Amino Acid Supply and Metabolism in Relation to Lactational Performance of Dairy Cows Fed Grass Silage Based Diets

Mikko Korhonen

Academic dissertation

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium 1041, Biocenter, Viikinkaari 5, on March 7th 2003, at 12 o’clock noon

Helsinki 2003
ABSTRACT
The main objective of the thesis was to study AA supply of dairy cows fed grass silage based diets with specific emphasis on the impact of changing individual and total AA supplies arising from individual AA supplementations or changes in the diet on milk production and AA utilisation. Mammary AA metabolism was also studied to broaden the understanding of the mechanisms underlying altered lactational performance.

Experiments documented in publications I and II were AA infusion studies in which the utilisation of infused His (I) and the role of BCAA as the second limiting AA on grass silage-cereal based diets (II) were investigated. Graded infusion of His (0, 2, 4 and 6 g/d) linearly increased milk and milk protein yields and the utilisation of infused His remained constant across infusion levels. This indicates that mammary gland is able to regulate nutrient uptake and that arterial supply is not the sole factor affecting mammary metabolism. Infusions of BCAA did not affect lactational performance suggesting that they are not second limiting AA on grass silage-cereal based diets.

The effects of barley and rapeseed meal supplementation of grass-red clover silage on omasal canal AA flow and the AA profile of liquid and particle associated bacteria, protozoa and the entire microbial protein were studied in publication III. Barley increased microbial AA flow and rapeseed meal increased the flow of dietary AA entering the omasal canal. The AA profiles of individual microbial fractions were different but the effect of diets on AA profiles was negligible. Diet had no effect on the AA profile of the entire microbial protein. Under the dietary conditions used, the assessment of microbial AA flow was more dependent on the accuracy of microbial protein flow measurements than the AA profile of individual AA fractions.

The effects of AA profile and rumen undegradable protein content of protein supplements on postruminal AA supply and lactational performance were investigated in publication IV. All protein supplements (fish meal, soybean meal and maize gluten meal) increased omasal canal AA flow and milk production. Higher dietary protein flow was also reflected in the AA profile of omasal digesta. Lactational responses and increases in AA flow were lower for soybean meal compared with fish meal and maize gluten meal owing to higher N losses in the rumen. The mammary gland appeared to be capable of regulating AA utilisation by changing the rate of extraction of individual AA.

The purpose of publication V was to assess the effect of silage harvest date (primary and secondary cuts) and the level of concentrate on postruminal AA supply and lactational performance. Lactational performance was higher for diets based on the secondary cut silage owing to greater nutrient supply. Increasing the concentrate level increased microbial protein supply but did not increase N capture in the rumen. Differences in AA flows between the concentrate levels were lower than predicted by the AAT/PBV system while ruminal degradation of barley was higher than current predictions.

Amino acid profiles of omasal digesta and microbial protein were similar between experiments. Some variation in total AA supply exists in spite of all basal diets were based on grass silage and cereal. This appears to be associated with differences in silage quality. Results demonstrated the importance of AA profile of absorbed protein in utilisation of dietary AA and also that AA supply can be altered by changing the AA profile and rumen undegradable protein content of protein supplements. Total AA supply appears in part, to compensate for the incomplete AA profile of digested protein because mammary gland is capable to regulate AA uptake.
ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor Pekka Huhtanen and Dr. Aila Vanhatalo for their guidance, unfailing support and inspiration during the work. I am indebted to my co-workers Professor Tuomo Varvikko and Dr. Seppo Ahvenjärvi for their contribution during the experimental work and fruitful discussions during the preparation of individual articles and thesis text. I want to greatly acknowledge, Aino Matilainen, Laila and Aaro Hakkarainen, Hannu Peltonen, Mirja Seppälä and Sanna Uusitalo for their assistance in conducting the animal experiments, and Vesa Toivonen and all laboratory staff for laboratory analysis. Financial support from the Academy of Finland and Rehuraisio Ltd. is gratefully acknowledged. I wish to also thank Dr. Kevin Shingfield for linguistic revision of this thesis. I am indebted to the referees appointed by the Faculty, Dr. Richard Dewhurst and Dr. David Chamberlain for their constructive criticism and useful suggestions for improving the thesis manuscript. My warmest thanks go to my family for their patience and support during this work.
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications subsequently referred to in the text by their Roman numerals:


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All experiments were conducted at the Animal Production Research, Agrifood Research Finland (MTT), Jokioinen.

All manuscripts were prepared by the author and revised according to the comments and suggestions of co-authors. The author participated in conducting experiments I and III and was responsible for calculation, statistical analysis and reporting of the data documented in publications I and III. Experiments II, IV and V were planned in conjunction with co-authors, while the author took full responsibility for conducting the studies, calculation, statistical analysis and reporting of the data documented in publications II, IV and V.
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<thead>
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<th>Term</th>
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<tr>
<td>AA</td>
<td>Amino acid</td>
</tr>
<tr>
<td>AAT</td>
<td>Amino acid absorbed from the intestine</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>AV</td>
<td>Arteriovenous</td>
</tr>
<tr>
<td>BCAA</td>
<td>Branched chain AA</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein (calculated as $N \times 6.25$)</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>EAA</td>
<td>Essential AA</td>
</tr>
<tr>
<td>FM</td>
<td>Fish meal</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>LAB</td>
<td>Liquid-associated bacteria</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>MG</td>
<td>Mammary gland</td>
</tr>
<tr>
<td>MGM</td>
<td>Maize gluten meal</td>
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<tr>
<td>MP</td>
<td>Microbial protein</td>
</tr>
<tr>
<td>MPS</td>
<td>Microbial protein synthesis</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral detergent fibre</td>
</tr>
<tr>
<td>NEAA</td>
<td>Non essential AA</td>
</tr>
<tr>
<td>NPN</td>
<td>Non-protein N</td>
</tr>
<tr>
<td>PAB</td>
<td>Particle-associated bacteria</td>
</tr>
<tr>
<td>PBV</td>
<td>Protein balance in the rumen</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>RDP</td>
<td>Rumen degradable protein</td>
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<tr>
<td>RSM</td>
<td>Rapeseed meal</td>
</tr>
<tr>
<td>RUP</td>
<td>Rumen undegradable protein</td>
</tr>
<tr>
<td>SBM</td>
<td>Soybean meal</td>
</tr>
<tr>
<td>TAA</td>
<td>Total AA</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>WSC</td>
<td>Water soluble carbohydrates</td>
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PUBLICATIONS I-V
1 INTRODUCTION

Feeding of dairy cows in Finland has long been based on a so called “Green Line” strategy, which refers to the majority of feed energy being derived from forages including grass silage, pasture and hay. In spite of the tendency for the increased usage of concentrate during recent years, the contribution of forages to total ME intake was 56% in 2001 (Maaseutukeskusten liitto, 2001). Concentrate supplements consisted largely of cereals (20% of total ME intake) in addition to commercial and other feeds (24% of the ME intake). The most common protein feed was rapeseed meal (RSM).

Consumption of dairy products has been fairly constant over recent decades, but the consumption and production of individual products has changed dramatically. This change results from a higher demand for cheese and processed dairy products at the expense of liquid milk and milk fat (Statistics Finland, 2001). Milk payment schemes have reflected the changes in demand for milk constituents such that milk protein is more valuable than milk fat. A tendency for decreasing income from milk production has also led to a situation where inputs need to be minimised. On the other hand, agriculture is the biggest environmental pollutant in rural areas of Finland and is the major source of the N and P entering inland and coastal waters (Hautala, 1990). These nutrients originate from fertilisers and manure from animal production systems (Tamminga, 1992). The utilisation of dietary N for milk production in Finland is on average 26 – 27% which is markedly lower than a theoretical maximum of about 40% (van Vuuren and Meijs, 1987). Therefore, improvements in N utilisation could be realised through developing more accurate dairy cow feeding strategies. However, this needs to be done without incurring a penalty on lactational performance. Improvements in dietary N utilisation can also reduce milk production costs because purchased feeds, including protein supplements, represented more than a third of the variable costs of milk production for Finnish recorded herds in 2001 (Maaseutukeskusten liitto, 2001).

Nitrogen losses from ruminants originate from rumen, faecal, urinary and maintenance losses (Tamminga, 1992). The contribution of maintenance to total losses is rather small and the potential to reduce it by increasing milk production is relatively limited. The same is true for faecal losses because the proportion of indigestible feed N in total faecal N excretion is low (about 30%) compared with that originating from endogenous and metabolic sources. However, there is potential for reducing urinary and rumen N losses.

Nitrogen requirement by the tissues is met by amino acids (AA) suggesting that altering dietary AA supply to match requirements could be used to increase milk and milk protein yields and reduce N losses (NRC, 2001). The importance of the AA profile of protein absorbed from the intestine for milk production is indicated by positive production responses to rumen protected or postruminally infused AA. On maize silage based diets lysine (Lys) and methionine (Met) have been considered as the most limiting for milk production (Guinard and Rulquin, 1995). Lack of responses to abomasal or intravenous infusions of Met (Chamberlain and Thomas, 1982, Varvikko et al., 1999), Lys (Varvikko et al., 1999), Met and Lys (Girdler et al., 1988b) and rumen protected Met and Lys supplements (Girdler et al., 1988a) in cows fed grass silage based diets suggest that in these cases other AA could be more limiting. Theoretical calculations presented by Varvikko et al. (1999) indicated that His and Leu could be first limiting AA for diets based on grass silage. The role of His as the first limiting AA for these diets has been verified in subsequent studies (e.g. Vanhatalo et al., 1999, Kim et al., 1999, Huhtanen et al., 2002a). The utilisation of infused His (at 6.5 g/d) was low in the studies of Vanhatalo et al. (1999) and Huhtanen et al. (2002a) suggesting that similar
lactational responses might be obtained at lower levels of His supplementation. Furthermore, different responses to various AA supplementations in milk and milk protein yields between the various types of the basal diets support the conclusions of Schwab et al. (1976) and Vanhatalo et al. (1999) that the composition of the basal ration may influence which AA is the most limiting for milk production and protein synthesis.

Milk production responses to infusions of casein and a mixture of AA have in many studies been higher than those obtained with infusions of individual AA (Schwab et al., 1976, Guinard and Rulquin, 1994) or mixtures with a different AA profile compared with casein (Choung and Chamberlain, 1992). Furthermore, infusions of Met and Lys on maize silage based diets (Schwab et al., 1976) resulted in higher responses compared with single AA infusions. These results suggest that by identifying the second and (or) third limiting AA, the efficiency of conversion of AA into milk protein could be further improved. This would also reduce urinary nitrogen losses, because surplus AA not converted into milk and tissue proteins are excreted in urine as urea (Tamminga, 1992).

Supplementing the basal diet with protein feeds can be used in practice to overcome limitations of AA supply from the basal diet. However, this strategy reduces the efficiency of N utilisation even when relatively high marginal production responses are obtained (e.g. Rinne et al., 1999a, Shingfield et al., 2002a). Different protein supplements have had variable effects on milk and milk protein yields (Rulquin and Verite, 1993, Khalili et al., 2001, Shingfield et al., 2002a). Of the individual protein feeds RSM has consistently resulted in good production responses in cows fed grass silage based diets (Huhtanen, 1998). Variable responses to protein feeds have been attributed to differences in the content and AA profile of rumen undegradable feed protein (RUP). This in addition to variable AA supply from the basal diet can result in both quantitative and qualitative differences in postruminal AA supply. In order to complement the AA supply with protein supplements it is important to define AA supply from the basal diet and also the impact of various protein feeds on AA supply.

In ruminants, microbial protein (MP) synthesised in the rumen and RUP are the two major sources of AA available for absorption from the small intestine. With grass silage based diets, the contribution of MP to the total postruminal protein flow is generally high (≥ 50%) because grass silage and cereal proteins are rapidly and extensively degraded in the rumen (Huhtanen, 1998). Microbial protein consists of liquid- (LAB) and particle-associated (PAB) bacteria and protozoa that can have markedly different AA profiles (Martin et al., 1996, Volden and Harstad, 1998, Volden et al., 1999). Furthermore, the contribution and AA profiles of individual fractions can be affected by the diet (Cecava et al., 1990, Faichney et al., 1997) such that using a mean AA profile for MP may result in erroneous estimates of postruminal AA supply (Clark et al., 1992).

As mentioned above, AA supply is dependent on the basal diet, but milk and milk protein yield responses to AA supplementation have been variable between the experiments despite similar basal diets (Vanhatalo et al., 1999, 2001). This suggests that AA supply may not be constant. The most likely explanation is that whilst diets appear similar, variations in silage quality (D-value, fermentation quality and characteristics and amounts of N constituents) affect MP synthesis (MPS) and energy and glucose supply, which can lead to variations in the ranking of the limiting AA.

In most studies conducted with maize silage based diets, milk and milk protein yields have been unaffected by increases in RUP supply (Santos et al., 1998). One potential explanation
for these findings is that increases in RUP reduce postruminal AA flow owing to a shortage of N for MPS in the rumen. It is clear that attempts to increase AA supply also have to maximise MP supply. On grass silage-cereal based diets, MPS is not generally limited by the availability of rumen degradable protein (Rooke and Armstrong, 1989, Ahvenjärvi et al., 1999). Often rumen degradable protein supply exceeds MP requirements leading to N losses from the rumen. This situation arises from extensive degradation of grass silage and cereal CP such that the extent and rate of ammonia-N production exceeds the capacity of rumen microbes to incorporate this into MP (Tamminga, 1992). Furthermore, cereal based concentrates may favour amylolytic bacteria which are thought to benefit from increased availability of preformed AA and peptides (Russell et al., 1992). The implication is that rumen microbial activity could be enhanced by provision of protein supplements in the diet. However, MP responses to protein have been inconsistent. Rapeseed meal has had no effect on MPS in some cases and in others had a positive effect (Ahvenjärvi et al., 1999, Oh et al., 1999). Fish meal (FM) supplements have also been reported to increase MPS (Dawson et al., 1988). These apparent discrepancies may be associated with differences in the type and quality of grass silage fed (Jacobs and McAllan, 1992, Jaakkola et al., 1993).

Another and probably additive reason for high ruminal N losses may be the low energy supply from silages associated with conversion of the carbohydrates in fresh herbage into lactic acid and volatile fatty acids (VFA) during ensiling, which have only a limited value as energy sources for rumen microbes (Chamberlain, 1987). One means to overcome this problem may be supplementation with barley because barley may enhance microbial N capture by providing more energy to the rumen microbes.

Tissues have a requirement for AA rather than protein per se. Traditional protein evaluation systems were based on digestible CP supply and did not consider MP as a source of AA. Because of the importance of rumen microbes to the supply of protein in ruminant animals, current protein evaluation systems (e.g. ARC, 1980, PDI; Verite and Peyraud, 1989, AAT/PBV; Madsen et al., 1995, NRC, 2001) have been developed to take into account both dietary and microbial sources of protein. Furthermore, the supply and requirements for protein are based on the total amount of AA absorbed from the small intestine. Results of studies in which AA supply have been altered with protein feeds or by supplementing the basal diet with individual AA have highlighted the role of individual AA in improving the utilisation of absorbed AA. However, to make further progress reliable in vivo data are required. Information concerning AA supply is also needed to develop dynamic models of ruminant AA metabolism at the whole animal or individual tissue level. Such models are useful tools to broaden the understanding of metabolism and interactions between individual nutrients.

The ultimate goal of the work reported in this thesis was to investigate AA supply on grass silage based diets and the influence of diet on the availability of AA for absorption. Data produced in experiments are intended for use in developing computer based dynamic models describing N metabolism of ruminants. Data also provides useful information to allow protein evaluation systems to be based eventually on the supply of individual AA. Metabolism of AA by the mammary gland (MG) was also studied to broaden the understanding of the mechanisms underlying changes in lactational performance. More specifically, the objectives of the experiments documented in the current thesis were

- Examine utilisation of the first limiting AA (I)
- Evaluate potential second limiting AA (II)
- Assess the effects of protein and energy supplementations on the AA profile of various microbial fractions, MP and postruminal AA supply (III)
- Evaluate the effect of AA profile and RUP content of protein supplements on post-ruminal AA supply and milk production (IV)
- Determine the effect of silage harvest date and concentrate level on post-ruminal AA supply and milk production (V)

Table 1. Summary of experiments.

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<th>Dietary Treatments</th>
<th>Objective</th>
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<tbody>
<tr>
<td>I 4 × 4</td>
<td>Grass silage (S)</td>
<td>S, C, His 0 g/d</td>
<td>Utilisation of infused His and Mammary AA metabolism</td>
</tr>
<tr>
<td></td>
<td>Cereal based concentrate 8 kg/d (C)</td>
<td>S, C, His 2 g/d</td>
<td></td>
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<tr>
<td></td>
<td>Abomasal glucose 250 g/d</td>
<td>S, C, His 4 g/d</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>S, C, His 6 g/d</td>
<td></td>
</tr>
<tr>
<td>II 5 × 5</td>
<td>Grass silage (S)</td>
<td>S, C, His, Ile, Val</td>
<td>Identification of the 2nd limiting AA for milk production</td>
</tr>
<tr>
<td></td>
<td>Cereal based concentrate 9 kg/d (C)</td>
<td>S, C, His, Ile, Leu</td>
<td></td>
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<tr>
<td></td>
<td>Abomasal glucose 250 g/d</td>
<td>S, C, His, Leu, Val</td>
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<tr>
<td></td>
<td>Histidine (His)</td>
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<td></td>
<td>Isoleucine (Ile)</td>
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<td>Leucine (Leu)</td>
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<td></td>
<td>Valine (Val)</td>
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<td>III 4 × 4</td>
<td>Grass-red clover silage (S)</td>
<td>S</td>
<td>Effects of energy and N supplements on AA profile of MP, LAB, PAB and protozoa and omasal canal AA flow</td>
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<td></td>
<td>Barley (B)</td>
<td>S, B</td>
<td></td>
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<td></td>
<td>Rapeseed meal (RSM)</td>
<td>S, RSM</td>
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<tr>
<td></td>
<td></td>
<td>S, B, RSM</td>
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<tr>
<td>IV 4 × 4</td>
<td>Grass silage (S)</td>
<td>S, B</td>
<td>Effects of AA profile and RUP content of protein supplements on omasal canal AA flow and lactational performance</td>
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<tr>
<td></td>
<td>Barley (B)</td>
<td>S, B, FM</td>
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<td></td>
<td>Fish meal (FM)</td>
<td>S, B, SBM</td>
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<td></td>
<td>Soybean meal (SBM)</td>
<td>S, B, MGM</td>
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<td></td>
<td>Maize gluten meal (MGM)</td>
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<tr>
<td>V 4 × 4</td>
<td>Primary growth grass silage (PGS)</td>
<td>PGS, B1, RSM</td>
<td>Effects of silage harvest date and concentrate level on omasal canal AA flow and lactational performance</td>
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<td>Secondary growth grass silage (SGS)</td>
<td>PGS, B2, RSM</td>
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<td></td>
<td>Barley 6 kg/d (B1)</td>
<td>SGS, B1, RSM</td>
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<td></td>
<td>Barley 10 kg/d (B2)</td>
<td>SGS, B2, RSM</td>
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<td></td>
<td>Rapeseed meal (RSM)</td>
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2. MATERIAL AND METHODS

2.1. Experimental animals and procedures

The work documented in publications I to V was conducted as five separate experiments (Table 1). Finnish Ayrshire dairy cows used in the experiments were fitted with rumen cannulae and had calved from 35 to 167 days prior to the start of experiments. Experiments were conducted as $4 \times 4$ (I, III and V) or $5 \times 5$ (II) Latin square designs with a $2 \times 2$ factorial arrangement of treatments in experiments III and V. For I and II, experimental periods lasted for 14 d and an additional 14 d period was conducted after the experiment to assess AA supply from the basal diet. The length of experimental period in these experiments was assumed to be sufficient since the basal diet did not change between the experimental periods and the changes in milk production occurs mainly within 24 h of the start of the infusion (Metcalf et al., 1996a). Experimental periods were 21 d for experiment III and 28 d for experiments IV and V.

Procedures used are described in detail within individual papers (I to V) and only a brief outline is provided here. Milk production was measured throughout the studies and samples for analysis of milk composition were taken from four consecutive milkings in each experimental period. Rumen fermentation was measured by sampling rumen fluid through rumen cannulae at regular intervals during one day. Digestibility of diets was measured using acid insoluble ash as a marker in experiments I and III and by total faecal collection in experiments II, IV and V. During the collection period, feeds were offered as two equal meals at 12 h intervals. Postruminal digesta samples were collected using the omasal sampling technique (Huhtanen et al., 1997a, Ahvenjärvi et al., 2000). Samples were taken from the omasum with the device inserted into the omasum through the reticulo-omasal orifice. Estimation of postruminal nutrient flow was based on a triple-marker method (France and Siddons, 1986). Chromium-mordanted straw or indigestible NDF, Yb and CoEDTA were used as markers for large particles, small particles and liquid phases, respectively. Bacterial samples were collected manually from reticular digesta in experiments II, III, IV and V and protozoal samples from the omasum in experiment III. Purines (Zinn and Owens, 1986, Makkar and Becker, 1999, Obisbo and Dehority, 1999) were used as a microbial marker in experiment II and $^{15}$N in experiments III, IV, and V. Blood samples were taken from one superficial epigastric (mammary) vein, considered to be venous blood and one coccygeal (tail) vessel considered to be arterial blood. Mammary metabolism of nutrients was studied according to the Fick principle and plasma Phe and tyrosine (Tyr) as markers to estimate mammary blood flow (Cant et al., 1993). In experiments I and II, AA and glucose were continuously infused into the abomasum.

2.2. Experimental treatments

Experiment I was conducted to investigate the effect of graded doses of postruminally infused histidine (0, 2, 4 and 6 g/d) on milk and milk protein yields, utilisation of supplementary His, plasma AA concentrations and mammary metabolism. Cows received grass silage ad libitum and 8 kg/d of a cereal-based concentrate. Glucose was infused into the abomasum of all cows at a rate of 250 g/d.

In experiment II, the basal diet (control) was similar to that in experiment I with the exception that concentrates were offered at 9 kg/d. The purpose of this study was to evaluate the role of branched-chain AA (BCAA) as second limiting AA for milk production on grass silage-cereal
based diets. Four infusion treatments consisted of a mixture of His, valine (Val), isoleucine (Ile) and leucine (Leu) or infusion of this mixture in the absence of Leu, Val or Ile. Glucose was infused into the abomasum on all treatments at a rate of 250 g/d.

In study III, the effect of protein and energy supplementation on postruminal AA supply and AA profile of individual microbial fractions (LAB, PAB and protozoa) and MP were investigated in cows fed a basal diet of grass-red clover silage. Treatments consisted of the basal diet, or the basal diet supplemented with 6 kg/d of barley, 2.1 kg/d of RSM or 6 kg/d of barley and 2.1 kg/d of RSM.

Study IV was conducted to investigate the effect of AA profile and the RUP content of protein supplements on milk production and postruminal AA supply. The basal diet (Control) consisted of a fixed amount of grass silage and barley (55:45 on a dry matter (DM) basis) such that daily DM intake was restricted to 95% of pre-experimental ad libitum intake. The amount of feed offered was maintained throughout the study. Treatments consisted of the basal diet, or the basal diet supplemented with FM, soybean meal (SBM) or maize gluten meal (MGM). Supplemented diets were formulated to be isonitrogenous while protein feeds were included in the diet to maintain the ratio of barley to grass silage (55:45 on a DM basis) across all treatments.

The effect of silage harvest date and concentrate level on milk production and postruminal AA supply was examined in experiment V. Grass silage was prepared from primary or secondary growths of timothy/ meadow fescue sward and ensiled with a formic acid additive. Silages were offered with 8.1 or 12.1 kg concentrate per day consisting of 2.1 kg RSM and 6 or 10 kg of barley.

For all experiments, cows were housed in individual stalls, milked twice daily and had continuous access to water. Mineral and vitamin supplements were provided with all diets and accounted for between 250 and 300 g/d.
3. RESULTS AND GENERAL DISCUSSION

3.1. Grass silage

In Finland, grass harvested for silage is generally ensiled with a high application rate (generally from 4 to 5 l of formic acid/t of grass) of a formic acid based additive. A high level of formic acid application reduces the pH of the ensiled material and restricts inherent fermentation and proteolysis in the silo (Jaakkola et al., 1993). In experiments I to V the application rates of formic acid based additives were from 5 to 6.1 l/t and the pH values of the silages (3.9 to 4.2) were typical of restrictively fermented silages. Typical features of formic acid treated silage are low lactate and high residual water soluble carbohydrate (WSC) concentrations as compared with bacterial inoculant or enzyme treated silages (Jaakkola and Huhtanen, 1990, Huhtanen et al., 1997b, Heikkilä et al., 1998, Shingfield et al., 2002b). In experiments I to V, lactate concentrations were accordingly low while WSC were variable (23 to 146 g/kg DM), probably reflecting large differences in the WSC concentration of ensiled herbage. In general, the fermentation quality of all silages was good as indicated by low pH, low ammonia-N content and negligible butyric acid content.

3.2. Effect of diet on rumen fermentation

Rumen fermentation patterns in experiments I to V were measured in order to determine the type of fermentation and to gain information on nutrient supply other than AA. Feeding restrictively fermented silage supplemented with moderate amounts of cereal-based concentrates favours a rumen fermentation pattern high in butyrate and acetate and low in propionate (van Vuuren et al., 1995, Huhtanen, 1998). Therefore, rumen fermentation is characterised by a high ratio of ketogenic to glucogenic acids. The patterns observed for diets based on grass silage and barley in experiments I to V (Table 2) were consistent with this pattern (Table 2).

Table 2. Rumen fermentation patterns of grass silage-cereal based diets in studies I - IV, mean for study V and the range observed in previously published Finnish studies.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Range 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate, mmol/mol</td>
<td>642</td>
<td>669</td>
<td>659</td>
<td>643</td>
<td>612</td>
<td>624 – 696</td>
</tr>
<tr>
<td>Propionate, mmol/mol</td>
<td>181</td>
<td>159</td>
<td>167</td>
<td>180</td>
<td>165</td>
<td>142 – 187</td>
</tr>
<tr>
<td>Butyrate, mmol/mol</td>
<td>129</td>
<td>125</td>
<td>141</td>
<td>141</td>
<td>132</td>
<td>113 – 158</td>
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<tr>
<td>Acetate/Propionate</td>
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<td>4.21</td>
<td>3.96</td>
<td>3.57</td>
<td>3.71</td>
<td>3.53 – 4.89</td>
</tr>
<tr>
<td>(Acetate+Butyrate)/Propionate</td>
<td>4.26</td>
<td>4.99</td>
<td>4.79</td>
<td>4.36</td>
<td>4.51</td>
<td>4.23 – 5.73</td>
</tr>
</tbody>
</table>

1From Huhtanen (1998). Data derived from Finnish studies (N = 34) on diets based on restrictively fermented grass silage.

Because AA have been infused either postruminally or intravenously and concentrate level has been fixed in most studies (Vanhatalo et al. 1999, Varvikko et al. 1999, Kim et al., 2000a, b, Huhtanen et al. 2002a), no changes in ruminal fermentation would be expected. Postruminal infusion of His did not change rumen fermentation pattern (I). Rumen fermentation also remained constant, except for the increased proportions of branched chain fatty acids, when grass silage and barley were replaced by SBM, FM or MGM (IV). These observations are in agreement with other studies that reported no changes in the proportions of acetic, propionic or butyric acids with diets supplemented with RSM (Aronen and Vanhatalo, 1992, Ahvenjärvi et al., 1999, Dewhurst et al., 1999), FM or SBM (Rooke et al., 1985, Aronen and Vanhatalo, 1992, Keady and Murphy, 1998, O’Mara et al., 1998, Dewhurst
et al., 1999). The effects of protein supplements on rumen fermentation appear to be restricted to variations in ammonia-N and total VFA and branched chain fatty acids concentrations associated with the amount and degradability of protein supplements.

Increases in barley supplementation increased the molar proportion of butyrate and slightly decreased that of acetate (V) which is consistent with changes observed in other studies (Thomas et al., 1980, Thomas and Chamberlain, 1982, Jaakkola and Huhtanen, 1993). Barley supplementation has been shown to increase the number of protozoa in the rumen (Chamberlain et al., 1983, Jaakkola and Huhtanen, 1993) which may account for these changes, since protozoa produce high amounts of butyrate (Hungate, 1966).

Chamberlain and Choung (1993) suggested that the rumen fermentation pattern is mainly controlled by silage fermentation type for grass silage based diets. This is supported by the findings of van Vuuren et al. (1995) and Martin et al. (1994a) which indicate elevated silage lactate concentrations associated with increases in the molar proportion of propionate in the rumen. In accordance with this, graded application of formic acid decreased silage lactate concentration and tended to decrease the proportion of propionate in the rumen (Jaakkola et al., 1993). The association between lactate and propionate metabolism was also demonstrated by Jaakkola and Huhtanen (1992) because graded ruminal infusion of lactate increased the proportion of propionate in the rumen of cattle. Silage WSC concentration is also known to be affected by the extent of fermentation, and ingestion of silage with high WSC is generally associated with increases in acetate or butyrate in rumen VFA (Vanhatalo et al., 1992, Jaakkola et al., 1993). Therefore, higher acetate and lower valerate and caproate concentrations with secondary compared with primary cut silage based diets (V) may reflect differences in the WSC concentrations of ensiled herbage.

3.3. Effect of AA supplementation on lactational performance from grass silage based diets

3.3.1. Milk yield

Milk production responses to Met and(or) Lys supplementations have been observed on maize and lucerne silage based diets (e.g. Schwab et al., 1976, King et al., 1991, Schwab et al., 1992a, b, Pisulewski et al., 1996) but not for grass silage based diets (Chamberlain and Thomas, 1982, Girdler et al., 1988a, b, Varvikko et al., 1999). In agreement with these results, infusions of a mixture of AA containing Met, Lys and tryptophan (Trp) (Kim et al., 2001b, c) or Met, Phe and Trp (Choung and Chamberlain, 1992) were shown to have no effect on milk production. Lactational performance also remained unchanged following infusions of Met and Lys in combination or separately with His (Vanhatalo et al., 1999, Kim et al. 2001b) or when Met was omitted from a mixture of AA containing His, Lys and Trp (Choung and Chamberlain, 1995).

In experiment I, graded doses of His infusion (0, 2, 4 and 6 g/d) linearly increased milk yield which is in agreement with the increases reported by Kim et al. (2001c) following infusions of 0, 3, 6 and 9 g/d and the responses attained with infusions of 6 and 6.5 g His per day (Vanhatalo et al., 1999, Kim et al., 2000a, Huhtanen et al., 2002a). In contrast, Kim et al. (2001c) did not observe a response to His infusion when the basal diet contained soybean meal. Histidine infusion alone (Vanhatalo et al., 2001) or in combination with BCAA (II) has also been shown to have no effect on milk production.
Increases in milk yield between the control diet and the 6 g His/d infusion treatment (Table 3) were lower than those observed by Kim et al. (2000a, 2001c) at similar infusion rates. In the studies of Vanhatalo et al. (1999) and Huhtanen et al. (2002a), responses were also found to be lower while simultaneous infusions of His and glucose (Huhtanen et al., 2002a) resulted in comparable responses to those in experiment I. Consequently, the most convincing explanation for these apparent discrepancies is variation in the supply of AA and glucose. In first mentioned studies (Kim et al., 2000a, 2001c) diets were supplemented with feather meal, which increases the supply of AA other than His making the basal diet clearly deficient in this AA. Furthermore, experimental cows were in positive energy balance which, together with a deficient His supply, enhances the potential of cows to respond infused His.

3.3.2. Milk protein yield and content

Milk protein yield and content were not affected by infusion of Met (Chamberlain and Thomas, 1982, Varvikko et al., 1999), Met and Lys (Girdler et al., 1988a, b, Kim et al., 2001b) or graded levels of Lys (Varvikko et al., 1999). Milk protein yield was not markedly increased in previous studies when Met or Lys were omitted from an infusion mixture containing His, Met, Lys and Trp (Choung and Chamberlain, 1995, Kim et al., 1999). Further studies have also shown that milk protein synthesis or content are not increased with infusions containing Met, Lys, Leu and His compared with His alone (Vanhatalo et al., 1999, Huhtanen et al., 2002a). Infusion containing His, Leu, Ile and Val and excluding Leu from the infusion mixture also had no effect on milk protein synthesis (II).

In experiment I, graded doses of His infusion increased milk protein yield but did not affect milk protein content which is in agreement with responses to graded doses (0, 3, 6 and 9 g/d) of His (Kim et al., 2001c) or infusion rates of 6.5 and 6 g His/d (Vanhatalo et al., 1999, Kim et al., 2000a). Consistent with this, Kim et al. (1999) also found that infusion of a mixture of His, Met, Lys and Trp increased milk protein yield, but the response diminished when His was omitted from the infusion mixture. In studies reported by Kim et al. (2001b) and Huhtanen et al. (2002a) His infusion increased both milk protein content and yield. Milk protein yield and content has also been increased when His was simultaneously infused (4 or 8 g/d) with Met and Lys (Kim et al., 2001a).

The increase in milk and milk protein yield with His infusions on grass silage-based diets alone or supplemented with feather meal in several experiments suggest that in these cases His is the first limiting AA for milk production. Increases in milk protein yield with His infusion in experiment I was higher than the mean response reported for His infusions (Table 3; 10.5 vs. 8.2 g/g) but was about half of that obtained when His was infused in combination with other amino acids (response mean of 16.3 g/g). This together with similar responses in milk yield, strongly supports the suggestion that identification of the second and even third limiting AA could allow further improvements in lactational performance to be gained. Progress in this respect has been slow, because of a very small margin between limiting AA (Schwab et al., 1976) or as a consequence of a variable ranking order of limiting AA such that even the first limiting AA can be different for similar basal diets (Kim et al., 2000a).

Theoretical calculations of AA supply and requirement presented by Varvikko et al. (1999) suggested that His and(or) Leu could be the first limiting AA in milk production on grass silage-cereal based diets. Furthermore, according to Schingoethe (1996) Leu is the second limiting AA in MP. Changes in lactational performances to infusion of other AA in
combination with His have been variable (e.g. Vanhatalo et al., 1999, Kim et al., 2000a, Huhtanen et al., 2002a, II). As a result there is no clear candidate for the second limiting AA.

Because the basal diets were similar in experiments I and II it was expected that infusion of BCAA and His (II) would result in the greatest milk and milk protein responses. In contrast to expectations, infusion of four EAA had no effect on milk production. A lack of response to 6.5 g/d infusion of His (Vanhatalo et al., 2001) also contradicts with the results of experiment I. However, Kim et al. (2000a) noted a similar variation in responses between two studies in cows offered grass silage based diets. In the first study, the highest increase in milk protein yield was obtained when His was infused in combination with Met and Lys, with His accounting for more than half of the response. In the second study, protein yield was increased by infusions of a mixture of His, Lys, Met and Trp, but this positive effect was diminished when either Met or Lys were omitted suggesting that these two AA were limiting. Furthermore, addition of His into the infusion mixture containing Met, Lys and Trp (Kim et al., 2001b) had no effect on milk protein yield or content. Inconsistencies between studies support the suggestion that the ranking of the first limiting AA can be variable in spite of similar basal diets (Kim et al., 2000a). Furthermore, responses to RSM were lower than expected, which together with the lack of response to His infusion suggest that in this case AA supply from the basal diet was relatively high. Based on the low rumen ammonia-N in relation to dietary CP content, MPS would appear to have been relatively efficient. Consequently, variation in responses to AA infusions probably arise from quantitative differences in postruminal AA supply. An abundant AA supply may also dilute the effect of AA profile of digested protein.

3.3.3. Milk fat yield and content

In experiment I, graded doses of His infusion decreased milk fat concentration, except with the infusion level of 4 g/d, in agreement with the observations of Kim et al. (2001c). Fat concentration also decreased when His was infused alone or in combination with other AA (Vanhatalo et al., 1999, Kim et al., 2000a, 2001a). In most cases, changes in fat concentrations have been negligible when His has been infused in combination with other AA (Table 3). The reason for the square effect in experiment I is difficult to explain. In contrast, Met has increased milk fat concentration when infused alone (Chamberlain and Thomas, 1982, Varvikko et al., 1999) or in combination with Lysine and(or) other AA (Girdler et al., 1988b, Kim et al., 2001b) or resulted in higher milk fat secretion (Chamberlain and Thomas, 1982, Girdler et al., 1988a, b, Varvikko et al., 1999). Responses in fat yield reported in other studies (Table 3) are more variable than those reported for milk fat content, primarily owing to AA infusions having a variable effect on milk yield. It is apparent that decreases in milk fat concentration are associated with dilution effects (I) because no direct effect of His on milk fat synthesis has been reported. Increases in milk fat content have been suggested to be associated with an imbalanced AA profile of absorbed protein (Choung and Chamberlain, 1992). The increases in milk fat content in response to Met are likely to be associated with fat synthesis because Met and its metabolites enhance de novo fatty acid synthesis, mammary uptake of fat precursors and fat absorption from the blood (Pisulewski et al., 1996, Varvikko et al., 1999).

3.3.4. Milk lactose yield and content

Lactose participates in the regulation of milk osmolarity and thus the volume of water passing into the mammary alveolus which determines the volume of milk secreted (Mather and
Keenan, 1983). Because of this, it has been speculated that milk lactose concentration remains constant despite changes in the diet. The absence of changes in milk lactose concentrations (I, II) support this suggestion. However, milk lactose concentrations have decreased (Vanhatalo et al., 1999, Kim et al., 2001a, b) or increased (Kim et al., 2000a) with infusion of His alone or in combination with other AA. The decrease in lactose also found with various infusions is most likely due to variation in glucose supply. Similar to the response in output of milk fat, lactose yield responses to AA supplementation have been variable and are primarily dependent on changes in milk yield (Table 3).
Table 3. Effect of AA infusion on milk production responses of cows fed grass silage based diets.

<table>
<thead>
<tr>
<th>Reference</th>
<th>AA infusion, g/d</th>
<th>Milk kg/d</th>
<th>Protein kg/d</th>
<th>Lactose g/kg</th>
<th>Fat g/d</th>
<th>Protein kg/g</th>
<th>Lactose g/kg</th>
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<td>Chamberlain and Thomas, 1982</td>
<td>Met 8</td>
<td>-0.2</td>
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<td>+4.4</td>
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<td>-11</td>
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<tr>
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<td>Met 12, Lys 18</td>
<td>-0.8</td>
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<td>+3.3</td>
<td>-16</td>
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<tr>
<td></td>
<td>Met, Lys 36</td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
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<td>+0.4</td>
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<td>+32</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>2AA3</td>
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<tr>
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<tr>
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<tr>
<td>Kim et al., 2001c</td>
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<td>-0.7</td>
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<tr>
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<td>+59</td>
<td>-1.5</td>
<td>+164</td>
<td>+3.5</td>
<td>+95</td>
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</table>

1EAA = His 8.5, Ile 14.9, Leu 27.9 and Val 18.3 g/d. –Leu = 4EAA –Leu, –Ile = 4EAA –Ile, –Val = 4EAA –Val.

2= His 6 g/d and corresponding amount of other EAA to achieve the EAA composition of casein, 2 = His, Met and Lys in the amounts of supplied by other treatments, 3 = His 6 g/d.

3EAA = His 9, Met 10, Lys 25.5 and Trp 4.8 g/d. –Met = 4EAA –Met, –Lys = 4EAA –Lys, –Trp = 4EAA –Trp.

4AA = Met 8, Lys 28 and Trp 2.5 g/d, 4AA = His 6, Met 8, Lys 28 and Trp 2.5 g/d.

5AA = Met 8 and Lys 28 g/d, 5AA = His 6, Met 8 and Lys 28 g/d.

6Concentrate consisted of barley and soybean meal.

73AA = Met 8, Lys 28 and Trp 2.5 g/d.
3.4. Effect of glucose supply on milk production

In ruminants, glucose supply is almost entirely met by hepatic gluconeogenesis for which propionic acid is the main precursor (Danfær, 1994). Propionate production is low for diets based on restrictively fermented grass silage, potentially limiting glucose supply and thereby compromising milk production owing to reduced lactose synthesis (Mather and Keenan, 1983). In addition to propionic acid, AA are also used in glucose synthesis (Tamminga, 1992, Oldham, 1994) which could reduce the utilisation of AA for tissue and milk protein synthesis, particularly when glucose supply is deficient. Milk and milk protein yield responses to His infusion were additive when glucose was simultaneously infused with His (Huhtanen et al., 2002a). Production responses also were additive when glucose was infused with casein (Vanhatalo et al., 2002a). Histidine infusions have also decreased milk lactose concentration (Vanhatalo et al., 1999, Kim et al., 2001a) suggesting a glucose deficiency. Kim et al. (2001a) also noted that milk yield only increased when glucose was infused with His. This together with enhanced plasma glucose concentrations following post-ruminal casein infusions (Vanhatalo et al., 2002a) further indicates a clear association between glucose and AA metabolism. Consequently, to avoid possible deficiency, glucose was infused with AA in experiments I and II. In contrast to the results mentioned above, glucose supplementation did not increase milk production in the studies of Kim et al. (2000b) and Vanhatalo et al. (2002b) suggesting that in these studies glucose supply was not limiting.

Oldham (1994) speculated about the existence of an interaction between glucose and AA metabolism and hypothesised that the translation of supplementary AA into milk protein may be dependent on glucose status. He proposed that when glucose supply is limiting, AA are utilised for gluconeogenesis and thus the responses observed are limited to increases in milk protein content. This is because AA can not fully account the requirements of C for gluconeogenesis. Alternatively, when an adequate glucose supply can be maintained without resorting to AA for gluconeogenesis, responses can be expected as an increase in milk yield and(or) milk protein concentration, and therefore, milk protein yield. Since His infusions have increased both milk and milk protein yields (I, Vanhatalo et al., 1999), milk, milk protein yields and protein concentration (Huhtanen et al., 2002) or only protein yield and concentration (Kim et al., 2001b) differences in the milk protein response may be related to variations in glucose status.

3.5. Utilisation of infused AA

Conversion efficiencies of infused His into milk protein have been low (0.10 and 0.11; Vanhatalo et al., 1999, Huhtanen et al., 2002a, respectively), in spite of increases in milk and milk protein yield. Utilisation of His more than doubled (0.24) when glucose was infused with His (Huhtanen et al., 2002a), while the utilisation of infused casein has also been enhanced by additional glucose supply (Vanhatalo et al., 2002a). It appears that the relatively high utilisation of infused His across the infusion levels in experiment I (mean 0.28) may be a consequence of the glucose infusion.

Energy to protein ratio is another important factor affecting utilisation of AA (Oldham, 1994). Milk protein response to casein infusions almost doubled when energy supply was increased from 85 to 100% of estimated requirements (Rulquin, 1982). In other studies (e.g. Whitelaw et al., 1986, Choung and Chamberlain, 1992, Vanhatalo et al., 2002a) casein infusions have enhanced lactational performance in animals in negative and positive energy balance. According to Whitelaw et al. (1986) cows experiencing negative energy balance aimed to
maintain a constant AA-N to metabolisable energy ratio by mobilising body fat and protein stores. Thus, the utilisation of supplementary AA may also depend on body fat and protein stores and the genetic capacity of animals to mobilise these tissues.

Based on a low utilisation of His at an infusion level of 6.5 g/d (Vanhatalo et al., 1999, Huhtanen et al., 2002a), it can be speculated that supply exceeded requirements. This suggests that similar milk production responses could be obtained using lower doses of His, particularly in the presence of additional glucose. Furthermore, utilisation of His could be expected to be high at low infusion levels. This hypothesis was tested in experiment I. Kim et al. (2001c) also carried out two similar experiments in which His was intravenously infused at levels of 0, 3, 6 and 9 g/d alone or in combination with a mixture of Met, Lys and Trp. Both in study I and that reported by Kim et al (2001c) His clearly remained the first limiting AA, because milk and milk protein yields increased linearly. However, conversion efficiencies of infused His were not affected by infusion level. In the studies of Kim et al. (2001c) the maximum utilisation of infused His for milk protein synthesis was 0.38 at 6 g/d and the mean conversion efficiency was not higher than 0.43 with a mixture of AA. These are higher than the respective value (0.28) in experiment I, but all these values are far below predictions (e.g. 0.67; NRC, 2001) within current protein evaluation systems.

Kim et al. (2001c) infused His intravenously while infusions into the abomasum were adopted in experiment I. This has the consequence that infused AA have to be absorbed from the digestive tract raising the possibility that splanchnic tissue metabolism and transport mechanisms through the gut wall may alter the profile of AA entering the blood both qualitatively and quantitatively. According to MacRae et al. (2000) there is evidence that the recovery AA is higher for intravenous than for post-ruminal infusions, although a recent direct comparison between infusion sites does not support this suggestion (Aikman et al., 2002). Increases in plasma His concentrations have also been similar in studies reported in Table 4 in spite of the variable infusion sites. It appears that the higher His utilisation observed in the studies of Kim et al. (2001c) compared with experiment I, may be associated with protein supply from the basal diet rather than infusion site. In experiment I, the basal ration consisted of grass silage and cereals without any protein supplement but in the study of Kim et al. (2001c) total AA supply was increased by feather meal supplementation. The concentration of His in feather meal is low and that of RUP is high making the basal diet clearly deficient in His. Another potential explanation may arise from differences in energy status which was clearly positive in the study of Kim et al. (2001c) but slightly negative in experiment I.

Histidine also has a specific role in haemoglobin and carnosine synthesis (Kim et al., 2001c) and therefore its accumulation in whole blood may be high. However, if mammary uptake of AA are mainly from plasma as is generally accepted (Bequette et al., 1996, 1999, Mackle et al., 2000) it may not provide an explanation for the low efficiency of utilisation, because plasma His concentrations have been very sensitive to increases in His supply (I, Kim et al., 2000a, b, 2001a, b, c, II). It has also been pointed out that His oxidation in tissues is one of the lowest compared with other AA (Black et al., 1990). Uptake of nutrients by the mammary gland (MG) regulated by the metabolism of the MG (Cant and McBride, 1996) and the MG is thought to maintain the uptake of AA commensurate with each other. If this is the case, then the low utilisation of His may be attributed to metabolism of AA by the MG. This is in agreement with findings that His is an AA that is taken up by the MG gland in a direct ratio to its output in milk (Mepham, 1982, Guinard and Rulquin, 1995, I).
When cereal based concentrates have been replaced by RSM in production trials the mean utilisation of supplementary AA absorbed from the small intestine has been close to 0.50 (Huhtanen, 1998). In agreement with this, the respective value (treatment means for TAA flow and milk protein yield and assuming a value of 0.84 for AA absorption from the intestine in experiments III, IV and V) was 0.46 when adjusted for between-study effects. Corresponding values for His, Met, Lys and Leu were 0.55, 0.40, 0.30 and 0.31, respectively. The higher utilisation of His than TAA, Met, Lys and Leu also supports the role of His as the first limiting AA for milk production on grass silage based diets.

Observations that infusion of Met, Lys and Trp simultaneously with His increased His utilisation (0.38 vs 0.43; Kim et al., 2001c) compared with His alone is likely to be explained by increased supply of the second limiting AA. Utilisation of His also was higher (0.55 vs 0.28; I, III, IV and V) when it originated from supplementary protein than from post-ruminal infusions. This is also most probably due to increased supply of the second limiting AA. On the other hand, higher AA supply may increase glucose supply and protein supplementation can also increase DM intake (Huhtanen, 2002) which increases energy supply that may also improve AA utilisation (Oldham, 1994).

3.6. Effect of AA infusions on plasma AA concentration

In studies conducted with Finnish cows fed grass silage based diets (Varvikko et al., 1999, Vanhatalo et al., 1999, Rinne et al., 1999a, Miettinen and Huhtanen, 1997, Huhtanen et al., 2002a, Vanhatalo et al., 2002a, b), plasma His concentration has varied from 17 to 42 µmol/l (mean 23 µmol/l; SD = 8.3). In experiment IV, the concentration was 12 µmol/l on the basal diet but in other experiments (I, II, III and V) His concentrations were within the expected range. In other studies (Kim et al., 2000a, 2001a, b, c) plasma His concentrations have ranged between 7 and 44 µmol/l (mean 20 µmol/l; SD = 12.8).

Two potential explanations can be provided for this variation. Postruminal AA supply from the basal diet may be different owing to variations in ruminal nitrogen metabolism which can alter the contribution of dietary and microbial protein to total postruminal protein flow (V). It is also possible that the AA profiles of these protein fractions vary between the diets or AA absorption was different. Alternatively, nutrient balance, primarily the protein to energy and the protein to glucose ratios, may affect tissue utilisation of absorbed AA.

Plasma Met concentrations were 21, 26, 24, 22 and 21 µmol/l in experiment I to V, respectively and are in good agreement with previously reported values (from 10 to 23 µmol/l; mean 19 SD = 4.0) for similar diets (Miettinen and Huhtanen, 1997, Rinne et al., 1999a, Vanhatalo et al., 1999, Varvikko et al., 1999, Kim et al. 2001a, b, c, Huhtanen et al., 2002a, Vanhatalo et al., 2002ab). In cases where SBM or feathermeal have been used as protein supplements (Kim et al. 2000a, 2001a, b, c) Met concentrations have varied from 11 to 23 µmol/l (mean 16 SD = 4.0).

Lysine is the third AA that has been put forward as a potential first limiting AA. Concentrations in arterial plasma of 71, 91, 62, 95 and 77 for experiments I-V, respectively, are consistent with previous values (mean 81 µmol/l; SD = 11.8) in cows fed similar basal diets, but higher than those (mean 58 SD = 13.1) reported when concentrate supplements have contained SBM or feather meal (Kim et al., 2000a, 2001a, b, c).
Mean plasma His concentrations of cows fed grass silage based diets (20 and 23 μmol/l) have been reported to be lower than half the values for maize silage based diets (Lescoat et al., 1996), while concentrations of Met and Lys have been reasonably similar. Assuming that plasma concentrations are indicative of AA supply, His supply appears to be limited on grass silage based diets. This is in agreement with milk and milk protein yield responses to His infusions (Vanhatalo et al., 1999, I, Kim et al., 2001c, Huhtanen et al., 2002a) and also the low His concentration of MP compared with milk protein or commonly used protein feeds (Schingoethe, 1996).

Variation in plasma concentrations has also been used to determine the limiting AA for milk production (Broderick et al., 1974). This method assumes that when AA are supplied there should be a clear difference between AA in the rate at which plasma concentrations increases. However, graded doses of His infusion linearly increased plasma His concentrations (Experiment I, Kim et al. 2001a, b, c) without any appearance of an inflexion point in plasma concentrations in spite of the increased milk and milk protein yields. Thus, the ranking of the limiting AA based solely on changes in plasma AA concentrations does not appear to be a reliable approach. In support of this, lactational performance was not affected by very low (10 μmol; Kim et al., 2001b), similar (22 μmol/l; experiment II) or higher (34 μmol/l; Vanhatalo et al., 2001) plasma His concentrations compared with those values often have been attained (I, Vanhatalo et al., 1999, Huhtanen et al., 2002a). Furthermore, His concentrations were high (39 and 32 μmol/l) in studies in which Met and Lys were infused (Varvikko et al., 1999) suggesting an increased supply of His compared with Met and Lys. However, in contrast to expectations increases in the supply of these AA had no effect on milk production.

<table>
<thead>
<tr>
<th>AA</th>
<th>Reference</th>
<th>N</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>His</td>
<td>Experiment I</td>
<td>15</td>
<td>(Y = 6.4 \text{ (SE} = 0.93) (X - 13.5 \text{ (SE} = 6.32)) (R^2 = 0.70)</td>
</tr>
<tr>
<td></td>
<td>Experiment II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kim et al., 2001b, c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Huhtanen et al., 2002a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>Fisher, 1972</td>
<td>19</td>
<td>(Y = 2.4 \text{ (SE} = 0.52) (X - 9.9 \text{ (SE} = 8.95)) (R^2 = 0.75)</td>
</tr>
<tr>
<td></td>
<td>Schwab et al., 1992a</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Aldrich et al., 1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pisulewski et al., 1996</td>
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<td></td>
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</tr>
<tr>
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<td>Kim et al., 2000a</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Kim et al., 2001c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>King et al., 1991</td>
<td>26</td>
<td>(Y = 2.4 \text{ (SE} = 0.24) (X - 34.4 \text{ (SE} =15.16)) (R^2 = 0.89)</td>
</tr>
<tr>
<td></td>
<td>Schwab et al., 1992b</td>
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</tr>
<tr>
<td></td>
<td>Varvikko et al., 1999</td>
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</table>
In experiments I, II and IV, plasma His concentration was sensitive to changes in supply derived from post-ruminal infusions or protein supplements which is consistent with other studies in which His was infused alone or in combination with other AA (Choung and Chamberlain, 1992, 1995, Kim et al., 2000a, 2001abc, Rulquin and Pisulewski, 2000a). Based on the overall regression between infused His (g/d) and plasma His concentration (µmol/l; Table 4), each gram of infused His increased plasma concentration by 6.4 µmol. Respective values for Met and Lys were 2.4 µmol. It appears that concentrations of His are more sensitive to changes in supply than those of Met or Lys.

The simplest explanation for this is that the rate at which His enters plasma is greater than the rate at which it is removed from plasma. However, in experiment I and in that reported by Kim et al. (2001c), His led to a linear increase in milk protein yield up to infusion level of 6 g/d indicating that across infusion levels, His remained the first limiting AA. These findings imply that the MG utilised additional His to increase protein synthesis and that the utilisation of His was not constrained by the supply of other AA. Kim et al. (2001c) speculated that the decreased protein yield at the higher rate of infusion (9 g/d) compared with 6 g/d could be associated with a reduction in Met availability because Met is a precursor of tetrahydrofolate a prerequisite for His catabolism. However, the catabolism of His while it remains first limiting may be low. The supply of other AA does not provide an explanation for the increases in plasma His concentration. One possible reason could relate to the utilisation of His by other tissues, since His has fewer metabolic requirements other than milk protein synthesis compared with other AA (Bequette et al., 1997) and is subjected to less tissue oxidation than other AA in lactating dairy cows (Black et al., 1990).

In experiment I, concentrations of other AA were unaffected by His infusion, with the exception of an increase in Lys concentration. In previous studies His infusion has decreased or tended to decrease plasma Leu concentrations but concentrations of other AA have remained similar or also tended to decrease (Vanhatalo et al., 1999, Huhtanen et al., 2002). Infusions of a mixture of His and BCAA (II) have only increased His and Val concentrations but decreased concentrations of arginine (Arg), Met and Lys were unexpected because infusions of BCAA elevated plasma concentrations of these AA (Hopkins et al., 1994, Mackle et al., 1999). Plasma concentration of Leu also increased following infusions in combination with His (Huhtanen et al., 2002a).

The inconsistency in the relation between plasma concentrations and AA supplies can be attributed to absorption or postabsortive metabolism of AA. Differences arising from absorption may be caused by competition between AA transport systems (II) or variable utilisation of individual AA in splanchnic tissues. Another possible reason is increased utilisation of AA by the MG (I, III, IV) and variable partitioning of AA between other tissues. Differences in the rate of liberation of AA during tissue protein degradation, as a result of variable energy supply, may also affect plasma AA concentrations. However, variability in plasma 3-Met-His concentrations, which are used as an indicator of tissue protein degradation were relatively small (I to V). In order to obtain realiable estimates of AA available for absorption it is necessary to estimate postruminal AA flow.

### 3.7. Effect of protein supplementation on milk protein yield

The effects of supplementary protein in the form of RSM (III), or FM, SBM and MGM (IV) on milk production were assessed. Milk protein yield responses to SBM (13 experiments; N = 33), FM (10 experiments; N = 21) and RSM (13 experiments; N = 38) within individual
studies, including studies III and IV, in cows fed grass silage based diets are shown in Figures 1 to 3. Adjusting responses for between-study effects, indicated mean milk protein yield responses to additional CP intake (g/g) of 0.12 for SBM ($Y = 0.12 \text{ (SE = 0.01) } X + 385 \text{ (SE = 41), } R^2 = 0.87$), 0.15 for FM ($Y = 0.15 \text{ (SE = 0.02) } X + 339 \text{ (SE = 73), } R^2 = 0.77$) and 0.16 for RSM ($Y = 0.16 \text{ (SE = 0.02) } X + 356, R^2 = 0.91$). The magnitude of responses is lower than that observed for RSM (0.22), FM (0.24), and SBM (0.19) in studies III and IV.

Figure 1. Relationship between CP intake and milk protein yield with soybean meal supplementation.
Figure 2. Relationship between CP intake and milk protein yield with fishmeal supplementation.

Figure 3. Relationship between CP intake and milk protein yield with rapeseed meal supplementation.
In agreement with Huhtanen (1998) responses do not appear to be dependent on the basal diet CP content because increases in protein yields have been reported across a wide range of dietary CP intakes (from 1500 to 3000 g/d). Protein feeds are thought to alleviate the negative associative effects of concentrate supplementation on diet digestibility and to stimulate a curvilinear effect on silage intake (Huhtanen, 2002). Therefore, energy supply is another factor that may have an influence on the response to protein supplements. In study IV, the effect of concentrate CP content on silage DM intake was eliminated by maintaining the same ratio of barley to silage across all treatments. This may have resulted in a higher supply of energy from concentrate supplements because the proportion of cereals in the diet was higher compared with cases where only cereals have been replaced with protein feeds.

Rather than considering the production responses per se, the main aim of experiment IV was to compare actual responses to different protein feeds under controlled conditions. Responses to SBM were lower than those with FM and MGM which were similar. These values are in agreement with higher responses to RSM than SBM (Shingfield et al., 2002), lower response to a mixture of SBM and MGM compared with RSM (Khalili et al., 2001) and lower responses to SBM compared with FM (Chamberlain et al., 1989, O’Mara et al., 1998). Cases where MGM is the sole protein supplement for cows fed grass silage based diets are rare. Feeding a protein supplement consisting of MGM and SBM resulted in 46 g/d reduction in milk protein yield compared with isonitrogenous amounts of rapeseed cake (Khalili et al., 2001). On maize and lucerne silage based diets, MGM and FM have been used to increase RUP supply compared with SBM. In most studies MGM has had a detrimental effect on milk production compared with lower RUP supplements (Santos et al., 1998). Thus, it is likely that lactational responses are affected by the quality (AA profile and RUP content; IV) and quantity of dietary protein. Manipulating the quality of protein supplements represents a realistic means of increasing milk and milk production yield in practice.

3.8. Effect of diet on postruminal protein supply

3.8.1. Protein supplementation

Most of the protein entering the duodenum of ruminants fed grass silage-cereal based diets is derived from MP because grass silage and cereal CP is rapidly and extensively degraded in the rumen (Huhtanen, 1998). This was confirmed by subsequent observations (II, IV and V) in which the contribution of MP flow to total protein flow entering the omasal canal consistently exceeded 60%. These estimates are comparable to previous observations based on the same digesta sampling technique and microbial markers (Ahvenjärvi et al., 1999, 2002). However, milk production has consistently been improved when protein supply has been increased with protein supplements (Figures 1 to 3). This suggests that MP and RUP supplies from grass silage and cereals alone are unable to satisfy the AA requirements of high yielding dairy cows.

On the basis of a large data set from studies conducted mainly with maize silage based diets protein supplements (mostly FM and MGM) with a higher RUP content generally did not affect post-ruminal N supply compared with lower RUP supplements of SBM (Santos et al., 1998). This was because RUP flow was unable to compensate for the reduction in MP flow associated with lowered MPS arising from limitations in rumen N availability.

In experiment IV, omasal N flow was higher across supplemented diets compared with the control diet because protein supplements increased dietary N flow but had no effect on
microbial N flow. Metcalf et al. (1994) observed a similar effect with FM supplements and Rooke et al. (1983) and Ahvenjärvi et al. (1999) with RSM supplements. In experiment IV, the concentration of ammonia-N in the rumen remained above the 3.6 mM threshold that has been considered to be the concentration below which MPS may be constrained by ammonia-N supply (Satter and Slyter, 1974). In agreement with this, Ahvenjärvi et al. (1999) reported no effect of urea, RSM or rapeseed cake supplementation on MPS. Rapeseed meal supplementation had no beneficial effects on MPS in experiment III (Ahvenjärvi et al., 2002) in spite of low ruminal ammonia-N concentration. The implication is that MPS does not appear to be limited by the supply of rumen degradable protein for diets based on good quality grass silage.

Rapeseed meal, FM and SBM supplementations have, however, increased MP supply in studies conducted with sheep or cattle when the basal diet consisted of grass silage alone or that supplemented with barley or barley starch (Rooke et al., 1983, Dawson et al., 1988, Jacobs and McAllan, 1992, Oh et al., 1999). Inconsistency probably arises from an increased supply of AA and peptides for MPS. Since MP flow was not affected by protein supplements in studies III (Ahvenjärvi et al., 2002) and IV the ruminal supply of these substrates appears to be adequate for diets based on formic acid treated silages. Restricting silage fermentation has been shown to increase MPS to a greater extent that would be expected based on ATP supply (van Vuuren et al., 1995). This finding probably reflects the higher energy supply from restrictively compared with extensively fermented silage, and a greater intake of soluble peptides (Nsereko and Rooke, 1993).

Of the protein supplements used in experiment IV, the increase in total N flow was lowest for SBM, highest for MGM and intermediate for FM supplementation, which is in accordance with findings of Santos et al. (1984), Titgemeyer et al. (1989), Robinson (1997) and O’Mara et al. (1998). Rooke et al. (1986) replaced barley with increasing amounts of SBM in cattle and demonstrated that postruminal N flow was only increased at the lowest level of inclusion. Supplements of SBM have also reduced post-ruminal N flow compared with FM (Metcalf et al., 1994) while the magnitude of the increase observed in experiment IV was lower than that reported for RSM (Ahvenjärvi et al., 1999). Increases in N intake from SBM are not realised as substantial increases in postruminal protein supply for diets based on good quality grass silage. This is most likely associated with the greater extent of protein degradation in the rumen for SBM than for FM or MGM (Broderick et al., 1992, Robinson, 1997, O’Mara et al., 1998, IV). Because MPS is not generally limited by RDP supply on grass silage based diets, high RDP protein supplements increase ruminal N losses (IV, Robinson, 1997). As judged from milk production responses, supplements that elevate postruminal RUP supply appear to be the most beneficial for grass silage based diets. This response arises from an increase in total AA supply and also to a certain extent from the variable AA profile of protein absorbed from the intestine (IV, V). Another factor affecting responses is the ratio of protein to energy supply (MacRae et al., 1985, Whitelaw et al., 1986, Oldham, 1994) because protein stimulate an increase in DM intake and energy supply.

3.8.2. Energy supplementation

On diets containing large proportions of grass silage microbial utilisation of silage N can be inefficient because dietary N degradation to ammonia exceeds the capacity of rumen microbes to utilise ammonia-N for MPS (Huhtanen, 1998). The limiting step in this process, particularly on diets with extensively fermented silage, is energy supply because carbohydrates of silage are fermented into lactic acid and VFA which have little value as energy sources for
rumen microbes (Chamberlain, 1987, van Vuuren et al., 1995). Thus, energy supplementation may be needed to optimise MP supply.

Barley is the most common energy supplement for grass silage based diets because it contains high amounts of starch and is extensively degraded by rumen microbes. In experiment III (Ahvenjärvi et al., 2002), increases in postruminal N flows were reasonably similar for barley and RSM supplements but the source of the increased N flow was different. Barley increased MP flow and RSM increased dietary N flow. Microbial N flow also increased with higher levels of concentrate supplementation in experiment V and in the study of McAllan et al. (1994). In contrast to the observations of McAllan et al. (1994), barley supplementation has not always increased total N flow (Rooke et al., 1985, 1992, V).

The effects of barley supplementation on omasal canal AA flows in experiments III and V were much lower than predictions based on the AAT/PBV system and Finnish feed table values (Tuori et al., 2000). One explanation for this is a lower energy supply for microbial metabolism in the rumen than would be expected owing to starch escaping the rumen. Decreases in NDF digestibility associated with increases in barley supplementation may also have a similar effect on energy supply (Ahvenjärvi et al., 2002). Increases in concentrate feeding did not elevate energy supply to the same extent as would be predicted in experiment V. It appears that the ruminal degradability of barley is higher than published feed table values because increases in the amount of N truly degraded in the rumen with barley supplementation exceeded the corresponding differences in N intake (III, V). This suggests that barley supplementation probably increases ruminal degradability of the whole diet. This is supported by the observations that postruminal dietary N flow did not increase when grass silage was replaced with a mixture (80:20) of barley and RSM (Jaakkola and Huhtanen, 1993).

Barley supplementation did not influence the efficiency of MPS in experiments III (Ahvenjärvi et al., 2002) and V. One potential explanation for this may be the level of barley offered. Chamberlain et al. (1989) suggested that rumen N losses were not prevented unless barley accounted for more than 50% of ingested DM. Alternatively, the lack of improvements in MPS may be associated with changes in rumen microbial populations since barley increases the population of rumen protozoa (Chamberlain et al., 1983, Jaakkola and Huhtanen, 1992). Higher numbers of protozoa in the rumen may result in increased intraruminal N recycling (Coleman, 1975) and increased microbial maintenance requirements (Russell et al., 1992). These changes may diminish the small beneficial effects of increased energy supply on the efficiency of MPS (Chamberlain et al., 1989). Since the efficiency of MPS remained unchanged in the experiments of Ahvenjärvi et al. (1999), and studies III (Ahvenjärvi et al., 2002) and V, increases in MP flows were related to more organic matter (OM) being fermented in the rumen (Ahvenjärvi et al., 1999, V). The lack of improvement in the efficiency of MPS suggests that increased use of barley will not result in higher N utilisation in the rumen of cows fed restrictively fermented grass silage. Furthermore, differences in rumen N balance (degraded N – synthesised microbial N) between two inclusion rates of barley were +10 g/d (primary cut silage based diets) and −9 g/d (secondary cut silage based diets) in experiment V. In addition, barley had no effect on rumen N balance in experiment III (−2 g/d silage and barley vs silage and −5 g/d silage, barley and RSM vs silage and barley). The current experimental findings suggest that the protein value of barley is overestimated and ruminal degradability of barley is underestimated by the current protein evaluation system adopted in Finland.
3.9. Postruminal AA supply

3.9.1. Microbial protein as a source of AA

Degradation of plant protein commences immediately after cutting and continues during ensiling resulting in increased proportions of non-protein N (NPN) in the total N (Chamberlain et al., 1989). Because of the rapid degradation of NPN into ammonia-N and the low energy supply from the silage, it has often been claimed that the MPS is lower on grass silage based diets compared with diets based on dried or fresh grass (e.g. Thomas and Thomas, 1985). These factors may increase ruminal N losses and reduce postruminal AA supply (Chamberlain et al., 1989). The results of Jaakkola and Huhtanen (1992) do not, however, support this because MPS and MP flow were not different between diets based on formic acid treated silage and those based on barn dried hay. Huhtanen (1998) also pointed out that the poor protein value often attributed to silages is only relevant to poor quality extensively fermented silage. An extensive and rapid degradation of silage and cereal CP result in MP being the major source of AA in cows fed grass silage supplemented with cereal based concentrates (experiments II to V). Enhancing lactational performance and optimising AA utilisation and supply require prior knowledge of the supply of AA derived from both RUP and MP. Because of the high contribution of MP to postruminal AA supply, the accuracy of estimates of AA supply are dependent on a reliable AA profile of MP (Martin et al., 1996).

Microbial protein leaving the rumen consists of LAB, PAB and protozoa (Broderick and Merchen, 1992, Martin et al., 1996, Volden et al., 1999) and these microbial pools can leave the rumen in different proportions. Furthermore, the marker to N concentration ratios of various microbial pools are extremely variable (Broderick and Merchen, 1992). Therefore, microbial samples containing only LAB, which is more often the case, are not truly representative of MP flowing from the rumen (Martin et al., 1994b). In order to estimate the effect of variable contributions of microbial fractions on MP and AA supply MP was fractionated into LAB, PAB and protozoa (III).

As reported by Ahvenjärvi et al. (2002) changes in the diet affected the proportion of individual fractions in total MP entering the omasal canal (III). This, together with variable marker concentrations between individual fractions, highlights the importance of distinguishing between individual fractions to accurately estimate MP supply (Ahvenjärvi et al., 2002). Furthermore, AA profiles between various fractions (III) were different for most individual AA, in agreement with previous observations (Martin et al., 1994b, 1996, Volden and Harstad, 1998, Volden et al., 1999). These changes, especially the differences in AA profiles between microbial fractions, have led to the conclusion that in order to accurately estimate microbial AA supply, all three fractions have to be taken into account. However, simultaneous measurements of ruminal outflow and AA profiles of individual microbial fractions are scarce. In spite of the changes in ruminal outflow of individual fractions and differences in AA profiles, AA supply across all treatments remained constant (III). Improving the accuracy of estimates of MP AA supply appears to depend more on the reliability of MP flow measurements than on estimates of the AA profile of individual microbial fractions (III). Using various combinations of microbial fractions (all three fractions, LAB or LAB and PAB) to represent MP showed that within diets (grass silage, energy and protein supplements) a mixed sample of LAB and PAB was not markedly different from that based on the entire microbial sample containing protozoa (III).
Diet had no effect on the AA profile of individual microbial fractions (III), in agreement with earlier observations (Martin et al., 1994a, 1996, Volden and Harstad, 1998, Volden et al., 1999). The AA profile of microbial fractions based on both LAB and PAB was also found to be independent of changes in protein (IV) and energy (V) supply and silage harvest date (V). These findings suggest that for grass silage based diets the AA profile of MP is maintained irrespective of changes in the diet. This suggestion is supported by the observations that dietary changes cause only minor differences in AA profile (Cecava et al., 1990, Volden et al., 1999). Amino acid profile also remained constant across a wide range of forage to concentrate ratios (Chamberlain and Thomas, 1979) or when protein supply was increased with RSM (Jacobs and McAllan, 1992).

Clark et al. (1992) concluded from an extensive review of the literature that the use of an average AA profile may result in erroneous estimates of microbial AA flow. However, the variability observed in these measurements can also arise through differences in experimental techniques (e.g. isolation of bacteria, sampling site, AA analysis, preparing of samples). The AA profile in study II and treatment mean values from studies III to V (Figure 4) were consistent, suggesting that between-experiment variations can be small when the same experimental techniques are used. The largest difference in concentration was for Met, which was much lower in study III compared with the other studies. One explanation for this is the inclusion of red clover in the silage fed in this study. Since the AA profiles measured in studies II, IV and V were generally similar it is apparent that estimates of MP supply may have a greater effect on the accuracy of microbial AA supply measurements than differences in microbial AA profile when similar basal diets are fed.

Figure 4. Mean AA profile of microbial protein for experiments II to V.

Lactational performance has been enhanced in several studies by post-ruminal infusion of individual AA, suggesting that MP may also be qualitatively suboptimal. If it is true that the AA profile of milk protein is constant irrespective of AA supply (Kirchgessner and Kreuzer, 1987) then the requirements of individual AA for milk protein synthesis (g/g milk protein) would also be expected to remain constant. Furthermore, at high milk production levels MG requirements may account for 90% of total AA requirements (Schingoethe, 1996) and in such
conditions the AA profile of milk protein may be indicative of the ideal AA balance. This may be true at least for AA for which the ratio of uptake and secretion in milk is close to one (His, Met, Thr, Phe, Tyr). The comparison of AA profiles between protein feeds and milk protein has long been called the biological value of protein feeds. Schingoethe (1996) reported this relationship as “milk protein scores” which were 0.78 for MP (AA profile of MP relative to milk protein). Of the individual AA the most limiting were ranked as His, Leu and Val. The ranking of His as first limiting AA is consistent with post-ruminal infusion studies (Vanhatalo et al., 1999, Kim et al., 1999, I, 2000a, 2001c, Huhtanen et al., 2002a). The fact that His is considered to be first limiting for grass silage-cereal based diets is likely to be associated with the high contribution of MP to total postruminial protein flow and also the low His content of MP. Synthesis and outflow of MP from the rumen is higher for restrictively than more fermented silages (Jaakkola et al., 1991, Choung and Chamberlain 1993) but this has not resulted in a significantly higher milk protein output (Huhtanen et al., 2002b). Based on an evaluation of an large data set (N = 234 from 47 experiments) Huhtanen et al. (2002b) demonstrated that restricting silage fermentation improved milk protein yield but the response was lower than expected. Lower responses were attributed to limitations in glucose supply or to suboptimal MP AA profile. In line with the AA profiles of protein feeds and MP reported in experiment IV, His content of MP has been noted as being lower than most commonly used protein supplements (Cecava et al., 1990, Kim et al., 2001c). In contrast to low His, MP is a reasonably rich source of Met and Lys (IV, Schingoethe, 1996, Santos et al., 1998). The low His supply from MP, grass silage and cereals may also explain the higher milk protein responses to RSM than to SBM supplements (Shingfield et al., 2002) since His is more abundant in RSM than SBM or MP.

3.9.2. Effect of basal diet on the AA profile of postruminal digesta

The AA profiles of digesta from cows fed grass silage-cereal based diets in experiments I, II, III and IV and the mean AA profile across treatments in experiment V were similar (Figure 5). Some differences were observed for His, Ile, Met and Phe for study III compared with the other studies probably because grass silage contained red clover while the other studies were all conducted with grass silages of similar fermentation characteristics. Compared with the AA profiles reported for basal diets containing maize and hay crop silage (Schwab et al., 1992a, b; Figure 6), it appears that digesta of cows fed grass silage contains less Leu and more Met and Ile. With respect to other EAA, concentrations in digesta are reasonably consistent despite between-study variations. These differences indicate that AA supply depend on the characteristics of the basal diet and as such provides the most probable explanation for variable milk production responses to AA infusions in cows fed maize compared with grass silage based diets (Schwab et al., 1992a, b, Pisulewski et al., 1996, Vanhatalo et al., 1999, Varvikko et al., 1999).
supplement and various protein feeds are mostly deficient in either Met or Lys. As mentioned (1996) who also pointed out that diets based on alfalfa, maize silage and maize as the grain both Met and Lys (Santos et al., 1998). This and reduced MP supply with high RUP 5.0 for Thr and 6.0 and 5.6 for Val in cows fed grass and maize silage based diets, Ile, 8.2 and 9.7 for Leu, 6.4 and 6.3 for Lys, 6.4 and 6.3 for Lys, 2.7 and 2.0 for Met, 5.8 and 5.3 for Phe, 5.3 and

Figure 5. Amino acid profile of omasal digesta on grass silage-cereal based diets (experiment I – IV) and the mean across all treatments for experiment V.

Figure 6. Differences in AA profiles of postruminal digesta between grass and maize silage based diets. Values (g/100 g AA) are 4.9 and 4.7 for Arg, 2.4 and 2.4 for His, 5.7 and 5.2 for Ile, 8.2 and 9.7 for Leu, 6.4 and 6.3 for Lys, 2.7 and 2.0 for Met, 5.8 and 5.3 for Phe, 5.3 and 5.0 for Thr and 6.0 and 5.6 for Val in cows fed grass and maize silage based diets, respectively.

Protein supplements most commonly used on maize based diets are rarely good sources of both Met and Lys (Santos et al., 1998). This and reduced MP supply with high RUP supplements often leads to Met and(or) Lys deficiency. This is supported by Schingoethe (1996) who also pointed out that diets based on alfalfa, maize silage and maize as the grain supplement and various protein feeds are mostly deficient in either Met or Lys. As mentioned
earlier, MP supply remains generally high on grass silage based diets in spite of the increased RUP supply indicating that limiting AA for milk production appear to be determined by the AA profile of MP. Practical means to increase lactational performance with individual AA supplementations appear to be lower in cows fed grass than maize silage based diets.

In experiment I, His was clearly the first limiting AA for milk production because it linearly increased milk production across all infusion levels, while infusion of a mixture of AA containing His did not improve milk or milk protein yields in study II. Lactational responses to AA infusions have also been reported to be variable in other studies (Kim et al., 2000a, 2001c). One probable explanation for this is variations in AA supply owing to changes in grass silage quality. Digesta AA profiles were reasonably consistent for the basal diets (I to V) and did not vary between the two silages (V) suggesting that the variable digesta AA profile may not be the sole reason for variation in milk and milk protein responses to infused AA. As observed in experiment V, the flows of total AA and of many individual AA were numerically higher for diets based on secondary compared with primary cut silages. Furthermore, despite feeding similar basal diets, some variation also existed in TAA flows (g/kg DMI) between studies I to V suggesting that variable responses to AA infusions and differences in the ranking of limiting AA reflect differences in AA supply from the basal diet.

3.9.3. Effect of protein supplementation on digesta AA profile

In spite of the higher His and lower Met concentrations of RSM compared with MP, the AA profile of digesta was not significantly altered by RSM supplementation compared with silage alone (2.7 vs 2.6 for His and 2.3 vs 2.4 for Met) in experiment III. Replacing barley with RSM has also had little impact on digesta AA profile (2.8 vs 2.7 for Met and 2.0 vs 2.1 for His; Korhonen, unpublished). In experiment IV, protein supplements altered digesta AA profile in accordance with the differences in AA content of MP and feed protein. Similarly digesta AA profiles have been altered when SBM has been replaced with FM or MGM (Rooke et al., 1983, Stern et al., 1983, Santos et al., 1984, Rooke and Armstrong, 1987, Keery et al., 1993, Robinson, 1997). Therefore, use of protein supplements of high RUP content with a AA profile complementary to that of MP represents a practical means of altering digesta AA profile. In experiment IV, lactational performance improved despite reductions in Met (2.6 vs 2.8) and Lys (5.2 vs 6.4) concentrations for SBM and MGM supplemented diets, respectively. This is in accordance with post-ruminal Met and Lys infusions having no effect on milk or milk protein yields (Varvikko et al., 1999) and supports the conclusion that Met and Lys are not the first limiting AA for milk production on grass silage-cereal based diets.

However, alterations in AA profile alone may not be the sole factor influencing milk production responses, since milk and milk protein yields have been improved by increased total AA supply rather than changes in digesta AA profile (O’Mara et al., 1998, III).

3.9.4. Amino acid degradability in the rumen

The AA profiles of intact feeds and those of residues after rumen incubation have been shown to be different (Varvikko, 1986, Erasmus et al., 1994, Vanhatalo et al., 1995, Weisbjerg et al., 1996, O’Mara et al., 1997). Therefore, the assumption that all AA are degraded in the rumen to a similar extent, which is used in current protein evaluation systems (e.g., AAT/PBV; Madsen et al., 1995), may result in errors in estimates of AA available for absorption from the intestine. The in situ technique has several shortcomings (e.g. particle losses from the bag;
Van Straalen and Tamminga, 1990, contamination of feed particles; Varvikko, 1986, and the validity of the assumption that degradation of the a-fraction occurs at an infinite rate; Dhanoa et al., 1999, Volden et al., 2002) that may result in misleading degradability values. However, differences in ruminal AA degradability have also been noted in vivo (Stern et al., 1983, Titgemeyer et al., 1989). Ruminal AA degradabilities appeared to be variable for studies III, IV and V (Figure 7) when calculated based on AA intake, dietary AA flow entering the omasal canal (TAA flow – microbial AA flow) assuming that all residual AA are entirely of dietary origin. It is difficult to draw firm conclusions from these observations because individual AA degradabilities were much more variable than those of total AA. In case that such variations are true, more emphasis should be directed towards the importance of rumen degradation on post-ruminal AA supply.

![Relative rumen degradability of individual AA across all treatments for experiments III - V](image)

Figure 7. Relative rumen degradability of individual AA across all treatments for experiments III - V.

### 3.9.5. Intestinal digestibility of AA

In addition to AA degradation in the rumen, digestibility of individual AA in the small intestine is assumed to be constant (0.85 for AA of microbial origin and 0.82 for AA of dietary origin) within the AAT/PBV system (Madsen et al., 1995). Cecava et al. (1990) pointed out that plasma AA concentrations do not always reflect changes in digesta AA flow and AA profile. A similar discrepancy was also observed between AA flows and plasma concentrations in experiment III (His, Ile, Leu, Lys) particularly for diet SB. The same was also true for experiment IV. Rapeseed meal had no effect on His concentration in digesta (III, V, Korhonen, unpublished) but plasma His concentrations have been reported to increase linearly in cows fed graded levels of RSM (Rinne et al., 1999a). These inconsistencies may be explained by a variable AA utilisation in the MG. Alternatively, this may arise from differences in the digestibility of individual AA. The mobile-bag method has been often used to estimate intestinal AA digestibility. Use of this technique has indicated that individual AA digestibility can vary and AA in RSM are less digestible than those in SBM (Vanhatalo et al., 1995). Digestibility of TAA across a wide range of protein feeds has been reported to be 0.9 while that of individual AA ranged between 0.877 (Lys) and 0.925 (Arg) (Weisbjerg et al.,
Storm et al. (1983) reported a value of 0.81 for digestibility of TAA in MP. Compared with this, digestibility for His (0.68) was lower and those for Arg, Met and Phe (0.89) were higher. Variation in apparent AA digestibility measured in vivo with dairy cows, cattle or sheep (Table 5) supports the view that individual AA digestibilities are different. The quantitative importance of this variation on estimated AA supply also merits further attention. If it can be established that this has a marked influence on AA supply, then protein evaluation system should be modified to account for individual AA digestibility.

Table 5. Apparent in vivo intestinal AA digestibility1.

<table>
<thead>
<tr>
<th>AA</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>0.76</td>
<td>0.078</td>
<td>0.56</td>
<td>0.88</td>
</tr>
<tr>
<td>His</td>
<td>0.72</td>
<td>0.079</td>
<td>0.57</td>
<td>0.87</td>
</tr>
<tr>
<td>Ile</td>
<td>0.64</td>
<td>0.091</td>
<td>0.45</td>
<td>0.79</td>
</tr>
<tr>
<td>Leu</td>
<td>0.72</td>
<td>0.065</td>
<td>0.58</td>
<td>0.84</td>
</tr>
<tr>
<td>Lys</td>
<td>0.72</td>
<td>0.073</td>
<td>0.53</td>
<td>0.87</td>
</tr>
<tr>
<td>Met</td>
<td>0.66</td>
<td>0.099</td>
<td>0.47</td>
<td>0.85</td>
</tr>
<tr>
<td>Phe</td>
<td>0.72</td>
<td>0.075</td>
<td>0.53</td>
<td>0.85</td>
</tr>
<tr>
<td>Thr</td>
<td>0.66</td>
<td>0.073</td>
<td>0.48</td>
<td>0.79</td>
</tr>
<tr>
<td>Val</td>
<td>0.69</td>
<td>0.069</td>
<td>0.50</td>
<td>0.83</td>
</tr>
<tr>
<td>Essential AA</td>
<td>0.71</td>
<td>0.059</td>
<td>0.56</td>
<td>0.83</td>
</tr>
<tr>
<td>Non essential AA</td>
<td>0.70</td>
<td>0.059</td>
<td>0.56</td>
<td>0.81</td>
</tr>
<tr>
<td>Total AA</td>
<td>0.70</td>
<td>0.056</td>
<td>0.56</td>
<td>0.82</td>
</tr>
</tbody>
</table>

1Data from Hvelplund and Möller (1976), Santos et al. (1984), Stern et al. (1985), Garrett et al. (1987), Waltz et al. (1989), Beever et al. (1990), Cecava et al. (1990), Hussein et al. (1991), Keery et al. (1993), Krastanova et al. (1995), Mabjeesh et al. (1996) and Kowalczyk and Zebrowska (1998)

3.10. Metabolism of nutrients in the mammary gland

3.10.1. Role of the mammary gland as a site of metabolism of absorbed nutrients

A substantial proportion of absorbed nutrients are utilised by the MG in high yielding dairy cows, such that milk protein synthesis accounts for between 20 and 40% of whole body protein synthesis (Mabjeesh et al., 2002). For milk production of 30 kg/d, blood flow across the MG is around 20000 l/d and to maintain this level of production about 1 kg of AA and 2 kg of glucose have to be transported into the MG.

3.10.2. Arteriovenous difference technique to measure nutrient uptake by mammary gland

The arteriovenous difference (AV) technique, which is based on the Fick principle (Fleet and Mepham, 1983), was used (I, II, IV) to assess nutrient uptake by the MG. This approach assumes that the uptake of substrates can be expressed as a difference between the quantity of nutrients delivered in arterial blood and quantity leaving in venous blood, i.e. as AV difference × blood flow rate. Blood is a major nutrient transporter and therefore, representative samples of arterial and venous blood have to be collected simultaneously to derive accurate uptake values. The main vessel supplying blood to the MG is the external pudic artery which is also the most important site for nutrient uptake by the MG (Fleet and Mepham, 1983). Arterial blood is, however, thoroughly mixed in the heart and lungs, and can therefore be sampled at any convenient site. The tail vessels (coccygeal vessels) were used because no catheters are needed unless regular samples of blood are required. Furthermore, it
does not matter whether the sample is taken from the artery or vein, because AV difference across the tail is negligible (Emery et al., 1965). The milk vein was used as a sampling site for venous blood and it was assumed that these samples were representative of blood leaving the udder because in straightforward AV difference studies the crossover of blood between draining vessels (external pudic vein and milk vein) may be of minor importance (Metcalf et al., 1992).

Table 6. Whole blood to plasma ratios based on mammary AV differences or the ratio of mammary uptake to secretion in milk.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Arg</th>
<th>His</th>
<th>Ile</th>
<th>Leu</th>
<th>Lys</th>
<th>Met</th>
<th>Phe</th>
<th>Thr</th>
<th>Val</th>
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<tbody>
<tr>
<td>Hanigan et al., 1991</td>
<td>1.20</td>
<td>4.78</td>
<td>0.72</td>
<td>1.39</td>
<td>1.25</td>
<td>1.10</td>
<td>1.16</td>
<td>1.35</td>
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</tr>
<tr>
<td>Cant et al., 1993</td>
<td>0.66</td>
<td>0.59</td>
<td>0.26</td>
<td>1.18</td>
<td>0.73</td>
<td>0.33</td>
<td>0.76</td>
<td>0.92</td>
<td>1.12</td>
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<tr>
<td></td>
<td>0.74</td>
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<td>0.26</td>
<td>1.05</td>
<td>0.72</td>
<td>0.48</td>
<td>0.59</td>
<td>0.82</td>
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<td></td>
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<td>0.46</td>
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<td>0.56</td>
<td>0.70</td>
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<td></td>
<td>0.47</td>
<td>1.22</td>
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<td>1.27</td>
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<td>0.43</td>
<td>0.72</td>
<td>1.01</td>
<td>0.68</td>
</tr>
<tr>
<td>Metcalf et al., 1996b¹</td>
<td></td>
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<tr>
<td></td>
<td>0.94</td>
<td>0.84</td>
<td>0.86</td>
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<td>1.13</td>
<td>0.79</td>
<td>1.41</td>
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¹ whole blood to plasma ratio for mammary uptake

3.10.3. The role of erythrocytes as nutrient transporters

Mammary utilisation of nutrients was assessed based on plasma concentrations (I, II, IV). The good agreement in the balance of mammary AA, C and N uptake based on plasma analysis with AA, C and N excreted in milk (Bickerstaffe et al., 1974) has indicated that the uptake of nutrients from plasma is representative of nutrient uptake from whole blood. If erythrocytes are involved in nutrient transport, uptake values based on plasma concentrations may be underestimated. Comparisons between plasma and whole blood AA arteriovenous differences across the MG and mammary uptake to milk output ratios of AA reported in different studies are summarised in Table 6.

Hanigan et al. (1991) concluded that erythrocytes are involved in the transport of nutrients across the MG, which is in accordance with studies where similar comparisons have been made across other tissues (Heitmnan and Bergman, 1980, Danilson et al., 1987). However, the negative AV difference reported for His indicate that there may have been problems in determining AA concentrations. In contrast, measurements of U-13C labelled AA have indicated that uptake of nutrients by MG, hepatic and portal drained viscera is primarily derived from plasma (Lobley et al., 1996, Bequette et al., 1997, 1999). Neither the results of Metcalf et al. (1996) nor those of Cant et al. (1993) support the role of erythrocytes in the transport of AA since AA uptake and AV difference from whole blood was lower than that from plasma. Mackle et al. (2000b) also pointed out that arterial concentrations and AV differences of all EAA from plasma and whole blood were highly correlated.
3.10.4. Measurement of mammary blood flow

Mammary uptake of nutrients is also dependent on the rate of blood flow across the MG. Therefore, determination of uptake of nutrients also requires reliable measurements of mammary blood flow that can be made either directly by using inserted flow probes or indirectly using markers.

The Fick principle (Fleet and Mepham, 1983) is the most common indirect method for measuring mammary blood flow. This is based on constant blood flow and the collection of representative arterial and venous samples (Fleet and Mepham, 1983). These assumptions are met more or less satisfactorily in vivo. Plasma Phe and Tyr were used as markers (I to V) according to Cant et al. (1993) with the exception that free Phe and Tyr in milk were neglected. It is assumed that these AA are stoichiometrically transferred from blood to milk. In studies I to V the mean mammary uptake to output ratios were 0.95 and 0.88 for Phe and Tyr, respectively. De Peeters and Cant (1992) reported the literature based ratios (6 experiments and 13 different treatments) which were 0.96 and 1.04 for Phe and Tyr, respectively. Blood flow was calculated from marker output in milk and AV marker differences (Fleet and Mepham, 1983) using the following equation: mammary plasma flow = marker output in milk / AV difference of marker. Direct comparisons of various methods are scarce, but there is evidence that the AV difference method results in reliable estimates of MBF (Davis et al., 1988, Bequette et al., 1996). Metcalf et al. (1994) also concluded that the AV difference method provided data that appeared to be biologically realistic. The purpose of this thesis and the studies reported therein was not to evaluate the accuracy of methods suitable for measuring MG nutrient metabolism. Estimated mean mammary uptake to output ratios of individual EAA across studies I to V (1.08 for His, 1.00 for Met, 1.35 for Lys, 1.13 for Leu, 2.57 for Arg, 1.01 for Thr, 1.38 for Ile and 1.30 for Val) are in line with previously reported values (Davis and Mepham, 1974). Current estimates are also consistent with findings that Lys, Arg, and BCAA are taken up in excess of their output in milk protein, but extractions of His, Thr, Met and Phe are almost equivalent to milk output (Guinard and Rulquin, 1994). Furthermore, mean glucose uptake (70.3 g/kg milk) is consistent with a value of 70 g/kg milk based on theoretical calculations and biochemical principles proposed by MacRae et al. (1988) and comparable with estimates of 75 and 71 g/kg milk based on the regression between milk yield and in vivo MG glucose flux rates and theoretical calculations, respectively (Danfær et al., 1994).

3.10.5. Amino acid uptake by the mammary gland

Nutrient supply for the tissues depends on the absorption of nutrients from the digestive tract and partitioning of absorbed nutrients between body tissues. A third factor affecting nutrient utilisation is the capacity of tissues to extract nutrients from vascular sources. At the mammary level, arterial nutrient concentration, rate of blood flow across the MG and extraction processes are the main factors thought to be associated with nutrient uptake (Mepham, 1982, Bequette et al., 2000). Understanding of mechanisms associated with these processes is required in attempts to assess milk and milk protein yield responses to increased supply of various nutrients and ultimately to increase milk protein synthesis.

Because reports show arterial concentration and AV difference of EAA to be positively correlated when milk production improved with post-ruminal casein infusions (Miettinen and Huhtanen, 1997, Mackle et al., 2000) mammary utilisation of AA can be considered to be driven by nutrient supply, often referred to as the “push” mechanism (Bequette and Backwell,
Rulquin and Pisulewski (2000a, b) also concluded that the uptake of His and Leu by the MG are dependent on arterial supply because mammary uptake of these AA increased in response to elevated plasma concentrations.

However, relationship between AV differences and arterial concentration of EAA were relatively weak for experiments I to V (R^2 = 0.11 - 0.36 n = 16 - 25) even though milk protein yields were increased by individual AA supplements (I) or dietary increases in protein intake (III, IV). The utilisation of infused His remained low irrespective of milk production responses and level of infusion (I). Furthermore, increasing plasma His concentrations has decreased mammary His extraction in several studies (Vanhatalo et al., 1999, I, Rulquin and Pisulewski, 2000a, Huhtanen et al., 2002a, II) while the extraction efficiency of His and Leu increased with infusions deficient in these AA (Bequette et al., 1996, 2000). Miettinen and Huhtanen (1997) also observed that extractions of several essential AA decreased when arterial supply was increased by changes in silage fermentation quality or casein infusions.

In experiment IV, the proportion of Arg in mammary EAA uptake was 14.4, 13.6, 15.7 and 13.6% for the control and FM, SBM and MGM supplemented diets. The respective values for Lys and Leu were 17.1, 17.0, 16.0 and 21.2 and 15.9, 18.2, 14.9 and 13.9. Graded infusions of Lys have been shown to vary the proportion of Arg, increase that of Lys and slightly decrease that of Leu in previous studies (Guinard and Rulquin, 1994, Varvikko et al., 1999). Uptake of these AA by the MG is greater than their secretion in milk. Variations in the proportions of these AA in total EAA indicates that by changing the uptake of these AA, the MG is able to balance N and C requirements for NEAA synthesis and to some extent prioritise the use of AA for protein synthesis at the expense of other purposes. Substantial increases in milk protein yield to MGM supplements occurred despite similar flows of Lys entering the omasal canal (IV). This suggests that high supplies of Leu were able to compensate for low Lys supply. Similarly, for SBM supplemented diets when increases in omasal flows of Leu and Lys were relatively small compared with the basal diet, the MG appeared to increase Arg utilisation. Consequently, the MG may be able to compensate for an unbalanced AA profile of absorbed protein by regulating the uptake of individual AA. In line with this speculation, Metcalf et al. (1996) and Cant et al. (2001) also noted that mammary AA uptake did not reflect increases in arterial supply but was more closely associated with the increases in milk protein synthesis.

In addition to changes in extraction, increases in plasma His concentration also numerically reduced MG blood flow (I). Methionine had similar effects on extraction and blood flow when arterial concentrations were increased by abomasal infusions (Guinard and Rulquin, 1995). In the studies of Bequette et al. (1996, 2000) AA supply was altered by infusing AA mixtures into the abomasum with or without His and Leu when the basal diet provided 77% or 75% of metabolisable protein requirements. Histidine and Leu deficient infusions increased mammary blood flow compared with complete AA infusions. It has been speculated that the reductions in blood flow induced by increased plasma His and Met concentrations could be mediated by the degradation products of these AA (Tau for Met and histamine for His; Guinard and Rulquin, 1995, Bequette et al., 1998). However, Leu also caused a similar depression in mammary blood flow (Bequette et al., 1996) suggesting that changes in mammary blood flow is one mechanism by which the MG controls nutrient uptake (Bequette et al., 1998, I). The endocrine system also appears to regulate AA utilisation by the MG since it has been claimed that insulin also stimulates an increase in milk protein synthesis, especially when infused with AA (Griinari et al., 1997, Mackle et al., 1999).
It is evident that AA uptake by the MG is driven by synthetic activity, and that the MG is capable of regulating this process. This regulation may occur to ensure an adequate supply of AA for milk protein synthesis and also to maintain the uptake of certain AA commensurate with other AA. To maintain these balances, the MG can alter extraction rates of individual AA and the blood flow across the MG. Consequently, the MG appears partly to be capable of compensating for imbalances in absorbed AA profile if TAA supply is sufficient. This does not, however, exclude the importance of AA profile of digested protein when the aim is to maximise AA utilisation in the MG and lactational performance.
4. GENERAL CONCLUSIONS

1. Positive milk and milk protein yield responses to post-ruminal His infusions together with a closer relationship between His supply and milk protein yield than for other AA provides convincing evidence that His is the first limiting AA for milk production based on grass silage-cereal diets. Milk protein yield responses to His infusion were comparable to those attained by replacing barley with 1 kg of RSM. Supplementing the diet with the first limiting AA represents a viable means of improving dietary N utilisation. However, it is questionable if this approach is economically attractive when the cost of protein supplements is relatively low.

2. Infusions of BCAA did not affect lactational performance suggesting that they are not second limiting AA on grass silage-cereal based diets. One reason could be the small margin between first and second limiting AA. Alternatively, the supplies of AA, glucose and energy may vary despite feeding similar basal diets.

3. In spite of improved lactational performance, utilisation of infused His was not associated with the level of infusion and remained low and constant across all infusion levels compared with the utilisation of individual AA derived from dietary supplements. This suggests that the utilisation of AA by the MG may be regulated by factors other than AA supply. Changes in mammary blood flow and AA extraction rates appear to be the mechanisms by which the MG controls nutrient uptake. This may reflect the desire to maintain the uptake of one AA commensurate with other AA and satisfy N and C requirements for milk protein synthesis.

4. Measurements of AA supply on grass silage based diets indicated that the flow of His and Lys were similar, that of Leu was lower and Met was higher compared with maize silage based diets. These differences are consistent with the ranking of limiting AA for milk production between diets based on these forages.

5. Omasal digesta AA profile was similar between studies but some variation existed in total AA flow and the ratio of EAA flow. This suggests that variable in milk production responses to postruminal AA infusions are associated with differences in total AA supply and to some extent the supply of individual AA derived from the basal diet. The most convincing explanation for this variation is differences in the quality of grass silage. Measurements of AA flow for diets based on a wider range of grass silages would allow the effect of silage quality on AA supply to be assessed.

6. The majority of AA flowing from the rumen originated from MP for grass silage based diets. Under these circumstances, barley supplementation did not improve nitrogen utilisation by rumen bacteria suggesting that the value of protein balance in the rumen for barley is close to zero. The increase in AA flow associated with barley supplementation was lower than predicted according to the current AAT/PBV system. Barley supplements also appeared to increase ruminal N degradability of the whole diet suggesting that ruminal degradation of barley CP is also higher than currently thought.

7. Amino acid profiles of MP and individual microbial fractions (LAB, PAB and protozoa) were independent of changes in the diet. In spite of the differences in AA profiles between individual microbial fractions, the accuracy of estimates of microbial AA supply are more dependent on MP flow measurements than on the AA profiles of distinct microbial entities.

8. Protein supplements (RSM, SBM, FM and MGM) did not affect postruminal MP flow but increased RUP flow, suggesting that the supply of rumen degradable nitrogen was adequate for MPS. Since all protein supplements increased milk production, it can be concluded that MP alone does not satisfy the AA requirements of high yielding dairy cows.
9. Differences in production responses between protein supplements demonstrated the importance of AA supply improving lactational performance and dietary nitrogen utilisation. Protein supplements containing relatively high amounts of RUP are needed to alter the AA profile of absorbed protein. An inbalanced AA profile may in part, be compensated for by increases in TAA supply. Lower responses to SBM than to FM, MGM and RSM reflected an increase in ruminal N degradability and lower AA flows entering the omasal canal.

10. Estimated AA degradability in the rumen indicated differences between individual AA. In addition to AA absorption from the small intestine, both represent potential sources of error when attempting to estimate AA availability at the tissue level. The quantitative effect of these on AA supply merits further investigation.
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