as milk energy. Small increases in milk energy output suggest that a large proportion of energy in starch was used for body tissue synthesis or oxidation. Replacing barley with maize in dairy concentrates would be a practical approach to increase glucose supply from increased digestion of starch in the small intestine, but milk protein yield responses (+20 g/d) have been minimal in high-yielding (35 kg/d) cows fed grass silage-based diets (Khalili *et al.*, 2001). Increased post-ruminal starch supplies tend to result in greater increases in lactose yield than in protein yield (Reynolds *et al.*, 2001; Khalili *et al.*, 2001). A small positive increase in milk protein output (30 g/d) with abomasal glucose infusions (300 g/d) compared with the control diet and corresponding infusions of starch (Vanhatalo *et al.*, 2003b) indicate that the use of post-ruminal glucose infusions may not necessarily mimic situations of increased supply of starch in the small intestine.

In addition to limited glucose supply, an imbalanced AA profile may limit milk protein yield responses from improved microbial protein synthesis with restrictively fermented silages. The concentration of His in microbial protein is markedly lower than in milk protein (21 vs. 27 g/kg AA). Plasma AA profile measurements (Miettinen & Huhtanen, 1997) suggest that improved microbial supply associated with silages of restricted fermentation may exacerbate the imbalance in AA supply to the mammary gland of cows fed grass silage-based diets. Restricting *in silo* fermentation and post-ruminal infusions of casein similarly increased plasma concentrations of branched-chain AA and lysine compared with extensively fermented high-lactate silage, but only casein infusion increased plasma His concentration and significantly enhanced milk protein output.

Conclusions and future perspectives

This review challenges the perception that the efficiency of N utilisation for milk production on grass silage-based diets is particularly poor. Part of this perception may have originated from observations based on indirect comparisons of EMPS, an overestimation in the range of ruminal protein degradability determined by current methods and kinetic models (*in situ*) and responses found with poorly-preserved silages being accepted as typical of all silages. Well-preserved restrictively-fermented silages and dried forages harvested from the same sward have generally resulted in comparable animal performance, despite estimates of MP supply sometimes being 0.20-0.25 lower for silage.

The AA profile of absorbed protein is relatively well-balanced in animals fed grass silage-based diets due to the high contribution of microbial protein and therefore reducing rumen ammonia-N losses represents the most promising means for improving overall efficiency of N use (milk N/dietary N). The easiest way to decrease N excess in the rumen is to alleviate an oversupply of RDP, but this is very difficult to achieve without compromising milk production. Partial replacement of early harvested grass silages with maize or other whole crop silages, forage management factors including N fertilisation and maturity at harvest all have a major impact on the N efficiency of milk production, but it should be noted that manipulating silage CP concentration by these two strategies results in very distinct effects on N utilisation.

Because modifications of the chemical composition of parent herbage during ensiling can have a major impact on the balance of nutrients available to rumen microbes and the host animal, dynamic and mechanistic substrate-based models could provide a more accurate prediction of nutrient supply than currently available models. The new Nordic dairy cow model Karoline (Danfær *et al.*, 2004) suggests that cell wall characteristics (e.g. indigestible NDF, rate of NDF digestion) and fermentation characteristics (e.g. lactic acid, VFA) of silage have a greater impact on the total supply of AA available for absorption than silage protein parameters. To monitor the efficiency of N utilisation at farm level, analysis of dietary CP and assessment of milk urea concentration are useful diagnostic tools. Low milk urea concentrations (<160 mg/l) are indicative of an RDP deficiency (Nousiainen *et al.*, 2004), whereas high concentrations (>300-350 mg/l) are associated with low marginal production responses and poor utilisation.

Manipulating the ratio of degradable protein and fermentable energy supply in the rumen provides the best opportunity to enhance the N efficiency of milk production. Excess RDP and ruminal N losses can be reduced by avoiding excessive levels of N fertilisation, and/or by replacing part of a grass silage-based diet with maize or whole-crop silages. Changes in chemical composition during ensilage have a major impact on the quantity of energy supplied to rumen microbes, which, together with changes in the N fraction, can result in substantial changes in microbial protein production and in the net absorption of ammonia from the rumen. Large amounts of supplementary protein are fed with grass silage-based diets, but despite substantial production responses, N utilisation is consistently compromised. However, differences in production responses between protein supplements do offer an opportunity to improve N efficiency. Attempts to balance nutrient supply by using various glucogenic substrates or AA supplements have had variable effects. Further research including both experimental studies and modelling of existing data needs to be targeted towards optimising the balance of absorbed nutrients. In the future it may be expected that plant breeders will develop new genotypes that result in grass varieties with lower CP content, and vegetable protein sources with reduced protein degradability characteristics and modified AA profile, which together may better complement microbial protein.

References

- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen & T. Varvikko, T. (1999). Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. *Acta Agriculturae Scandinavica, Section A, Animal Science*, 49, 179-189.
- Ahvenjärvi, S., A. Vanhatalo & P. Huhtanen (2002). Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. *Journal of Animal Science*, 80, 2176-2187.
- ARC (1984). The Nutrient Requirements of Ruminant Livestock, Supplement 1. Commonwealth Agricultural Bureaux, Farnham Royal, 45 pp.
- Bertilsson, J. (1983). Effects of conservation method and stage of maturity upon the feeding value of forages to dairy cows. Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård. *Rapport*, 104, 21 p.
- Chamberlain, D.G. (1987). The silage fermentation in relation to the utilization of nutrients in the rumen. *Process Biochemistry*, 22, 60-63.
- Chamberlain, D.G. & J.-J. Choung (1993). The nutritional value of grass silage. In: P. O'Kiely *et al.* (eds.). *Silage Research 1993. Proceedings of the 10th International Conference on Silage Research. Dublin, Ireland*, 131-136.
- Chamberlain, D.G. & J.-J. Choung (1995). The importance of rate or ruminal fermentation of energy sources in diets for dairy cows. In: P.C. Garnsworthy *et al.* (eds.) *Recent Advances in Animal Nutrition*. Butterworths, London, 3-27.

- Chamberlain, D.G., P.A. Martin & S. Robertson (1989). Optimizing compound feed use in dairy cows with high intakes of silage. In: W. Haresign & D.J.A. Cole (eds.) *Recent Advances in Animal Nutrition*. Butterworths, London, 175-193.
- Chamberlain, D.G., P.C. Thomas, W. Wilson, C.J. Newbold & J.C. McDonald (1985). The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. *Journal of Agricultural Science*, 104, 331-340.
- Choi, C.W., S. Ahvenjärvi, A. Vanhatalo, V. Toivonen & P. Huhtanen (2002). Quantification of the flow of soluble non-ammonia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. *Animal Feed Science and Technology*, 96, 203-220.
- Danfær, A., P. Huhtanen, P. Udén, J. Sveinbjörsson & H. Volden (2004). Karoline - a Nordic cow model for feed evaluation: model description. J. Dijkstra. (ed) Sixth International Workshop of Modelling Nutrient Utilisation in Farm Animals, Abstracts, p. 45.
- Dewhurst, R.J., K. Aston, W.J. Fisher, R.T. Evans, M.S. Dhanoa & A.B. McAllan (1999). Comparison of energy and protein sources offered at low levels in grass-silage-based diets for dairy cows. *Animal Science*, 68, 789-799.
- Dewhurst, R.J., D.R. Davies & R.J. Merry (2000). Microbial protein supply from the rumen. *Animal Feed Science and Technology*, 85, 1-21.
- Egan, A.R. (1970). Nutritional status and intake regulation in sheep. VI. Evidence for variations in setting of an intake regulatory mechanism relating to the digesta content of the reticulorumen. *Australian Journal of Agricultural Research*, 21, 735-746.
- Givens, D.I. & H. Rulquin (2004). Utilisation by ruminants of nitrogen compounds in silage-based diets. *Animal Feed Science and Technology*, 114, 1-18.
- Hristov, A.N. & G.A. Broderick (1996). Synthesis of microbial in ruminally cannulated cows fed alfalfa silage, alfalfa hay, or corn silage. *Journal of Dairy Science*, 79, 1627-1637.
- Huhtanen, P. (1998). Supply of nutrients and productive responses in dairy cows given diets based on restrictively fermented silage. *Agricultural and Food Science in Finland*, 7, 219-250.
- Huhtanen, P. (2005). A review of the 2001 dairy cattle NRC protein and amino acid model – A European Perspective. *Journal of Dairy Science*, 88, Supplement (In press)
- Huhtanen, P., S. Ahvenjärvi, M.R. Weisbjerg & P. Nørgaard (2005). Digestion and passage of fibre in ruminants. *Proceedings of the 10th International Symposium in Ruminant Physiology* (In press).
- Huhtanen, P., J.I. Nousiainen, H. Khalili, S. Jaakkola & T. Heikkilä (2003). Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. *Livestock Production Science*, 81, 57-73.
- Huhtanen, P., A. Vanhatalo & T. Varvikko (2002). Effects of abomasal infusions of histidine, glucose and leucine on milk production and plasma metabolites of dairy cows fed grass silage diets. *Journal of Dairy Science*, 85, 1, 204-216.
- Hvelplund, T. & M.R. Weisbjerg (2000). *In situ* techniques for the estimation of protein degradability and postrumen availability. In: D.I. Givens, E. Owen, R.F.E. Axford & H.M. Omed (eds.) Forage Evaluation in Ruminant Nutrition. CABI Publishing, Wallingford, 233-258.
- Jaakkola, S. & P. Huhtanen (1992). Rumen fermentation and microbial protein synthesis in cattle given intraruminal infusions of lactic acid with a grass silage based diet. *Journal of Agricultural Science, Cambridge*, 119, 411-418.
- Jaakkola, S. & P. Huhtanen (1993). The effects of the forage preservation method and the proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. *Grass and Forage Science*, 48, 146-154.
- Jaakkola, S., P. Huhtanen & V. Kaunisto (1993). VFA proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. In: P. O'Kiely *et al.* (eds.) Silage research 1993. *Proceedings of the 10th International Conference on Silage Research, Dublin, Ireland*, p.139-140.
- Keady, T.W.J., C.S. Mayne & M. Marsden (1996). The effects of concentrate energy source on silage intake and animal performance with lactating dairy cows offered a range of grass silages. *Animal Science*, 66, 21-34.
- Keady, T.W.J., C.S. Mayne, D.A. Fitzpatrick & M. Marsden (1999). The effects of energy source and level of digestible undegradable protein in concentrates on silage intake and performance of lactating dairy cows offered a range of grass silages. *Animal Science*, 68, 763-777.
- Khalili, H. & P. Huhtanen (1991). Sucrose supplements in cattle given grass silage-based diet. 1. Digestion of organic matter and nitrogen. *Animal Feed Science and Technology*, 33, 247-261.
- Khalili, H. & P. Huhtanen (2002). Effect of casein infusion in the rumen, duodenum or both sites on factors affecting forage intake and animal performance of dairy cows fed red clover-grass silage. *Journal of Dairy Science*, 4, 909-918.
- Khalili, H., A. Sairanen, K. Hissa & P. Huhtanen (2001). Effects of type and treatment of grain and protein source on dairy cow performance. *Animal Science*, 72, 573-584.

- Kim, C.H., T.G. Kim, J.J. Choung & D.G. Chamberlain (1999). Determination of the first limiting amino acid for milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal. *Journal of the Science of Food and Agriculture*, 79, 1703-1708.
- Kim, C.H., T.G. Kim, J.J. Choung & D.G. Chamberlain (2000). Variability in the ranking of the three most-limiting amino acids for milk protein production in dairy cows consuming grass silage and a cereal-based supplement containing feather meal. *Journal of the Science of Food and Agriculture*, 80, 1386-1392.
- Kim, C.H., T.G. Kim, J.J. Choung & D.G. Chamberlain (2001a). Estimates of the efficiency of transfer of L-histidine from blood to milk when it is the first-limiting amino acid for secretion of milk protein in the dairy cow. *Journal of the Science of Food and Agriculture*, 81, 1150-1155.
- Kim, C.H., J.J. Choung & D.G. Chamberlain (2001b). The effects of intravenous infusion of amino acids in dairy cows given diets of grass silage and cereal-based concentrates. *Journal of Animal Physiology and Nutrition*, 85, 293-300.
- Korhonen, M. (2003). Amino acid supply and metabolism in relation to lactational performance of dairy cows fed grass silage based diets. Dissertation, 45 p. + encl. University of Helsinki.
- Korhonen, M., A. Vanhatalo, & P. Huhtanen, P. (2002a) Effect of protein source on amino acid supply, milk production and metabolism of plasma nutrients in dairy cows fed grass silage. *Journal of Dairy Science* 85, 3336-3351
- Korhonen, M., A. Vanhatalo, T. Varvikko & P. Huhtanen (2000). Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. *Journal of Dairy Science*, 83, 2596-2608.
- Korhonen, M., A. Vanhatalo, T. Varvikko & P. Huhtanen. (2002b). Evaluation of isoleucine, leucine, and valine as a second-limiting amino for milk production in dairy cows fed grass silage diet. *Journal of Dairy Science*, 85, 1533-1545.
- Lock, A.L & K. Shingfield (2004). Optimisation milk composition. In: E. Kebreab, J. Mills & D. Beever (eds.) UK Dairying: using science to meet consumers' needs. Nottingham University Press, Nottingham, UK. pp. 107-188.
- Miettinen, H. & P. Huhtanen (1997). Effects of silage fermentation and postruminal casein supplementation in lactating dairy cows. 2. Blood metabolites and amino acids. *Journal of the Science of Food and Agriculture*, 74, 459-468.
- Nocek, J.E. & S. Tamminga (1991). Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *Journal of Dairy Science*, 74, 3598-3629.
- Nousiainen, J., K.J. Shingfield & P. Huhtanen (2004). Evaluation of milk urea concentration as a diagnostic of protein feeding. *Journal of Dairy Science*, 87, 386-398.
- NRC (2001). Nutrient requirements of dairy cattle. Seventh Revised Edition. National Research Council. 381 p.
- Nsereko, V.L. & J.A. Rooke (1999). Effects of peptide inhibitors and other additives on fermentation and nitrogen distribution in perennial ryegrass silage. *Journal of the Science of Food and Agriculture*, 79, 679-686.
- Oldham, J.D. (1984). Protein-energy interrelationships in dairy cows. *Journal of Dairy Science*, 67, 1090-1114.
- Ørskov, E.R. & I. McDonald (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science*, 92, 499-503.
- Peltekova, V.D. & G.A. Broderick (1996). *In vitro* ruminal degradation and synthesis of protein from fractions extracted from alfalfa hay and silage. *Journal of Dairy Science*, 79, 612-619.
- Reynolds, C.K., S.B. Cammell, D.J. Humphries, D.E. Beever, J.D. Sutton & J.R. Newbold (2001). Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. *Journal of Dairy Science*, 84, 2250-2259.
- Rigout, S., C. Hurtaud, S. Lemosquet, A. Bach & H. Rulquin (2003). Lactational effect of propionic acid and duodenal glucose in cows. *Journal of Dairy Science*, 86, 243-253.
- Rinne, M., P. Huhtanen & S. Jaakkola (2002). Digestive processes dairy cows fed silages harvested at four stages of maturity. *Journal of Animal Science*, 80, 1986-1998.
- Rinne, M., S. Jaakkola & P. Huhtanen (1997). Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. *Animal Feed Science and Technology*, 67, 1-17.
- Rinne, M., S. Jaakkola, K. Kaustell, T. Heikkilä & P. Huhtanen. (1999a). Silage harvested at different stages of grass growth versus concentrate foods as energy and protein sources in milk production. *Animal Science*, 69, 251-263.
- Rinne, M., S. Jaakkola, T. Varvikko & P. Huhtanen. (1999b). Effects of the type and amount of rapeseed feed on milk production. *Acta Agriculturae Scandinavica, Section A, Animal Science*, 49, 137-148.
- Satter, L.D., H.G. Jung, A.M van Vuuren & F.M. Engels (1999). Challenges in the nutrition of high-producing ruminants. In: H.J.G. Jung & G.C. Fahey, Jr. (eds.) Nutritional Ecology of Herbivores. *Proceedings of the Vth International Symposium on the Nutrition of Herbivores*, pp. 609-646.
- Santos, F.A.P., J.E.P. Santos, C.B. Theurer & J.T. Huber (1998). Effects of rumen undegradable protein on dairy cow performance. *Journal of Dairy Science*, 81, 3182-3213.

- Shingfield, K.J. (2000). Estimation of microbial protein supply in ruminant animals based on renal and mammary purine metabolite excretion. A. Review. *Journal of Animal and Feed Sciences*, 9, 169-212.
- Shingfield, K.J., S. Jaakkola & P. Huhtanen (2001). Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. *Animal Science*, 73, 541-554.
- Shingfield, K.J., A. Vanhatalo & P. Huhtanen (2003). Comparison of heat-treated rapeseed expeller and solvent-extracted soya-bean meal protein supplements for dairy cows given grass silage-based diets. *Animal Science*, 77, 305-317.
- Schwab, C.G., P. Huhtanen, C.W. Hunt & T. Hvelplund (2005). Nitrogen Requirements of Cattle. In: E. Pfeffer & A.N Hristov (eds), *Interactions between Cattle and the Environment*. CABI publishing (In Press).
- Tamminga, S. (1992). Nutrition management of dairy cows as a contribution to pollution control. *Journal of Dairy Science*, 75, 345-357
- Vanhatalo, A., E. Pahkala, P. Salo-Väänänen, H. Korhonen, V. Piironen & P. Huhtanen (2003). Rapeseed and soyabean as protein supplements of dairy cows fed grass silage based diets. In: Hilmer Sorensen et al. (eds.) *Proceedings of the 11th International Rapeseed Congress*. The Royal Veterinary and Agricultural University, Copenhagen, 1238-1240.
- Vanhatalo, A., P. Huhtanen, V. Toivonen & T. Varvikko (1999) Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *Journal of Dairy Science*, 82, 2674-2685
- Vanhatalo, A., T. Varvikko & P. Huhtanen (2003a). Effects of casein and glucose on responses of cows fed diets based on restrictively fermented grass silage. *Journal of Dairy Science*, 86, 3260-3270.
- Vanhatalo, A., T. Varvikko & P. Huhtanen (2003b). Effect of various glucogenic sources on production and metabolic responses in dairy cows fed grass silage-based diets. *Journal of Dairy Science*, 86, 3249-3259.
- Van Bruchem, J., H. Schiere & H. Van Keulen (1999). Dairy farming in the Netherlands in transition towards more efficient use of nutrients. *Livestock Production Science*, 61, 145-153.
- Van Vuuren, A.M., P. Huhtanen & J.P. Dulphy 1995. Improving the feeding and health value of ensiled forages. In: Journet, M. et al. (eds.). *Recent Developments in the Nutrition of Herbivores*. INRA, Paris. p. 297-307.
- Verbic, J., E.R. Ørskov, J. Zgajnar, X.B. Chen & V. Znidarsic-Pongrac (1999). The effect of method of forage preservation on the protein degradability and microbial protein synthesis in the rumen. *Animal Feed Science and Technology*, 82, 195-212.
- Volden, H., L.T. Mydland & V. Olaisen (2002). Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. *Journal of Animal Science*, 80, 2704-2716.
- Wahlroos, M.T. (2004). Modification of histidine content in transgenic *Brassica rapa* plants. Dissertation, 49 p. + encl. University of Turku.
- Weiss, W.P., D.G. Chamberlain & C.W. Hunt (2003). Feeding silages. In: D.R Buxton, R.E. Muck & J.H. Harrison (eds.) *Silage Science and Technology*. American Society of Agronomy, Madison, USA, 469-504.