EFFECTS OF SUPPLEMENTARY AMINO ACIDS ON MILK PRODUCTION IN DAIRY COWS CONSUMING DIETS LOW IN HISTIDINE

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SUMMARY

1. Experiments in this thesis were conducted to investigate effects of supplementary amino acids on milk production in dairy cows consuming grass silage and a supplement containing feather meal which is low in histidine. The primary object was to identify a basal diet showing a clear deficiency of the first-limiting amino acid for milk production, such that supplementing the supply of this AA, either direct into the abomasum or intravenously, would evoke sizable increases in the yield of milk protein so allowing the efficiency of incorporation into milk protein to be calculated.

2. A series of four experiments was conducted to identify the first-limiting AA for milk production, and to determine the magnitude of the response of milk production to supplementation with the limiting AA. Experiment 1 examined responses of milk production to continuous intravenous infusion of 9.7 g/d histidine, 9.1 g/d methionine, 30.0 g/d lysine and 2.6 g/d tryptophan (4AA); infusion of the AA mixture without histidine (-His); and infusion of the AA mixture without lysine (-Lys). Since methionine has been shown to be not limiting in a previous experiment with this basal diet, infusion of the AA mixture without methionine was not included in the experimental treatments. The yields of milk protein were 740, 874, 715 and 851 g/d (SED 32.8) for the basal, 4AA, -His and -Lys treatments respectively. The response was not diminished by omission of lysine but was cancelled by omission of histidine, so indicating that histidine was the first-limiting amino.

In Experiment 2 a single dose (9.7 g/d) of histidine was given intravenously but there was no significant effect of histidine supplementation on milk production.

Experiment 3 was conducted to examine responses of milk production to increasing intravenous doses (3, 6 and 9 g/d) of histidine alone. All levels of histidine increased the

yield of milk protein over the basal treatment, the respective values being 670, 700, 781 and 699 g/d (SED 22.2) for Basal, 3, 6 and 9 g/d of histidine supplementation.

In Experiment 4, increasing intravenous doses of 0, 3, 6 and 9 g/d of histidine were supplemented with the combination of 8 g methionine, 28 g lysine and 2.5 g tryptophan. The addition of the other acids was aimed at ensuring that histidine remained first-limiting as its level of infusion was increased. Increasing doses of histidine produced linear increases in the yield of milk protein [727, 785, 826 and 879 g/d (SED 18.6) for the 0, 3, 6 and 9 g/d levels, respectively].

3. Two experiments were conducted to test the hypothesis that an increase of milk production would follow from delivery of extra histidine into the abomasum as a result of dietary addition of an effective rumen-protected form of the AA or from dietary addition of proteins rich in histidine and of low rumen degradability. In Experiment 5 effects of intravenous and intra-abomasal administration of two levels (3 and 6 g/d) of histidine were compared. None of treatments affected the yield of milk or milk constituents.

Experiment 6 examined responses of milk production to progressively substituting avian blood meal (rich in histidine and poor in methionine) for part of the feather meal in its supplement. Blood meal cubes were substituted for 0, 0.10, 0.20 and 0.40 of the feather meal cubes in the supplement. Substitutions of blood meal cubes increased the yield of milk protein [593, 679, 681 and 701 g/d (SED 25.2) for 0, 0.10, 0.20 and 0.40 of blood meal substitution for feather meal, respectively] but there was no further increase beyond the first level.

4. Two experiments were conducted to characterize more fully the dietary 'model'. Experiment 7 was designed to determine the sequence of limitation of the AAs for milk production when each of three AAs was omitted in turn from a continuous intravenous

infusion supplying a mixture of the group of probable next-limiting AAs after histidine (methionine, lysine and tryptophan), histidine being retained in all infusates. The yields of milk protein were 771, 872, 784, 812 and 897 g/d (SED 27.5) for the basal, 4AA, methionine-free, lysine-free and tryptophan-free treatments, respectively. The results indicated that methionine was likely to be the co-limiting AA or the very close second-limiting AA.

Experiment 8 was carried out to determine how much of the milk production response to addition of all the essential AAs could be obtained by histidine alone or the group of the next 3 most-limiting AAs. The four treatments were the basal treatment (Basal), continuous infusion supplying a mixture of essential AAs (EAA), infusion of a AA mixture of histidine, methionine and lysine (3AA), and infusion of histidine alone (His). The yields of milk protein were 518, 625, 626 and 578 g/d (SED 24.4) for the basal, EAA, 3AA and His treatment, respectively. No extra response was seen to EAA over that seen with 3AA and histidine alone accounted for about 56% of the maximum response.

5. When the results of all the experiments are considered together, reasons for the inconsistent response to supplements of histidine alone became apparent. The most likely cause was variation in the background supply of methionine whose status varied between that of second-limiting or co-limiting with histidine. From an examination of the literature, the most likely cause of the variation in methionine status was variation in the output of microbial protein from the rumen, compounded with fluctuations in the concentrations of methionine and histidine in the microbial protein.

The linear response of milk protein output in Experiment 4 allowed the calculation of an efficiency of transfer of histidine into milk of 0.43. The model could be modified to determine the efficiency of transfer of methionine and lysine, when they are first-limiting, by manipulating the intravenous infusion of the 3 AAs, histidine, lysine and methionine.

DECLARATION

All animal experimentation was carried out by me. All routine laboratory analysis was carried out by Mrs I Stewart and Mr J Davidson. With this exception, I declare that the work contained in this thesis is my own, undertaken under the supervision and guidance of Drs DG Chamberlain and J-J Choung.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
ATP	Adenosine triphosphate
BMC	Blood meal cubes
DM	Dry matter
DNases	Deoxyribonucleases
DUN	Digestible undegraded nitrogein
DUP	Digestible undegraded protein
EAA	Essential amino acid
FMC	Feather meal cubes
FME	Fermentable metabolisable energy
GIT	Gastrointestinal tract
GRF	Growth hormone releasing factor
ISTD	Internal standard
MCP	Microbial crude protein
MDV	Mesenteric drained viscera
ME	Metabolisable energy
MP	Metabolisable protein
MPS	Microbial protein synthesis
NDF	Neutral detergent fibre
NEAA	Nonessential amino acid
OMDR	Organic matter digested in the rumen
PDV	Portal-drained viscera
RDP	Rumen-degradable protein

RNases	Ribonucleases
TCA	Tricarboxylic acid
UDN	Undegraded nitrogen
VFA	Volatile fatty acids
WSC	Water-soluble carbohydrate

PREFACE

Ruminant animal production is an important component of sustainable agricultural systems throughout the world. Large tracts of land are unsuitable for cereal production and can only be used for grazing by ruminants, which are able to utilize forages as their principal source of nutrients. In ruminants grazing temperate pastures or receiving concentrate rations, the conversion of plant feedstuffs into animal products is a wasteful process and only about 30% of the protein ingested is used for meat, milk or wool production. This reduces profitability and also causes pollution problems in the disposal of nitrogen-rich excreta. There is therefore considerable scope for increasing the efficiency with which nitrogen is utilized by ruminants, and also for increasing the nutritional value of the end-products. In addition, there is pressure to enhance the health properties of meat and milk products, such as by increasing their protein:fat ratio.

Inefficient use of amino acids (AAs) by ruminants arises partly from losses during fermentation and eventual production of ammonia in the rumen and partly from their deamination during metabolism in the liver and other tissues. A better understanding of AA metabolism could lead to more efficient use of AAs for milk protein synthesis. Recent studies on high yielding dairy cows suggest that intestinal AA profile may vary more than previously assumed (Clark *et al.*, 1992), and can be deficient in specific AAs, as indicated by responses to supplementation of AAs.

Improved efficiency of milk production in dairy cows, and hence a more cost effective industry, depends upon a better understanding of the digestion of food, the metabolism of absorbed nutrients in the tissues and the utilization of nutrients for milk production. The experiments described in this thesis examined the response of the dairy cow, in terms of changes in milk production, to supplements of AAs with the particular

aim of obtaining estimates of the efficiency of use of limiting AAs for secretion of milk protein.

CHAPTER ONE

REVIEW OF THE LITERATURE

1.1 DIGESTION OF PROTEIN IN THE RUMINANT

Digestion of protein and metabolism of AAs following absorption appear to be similar for ruminants and non-ruminants. However, the ruminant animal has the unique ability to subsist and produce without a source of dietary protein due to the synthesis of microbial protein from non-protein nitrogen (N) within the rumen. Ruminal microorganisms and dietary protein that escapes degradation in the rumen supply the small intestine with protein for digestion and absorption. Therefore, this section will discuss the features of ruminant digestion of protein and Figure 1.1 shows a schematic representation of nitrogen digestion in the ruminant.

1.1.1 Rumen

1.1.1.1 Degradation of dietary protein in the rumen

The extent to which protein is degraded mainly depends upon microbial proteolytic activity in the rumen; the nature (i.e. chemical and physical structure) of protein; microbial access to the protein; and ruminal retention time of the protein.

Most of the proteolytic activity in the rumen is associated with cells; there is little proteolytic activity in particle-free (centrifuged) rumen fluid (Nugent and Mangan, 1981). Warner (1956) and Annison (1956) concluded that at least half of this activity was associated with the bacteria; indeed, 30-50% of species commonly isolated have proteolytic activity. Bacteria classified as *Prevotella ruminicola* and *Butyrivibrio fibrisolvens* are commonly thought to play a major role in ruminal protein degradation (Wallace and Cotta, 1988; Wallace, 1994), with *P. ruminicola* isolates possessing an activity profile most similar to that of total ruminal contents (Wallace and Brammall,

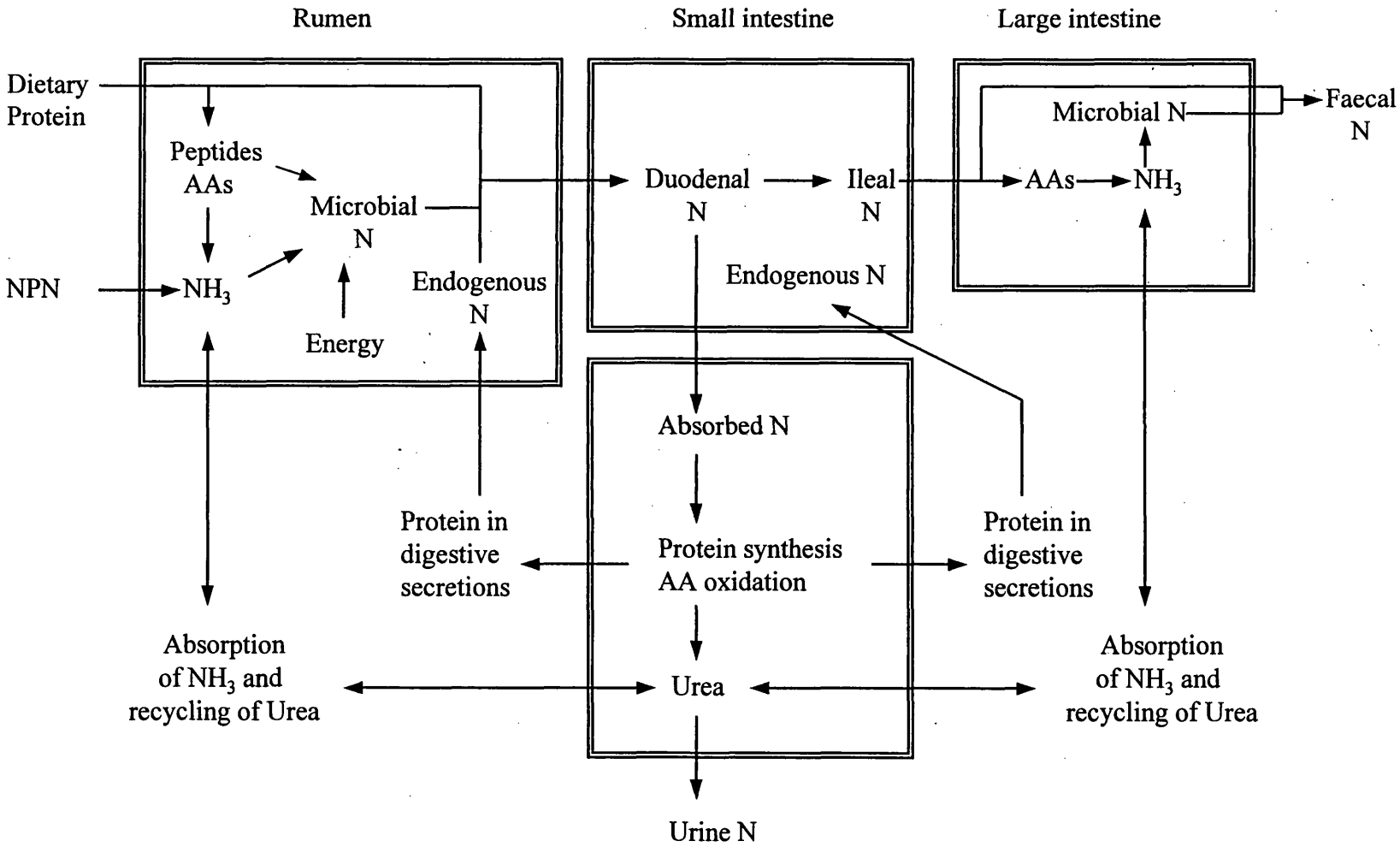

Figure 1.1 Schematic representation of digestion of N in the ruminant (modified from Thomas and Chamberlain, 1981)

1985). From studies of the rate of degradation of [^{14}C]-labelled fraction 1 leaf protein from lucerne, Nugent and Mangan (1981) concluded that protozoa were responsible for only 10-20% of the proteolytic activity in bovine rumen fluid. The anaerobic fungus *Neocallimastix frontalis* possesses a metalloprotease (Wallace and Joblin, 1985), but proteolytic activity was not readily detectable in other species (Michael *et al.*, 1993).

The rate and extent of protein degradation depends on the accessibility of the hydrolysable sites in the polypeptide chain; this accessibility depends on the types of enzyme involved, and on conditions at the site of binding to the cell wall (i.e. pH, availability of metal co-factors, etc.). Moreover, the surface area of protein or other water-insoluble materials, and disruption of these associations may increase protein degradation rate. The degree of secondary and tertiary structure and the density of disulphide cross-linkages (which may bind together sections within a single polypeptide chain or link two different chains), also appear to correlate closely with lower degradation rates (Mahadevan *et al.*, 1980).

Soluble compounds in the rumen are attacked more rapidly and digested more completely than are insoluble compounds due in part to differences in microbial access. With most feedstuffs, soluble protein is only a small fraction of the total protein degraded in the rumen, so when comparing the extent of degradation of different feedstuffs, differences in degradation of the insoluble fraction account for more of the variation than solubility alone. Estimates of ruminal degradation of protein insoluble in ruminal buffers generally range from 30 to 50%. This supports the concept that solubility alone is a poor predictor of the extent of ruminal degradation across a variety of diets and feeding conditions. Yet, if limited to high feed intakes of a high-concentrate diet, solubility alone may prove useful as an index of the extent of degradation (see Owens and Zinn, 1988). The extent of degradation for some of the AAs may be greater than for others because, for a given protein, the AA composition of the soluble fraction can differ from that of the

insoluble fraction (see Owens and Zinn, 1988).

Another factor that can influence the rate of degradation of a protein is the extent to which the structure is chemically modified. Bovine submaxillary mucoprotein, for example, is structurally modified with *N*-acetyl galactosamine and sialic acid residues, and is degraded extremely slowly in the rumen (Nugent *et al.*, 1983); indeed, its resistance to breakdown and the resultant stabilization of foam appear to be causative factors in bloat (Jones *et al.*, 1978). The same parameters will obviously influence the degradation of heterogeneous protein supplements as well. Treatment of fishmeal with mercaptoethanol to break disulphide bonds caused a large increase in degradability (Mahadevan *et al.*, 1980), but there is no similar evidence available for other supplements. It is tempting to suggest that the resistance shown by animal proteins might be caused by cross-linked connective proteins analogous to elastin, but there is no evidence to support this. Other, nonprotein factors such as the association between proteins and materials like oils, starch and cell wall polysaccharides must also be involved. The resistance of associated polymers, rather than the protein itself, to microbial attack probably often limits the degradation of a protein.

Agricultural Research Council (ARC; 1980) recognized the probability that feedstuffs did not have a constant degradability value but the value would vary with the nature of the basal diet and the level of feeding. Variation in feed intake, proportion of roughage to concentrate and ionic concentration of the diet, particle size of feed and environmental temperature are known to markedly alter the rumen liquid turnover rate. Consequently, the mean retention time in the rumen of small particles is likely to vary with the same factors and the extent of degradation of dietary protein can be expected to vary with its mean retention time.

1.1.1.2 Microbial protein synthesis

Availabilities of energy and N are the major determinants of the amount of microbial

protein synthesized in the rumen but other nutrients such as sulphur (S), minerals and vitamins are also required (Owens and Zinn, 1988).

A mixture of structural (cellulose, hemicellulose) and nonstructural carbohydrates (sugars, starch) is normally the best source of energy for growth of bacteria because, upon fermentation, carbohydrates yield more energy per unit of weight than protein (Stern *et al.*, 1978; Russell *et al.*, 1983; Nocek and Russell, 1988). Tamminga (1992) suggested that adenosine triphosphate (ATP) yield from AA fermentation is considerably lower and per unit of weight fermented approximates to 50% of that of fermented carbohydrates. The growth of microbial cells is dependent on degradation of organic matter to provide the necessary monomers from which the array of cell constituents (proteins, polysaccharides, lipids, nucleic acids) are synthesized and from which, by fermentation, ATP is generated to provide the energy for these syntheses.

In most cases, ammonia acts as the principal N source. Depending on the carbohydrate source from which they obtain the energy necessary for their growth, bacteria markedly differ also in terms of N utilization. Thus, most cellulolytic bacteria have an absolute requirement for ammonia (Hespell, 1984), however, starch-digesting bacteria have been shown to obtain 66% of their own N from AAs and peptides and only 34% from ammonia (Russell *et al.*, 1983). Findings such as these imply that, although cellulolytic bacteria may need only small amounts of AA in ruminal fluids provided their requirements for branched-chain AAs are met (Bryant, 1973), starch-digesting bacteria may need significant amounts of AAs and peptides if they are to achieve their maximum growth rate (see Russell *et al.*, 1992).

Sulphur is also a required nutrient to ensure optimal growth of microbes (Bird, 1973) and significant quantities can be obtained from preformed S-AAs when feeding conventional diets. According to Durand and Kawashima (1980), other inorganic elements that may limit microbial activity are the trace elements copper (Cu) and cobalt (Co). Co is

required as a co-factor in the synthesis of vitamin B_{12} which seems more essential to the host-animal than to the microbes. Other B-vitamins believed to be important are thiamine and niacin (Brent and Bartley, 1984).

To further optimize microbial growth in the rumen, Nocek and Russell (1988) suggested synchronizing the supply of energy and protein. However, Chamberlain and Choung (1995) concluded that reliable experimental evidence to support a need for close synchronization was not available.

1.1.2 Intestines

The ruminant appears to possess the same complement of proteases and peptidases within the small intestine as does the non-ruminant except for an abundance of pancreatic ribonucleases (RNases; Armstrong and Hutton, 1975; Bergen, 1978; Chalupa, 1978). The principal proteases of bovine pancreatic fluid are chymotrypsin, trypsin, pancreatopeptidase, elastase and the carboxypeptidases A and B, the first three being classed as endopeptidases attacking at centrally located peptide bonds, and the last two exopeptidases which cleave only the terminal bonds of proteins or peptides. All are hydrolytic in action, show maximal activity at a pH of 7.5 or above and are secreted as inactive zymogens; activation is achieved by trypsin, following activation of trypsinogen to trypsin by the action of enterokinase secreted by the intestinal mucosa. The hydrolytic actions of the pancreatic proteases are complemented and further extended by peptidases present in the mucosal cells of the small intestine (see Armstrong and Hutton, 1975).

Breakdown of nucleic acids is achieved by deoxyribonucleases (DNases), RNases, phosphodiesterases, and phosphomonesterases (Bergen, 1978; Roth and Kirchgessner, 1980). An important role for abundant pancreatic RNase in the ruminant is release of nucleic acid phosphorus for recycling to the rumen via saliva (Barnard, 1969). It appears that the products of nucleic acid digestion that are absorbed are nucleotides, nucleosides,

and bases (Bergen, 1978; Smith, 1979).

From earlier studies on low-yielding ruminants it was concluded that intestinal AA composition is almost constant and reasonably adequate for milk protein synthesis (Oldham and Tamminga, 1980; Tamminga and Oldham, 1980; Smith, 1984; Buttery and Foulds, 1985). However, recent studies on high-yielding dairy cows suggest that intestinal AA profile varies more than previously assumed (Clark *et al.*, 1992; Rulquin and Vérité, 1993). Extensive literature studies on the extent and origins of the variation in intestinal AA composition were carried out using 105 data for sheep (Le Henaff, 1991) and 133 data for cattle (Guinard *et al.*, unpublished; cited by Rulquin and Vérité, 1993). The coefficient of variation for individual essential AA (EAA) concentration (g/100 g total AA) ranged from 6-11% for lysine, threonine, phenylalanine and branched-chain AAs, 18% for histidine and arginine (only for sheep) and 18-24% for methionine (Table 1.1). In other words, the concentration of each individual AA could vary two or threefold under different feeding conditions.

Microbial proteins supply the majority of AAs entering the small intestine, accounting for 35-66% in dairy cows (Clark *et al.*, 1992) and even more in sheep, up to 60-90% (Smith, 1975). AA composition of bacteria isolated from the rumen has been reported to be constant (Weller, 1957; Purser and Buechler, 1966; Bergen *et al.*, 1968; Storm and Ørskov, 1983). However, a summary of AA composition of 441 bacterial samples from animals fed 61 dietary treatments in 35 experiments, expressed as grams of individual AA per 100 g of AA, indicated that differences in AA composition were large (Clark *et al.*, 1992; Table 1.2). This could partly arise from differences in isolation and analytical procedures, but such variations are also found within laboratories and their origins are unknown.

1.2 ABSORPTION OF END PRODUCTS OF PROTEIN DIGESTION

Table 1.1 Variability of amino acid composition (g/100 g total AA) of intestinal contents in ruminants (After Rulquin and Vérité, 1993)

Species	Cattle[1]				Sheep[2]				
	Mean	CV	Minimum	Maximum		Mean	CV	Minimum	Maximum
His	2.11	11.9	1.34	2.89	His	2.55	17.8	1.63	3.89
Thr	5.32	7.0	4.36	6.16	Thr	6.00	11.2	4.38	8.46
Arg	4.96	10.6	3.76	7.07	Arg	5.46	18.2	2.92	9.65
Met	1.97	17.6	1.27	2.99	Met	2.35	24.1	0.78	3.58
Phe	5.12	7.1	4.13	6.06	Phe	5.75	9.0	4.44	6.89
Val	6.01	10.8	4.03	7.33	Val				
Ile	5.45	8.0	4.44	6.73	Ile	21.48	6.0	18.59	24.49
Leu	8.87	10.5	6.77	11.90	Leu				
Lys	6.88	9.7	4.82	8.42	Lys	7.46	10.6	5.03	9.69
Asp	10.94	6.6	8.90	12.23	Asp				
Ser	5.12	8.8	4.01	7.19	Ser				
Glu	14.52	8.5	11.93	18.08	Glu	48.95	2.5	44.78	52.52
Gly	6.24	19.2	3.20	10.10	Gly				
Ala	6.92	5.6	5.54	7.79	Ala				
Tyr	4.47	11.9	2.98	5.87	Tyr				
Pro	5.01	15.3	3.63	7.56	Pro	nd			

nd = not determined

CV = coefficient of variation (%).

[1] Literature review on 133 diets (Guinard *et al.*, unpublished, cited by Ruquin and Vèritè, 1993)

[2] Literature review on 105 diets (Le Henaff, 1990)

Table 1.2 Amino acid composition (g/100 g of AA) of ruminal bacteria (After Clark *et al.*, 1992)

Amino acid	Mean[1]	Minimum	Maximum	SD	CV
Histidine	2.0	1.2	3.6	0.4	21.3
Threonine	5.8	5.0	7.8	0.5	8.9
Arginine	5.1	3.8	6.8	0.7	13.2
Methionine	2.6	1.1	4.9	0.7	25.6
Valine	6.2	4.7	7.6	0.6	10.1
Phenylalanine	5.1	4.4	6.3	0.3	6.4
Isoleucine	5.7	4.6	6.7	0.4	7.4
Leucine	8.1	5.3	9.7	0.8	10.3
Lysine	7.9	4.9	9.5	0.9	11.9
Aspartic acid	12.2	10.9	13.5	0.6	4.8
Glutamic acid	13.1	11.6	4.4	0.7	5.3
Serine	4.6	3.4	5.4	0.4	8.9
Glycine	5.8	5.0	7.6	0.5	8.2
Alanine	7.5	5.0	8.6	0.6	7.3
Tyrosine	4.9	3.9	7.7	0.6	13.2
Proline	3.7	2.4	5.3	0.5	13.2

SD = standard deviation.

CV = coefficient of variation (%).

[1] Average composition of 441 bacteria samples from animals fed 61 dietary treatment in 35 experiments.

Most N absorbed from the forestomach is as ammonia, although, recently, it has been suggested that AAs and small peptides may be absorbed from the forestomach (see below). Again, irrespective of site of absorption, peptides are more rapidly absorbed than free AAs. Absorption of AAs and peptides from the small intestine of the ruminant animal shows a similar pattern to that in the non-ruminant animal. This section will discuss some of the current concepts of mechanisms of absorption and transport of AAs and peptides and quantitative estimations of absorption of AAs.

1.2.1 Rumen

1.2.1.1 Ammonia absorption in the rumen and recycling of endogenous nitrogen

Ammonia N is removed from rumen fluid in several ways: by incorporation into microbial matter which then passes out of the rumen; by absorption through the rumen wall; and in fluid flowing to the lower digestive tract.

Uptake of ammonia across the epithelium of the reticulo-rumen appears to be a passive process. Therefore rates of absorption are regulated by ammonia concentration in the digesta (Leng and Nolan, 1984), and by the pH of the rumen liquor. A linear relationship between the concentration of un-ionized ammonia and ammonia disappearance from the digesta was found (Siddons *et al.*, 1985). This is consistent with the suggestion by Hogan (1960), that un-ionized ammonia diffuses through the rumen wall more freely than the ionized form.

When ruminal production rates are very high, for instance when high levels of non-protein nitrogen (NPN) are fed, the rapid ammonia absorption can result in high levels of ammonia reaching the extra-hepatic circulation, with the animal suffering recumbency and eventually coma caused by the hyperammonaemic toxicity (Bartley *et al.*, 1976, 1981).

Absorbed ammonia is converted to urea in the liver before being excreted in the urine or recycled to the rumen via the saliva. The recycling of urea to the forestomach is an

important source of N for synthesis of microbial protein, which in turn provides a potential supply of AAs for the host animal after passage to the small intestine. There is also evidence that a large amount of endogenous urea enters the postruminal gut (Nolan and Leng, 1972; Dixon and Nolan, 1982, 1986).

Urea enters the reticulo-rumen via diffusion from the blood into saliva (Obara and Shimbayashi, 1980) and via diffusion across the reticulo-ruminal epithelium (Ford and Milligan, 1970; Cheng and Wallace, 1979; Owens and Bergen, 1983). The recycling of urea via the saliva is the product of salivary flow and salivary urea concentration. Salivary urea concentration is positively correlated to blood urea concentration (Somers, 1961). Factors which alter the salivary flow rate, such as feed intake (Bartley, 1976) and rumen ammonia concentration (Obara and Shimbayashi, 1979) as well as the urea concentration in the blood will also affect the salivary contribution to urea transfer.

In ruminants given energy-rich supplements there is usually a marked increase in the amount and/or proportion of urea recycled to the rumen (Potthast *et al.*, 1977; Engelhardt *et al.*, 1978; Obara and Shimbayashi, 1980, 1987, 1988). The increase in urea transfer to the rumen of animals given energy-rich supplements is due to an increase in permeability of the rumen wall. It is apparent that intake of high energy supplements is often associated with an increased influx of urea into the rumen regardless of plasma urea concentration, but that rumen ammonia levels are decreased because of increased uptake of ammonia by microbes. This suggests that the transfer of urea across the rumen wall is independent of either plasma urea or rumen ammonia concentration (Norton *et al.*, 1982; Obara *et al.*, 1991).

1.2.1.2 Amino acid and peptide absorption in the forestomach

It is accepted that forestomach epithelia have the ability to absorb VFA, electrolytes, ammonia, and water (Van Soest, 1987). The ruminal epithelium of goats and sheep appears

to possess the ability to absorb free AAs (Cook *et al.*, 1965; Leibholz, 1971; Bird and Moir, 1972; Fejes *et al.*, 1991). A study (DiRienzo, 1990) measuring the arterial venous flux of free and peptide-bound AAs across non-mesenteric viscera (mainly stomach) of wethers and steers showed that forestomach tissues may have the ability to absorb AAs and peptides. From a recent *in vitro* study (Matthews and Webb, 1995) to evaluate forestomach absorption of AAs and dipeptides, omasal tissue appeared to have a greater overall capacity for methionine and methionylglycine (Met-Gly) absorption than does ruminal tissue. Both substrates passed through omasal tissue in greater quantities than through ruminal tissue, both tissues displayed a slightly greater tendency to absorb methionine than Met-Gly and the flux across the tissues was not saturable, suggesting non-mediated uptake of methionine, Met-Gly and carnosine (N-β-alanyl-L-histidine).

Proton gradients have been used to demonstrate the presence of carrier-mediated dipeptide transport by intestinal and renal epithelial brush-border-membrane vesicles (Ganapathy and Leibach, 1983; Daniel *et al.*, 1991) and in cultured colon cells (Saito and Inui, 1993). Forestomach tissues may absorb peptide-bound AAs by a mediated process because omasal liquor is acidic (Prins *et al.* 1972) and ruminal liquor can develop pH levels of 5.5 or less (Whitelaw *et al.*, 1970). A study (Matthews *et al.*, 1996a) demonstrated that sheep omasal epithelial cell express mRNA encoding for proteins that are capable of H^+-dependent transport activity.

The demonstration *in vitro* (Matthews and Webb, 1995) that ruminal and omasal epithelia have the ability to absorb AAs and peptides by a non-mediated process indicates that *in vivo* absorption of free and peptide-bound AAs is likely to depend on their relative concentrations in forestomach liquor. It has been reported that concentrations of peptide N are higher than those of free AAs in ruminal liquor (Matthews *et al.*, 1996b). If, therefore, extracellular substrate concentration affects absorption, a greater driving force for peptide absorption than for free AA absorption can be expected to exist in ruminal liquor both

before and after the feeding of common diets (see Webb and Matthews, 1998). However, because omasal liquor AA and peptide N concentrations are unknown, it is not possible to predict the potential relative driving forces for AA absorption across the omasal epithelium.

1.2.2 Small intestine

1.2.2.1 AA and peptide absorption

The duodenal, jejunal and ileal regions of the small intestine appear to have different abilities to absorb AAs; thus, absorptive activities are not uniformly apportioned throughout the length of the small intestine. The jejunal region of animals such as the rat and guinea pig has the greatest potential for AA absorption (Spencer and Samiy, 1961; Cohen and Huang, 1964; Larsen *et al.*, 1964; Matthews and Laster, 1965; Baker and George, 1971).

Williams (1969) studied the absorption of AAs from the intestine of the sheep and found that the order of absorption of AAs varied with intestinal location and that transport systems at each site had different affinities for individual AAs. Maximal lysine absorption was observed to occur in the ileum of the sheep (Johns and Bergen, 1973). In sheep, the greatest absorption of threonine and valine occurred in the ileum, and methionine was absorbed about equally from both the jejunum and ileum (Phillips *et al.*, 1976). None of these AAs was absorbed from the duodenum. In a subsequent study with perfused intestinal segments from sheep, it was confirmed that the ileum is the dominant site of AA absorption (Phillips *et al.*, 1979). Wilson and Webb (1990) reported that total methionine and lysine uptake was higher by ileal brush border membrane vesicles than by jejunal brush border membrane vesicles. Also, methionine transporters had lower affinities and higher capacities for methionine than the corresponding lysine transporters had for lysine in both ileal and jejunal tissue.

The significance of these site differences in AA absorption is not clear. Species of animal seems to be important in determining the predominant site of AA absorption. Impacts of variables such as diet and physiological state on the site of AA absorption remain largely unknown. The optimal pH for most intestinal proteases is in the range of pH 7.2 to 9 (Bondi, 1987). The contents of the ruminant small intestine usually do not reach this pH range until near the latter one-half to one-third of the small intestine (Ben-Ghedalia *et al.*, 1976). Because the luminal environment of the ileal region of the small intestine of the ruminant is more compatible with optimal proteolytic activity, one might expect transport capabilities to be concentrated there. Little is known about the ability of intestinal transport systems to adapt to transitions in dietary intake and content or physiological status of the animal (Karasov and Diamond, 1987).

When absorption rates of AAs from equimolar mixtures of 18 common dietary AAs or of eight EAA were studied in humans, methionine and the branched-chain AAs were absorbed most rapidly (Adibi and Gray, 1967; Adibi *et al.*, 1967). As a group, EAA were absorbed more rapidly than nonessential AAs (NEAA). Similar results with human subjects were obtained by Silk *et al.* (1980). They perfused the upper jejunum with a mixture of 16 AAs simulating lactalbumin. The top six AAs in order of absorption, measured by percentage of AA absorbed, were methionine, leucine, valine, phenylalanine, arginine and isoleucine. The disappearance was greater for essential than for nonessential AAs from the small intestine of sheep (Coelho da Silva *et al.*, 1972a,b; MacRae and Ulyatt, 1974; Armstrong *et al.* 1977; Christiansen and Webb, 1990a) and cattle (Armstrong *et al.*, 1977; Christiansen and Webb, 1990b). In all the above studies with sheep and cattle, absorption rate was greater for methionine than for other AAs.

The complex pattern of absorption of AAs probably is attributable to differences in the affinities of carrier systems for individual AAs and to the fact that competition for transport is greater among AAs for which a carrier has a greater affinity (Adibi, 1969).

Johns and Bergen (1973) reported a 48% reduction in lysine absorption by ovine intestinal rings when leucine was present at three to four times the lysine concentration. More methionine was absorbed than either threonine or valine from everted sacs of ovine intestine and methionine was a strong inhibitor of the absorption of both AAs when they were perfused through isolated segments (Phillips *et al.*, 1976, 1979).

Not all the interactions that are observed among AAs in the absorptive process are negative. Some AAs stimulate the transport of others. Examples include the ability of alanine and phenylalanine (Reiser and Christiansen, 1969) and leucine (Munck, 1966) and methionine (Reiser and Christiansen, 1971) to increase the intestinal transport of the basic AAs arginine, lysine and ornithine. Phillips *et al.* (1979) also noted that methionine stimulated the absorption of threonine when threonine concentrations were low.

Several carriers are involved in the movement of AAs across the intestine and all carriers require the presence of an amino or imino group and a carboxyl group (Spencer *et al.*, 1962; Schultz *et al.*, 1972). Small molecular weight hydrophilic neutral AAs are taken up less readily than the larger hydrophobic AAs, the transport of basic AAs is intermediate (Sepúlveda and Smith, 1978, 1979). Some carriers are located specifically on the brush border, some on the basolateral membrane, and some on both (Bender, 1985; Hopfer, 1987). Some of these carriers require energy to function and are said to be active transporters and Na^+-dependent. The carrier complex of the active transport system must co-transport a Na^+ ion and an AA. The Na^+ gradient, high Na^+ extracellularly and low Na^+ intracellularly is maintained by Na^+/K^+ ATPase located in the basolateral membrane of the enterocyte (Quigley and Gotterer, 1969; Fujita *et al.*, 1972). Transport systems not coupled with Na^+ are called Na^+-independent and do not require metabolic energy to transport AAs against a concentration gradient.

Another issue of increasing concern is the question of whether peptides may be absorbed. Direct comparisons show that AAs in peptides are absorbed from the intestinal

lumen more rapidly than are free AAs (Adibi and Phillips, 1968; Craft *et al.*, 1968; Adibi, 1971; Cheng *et al.*, 1971; Burston *et al.*, 1972). Absorption of AAs from rat jejunum *in vivo* was greater from pancreatic hydrolysates of four proteins (casein, albumin, lysozyme and lactalbumin) than from equivalent mixtures of free AAs (Matthews, 1972). The fact that peptides are more rapidly transported than free AAs suggests that independent transport systems for peptides may exist or that there is a competitive advantage to the peptides for carriers. Studying several different AAs and peptides, Rubino *et al.* (1971) demonstrated that free AAs had no effect on the transport of dipeptides, and vice versa.

Peptides are transported against a concentration gradient by a system(s) requiring energy (Addison *et al.*, 1972, 1975; Matthews *et al.*, 1974; Nutzenadel and Scriver, 1976). Takuwa *et al.* (1985) demonstrated the active accumulation of a peptide in brush border membrane vesicles in the presence of a H^+ gradient. Unequivocal evidence was provided for direct transport and for energization of peptide transport by electrical as well as chemical components of the proton-motive force when a membrane potential and a H^+ gradient were employed simultaneously (Miyamoto *et al.*, 1985).

Most of peptide absorption has been concerned with the entry of peptides across the brush-border membrane into enterocytes, a process now being understood to be by proton-linked active transport, where peptides are hydrolysed, rather than by passage of intact peptides across the whole epithelial 'barrier' (Alpers, 1986; Webb, 1990; Webb and Bergman, 1991; Fei *et al.*, 1994; Gardner, 1998). Other peptide fractions resulting from tissue protein turnover could be translocated to the venous blood and contribute to a net efflux of peptide-N from the gut (Seal and Parker, 1996). The possibility, however, that some peptides may enter the circulation intact, where they could represent a significant means of interorgan transport of AAs (Webb *et al.*, 1992; Backwell, 1994), remains the subject of much controversy, mainly due to the problems associated with accurate and reproducible quantification of peptide concentrations in blood and plasma. The possibility

that the differences between studies are due to differences in diet or physiological state of the animals cannot be ruled out and this also must be further investigated (Backwell *et al.*, 1997). Backwell *et al.* (1997) concluded that the absorption of intact peptides into the portal circulation is not a major route by which dietary N is absorbed. However, this does not detract from the importance of intestinal peptide transport systems in the absorption of dietary derived peptides into the enterocyte, where they are hydrolyzed before release of their constituent AAs to the circulation. Also to be remembered is that Gardner (1984, 1998; Gardner and Wood, 1988) considered that a paracellular route via 'tight junctions' and extrusion zones might be available for passage of peptides, especially small ones.

1.2.2.2 Quantitative estimates of AA absorbability

AAs available for absorption and ultimately for production are supplied by microbial protein synthesized in the rumen, dietary protein escaping ruminal degradation and endogenous secretions into the digestive tract (Richardson and Hatfield, 1978). On the evidence available, ARC (1980) used a value for AA apparent absorption of 0.70 to apply both to microbial and undegraded dietary AAs. This was changed in ARC (1984) to an assessment of true absorption of AAs, largely on the grounds that this was consistent with moves to take account of truly endogenous N losses from the body, not only in urine (ARC, 1980), but also in faeces (ARC, 1984). AFRC (1992) retained the use of coefficients for the true absorption of AAs from the gastrointestinal tract.

Salter and Smith (1977) showed that the true digestibility of microbial N in the small intestine was 0.79 and of sulphur containing AAs 0.85, a value similar to that found by Storm and Ørskov (1982). The true digestibility in the small intestine of rumen microbial AA nitrogen (AAN) was determined by infusion of isolated rumen microorganisms into the abomasum. The average true digestibility of the AAN was 0.847 and the difference in digestibility between individual AAs was generally very small (Storm and Ørskov, 1982;

Storm *et al.*, 1983a,b). Tas *et al.* (1981) found a value of 0.87 for true digestibility of microbial AAN. They excluded several AAs, namely diaminopimelic acid (DAPA), cystine, proline and tryptophan. Therefore the true digestibility value obtained by Tas *et al.* (1981) may be a slight overestimate of the true mean value; even so, it is close to the value derived by Salter and Smith (1977), Storm and Ørskov (1982) and Storm *et al.*(1983a,b). AFRC (1992) accepted a constant value (0.85) for the true absorption coefficient for microbial AAs.

ARC (1984) acknowledged that AAs of dietary origin would not always be truly absorbed with the same efficiency. In particular it was suggested that nitrogen bound to indigestible fibre might be of low absorbability, but in the light of few data, or of evidence that such correction will improve assessment of undegraded nitrogen (UDN) needs, a constant value of 0.85 was adopted.

However, Wilson and Strachan (1981) suggested that N leaving the rumen closely bound to indigestible fibre would be virtually unavailable for digestion in the small intestine and should not, therefore, be included as UDN. They proposed subtracting the N present in acid detergent fibre (ADF-N) from estimates of UDN to permit an estimate of digestible undegraded protein (DUP) which they argued, at least for forages, should replace UDP in the calculations. Webster *et al.* (1986) have shown that the true absorption of N from the UDN fraction of feeds is closely and inversely related to the proportion of that N which is insoluble in acid detergent (ADIN). Some by-products such as distillers grains and maize gluten which have been subjected to prolonged heat and moisture undergo Maillard reactions which substantially increase ADIN concentration above that in the original grain. Accordingly, AFRC (1992) chose an equation to calculate true absorption of UDN, which is Digestible undegraded N, [DUN] = 0.9([UDN] – [ADIN]). Beever and Cottrill (1993) suggested that for distillers products and other processed materials containing significant amounts of ADIN formed during processing, DUN can be

estimated as DUN = 0.9(UDN – 0.5ADIN) because the added ADIN may, in part, be degraded in the rumen. O'Connor *et al.* (1993) proposed a scheme, the Cornell Net Carbohydrate and Protein System, based largely on the model of Sniffen *et al.* (1992). They assigned a true digestibility of 100, 100, and 80% for the B1, B2 and B3 insoluble protein fractions (i.e. the B1 and B2 fractions are completely digested in the intestine but the B3 protein fraction was assumed to be less than completely digested.) and the bound protein fraction is not digested.

1.2.3 Large intestine

Nitrogen enters the caecum plus large intestine from the ileum and by diffusion through the intestinal wall. Input from the ileum consists of undigested feed protein, indigestible feed protein, undigested bacterial protein, plus endogenous N secreted or sloughed from the earlier sections of the intestinal tract. Amounts of free AAs or peptides entering the large intestine are insignificant (Clarke *et al.*, 1966). Under most feeding conditions, more N enters the large intestine from the ileum than leaves as faecal protein leading to a net absorption of 0.5 to 2 g daily in sheep (Clarke *et al.*, 1966; Hecker, 1971; Ørskov *et al.*, 1971; Coelho da Silva, 1972a; Thornton *et al.*, 1970) and 0 to 5 g in cattle (Van't Klooster and Boekholt, 1972; Zinn and Owens, 1982). Nitrogen absorption from the caecum and large intestine into the blood stream or through diffusion to other organs is enhanced by the high large intestinal pH (7 to 9) with roughage rations and is thought to be primarily as ammonia. Ammonia can be utilized by bacteria in the large intestine for microbial protein synthesis, be passively absorbed into the portal blood system, or passed with faecal protein.

Although AA absorption from the large intestine is negligible there is some evidence that AAs can be absorbed from the isolated perfused sheep cecum (Demaux *et al.*, 1961) and *in vitro* from rat and rabbit colon (Binder, 1970) and that the mechanism may be

simple diffusion. Such results do not prove net absorption of AAs *in vivo* but they do show that it is possible. However, the low concentrations of free AAs in the caecum and large intestine might be interpreted to suggest that sufficient quantities of AAs are not available in the free form for absorption. Low concentrations of AAs in the large intestine reflect the rapid uptake and catabolism of AAs by intestinal microbes. Wrong *et al.* (1981) concluded that AA absorption from the large intestine, except in the newborn animal, is quantitatively insignificant.

1.3 METABOLISM OF AAs BY THE GUT DURING ABSORPTION

AAs do not simply move out from the digestive tract to nourish the various tissues. Instead, the gut epithelium actually metabolizes a portion of AAs during absorption. Tagari and Bergman (1978) observed that the quantity of AAs appearing in the portal blood of sheep did not balance the amount disappearing from the intestinal lumen, and this report and various others since have suggested that between 30 and 80% of AAs disappearing from the lumen do not appear in the portal vein. In steers given postruminal casein (Guerino *et al.*, 1991) only 28% of the casein N infused abomasally appeared in the portal blood as α-amino N. Piccioli Cappelli *et al.* (1993) similarly demonstrated that up to 48% of [^{13}C] leucine infused directly into the duodenum of sheep could not be recovered in the portal vein when measured on a net transfer basis. However, if the sequestration of arterial ^{13}C tracer by the gut tissues is taken into account, recovery of [^{13}C] leucine administered to the small intestine may be nearer 70% (MacRae *et al.* 1993). Again, more recently when sheep were prepared with A-V preparations across the mesenteric drained viscera (MDV) and the portal-drained viscera (PDV) and with either duodenal and/or jejunal plus ileal cannulas, a much smaller imbalance of absorbed EAA reaching the liver was found (MacRae *et al.*, 1995).

High rates of protein turnover within the gastrointestinal tract (GIT), together with

the use of AAs as energy substrates within the mucosa, are cited as possible fates of those AAs lost across the gut wall, substantially affecting the pattern of AAs available to the liver and peripheral tissues (Lobley *et al.*, 1980). However, recently MacRae *et al.* (1995) indicated that as the majority of the AA appear to be derived from arterial rather than luminal sources, the GIT acts as a competitive peripheral tissue, extracting requirements for protein metabolism from the same systemic AA pools available to other tissues, including skeletal muscle and mammary gland. The rates of protein synthesis (35-45%) in gastrointestinal tissues of steers compared with whole body are disproportionately high relative to protein mass (5% of whole body mass) or rate of daily protein accretion (MacRae *et al.*, 1995). In the human intestinal wall, arginine is metabolized with the appearance of urea, ornithine and citrulline (Jungas *et al.*, 1992). In rats, enterocytes metabolize the carbon skeleton of glutamine to CO_2 (64%) and lactate (11%), and the nitrogen is exported as ammonia (38%), citrulline (28%) and alanine (24%), thus providing a major energy source for these tissues (Windmueller and Spaeth, 1974, 1975, 1980; Windermuller, 1982). Furthermore, as much as 33% of this glutamine uptake is transaminated and released as alanine. It is likely that similar reactions occur in ruminant tissues, since both sheep and lambs showed a net export of both alanine and citrulline from the PDV (Wolff and Bergman, 1972; Heitmann and Bergman, 1978; Burrin *et al.*, 1991). Additionally, gut protein synthesis rates vary with feed intake and physiological state (see Baracos *et al.*, 1991) and change during postnatal growth and development (Reeds *et al.*, 1993).

Measurements of net PDV α-amino N flux over a wide range of N intakes and different diets suggest that AA release by PDV is not correlated with dietary N supply (Figure, 1.2; Seal and Reynolds, 1993). Within individual experiments it has been suggested, however, that net α-amino N release by PDV is correlated with metabolizable energy (ME) intake (Huntington *et al.*, 1988; Huntington, 1989; Reynolds *et al.*, 1991b),

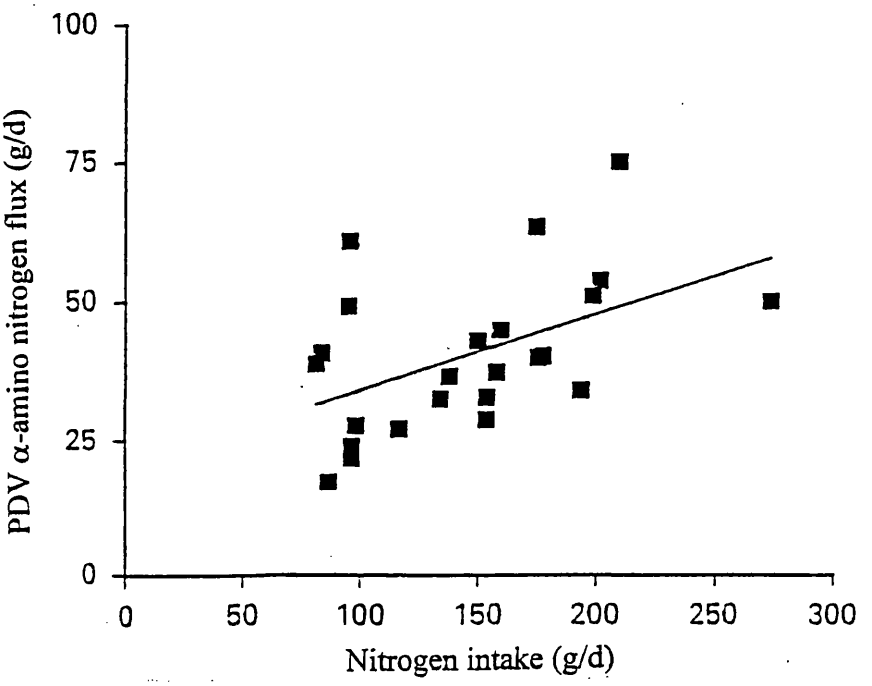

Figure 1.2. The relationship between portal drained viscera (PDV) flux of α-amino N in whole blood (g/d) and N intake (g/d). Each point represents animal means from individual experiments (from Seal and Reynolds, 1993). The equation of the regression line is $y = 20.429 \pm 0.136x$ ($R^2 = 0.235$). Data are from the experiments of Harmon *et al.* (1988), Huntington *et al.* (1988). Reynolds and Huntington (1988), Eisemann and Nienaber (1990), Guerino *et al.* (1991), Reynolds *et al.* (1991b), Reynolds and Tyrell (1991), Reynolds *et al.* (1992b) and Harmon *et al.* (1993).

the principal determinant of microbial protein synthesis and therefore duodenal protein flow (Clark *et al.*, 1992). However, comparisons between experiments again show poor correlation, perhaps due to differences between experiments in sample processing and analytical techniques (Maltby, 1993). This confirms the complex interaction between gastrointestinal tissues and the diet, which may be a direct response to changes in gut energy expenditure or patterns or rumen fermentation. Changing propionate supply by intraruminal infusion of propionic acid in steers resulted in higher circulating AA levels and elevated net absorption rates of AAs across the MDV and PDV (Seal and Parker, 1991, 1996). Subsequent experiments with cannulated sheep infused with propionate (Seal *et al.*, 1993) showed that these responses were not associated with changes in overall nitrogen digestibility or alterations in rumen microbial metabolism and the flow of microbial nitrogen into the small intestine. Circulating AA levels were also increased in these animals, and the results imply that AA utilization within the intestinal tissues had been reduced as a direct response to increased ruminal propionate availability. In contrast, Harmon (1991) showed that increased butyrate availability in animals maintained by intragastric infusion reduced net PDV α-amino N flux. A similar response was observed in steers (Krehbiel *et al.*, 1992), where increasing ruminal butyrate tended to lower PDV α-amino N flux. It was suggested that this was due to increased tissue AA requirements in response to the known trophic stimulus of butyrate (Sakata and Tamate, 1978).

1.4 METABOLISM OF AAs BY LIVER

The liver accounts for 25% of the whole body O_2-uptake while representing only 1.5% of total body mass in lactating cows (Huntington, 1990). This high-energy activity is linked to a high rate of AA metabolism. The studies of Bergman and co-workers demonstrated both net and unidirectional metabolism of individual AAs by liver of sheep, as well as the impact of fasting, acidosis and hormonal manipulation on splanchnic AA

metabolism (Bergman and Heitmann, 1978; Bergman and Peel, 1984; Bergman, 1989). Most AAs are removed on a net basis by the liver, reducing the availability to other body tissues of AAs absorbed across PDV. Exceptions include the branched-chain AAs and glutamate, which are usually released by liver. These studies also identified a number of interorgan AA cycles in which the liver is a central player. Participants include glutamate and glutamine, glycine and alanine and also arginine, ornithine and citrulline. These cycles shuttle carbon and nitrogen between the liver (as a site of glucose, protein and urea synthesis) and peripheral tissues (as sites of glucose utilization and protein synthesis and turnover).

A vital hepatic function in all mammals is detoxification of ammonia, produced from bacterial fermentation or metabolism within the gastrointestinal tract and peripheral tissues and also arising from the catabolism of AAs, nucleic acids and other nitrogenous compounds (Huntington, 1986). The main fates of the removed ammonia are, in sheep, directed towards synthesis of urea and to a lesser extent, glutamine (Lobley *et al.*, 1995, 1996). From several observations in cattle hepatic removal of ammonia N only accounted for 0.5-0.6 of urea-N output (see Lobley *et al.*, 1995) and Reynolds (1992) suggested that transamination of AAs contributes nitrogen to ureagenesis via glutamate and aspartate. In growing cattle fed diets high in rumen-soluble nitrogen, excessive ammonia absorption has been associated with increased net removal of AAs by liver (Huntington, 1989; Reynolds *et al.*, 1991c). This response was attributed to an increase in urea cycle requirements for cytosolic aspartate and glutamate, which cannot be met by mitochondrial capture of ammonia as glutamate (Reynolds, 1992); however, other studies failed to confirm this effect of ammonia uptake on liver AA removal (Reynolds *et al.*, 1992a; Maltby, 1993). More recently, Wessels and Titgemeyer (1998) concluded that the abomasal infusion of aspartate in steers had no effect on N balance.

A second aspect of liver N metabolism in ruminants reflects the constant demand of

liver gluconeogenesis for glucose precursors. Bergman *et al.* (1970) have shown that glucose production by the sheep liver accounts for about 85% of the body's glucose turnover. Ruminants absorb relatively little dietary carbohydrate as glucose; therefore, liver synthesis must meet most, if not all, body glucose requirements. Under most conditions ruminant animals have an obligate need to synthesize glucose. In the fed animal propionate (32-73%) and AAs (6-35%) are the predominant precursors (Seal and Reynolds, 1993), but during fasting other sources such as glycerol and lactate become more important (Bergman *et al.*, 1968; Lomax and Baird, 1983). It is known that AAs contribute carbon to liver glucose synthesis, but precise measurements of glucose carbon originating from AAs is lacking. Many AAs must enter the tricarboxylic acid (TCA) cycle to be used for gluconeogenesis; therefore a loss of carbon as CO_2 accompanies their contribution to glucose. Bergman and colleagues demonstrated that 11 to 18% of glucose flux (approximately equivalent to 50 to 100% of glucose oxidation; Lobley, 1991) could be attributed to synthesis from the major gluconeogenic AAs, glutamate, glutamine, and alanine, with lesser contributions from aspartate, serine, and glycine (Wolff and Bergman, 1972; Heitmann and Bergman, 1978). The involvement of the gluconeogenic EAAs, such as threonine, seems to be relatively trivial (Egan *et al.*, 1983). The exact contribution of AAs to glucose synthesis has been difficult to determine precisely, in part due to the problems of label randomization in carbon exchange studies using ^{14}C-labelled AAs, but total gluconeogenic AA removal maximally accounted for 30% of liver glucose release in sheep at maintenance (Wolff and Bergman, 1972). Table 1.3 shows maximal net contributions of precursors released by ruminant liver with different animals and diets (Seal and Reynolds, 1993). Danfær *et al.* (1995) suggested that AA use for gluconeogenesis by the liver is highly variable depending on the availability of gluconeogenic nutrients and maybe also on hormonal concentrations.

Dietary changes may affect liver metabolism of AAs. In beef steers fed alfalfa

Table 1.3 The percentage contribution of various glucogenic precursors to the net hepatic release of glucose (After Seal and Reynolds, 1993)

Animal	Diet	Glucose Release (mmol/h)	Percentage of net glucose release from			Reference
			Propionate	AAs	L-Lactate	
Mature sheep	Lucerne	21.7	40.3	29.0		Bergman *et al.* (1970); Bergman and Wolff (1971); Wolff and Bergman (1972)
Growing steers	Concentrate	180	72.8	12.1	13.1	Huntington and Eisemann (1988)
Growing steers	Fescue hay	147	46.3	21.4	10.9	Harmon *et al.* (1991)
Growing steers	Concentrate					
	Low intake, saline	123	58.5	11.6	10.7	Reynolds *et al.* (1992b); Reynolds *et al.* (1992a)
	Low intake+GRF[1]	105	56.1	5.8	14.2	
	High intake, saline	245	53.9	15.1	1.4	
	High intake + GRF	237	67.3	13.2	1.6	
Growing steers	Concentrate:Hay	116	52.6	28.4	44.0	Krehbiel *et al.* (1992)
Growing steers	Lucerne	227	42.5	19.6	15.0	Harmon *et al.* (1993)
Mature steers	Concentrate	190	32.2	20.0	9.9	Reynolds *et al.* (1992c)
Growing heifers	Lucerne					
	Low intake	119	52.2	32.4	7.0	Reynolds *et al.* (1991b); Reynolds *et al.* (1993)
	High intake	263	59.9	35.3	9.3	
	Concentrate					
	Low intake	116	46.6	23.9	9.1	
	High intake	285	59.8	24.6	1.9	
Mature heifers	Forage	129	75.8	26.0	8.2	Reynolds and Tyrrell (1991); Reynolds *et al.* (1991a)
	Concentrate	214	59.6	23.5	6.6	
Lactating cows	*Ad libitum*	713	55.4	16.5	17.5	Reynolds *et al.* (1988)

[1] GRF: growth hormone releasing factor.

containing 17% crude protein or concentrate containing 12% crude protein at equal metabolizable energy intake, net PDV absorption of α-amino N was similar between the two diets, but net PDV absorption and liver removal of ammonia more than doubled when steers were fed alfalfa compared with concentrate. This increase in liver removal of ammonia resulted in a doubling of liver urea production and a threefold increase in liver α-amino N removal, such that total splanchnic release of α-amino N to other body tissues was markedly reduced on the alfalfa diet (Huntington, 1990). Similar trends were evident in beef heifers fed isonitrogenous diets differing in forage:concentrate ratio at equal metabolizable energy intakes (Reynolds *et al.*, 1991c). Heifers fed the high forage diet, which had a lower metabolizable energy density, consumed and digested more N. As a consequence of greater N digestion, heifers fed the high forage diet absorbed more ammonia and their livers produced more urea and removed more α-amino N than when fed the high concentrate diet. In addition, the livers of heifers fed the high forage diet released less glutamate.

Liver AA utilization may also support ureagenesis by providing a source of energy. When considered in isolation, the urea cycle requires four high-energy phosphate bonds. In dairy cattle, the reduced net energy available from high-protein diets has been attributed to increased heat production resulting from urea production and recycling via the digestive tract (Tyrrell *et al.*, 1970; Oldham, 1984). The true cost of increased ureagenesis and the reasons for decreased energy availability in ruminants fed excess protein appears more complex than an increase in liver energy requirement *per se* and may relate to increased AA utilization or effects on gluconeogenesis and exchanges of carbon via the TCA cycle (Prior *et al*, 1970).

There is growing interest in the role of plasma proteins and peptides as sources of AAs for extrahepatic tissues i.e. although it may appear that liver is removing AAs from tissue use, the AAs may be supplied to tissue as peptide or protein synthesized in the liver.

Recently Taniguchi *et al.* (1995) reported splanchnic release of α-amino N in beef steers which accounted for only 0.3-0.6 of body N retention. Thus it would appear that the liver must convert some of the free EAA to bound forms for peripheral tissue metabolism. One obvious source is the export plasma proteins that in man account for 30% of hepatic protein synthesis (Ballmer *et al.*, 1990) and albumin contributes 30-35% of total hepatic protein synthesis in sheep (Connell *et al.*, 1997). It is possible, of course, that lower molecular weight peptides may be involved also in this transfer of EAA (MacRae *et al.*, 1995).

1.5 UTILIZATION OF AAs BY EXTRAHEPATIC TISSUES

Metabolism in the gastrointestinal tract (MacRae *et al.*, 1997) and liver (Lobley and Milano, 1997) has been shown to be important in determining the amount and pattern of AAs available post-hepatically for mammary gland utilization as well as for muscle and skin. Comparison of data on protein synthesis in the various major organs of the ruminant shows that muscle and skin contribute 16-22% and 8-16% of total body synthesis while their protein mass constitutes 50% and 20% of total body, respectively, indicating lower metabolism of AAs in these sites (MacRae, *et al.*, 1995). However, muscle tissues modify the AA profile (Teleni, 1993) and in early lactation the mobilization of AAs from the muscles can be substantial (Andrew *et al.*, 1994). Muscle, like the liver, appears to participate, in part, as a 'short term buffer' for the AA supply and thus helps to maintain homeostasis in the organism, even though AA metabolism is significant in this tissue (see Lescoat *et al.*, 1996). This implies that the pattern of AA release differs largely from that of uptake, as observed previously in rats (Chang and Goldberg, 1978).

The lactating mammary gland is a site of considerable metabolic turnover, with high rates of protein synthesis and degradation and continuous AA catabolism and anabolism. High rates of metabolism by the non-mammary tissues have been suggested as one reason

for the low efficiency (approximately 20%) of conversion of dietary N into milk protein (MacRae *et al.*, 1995). Thus, ways to reduce these losses could lead to a larger proportion of AAs being partitioned to the mammary gland for milk protein synthesis. However, studies in dairy goats and cows indicate that total protein synthesis in the lactating mammary gland is 1.3-2.5-fold the rate of milk protein secretion (Oddy *et al.* 1988; Champredon *et al.*, 1990; Baracos *et al.*, 1991; Bequette *et al.*, 1996). These results suggest that once AAs are extracted by the mammary gland, considerable losses in efficiency of conversion of these AAs into milk protein also occur (i.e. net mammary AA uptake greater than milk AA outputs). In a subsequent experiment with dairy cows, Metcalf (1994, unpublished data; cited by Bequette *et al.*, 1998) demonstrated the relationship (2.5:1) between total protein synthesis and milk protein secretion when he employed the [1-^{13}C] leucine kinetic model across the mammary gland and found that jugular infusion (208 g/d) of a mixture of 10 EAA increased total protein synthesis in the mammary gland according to a fixed (2.5:1) relation with milk protein output, which was increased by 92 g/d.

As early as 1966, it was shown that mammary arteriovenous (A-V) AA uptake from plasma by the lactating mammary gland was sufficient to provide N in mammary-synthesized milk proteins of the goat (Mepham and Linzell, 1966). When quantities of individual AA were considered, EAA uptake was approximately equal to that secreted in milk. Mammary uptake of NEAA was variable, with some AA extracted in quantities much greater than that secreted in milk. The net extraction rate, by the gland from blood plasma, averaged 36% for EAA, the highest being for methionine at 77% (Mepham and Linzell, 1966). In contrast NEAA extraction averaged only 24%. Some of the theories put forward regarding mammary AA metabolism were that excess uptake of some EAA might be transaminated within the gland to supply the requirements for those NEAA which were not extracted in sufficient quantities to supply output in milk (see Figure 1.3). On average, 25% of plasma flux (irreversible loss rate) was partitioned to the mammary gland in mid-

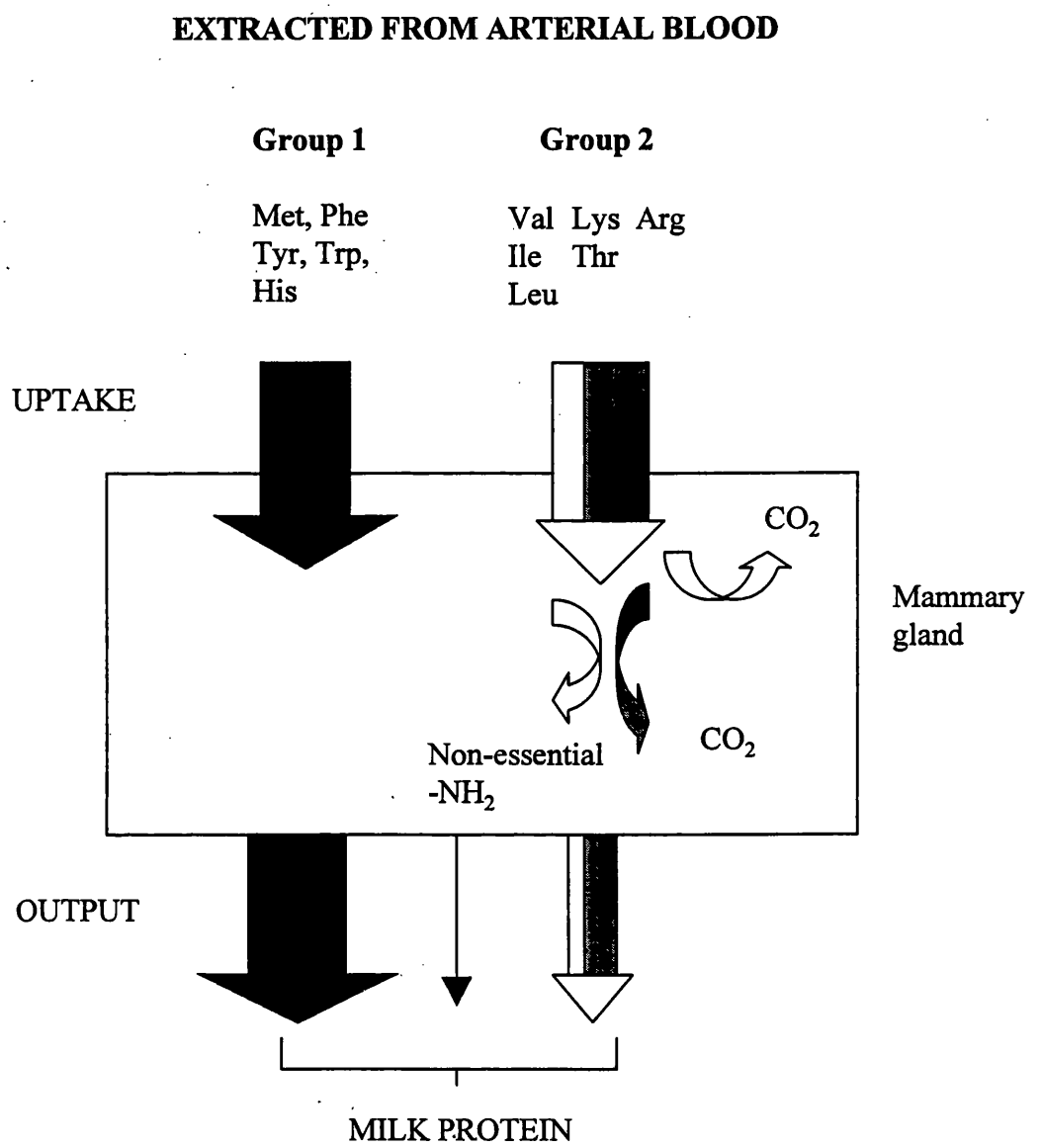

Figure 1.3 A simplified representation of essential amino acid (EAA) metabolism within the mammary gland (modified from Mepham, 1979)

lactation (week 16) goats in a study using a mixture of [U-^{13}C] labelled AA as a tracer, with values for histidine, serine, phenylalanine and alanine being less than 20%, arginine, threonine, tyrosine and leucine from 20 to 30% and proline, isoleucine, lysine and valine from 30 to 40% (Bequette *et al.*, 1997).

Numerous studies in dairy animals, under basal feeding conditions and when given supplemental sources of AAs, have demonstrated that for several AAs (leucine, valine, isoleucine, arginine, lysine, threonine, histidine) net extractions by the mammary gland are in excess of milk protein outputs (Bickerstaffe *et al.*, 1974; Guinard and Rulquin, 1994; Metcalf *et al.*, 1994, 1996b). Using radio-labelled AAs, Roets *et al.* (1979, 1983) found that 36% of leucine, 0% of phenylalanine, 15% of threonine, 0.3% of tyrosine and 30% of valine taken up by the gland were oxidized to CO_2, and 10% of valine and 8% of leucine found in mammary venous blood had been reversibly transaminated, i.e. converted to another AA, and then back to the original AA, during a single pass through the gland. In studies conducted with perfused glands (Verbeke *et al.*, 1968; Roets *et al.*, 1974) and mammary explants (Wohlt *et al.*, 1977) these AAs were found to be catabolized along pathways similar to those found in other tissues, utilized in the synthesis of NEAA and specialized compounds, as well as being used for casein synthesis. Methionine could supply S for the *de novo* mammary synthesis of cysteine. However, in recent experiments conducted in New Zealand, this contribution in the goat was found to be small (Lee *et al.*, 1997). The net uptake of methionine rarely exceeds milk outputs, even under supplemental conditions (Guinard and Rulquin, 1995); thus, transport of methionine into the mammary cell and the contribution of its S group via trans-sulfuration could be the limiting step in the synthesis of cysteine (see Bequette and Backwell, 1997). Tyrosine, also, is not extracted in adequate quantities. It is assumed, but not proven *in vivo*, that this deficit can be made-up through the mammary conversion of phenylalanine to tyrosine by the enzyme phenylalanine hydroxylase (Jorgensen and Larson, 1968).

The branched-chain AAs are taken up in excess by the mammary gland and would appear to be candidates to contribute some of this N because of their involvements in transamination reactions. Arginine, also extracted in quantities three times greater than its output in milk, and ornithine and citrulline, which are not incorporated into milk protein but are extracted in large quantities, have been shown to contribute 20% of the proline residues in casein via a partial urea cycle (Verbeke *et al.*, 1968; Roets *et al.*, 1974). The uptakes of both tyrosine and cysteine from blood can be increased to match or exceed their outputs in milk protein when dairy cows are given additional dietary protein or post-ruminal infusion of casein (Guinard *et al.*, 1994; Metcalf *et al.*, 1994, 1996a). Despite the increased arterial supply of AAs, the extractions of serine, proline, glutamine, glutamate, glycine and aspartate, although increased, were still insufficient to balance their outputs in milk protein. Many of these AA can apparently be synthesized from the C skeletons of some of the EAA (Wohlt *et al.*, 1977).

Recent studies on a simple A-V difference basis (Metcalf, *et al.*, 1994; Shennan and McNeillie, 1994; Backwell, *et al.*, 1994, 1996) have shown that the lactating mammary gland is able to utilize plasma-derived dipeptides for milk protein synthesis. However, Shennan *et al.* (1998) demonstrated that the uptake of the hydrolysis resistant dipeptides D-[^{3}H]phenylalanine-L-glutamine and D-[^{3}H]phenylalanine-L-glutamate by the rat mammary gland was low and concomitant addition of L-leucine-L-alanine (50 mM) had no effect on the clearance of either labelled dipeptide suggesting that the small, albeit significant, uptake of the dipeptides is not via a high affinity peptide transporter (PepT1/PepT2), which led them to conclude that the peptides were probably being hydrolysed extracellularly followed by the uptake of free anionic AAs via the mammary tissue high affinity, Na^+-dependent anionic AA carrier.

1.6 LIMITING AAs FOR MILK PROTEIN SECRETION

Absorbed AAs are the building blocks for the synthesis of milk proteins. AAs are joined together according to a predetermined genetic code during *de novo* synthesis of proteins i.e. the AA composition of a protein is the same every time it is synthesized. As a result, AAs are required in proportion to one another. Research with non-ruminants indicates that when EAA are absorbed in the correct profile (i.e. all are equally limiting), their efficiency of use for protein synthesis is maximized (Heger and Frydrych, 1989). In contrast, use of absorbed AAs for protein synthesis is less than maximum when they are absorbed in a profile that is less than ideal. In this case, it is the quantity of the first-limiting AA (the AA in shortest supply relative to requirements) that determines the extent of protein synthesis.

1.6.1 Methods to identify limiting AAs

Several methods have been used to identify limiting AAs, and there are considered below. However, it should be recognized that the AAs identified as limiting in any particular study will be determined by the combination of the nutritional and physiological status of the animals and the experimental techniques used.

Prediction of limiting AAs in ruminants is often derived from theoretical calculations based on comparisons of AA profiles of animal products and digesta. Factorial approaches have been used in an attempt to identify the AAs that are most likely to limit milk production with grass silage diets. For diets based on grass silage and cereal supplements, Thomas and Chamberlain (1982) calculated the average composition of the mixture of indispensable AAs absorbed, values for the AA composition of carcass tissue and milk, using the values for the AA composition of duodenal digesta. They speculated that methionine, lysine and threonine in the growing animal and methionine, lysine and valine in the lactating animal are the limiting AAs for protein synthesis, though it should be stressed that such ratios must be interpreted with caution since they do not allow for

differences between AAs in their efficiency of utilization in the body tissues. Using a diet of grass silage and a cereal supplement containing feather meal, Choung and Chamberlain (1995) set out to obtain an indication of the likely order of limitation within the group of four AAs (methionine, lysine, tryptophan and histidine). The amounts of the various protein fractions reaching the duodenum of cows fed grass silage supplemented with feather meal were calculated, using the factors in AFRC (1992) and assuming a value of 0.20 for the ruminal degradation of feather meal (Chamberlain *et al.* 1992). The contribution of each fraction and its AA composition were used to calculate the AA composition of the total digesta. The concentrations of the four AAs in duodenal digesta, expressed relative to their concentrations in milk protein, indicate histidine to be clearly first-limiting (Table 1.4).

Mammary A-V difference studies can be useful in understanding the contribution of individual blood AAs to milk protein synthesis because of the ease of measurement of the final product in milk. One criterion often applied to identify potentially limiting AAs at the mammary gland is to assume that the AAs that are in shortest supply relative to demand (and therefore potentially limiting) will be extracted with highest efficiency from the arterial blood. This would imply that methionine, phenylalanine, leucine and threonine are the most limiting AAs (Oldham, 1980). Clark *et al.* (1977) and Spires *et al.* (1975) also suggested that methionine and lysine were the most critical AAs with a larger percentage of extraction. Another similar criterion is to assume that AAs in shortest supply relative to demand will undergo minimal metabolism in the gland. Using this method, measuring AA uptakes and calculating the theoretical milk protein production, led Derrig *et al.* (1974) to place phenylalanine and methionine as the first-limiting and second-limiting AAs, respectively.

Many experiments have been conducted in which plasma AA profiles have been determined in order to define those AAs that limit milk production. Broderick *et al.* (1974)

Table 1.4 The concentrations (relative to those in milk protein[1]) of histidine, methionine, tryptophan and lysine in the protein fractions reaching the duodenum and the predicted value for whole duodenal digesta[2] for animals consuming grass silage and barley and feather meal supplement (After Choung and Chamberlain, 1995)

	Microbial[3]	**Feather meal[4]**	**Silage[5]**	**Barley[5]**	**Whole digesta**
Histidine	0.65	0.16	0.62	1.00	0.50
Methionine	0.95	0.25	0.62	0.73	0.68
Tryptophan[6]	1.00	0.28	1.07	0.78	0.79
Lysine	0.98	0.17	0.43	0.53	0.63

[1] From Kaufmann (1980).

[2] See text for details of the calculation.

[3] From Storm and Ørskov (1983).

[4] From Chamberlain *et al.* (1992).

[5] From Thomas *et al.* (1980).

[6] Values for feather meal barley from Buttery *et al.* (1978) and for silage from Ohshima and McDonald (1979).

suggested that an observed elevation of plasma methionine, lysine and valine concentrations that were due to increases in dietary protein and accompanied by increased milk yield implicated these AAs as being limiting. Bouchard and Conrad (1973) classified 150 aminograms based on the assumptions that a deficient AA will decrease in plasma while other EAA increase and that improved availability of a deficient AA will cause it to increase in plasma while other EAA drop. They determined threonine and methionine to be most limiting to milk yield. Choung and Chamberlain (1992a) also comparing the changes of plasma AA concentrations, suggested that methionine, tryptophan and phenylalanine were in shortest supply relative to demand when they compared responses of milk production to abomasal infusions of casein, soya protein isolate (SPI) and fishmeal-based product on a grass silage-based diet. A major problem with using plasma AA variations to predict limitation is that this pool is very small relative to total body pool size and, thus, is easily influenced by a number of processes.

The magnitude of oxidation of AAs is related to the composition of AAs absorbed and to the supply of the main limiting EAA for milk protein synthesis (Rulquin and Journet, 1987). AAs derived from dietary protein and mobilization of body protein can be oxidized to CO_2 at different rates for each AA after their release into the blood. Whenever carbon from an AA is oxidized to CO_2, that AA is irretrievably lost as a precursor for protein synthesis. Glutamate, aspartate, alanine and glutamine were extensively oxidized in the cow (Black *et al.*, 1990) and histidine was the AA least oxidized when rats (Aguilar *et al.*, 1972) and cows (Black *et al.*, 1990) were fed several [^{14}C] AAs. Limited oxidation of histidine would favour more extensive reutilization of it for protein synthesis (Irwin and Hegsted, 1971). The percent oxidation of AAs provides a basis for evaluating nutrient partitioning, especially when the efficiency of utilization of AAs for protein synthesis is the primary interest. Recently, Mnilk *et al.* (1996) estimated the efficiency of utilization of absorbed lysine in growing pigs given AA-deficient diets, measuring the rate of lysine

oxidation, to be 0.85. $^{14}CO_2$ recovery may also be useful for confirming the identification of the most limiting AA for production by measuring [l-^{14}C] leucine oxidation in control cows and then administering in sequential experiments each of the potentially limiting AAs. It would then be expected that the most limiting AA would have the greatest effect in reducing leucine oxidation and increasing its recovery in protein (Black *et al.*, 1990).

Although tedious, one method to decipher which AA is most limiting is to increase or decrease the supply of each one separately. Schwab *et al.* (1976) abomasally infused various combinations of EAA into cows fed 65 to 84% of their CP requirement. Milk N secretion was improved with lysine but not methionine infusion; methionine plus lysine induced a further response. Fraser *et al.* (1991) concluded that lysine, methionine and histidine were the first, second and third limiting AAs in the lactating cow, where casein was the sole protein source when lysine, methionine and histidine, respectively, were removed individually from a EAA mixture.

1.6.2 Effect of supplements of limiting AAs

Rumen-protected AAs have been used to supplement diets, the most widely used to date being methionine and lysine because of the commercial availability of these AAs and the fact that they are often indicated to be first limiting AAs (e.g. Schwab *et al.*, 1992a, b). Feeding rumen-protected methionine in the diets of lactating dairy cows has increased milk protein content in some experiments (Illg *et al.*, 1987; Rulquin and Delaby, 1997, 1994a,b) and has also increased yields of both milk and protein (Robert *et al.*, 1994). However, responses to feeding individual AAs are not consistent (Depeters and Cant, 1992). In other studies, protected methionine did not influence milk yield or the protein content of milk (e.g. Papas *et al.*, 1984 a, b; Yang *et al.*, 1986; Rogers *et al.*, 1989). Responses of dairy cows can be greater when provided with rumen-protected methionine and lysine combinations (Rogers *et al.*, 1987, Xu *et al.*, 1998) but, again, responses are inconsistent

(e.g. Guillaume *et al.*, 1991; Piepenbrink *et al.*, 1996). Differences in response to protected AAs are likely to be due to differences in the quantity and proportion of AAs in the microbial and dietary protein digested and absorbed from the small intestine on the various experimental diets. The inconsistency of responses to supplemental protected methionine and lysine may indicate that these acids are not always clearly first-limiting.

When AAs are supplied by infusion techniques, a similar picture also emerges with respect to methionine and lysine with responses in some experiments (Schwab *et al.*, 1976; Rogers and McLeay, 1977; Rulquin, 1987; Pisulewski *et al.*, 1996) but not in others (Fisher, 1969; Champredon and Pion, 1979; Chamberlain and Thomas, 1982; Wong, 1984; Choung and Chamberlain, 1992a; Mabjeesh *et al.*, 1998). Again the probable explanation is that these two acids are not always clearly limiting. In this content, it is interesting to note that Fraser *et al.* (1991) in cows given abomasal infusions of AA mixtures, showed significant reductions in milk yield and milk protein yield from the removal of lysine and histidine, but not from the removal of methionine the mixture of AAs infused.

It has been suggested from time to time, that NEAA supply may limit milk production (Halfpenny *et al.*, 1969). However, infusion data of Oldham *et al.* (1984) using aspartic acid or of Fraser *et al.* (1991) with several NEAA did not support the idea that NEAA could be limiting. On the other hand, increasing proline supply has been reported to increase fat yield (Bruckental *et al.*, 1991); it could have been associated with a modification of the mammary metabolism of arginine but the exact mechanism is not yet understood although it should be noted that the dose level of proline, or 80 g/d, was very high in physiological terms. Glutamine has been suggested as potentially limiting because of its low blood level in early lactation (Meijer *et al.*, 1992) but supplementation with glutamine in a subsequent experiment did not affect milk production (Meijer *et al.*, 1995).

Responses to AA supplementation may be influenced by the animal's lactational stage. Some studies of lysine supplementation suggested that the lactational response

depends not only on the basal supply of limiting AA, but also on the stage of lactation, the increase in secretion of milk protein and improvements in milk yield of dairy cows at peak lactation being greater in response to the duodenal infusion of lysine (King *et al.*, 1991; Schwab *et al.*, 1992b) whereas the volume of milk did not improve in response to added lysine at later stages of lactation (week 8 to 12, 17 to 21, and 27 to 31; Schwab *et al.*, 1992b).

1.6.3 Overall conclusions

Lysine and methionine have long been regarded as potentially rate-limiting for milk production, either alone or in combination (Derrig *et al.*, 1974; Spires *et al.*, 1975; Schwab *et al.*, 1976; Clark *et al.*, 1977; Rogers and McLeay, 1977; Casper *et al.*, 1987; Rulquin, 1987; Schwab, 1996; Rulquin and Vérité, 1993; Sloan, 1997). However, these two AAs are not always the most limiting acids for milk production. Apart from the earlier studies of Schwab *et al.* (1976), in which histidine appeared to stimulate milk yield, this AA has not been widely implicated as a limiting AA for lactation. However, work by Schingoethe and his group indicated that histidine may be first-limiting (Yang *et al.*, 1986; Casper *et al.*, 1987; Illg *et al.*, 1987; Schingoethe *et al.*, 1988) and Robinson *et al.* (1998) also suggested that histidine and isoleucine may be limiting acids.

Another consideration in studies to investigate the response to supplementation of AAs is which technique we should use to supply the AAs. Feeding rumen-protected AAs or supplementation of AA into the small intestine has the uncertainty of the absorbability of the supplementary AAs. AAs are absorbed at different rates and the disappearance is greater for essential than non-essential AA from the small intestine of sheep (Coelho da Silva *et al.*, 1972b; MacRae and Ulyatt, 1974; Armstrong *et al.* 1977; Christiansen and Webb, 1990a) and cattle (Armstrong *et al.*, 1977; Christiansen and Webb, 1990b). Additionally, between 30 and 80% of AAs disappear before reaching the portal vein owing

to metabolism of AAs by the gut. On the other hand, the intravenous route avoids uncertainty over the absorbability of the supplementary AAs but has the drawback that it may be difficult to extrapolate to the response to be expected from dietary addition of AAs.

1.7 EFFICIENCY OF USE OF AAs FOR MILK PROTEIN SECRETION

The efficiency of converting dietary N into milk protein output is poor at 20% to 30% at most. Milk protein content and yield can be increased by AA supplementation; however, the responses attained are often unpredictable and are considerably less than would be predicted by current feeding schemes for dairy cows (Madsen, 1985; NRC, 1985; Vérité *et al.*, 1987: AFRC, 1992). The modern protein evaluation systems (Madsen, 1985; NRC, 1985; Vérité *et al.*, 1987: AFRC, 1992) assume fixed values for the efficiency of AA utilization for milk protein production. Differences from 0.64 to 0.75 occur in these fixed values for the various systems. Differences between the schemes can partly be explained by differences in estimating the protein value of feedstuffs. Differences might also be caused by differences in feedstuffs, milk production level and experimental design (Hvelplund and Madsen, 1990; Van Straalen *et al.*, 1994). Jarrige (1989) indicated that the estimation of true protein requirement derived from feeding trials or nitrogen-balance experiments (INRA, 1978) can differ from true protein-requirements estimated by a factorial approach (ARC, 1980, 1984; NRC, 1985).

A fixed factor (0.64 to 0.75 on an incremental basis) has been adopted despite evidence that the observed response to supplemental AAs is curvilinear [i.e., diminishing partial efficiencies (Wilde *et al.*, 1989; Molenaar *et al.*, 1992; Guinard and Rulquin, 1995)]. For example, Guinard *et al.* (1994) observed that the conversion of absorbed EAA into milk protein decreased from 0.44 to 0.34 as levels of duodenal infusion of casein increased up to 762 g/d. A large proportion of this loss occurred across the mammary gland such that the udder conversion of EAA decreased from 0.88 to 0.49 with infusion. The

current protein feeding schemes would not have predicted these responses. Thus, in the future, the dynamics of postabsorptive metabolic events, especially those governing the availability of AAs to the mammary gland and the utilization of AAs for milk protein synthesis, will need to be represented so that responses in milk protein output from changes in dietary nutrient availability can be predicted more accurately.

Due to methodological problems, our knowledge of AA utilization is still fragmentary. Values for efficiency are valid only when protein is limiting and when the use of AAs is not constrained by other features of the diet. There are some factors of importance that may influence the amount of AAs available for milk protein synthesis. A lack of supply (compared with demand) of non-amino nutrients whose role can be substituted by AAs, can have a bearing on the way in which even an ideally balanced AA mixture is used. Thus, the supply of total energy-yielding nutrients if inadequate, might influence the efficiency of use of an ideally balanced AA mixture. The efficiency of AA use can be expected to fall when the ratio ME : metabolizable protein (MP) falls below a certain level (e.g. 96 MJ ME/kg MP for cows producing 40 kg/d milk and allowing for body fat loss; Oldham, 1994). Amino acids will also be needed for milk lactose or fat synthesis if there is an absolute requirement for AAs to be used for glucose production, fat membrane formation, or precursor synthesis (Dado *et al.*, 1993). If lactational performance is truly limited by AA supply, the net yield of glucose from AA catabolism can only be small because the available AA supply is being used for protein synthesis with a relatively high efficiency. Thus, Webster (1992) calculated that the conversion of metabolizable AAs, by catabolism into glucose, above maintenance requirements for protein, would, at a maximum, be capable of yielding only sufficient glucose to supply 17% of the observed lactose secretion.

Although there is no measurement in production studies, the mobilization or retention of body protein might influence the AA utilization for milk production (NRC,

1985; Tamminga *et al.*, 1994). Tissue protein may be subject to net catabolism whilst milk protein secretion progresses; this is usually referred to as net tissue protein mobilization to support lactation. Under extreme circumstances around 20% of fully replete maternal protein mass might be available for mobilization (Botts *et al.*, 1978) although under more conventional production circumstances such losses might not be so great (Gibb *et al.*, 1992). Whitelaw *et al.* (1986) demonstrated that under severely protein-limiting conditions for lactational performance, supplements of casein given by abomasal infusion were partitioned approximately equally between enhanced protein secretion in milk and nitrogen retention at low levels of casein supplementation. There is also no information available on the amount of absorbed AAs used for specific body processes, for example the use of methionine in formation of lipoproteins, which play a role in transport of lipids from the liver (Daugaard, 1978).

Responses, in milk protein output, to the infusion of proteins into the abomasum (usually casein) have frequently been found to conform to some sort of diminishing return pattern (Figure. 1.4). In every case shown in Figure 1.4 a small increase in abomasal casein supply yielded a high fractional rate of return in terms of enhanced milk protein output, although incremental responses were not maintained at a high rate across the full range of supplement levels of casein, because in the different circumstances other factors would have come into play to limit performance. In each of the experiments represented in Figure 1.4, the responses to casein infusion over at least part of the supplemented range were associated with an increased output of energy in milk which far outweighed the additional ME supplied as casein (or casein plus glucose, for Ørskov *et al.*, 1977). Such responses imply a repartitioning of nutrient use between body tissues, milk synthesis and catabolism. Choung and Chamberlain (1993) concluded that effects of protein supply on the efficiency of use of ME for milk synthesis were unlikely and that a repartitioning effect on the use of energy-yielding substrates was more likely. The regulation of such a response might be

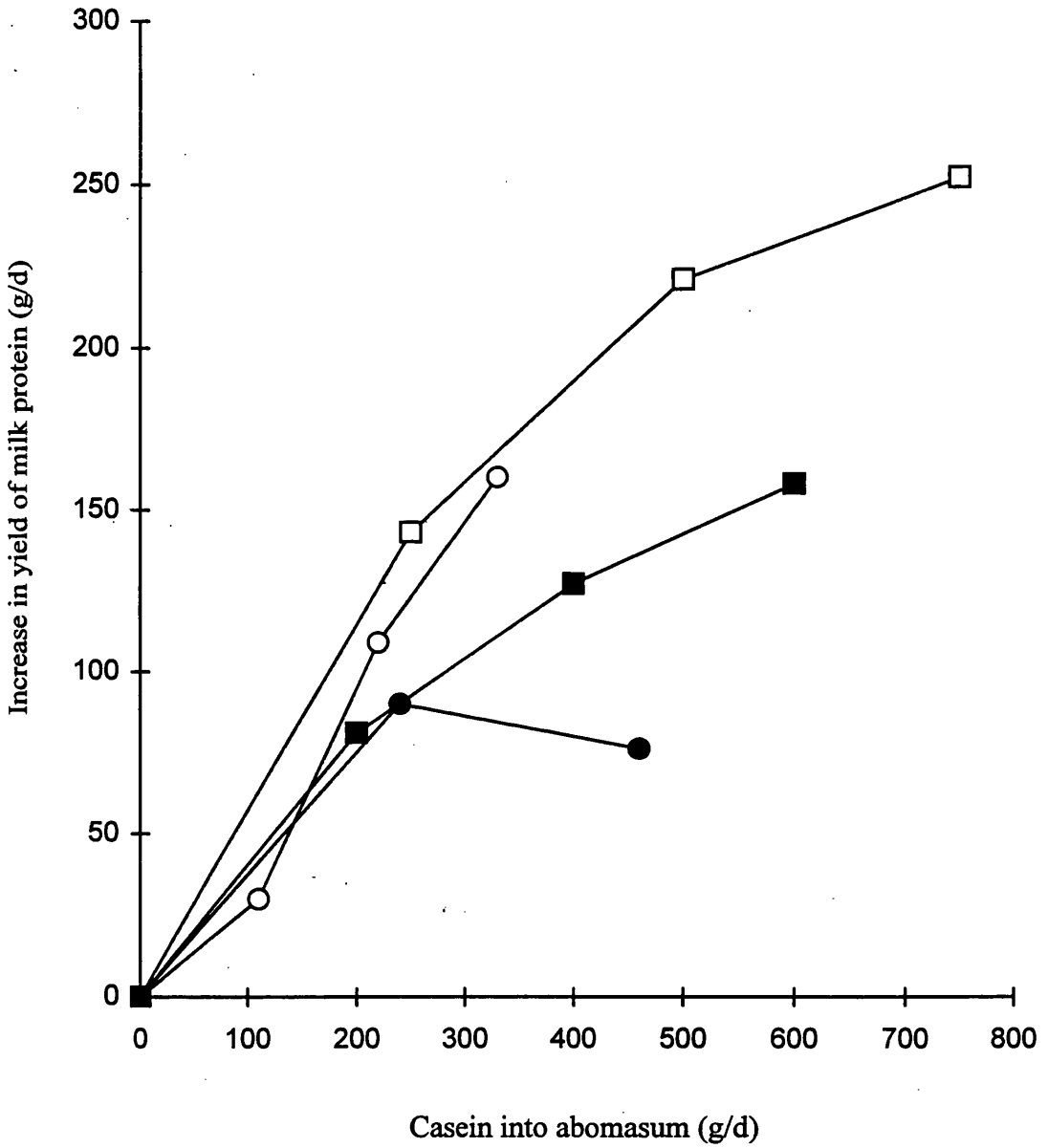

Figure 1.4 Patterns of response in milk protein output to the infusion of casein directly into the abomasum of dairy cows (Data from: Ørskov *et al*., 1977, □; König *et al*., 1984, ●; Whitelaw *et al*., 1986, ■; Choung and Chamberlain, 1993,○; from Oldham, 1994)

associated with increases in circulating levels of growth hormone, which can follow the enhancement of protein status in ruminants (Oldham *et al.*, 1978, 1982) sometimes also with reduced insulin concentrations in the circulating blood (Choung and Chamberlain, 1992b).

Large differences in efficiency of use of AAs exist for individual AAs when efficiency is expressed in terms of AA in milk/AA at duodenum. Low values (0.1 to 0.2) are observed for some NEAA such as aspartic acid, alanine, glycine, arginine which are oxidized to a large extent or used for gluconeogenesis. In contrast, high values are observed for AA that are formed in milk such as proline; the efficiencies of utilization for EAA are 20% (0.39 vs. 0.33) higher than for NEAA (Rulquin, unpublished; cited by Rulquin and Journet, 1987). But a large range for efficiencies seems to occur for the same AA according to the characteristics of the diet. The ratio AA supply: energy supply markedly affects values of efficiency, the lower values being observed for high protein and low energy supplies. A large range of efficiencies is recorded for some EAA, mainly for methionine, which is very sensitive to the level of energy supply and to the supply of other limiting AAs, mainly lysine (Rulquin, 1986, 1987).

From the discussion above it is clear that many factors can influence the net efficiency of utilization of AAs for milk protein production and that there are many interactions because many influences can be present simultaneously. More research is necessary to quantify the effect of each of the influences mentioned above and the interactions between them in order to determine the possible extent of improvement of utilization of protein for milk protein production.

In the absence of tracer techniques, a prerequisite of studies to examine the efficiency of use of AAs for milk production in the dairy cow is that clear, sizable increases of milk production be obtained in response to supplementation with the first-limiting AA. With diets typical of those used in practice, the margin between first, second

and even third-limiting AAs can be very small (Schwab *et al.* 1976) such that responses in the yield of milk protein to supplements of the first-limiting acid are usually small.

1.8 AIMS AND OBJECTIVES

The limiting AAs for milk production in dairy cows are not only difficult to identify but also vary from experiment to experiment. The order of limitation of AAs would be expected to depend on nutritional, and probably physiological circumstances. However, apart from comparison of diets of markedly different compositions, no systematic relation between type of diet and the identity of the first-limiting AA is apparent in the literature. Because, with most diets used in practice, the margin between the group of limiting AAs in terms of which is first-, second- or third-limiting is likely to be small, the sequence of limitation could be altered by background variations in the ruminal synthesis of microbial protein and degradation of dietary proteins that could occur with diets of similar composition. This makes it difficult to study the utilization of limiting AAs in a systematic way because, with diets typical of those used in practice, reproducible responses between experiments are not usually obtained.

The objectives of this thesis were:

(1) To devise a diet for which (a) the first-limiting (and, if possible, also the 2nd and 3rd-limiting) AAs could be clearly identified , and (b) sizable and reproducible increases in the yield of milk protein could be obtained in response to intravenous or abomasal additions of the first-limiting AA.

(2) To use this diet to obtain information on the efficiency with which the first-limiting AA is used for the secretion of milk protein when it is supplied direct into the abomasum or into peripheral blood.

CHAPTER TWO

MATERIALS AND METHODS

2.1 THE ANIMALS

2.1.1 Preparation of surgically and experimentally modified animals

2.1.1.1 Rumen cannulation

The animal was fasted and denied access to water for 24 hours prior to the operation. Hair was removed from the area of operation by clipping and shaving. Immediately prior to the operation the animal was weighed and anaesthesia was introduced into vertebrae of the animal by injection of local anaesthetic (Lignavet, C-Vet Veterinary Products, Leyland, Lancashire, UK).

The operation was performed with the cow in a standing position. The area of operation was scrubbed with an antiseptic solution. For the insertion of the cannula a 12 cm long incision was made with a scalpel below the transverse processes of the lumbar vertebrae and 10 cm posterior to the last rib of the cow. The muscle layers of the abdominal wall were separated along the direction of travel of their muscle fibres and retracted to expose the peritoneum which was incised to expose the rumen wall. A pouch of ventral rumen wall was exteriorized and clamped completely. The rumen wall, attached layers and skin were stitched together paying special attention to stitching securely at the lower end of the incision. The exteriorized rumen was wrapped with swaps and tape. Ten days later, the clamp and the exteriorized rumen were removed. A bung made from cotton wool in a plastic bag was inserted into the hole and secured with elastic bandage over the area and covered body wall. The bung was left in position for 3 days or, if necessary until the fistula was large enough to allow insertion of a rumen cannula with rolled inner flange (Bar Diamond Lane, Parma, Idaho, USA). The flange of the cannula was introduced into the rumen; the stem was exteriorized through the body wall; and the rubber stopper was

inserted into the cannula.

2.1.1.2 Jugular catheterization

The site of venipuncture on the neck of the animal was sterilized with Hibitane solution of 10% (v/v) Hibitane 5% concentrate (v/v; Zeneca Ltd, Macclesfield, Cheshire, UK), 75% ethanol (v/v) and 15% distilled water (v/v). Intravenous catheterization was performed about 18 hours before the start of infusion and blood sampling. Using a Medicut (14g; Sherwood Medical, Tullamore, Ireland) one of jugular veins was punctured and a stainless steel guide wire (Meadox Ltd, Dunstable, Bedfordshire, UK) was inserted into the lumen of the jugular vein through the Medicut until approximately 20 cm lay in the vein. An indwelling polyethylene catheter (14g x 8 cm; Arrow International Inc, Reading, Berkshire, UK) was inserted into the lumen, being guided by the wire and the catheter clamp was connected with a tubing line (200 cm; Kimal scientific products Ltd, Uxbridge, Middlesex, UK) and attached to the skin using superglue. An adhesive bandage (5 cm, Treatplast; Animalcare, Dunnignton, York, UK) was used to cover and secure the catheter. The catheter was changed every treatment period and the bandage was changed every two days. Patency of catheter was maintained by flushing it with a sterilized sodium citrate saline solution of 0.9% (w/v) sodium chloride and 0.5% (w/v) trisodium citrate every morning before the start of infusion.

2.1.1.3 Insertion of abomasal infusion catheter

A large rumen cannula allowed the positioning by hand of an infusion line into the abomasum, secured in position by a plastic bottle and rubber cuff (Derrig *et al.*, 1974). The pH of withdrawn digesta was routinely checked twice daily to ensure that the catheter was positioned in the abomasum and the cannula and the infusion tubing was flushed with warm water every morning prior to the start of infusion.

2.1.2 Management of animals in the experiments

The cows were housed individually in metabolism stalls with free access to drinking water. Food was given twice a day. Feed intake was determined daily prior to the afternoon milking and the silage offered was adjusted to ensure a refusal of approximately 15% of that offered. The animals were milked each day at 06:00 and 15:00 h.

2.2 EXPERIMENTAL TECHNIQUES

2.2.1 Infusion systems

2.2.1.1. Intravenous infusion

The solution of AA(s) in a volume of 4 litres/day was infused into the jugular vein using a volumetric infusion pump (Smith and Nephew Medicals Ltd, Hull, Humberside, UK). Pumping speed was set to deliver the required volume within a 23 hour period. The solution was changed daily at 10:00 h. The A26 solution administraton set (Avon Medicals, Hythe, Kent, UK) was replaced every treatment period.

2.2.1.2 Intra-abomasal infusion

The solution of histidine in a volume of 4 litres/day was infused into the abomasum using a single-channel peristaltic pump (Watson and Marlow 502S; Watson and Marlow Ltd, Falmouth, Cornwall, UK) with 1.6mm internal diameter tubing (Watson and Marlow Ltd, Falmouth, Cornwall, UK). An infusion period of 23 hours each day was allowed for delivery of all infusates. Silicon rubber tubing at the peristaltic pump head was replaced every treatment period regardless of apparent condition.

2.2.1.3 Preparation of infusates

For all experiments, all AAs administered were as the L-isomers. For abomasal

infusion, histidine was dissolved in distilled water to a final volume of 4 litres. Solutions of AA(s) for intravenous infusion were prepared by the gradual addition of the acids to distilled water, adding NaOH to complete dissolution, adjusted to pH 7.4 and made up to 4 l. All solutions were filtered through a Whatman cellulose-nitrate membrane filter (pore size in 0.45μm; Whatman Ltd, Maidstone, Kent, UK) and sterilized at 121°C for 15 minutes.

2.3 COLLECTION AND PREPARATION OF SAMPLES

2.3.1 Feedstuffs

Silage samples were taken from the silo at least once during each treatment period and a subsample of 1500 g was dried and ground through a 1 mm screen for dry sample analysis. Another subsample of 1500 g was minced through a 100 mm dye (Crypto Ltd, London, UK) and stored frozen for analysis.

Feedstuffs included in the rations were also sampled at least once during each treatment period. Subsamples were dried in triplicate at 60°C in a forced-draught oven for dry matter determination and ground through a 1 mm screen and stored for analysis.

2.3.2 Blood

Samples of blood were withdrawn from either a jugular vein or tail vessel. For jugular samples, blood was taken via the intravenous catheter inserted prior to the start of the infusion. The infusion was stopped and the catheter flushed with sterile citrate saline before withdrawing the blood sample. Blood samples were taken into 20 ml syringes and transferred into 15 ml heparinized tubes which were prepared by distribution of 143unit heparin/100μl and dried at 60°C.

The tail blood samples were withdrawn directly from the tail vessel into heparinized vacutainer tubes (Becton and Dickinson, Vacutainer System Europe, Meylan-Cedix,

France) through a small-bore needle (20G) while animals were restrained in their stalls. Samples were centrifuged at 1500 g for 15 minutes and the blood plasma was removed shortly after collection. Plasma was harvested and stored immediately at -20°C prior to analysis.

2.3.3 Milk

Milk samples were collected from the last four consecutive milkings of each treatment period. Approximately 300 ml of milk were collected into bottles containing 180 mg potassium dichromate (Thompson and Capper Ltd, Runcorn, Cheshire, UK), mixed thoroughly to dissolve the preservative and stored at 4°C until analysed. At the end of each sampling period, milk samples were gradually warmed to 40°C in a water bath to disperse the fat globules. Successive samples from individual cows were then bulked according to milk yield.

2.4 ANALYTICAL METHODS

All routine methods were based on well established techniques that had been validated by technicians in the analytical laboratory.

2.4.1 Dry matter and ash

Dry matter and ash contents in all samples of feedstuffs with the exception of silage, were determined by standard methods. A known weight of sample was oven dried at 100°C to constant weight and the dry matter expressed as a percentage of fresh weight.

For the silage the dry matter content was determined by distillation of a minced silage sample with toluene following the procedure of Dewar and McDonald (1961).

Reagents

1) Toluene, distilled (100 – 120°C) to remove any water

2) Neutral ethanol (25 ml ethanol was titrated against 0.1 M NaOH and then a stock solution prepared according to the values obtained)

Procedure: Twenty-five grams of minced silage were placed in a round-bottomed 1 litre flask and immediately covered with 300 ml of redistilled toluene. The refluxing was continued until the level of water in the receiver did not change over a period of 15 minutes. The water receiver was then disconnected and left for 1 hour, after which the volume of water was read and toluene removed using a Pasteur pipette. Ten ml of water were pipetted into a 25 ml volumetric flask and diluted to volume. The acidity of the water was measured using 10 ml of the diluted liquid. Forty ml of neutral ethanol were added and titrated with 0.1 M NaOH using phenolphthalein as an indicator.

Calculation

Volume correction = 2.5 x V/10 x T x 0.0055 ml = 0.001375VT ml

At 20°C wt of water = 0.998V (1 – 0.01375T)

wt of silage DM = W – 0.998 (1 – 0.001375T)

% of Dry matter = 100 x {W – 0.998V (1 – 0.001375T)}/W

where W = weight of fresh silage (g)

V = observed volume of water (ml)

T = titre of 0.1 M NaOH (ml)

Ash content was determined by ignition of a known weight of dry matter in a muffle furnace at 550°C for at least 3 hours, and expressed as a percentage of the dry sample.

2.4.2 pH of silage

A representative sample of 20 g of wet silage was taken and mixed with 20 ml of distilled water and the pH was read using a pH meter (Hanna instruments Ltd, Leighton Buzzard, Bedfordshire, UK).

2.4.3 Total nitrogen

The N content of feed samples was measured by a macro-Kjeldahl method using a Kjeltec Auto 1030 analyser (Foss UK Ltd, Didcot, Oxon, UK). A sample containing 1-2 mg nitrogen was digested (420°C) with 98% (w/v) sulphuric acid (N free) and catalyst tablets containing 3.5 g potassium sulphate and 0.4 g copper sulphate. The digested sample was placed in a Kjeldahl digestion tube in the Auto analyser where it was distilled with sodium hydroxide and titrated with 0.02 M HCl using basic acid indicator solution containing bromocresol green/methyl red. Crude protein content was calculated by multiplying the total nitrogen content of the sample by 6.25.

2.4.4 True protein and non-protein nitrogen (NPN) in silage

The true protein content of silage was determined by Kjeldahl analysis of the material precipitated by tannic acid (Van Roth, 1939). NPN content was calculated by subtracting the true protein content from the crude protein content.

Reagents: Tannic acid solution was prepared by dissolving 4.45 g tannic acid in water and adding 0.1 ml of concentrated sulphuric acid. The mixture was made up to 100 ml with distilled water. This was allowed to stand for 24 hours and then filtered through Whatman No. 42 filter paper.

Procedure: Wet minced silage (1 g) was weighed accurately into a centrifuge tube and 20 ml boiling tannic acid solution were added. The tube was placed in a boiling water bath for 15 minutes, cooled for 15 minutes and then centrifuged at 1500 g for 10 minutes. The supernatant was removed by suction through a tube covered at the end with a layer of fine, washed muslin. Any particles of sample were washed from the muslin back into the tube and the volume made up to 25 ml with distilled water. The residue was resuspended and the tube centrifuged as before. The washing and centrifuging was repeated twice more and the residue was finally washed into a Kjeldahl digestion tube for nitrogen determination as

described above.

2.4.5 Ammonia nitrogen in silage

This was determined on a water extract of the sample. The extract was prepared by placing 20 g wet minced silage and 200 ml distilled water in a beaker in a water bath at 40°C for 30 minutes, stirring intermittently. The extract was filtered by squeezing the silage juice through muslin, and was centrifuged at 1500 g for 20 minutes.

Procedure: Ten ml of silage extract were pipetted into a Kjeldahl digestion tube and placed in the Kjeltec 1030 analyser, and the ammonia released after addition of NaOH was titrated as described for the determination of total N.

2.4.6 Lactic acid in silage

Lactic acid was determined by the method of Elsden and Gibson (1954) in which lactic acid is oxidized to acetaldehyde which combines with sodium metabisulphate and is determined iodimetrically. Sugars, which may give rise to carbonyl compounds, and nitrogenous compounds such as protein are removed with copper sulphate and calcium hydroxide.

Reagents

1) 2% (w/v) ceric sulphate solution in 0.5 M sulphuric acid.
2) 0.05 M iodine solution, prepared by dissolving 20 g potassium iodide in 35 ml water and then dissolving 13 g of iodine in this solution and making up to 1 l with water.

Procedure: 9.5 ml silage extract were transferred into a 15 ml centrifuge tube and 0.5 ml copper sulphate and 1 g calcium hydroxide were added. These were mixed and allowed to stand for 30 minutes and then centrifuged until clear. One ml of the clear solution was transferred into 100 ml flask with 0.5 ml 5 M sulphuric acid and a few anti-bumping granules and steam distillation was commenced, 5 ml ceric sulphate being added through

the separating funnel. Fifteen ml of distillate were collected in a 50 ml conical flask containing 2 ml of 0.5% (w/v) sodium metabisulphate. One ml of 2% (w/v) starch solution and 0.05M iodine were added until a permanent blue colour was obtained and then decolourization was achieved by adding 5 mM iodine until the pale blue colour persisted. This was titrated with 5 mM iodine after adding 1 g sodium hydrogen carbonate.

2.4.7 Total soluble sugars in silage

These were determined by a method similar to that of Somogyi (1945).

Reagents

1) Arsenomolybdate reagent, prepared by dissolving 25 g ammonium molybdate in 450 ml water then adding 21 ml Analar concentrated sulphuric acid. A solution of 3 g (12%) di-sodium hydrogen arsenate in 25 ml water was added in a water bath at 55°C for 25 minutes with continuous stirring. The mixture was transferred to a brown bottle, incubated for 24 hours at 37°C , cooled and held at 4°C until used.

2) Reagent A, B and C

i) Reagent A, prepared by dissolving 25 g sodium carbonate, 25 g Rochelle salt (potassium sodium tartrate) and 20 g anhydrous sodium sulphate in 800 ml water and diluting to 1 l.

ii) Reagent B, 15% (w/v) copper sulphate solution containing 1 or 2 drops of concentrated sulphuric acid per 100 ml.

iii) Reagent C, reagents A and B were made up as 1 part B to 25 parts A.

Procedure: A sample (5 ml) of silage extract was pipetted into a glass stoppered test tube for hydrolysis. 0.1 ml of 1 N sulphuric acid was added and the tube and its contents were boiled in a boiling water bath for 30 minutes. The tubes were cooled in a water bath and then 0.1 ml of 1 M NaOH was added. Two ml of hydrolysate were transferred to duplicated 15 ml centrifuge tubes and deproteinized by adding 4 ml of 5% (w/v) zinc sulphate

solution and 4 ml of 0.3 M sodium hydroxide. After mixing the tube and its contents were centrifuged at 1500 g for 10 minutes. Two ml of supernatant or standard were transferred to a glass-stoppered test tube containing 2 ml of reagent C. The tube was heated in a boiling water bath for 10 minutes. After cooling 2 ml of arsenomolybdate reagent were added, the solution transferred to a 50 ml volumetric flask and made up to volume with water. The absorbance was read on a spectrophotometer (Cecil instruments, Cambridge, UK) at 500 nm against a blank of distilled water. The total soluble sugars in samples were calculated by reference to a calibration graph derived from standard solutions of D-glucose containing 50 to 250 ml/l.

2.4.8 Ethanol in silage

Ethanol was determined by gas chromatography by the method of Huida (1982) using methanol as an internal standard. Thirty ml of dry methanol were added to 5 ml of silage extract and 1 µl injected on to the column of a Shimadzu GC-8A gas chromatograph (Shimadzu Europe Ltd, Milton Keynes, Buckinghamshire, UK) fitted with a flame ionization detector. The columns were 2 m long and of 2 mm internal diameter and were packed with Chromosorb 101. The oven setting was 100°C and the carrier gas (N_2) flow was 60 ml/min.

2.4.9 Total and individual volatile fatty acids (VFA)

The VFA in the silage were determined by gas chromatography by the procedure of Cottyn and Boucque (1968).

Reagents

1) Preservative mixture, containing 30 ml metaphosphoric acid (25% w/v), and 20 ml distilled water.
2) Internal standard, hexanoic acid (2 g) dissolved in 1 l distilled water.

3) VFA standard solution, prepared by pipetting 4 ml acetic acid (6 g/100 ml water), 2 ml propionic acid (7.2 g/100 ml), 2 ml butyric acid (8.4 g/100 ml), 2 ml isobutyric acid (0.8 g/100 ml), 2 ml valeric acid (0.96 g/ml) and 2 ml isovaleric acid (0.96 g/100 ml) into a 100 ml volumetric flask and diluting to volume with water.

Procedure: Two ml of silage extract were transferred to a 10 ml test tube and 1 ml preservative and 2 ml hexanoic acid were added and the contents mixed well. The tube was shaken and allowed to stand for 20 minutes and then centrifuged at 1500 g for 20 minutes. The supernatant was analysed using a Shimadzu GC-8A gas chromatograph. The sample (1-3µl) was injected onto a glass column packed with 5% Carbowax 20M/TPA on Chromosorb G 80/100 mesh. The oven temperature was 100-120°C and carrier gas (N_2) flow rate was 60 ml/min.

The molar concentration was calculated for each acid from the peak area on the chromatograph relative to that of hexanoic acid. Corrections were made for the differences in the response of the detector to each acid using factors derived from the analysis of a standard VFA solution.

2.4.10 Neutral detergent fibre (NDF) and acid detergent fibre (ADF)

The NDF and ADF contents in food were determined by the method of Goering and Van Soest (1970).

Reagents

1) Neutral detergent (ND) solution contained 30 g sodium lauryl sulphate, 18.6 g disodium ethylenediaminetetraacetic acid dihydrate, 6.81 g sodium borate decahydrate, 4.56 g anhydrous di-sodium hydrogen phosphate and 10 ml 2-ethoxyethanol in 1 l of solution.
2) Acid detergent (AD) solution consisted of 20 g cetyl-trimethyl ammonium bromide per 1 of 0.5 M sulphuric acid.

Procedure: Approximately 1 g of sample was added to a 500 ml round-bottom flask to

which 100 ml of ND solution, 2 ml Dekalin and 0.5 g sodium sulphate for NDF determination or 100 ml AD solution and 2 ml Dekalin for ADF determination were added. The flask was then refluxed for 60 minutes after the onset of boiling and the contents were then transferred to a pre-weighed sintered glass crucible (porosity 1) which had been previously set on a filter manifold. The flask and the inside of the crucible were washed twice with boiling water and twice with acetone. The crucible and its contents were then dried overnight in an oven at 100°C and re-weighed after cooling in a desiccator.

2.4.11 Determination of individual amino acids

The AA composition of feeds and blood plasma was determined by the modified method of Umagat *et al.* (1982) using high-performance liquid chromatography (HPLC) with ortho-phthaldialdehyde/2-mercaptoethanol (OPA/MCE) precolumn derivatization.

The OPA/MCE reagent reacts with the primary amine function of the AA, to form a fluorescent 1-alkyl-thio-2-alkyl-substituted isoinodol. The OPA reaction is specific for primary amines, and HPLC analysis of the OPA/amine derivatives permits high sensitivity of detection and offers the advantages of relatively short analytical run times and no interference from ammonia.

***Apparatus*:** The HPLC system used consisted of a Spectra-Series P200 solvent delivery system (Thermo Quest, Herts, UK) coupled to a Gilson Model 121 filter fluorimeter (Anachem Ltd, Luton, Bedfordshire, UK) with wavelength of 305-309 nm excitation filter and a 430-470 nm emission filter. Separations were carried out on a 250 x 4.6 mm ID Apex II column prepacked with 5 μm octadecyl particles (Jones Chromatography, Hengoed, Mid Glamorgan, UK). Sample injections were made using a Gilson Model 401 sample dilutor and a Gilson Model 231 sample injector. The chromatographic data were processed by a Shimadzu C-R1B integrartor.

Reagents

1) OPA/MCE derivatizing reagent, prepared by dissolving 125 mg OPA with 2.5 ml HPLC grade methanol in a 25 ml volumetric flask and then made up to volume with 0.4 M sodium borate buffer (pH 9.5). 100 µl MCE was added to the solution and stored in dark and allowed to stand for 24 hours before use. Every day 10 µl of MCE were added.
2) 0.4 M sodium borate buffer (pH 9.5), made by dissolving 24.732 g boric acid in 970 ml water and pH adjusted to 9.5 with 4 M NaOH. The solution was made up to 1 l with water and filtered through 0.45µm pore size Whatman filter.
3) 6 M HCl solution, prepared by adding 501 ml concentrated HCl (specific gravity 1.18) to 499 ml water and then 0.5 ml of MCE was added.
4) Citrate buffer (pH 2.2), prepared by dissolving 19.6 g sodium citrate in 700 ml water and adding 16.5 ml concentrated HCl, 20 ml thiodiglycol, 2 ml Brij-35 solution and 0.1 ml octanoic acid and made up to 1 l with water.
5) Solvent A was 0.05 M sodium acetate (pH 5.7), HPLC grade tetrahydrofuran and HPLC grade acetonitrile in a ratio of 96:1:3.
6) Solvent B was HPLC grade methanol.
7) AA standard, prepared by adding asparagine, ornithine, taurine, α-aminobutyric acid, γ-aminobutyric acid, tryptophan and cysteic acid solution to the commercial Sigma standard (AA-S-18) for plasma analysis. The commercial Sigma standard alone was used for feed analysis. The concentration of standard was 500 nM and the standard was kept at -20°C until analysed. For feed analysis, standard was diluted to a concentration of 100 nM with citrate buffer and with 0.01 M HCl for plasma analysis.
8) For feed analysis, internal standard (500 nM) was prepared by dissolving 0.0234 g ethanolamine in 100 ml water and was stored at 4°C. This was diluted to a concentration of 200 nM with citrate buffer. For plasma analysis, internal standard (2500 nM) was prepared by dissolving 0.0298 g homoserine in 0.01 N HCl. This was diluted to a

concentration of 100 nM by taking 1 ml and making up to 25 ml in 0.01 HCl.

Preparation of samples

Feeds

Feed samples were prepared using acid hydrolysis. The sample containing approximately 15 mg total nitrogen was transferred into 80 ml Quickfit tube, 70 ml of 6 M HCl were added and the tube stoppered. The flask was maintained for 24 hours at 105°C and then cooled to 4°C. The contents were filtered through Whatman No. 42 filter paper into a 200 ml volumetric flask, and washed and made up to volume with water. One ml of filtrate was diluted with 1 ml of 2 M NaOH and 8 ml water, and 2 ml of internal standard was added to 2 ml of dilutant. This mixture was filtered through a 0.2 μm syringe filter (Whatman Ltd, Maidstone, Kent, UK).

Plasma

Plasma samples were deproteinized using 5-sulphosalicylic acid (SSA). Equal volumes of plasma and SSA were mixed in the centrifuge tube and centrifuged at 4°C for 30 minutes at 1500 g. One ml of supernatant was added to 1 ml of internal standard (homoserine 200 nM). This mixture was filtered through a 0.2 μm syringe filter.

Chromatographic conditions: OPA/MCE derivatization was conducted in the dilutor at room temperature and 20 μl of the derivatized mixture was injected on to the column by the automatic sample injector. The solvents used were degased with helium throughout the analysis and the gradient program was applied (Table 2.1). The flow rate was maintained at 1 ml/min. The sensitivity of the fluorimeter was set at 0.5 to reduce the base line noise and the attenuation of integrator was set at 6.5. Analytical run time was 42 minutes.

Procedure: The AAs in feed and deproteinized plasma were separated by elution with solvents in order of hydrophobicity. The OPA/MCE reagent vial, a 100 μl standard or sample vial and a mixing vial were placed on the sample rack in the automatic sample injector. A few minutes after starting the gradient program, the analysis program was

Table 2.1 Chromatographic gradient conditions for HPLC analysis

Time (min)	Solvent A (%)	Solvent B (%)	Flow rate (ml/min)
0	90	10	1
2	90	10	1
3	80	20	1
25	55	45	1
40	20	80	1
42	20	80	1
43	0	100	1
50	0	100	1

memorized through the control panel using the following commands: tubing volume = 220, air gap volume = 2, sample No. = n, sample volume = 30, injection volume = 20, flush volume = 500, injection valve rinse volume = 500. Identification of the individual AAs was made by reference to their retention times measured under the given conditions when a standard mixture of AAs was analysed. Typical chromatograms of AA standard for plasma and feed analyses are shown in Figures 2.1 and 2.2.

2.4.12 Milk fat

Milk fat was determined by the Gerber method according to British Standard 696 (1969). Fat was separated from the milk by the addition of concentrated sulphuric acid and measured directly using a Gerber butyrometer.

2.4.13 Milk protein

Total nitrogen was determined by a macro-Kjeldahl method (Association of Official Agricultural Chemists, 1975). This value was multiplied by 6.38 to obtain the crude protein.

2.4.14 Milk lactose

Milk lactose was determined by polarimetry (Grimbleby, 1956).

Reagents: Grimbleby's solution was prepared by dissolving 2.5 g zinc acetate and 12.5 g dodeca-tungstophosphoric acid in water, 20 ml of glacial acetic acid was added and the mixture diluted to 200 ml. The diluted mixture was filtered through Whatman No.42 filter paper.

Procedure: A 50 ml wide-necked volumetric flask was weighed before and after 20 ml of milk sample was added. Five ml of Grimbleby's solution was pipetted into the flask and the mixture was diluted to volume with water. The flask was stoppered, shaken vigorously

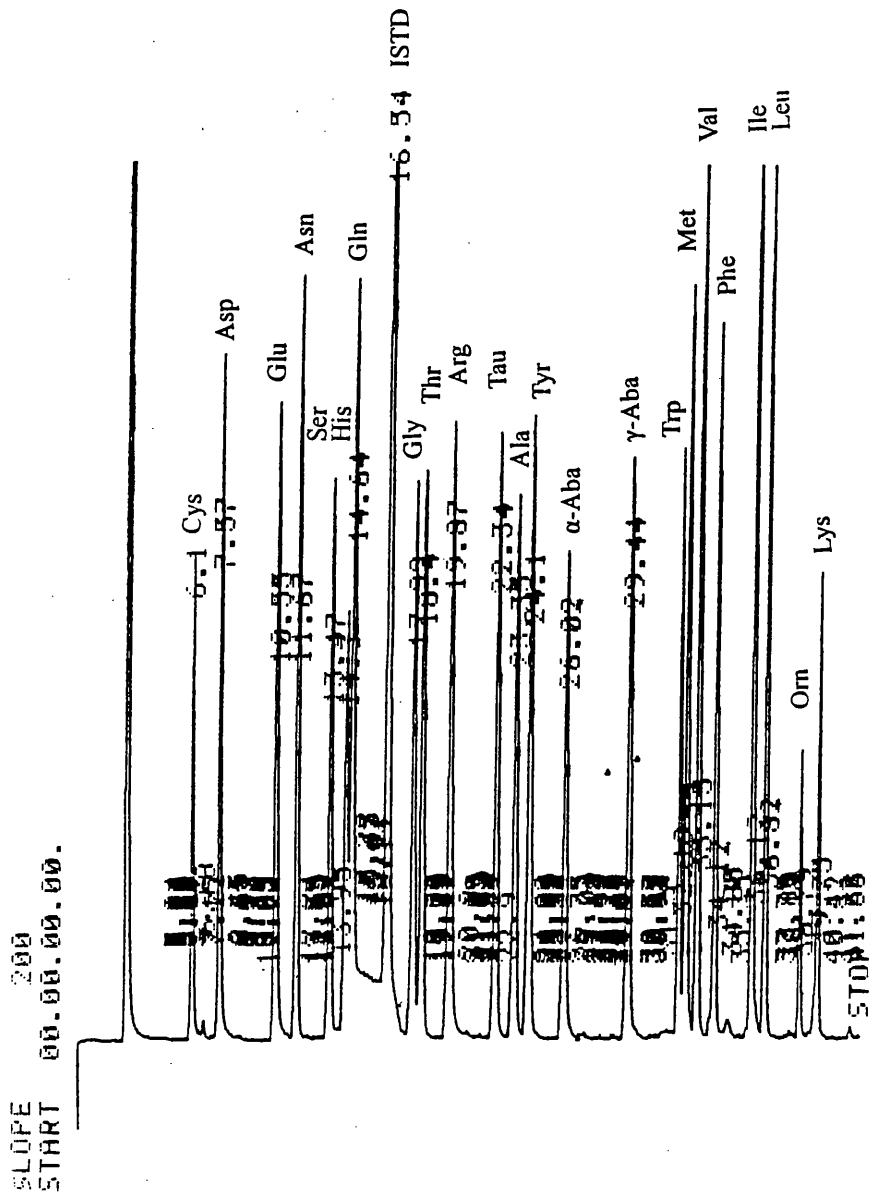

Figure 2.1 Chromatogram of an amino acid standard for plasma analysis (Cys, Cysteic acid; Asp, aspartic acid; Glu, glutamic acid; Asn, asparagine; Ser, serine; His, histidine; Gln, glutamine; ISTD, internal standard; Gly, glycine; Thr, threonine; Arg, arginine; Tau, taurine; Ala, alanine; Tyr, tyrosine; α-Aba, α-aminobutyric acid; γ-Aba, γ-aminobutyric acid; Trp, tryptophan; Met, methionine; Val, valine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; Orn, ornithine; Lys, lysine).

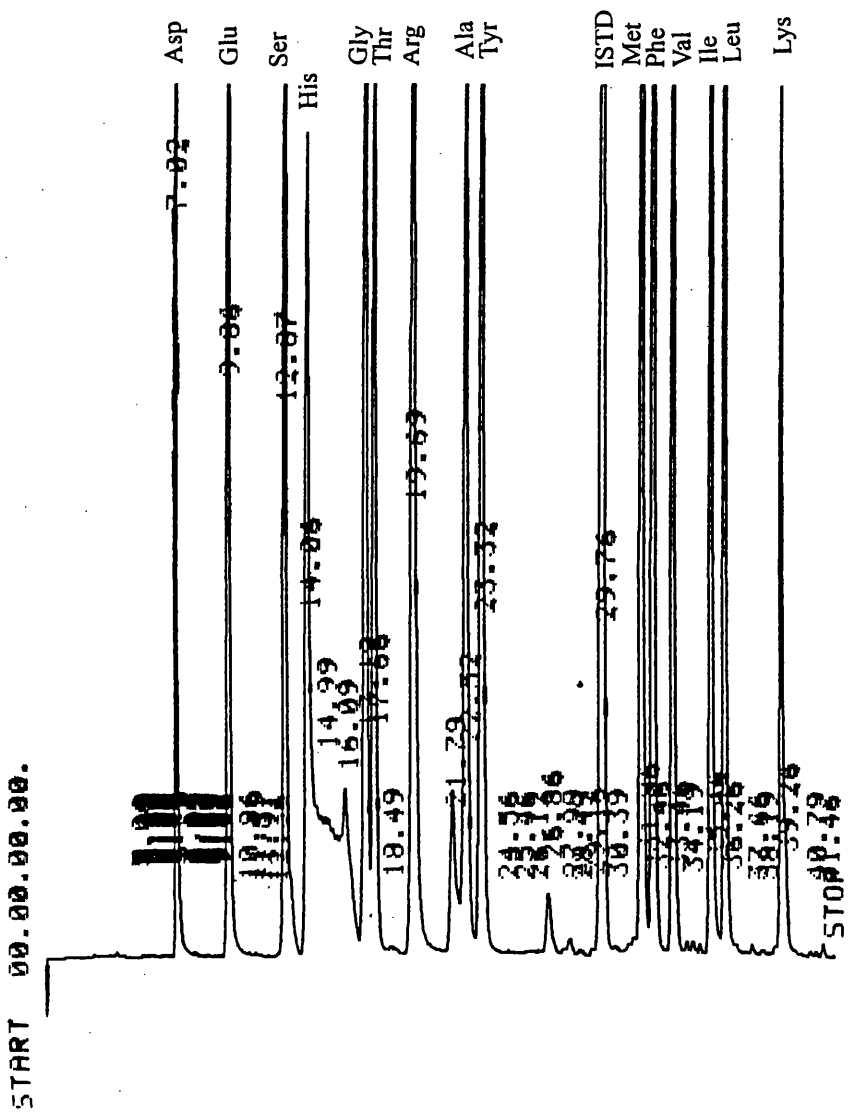

Figure. 2.2 Chromatogram of an amino acid standard for feed analysis

and then left to stand at room temperature for 15 minutes and the mixture was filtered through Whatman No. 42 filter paper. An aliquot of the filtrate was taken for determination of the rotation using an automatic polarimeter (Thorn Automation Ltd, Nottingham, UK). The polarimeter was standardized with sucrose solution (2.6 g Aristar sucrose per 100 ml water) and this gave a rotation of 0.4069 at 20°C.

Calculation: A correction was made for the fat and protein by multiplying their percentages in the milk sample by standard factors (Giggs and Szigarto, 1963).

% Lactose = {(A x V)/(L x 61.9)} / (100/wt of milk sample)

where A = observed rotation (degrees)

V = corrected volume of solution

L = length of tube with sample (dm)

2.4.15 Plasma urea

Determination of the urea concentration of blood plasma was carried out using an enzymatic procedure in a commercial kit (Sigma Diagnostics, St.Louis, MO, USA). Urea is hydrolyzed by urease to ammonia and carbon dioxide. Ammonia then reacts with alkaline hypochlorite and phenol in the presence of a catalyst, sodium nitroprusside [$(Na_2Fe_9CN)_5$ NO $2H_2O$], to form indophenol (Weichselbaum *et al.*, 1969; Horn and Squire, 1967). The concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured spectrophotometrically at 570 nm.

Reagents

1) Phenol nitroprusside solution contained 50 g/l phenol, sodium nitroprusside and stabilizer.
2) Alkaline hypochlorite solution contained 0.2% sodium hypochlorite in alkali.
3) Urease buffer reagent contained buffered 100 unit urease from jack beans and sodium azide added as preservative.

4) Urease solution was prepared by reconstituting a vial of urease buffer reagent with 30 ml water.

5) Urea nitrogen standard solution contained urea at a urea N level of 30 mg/dl (10.7 mmol/l) and benzoic acid as preservative.

Procedure: An aliquot of plasma (10 µl) was added to 0.5 ml urease solution. One blank and one standard were also prepared containing 0.5 ml urease solution and 10 µl distilled water or 10 µl urea N standard solution. The mixture tubes were placed in a 37°C water bath for 5 – 10 minutes while urea was hydrolyzed to ammonia prior to the addition of 1.0 ml phenol nitroprusside solution, 1.0 ml alkaline hypochlorite solution and 5.0 ml distilled water. The mixture was mixed and allowed to develop colour at room temperature for 20 – 30 minutes. After correction for the blank, the absorbance of the sample was divided by the absorbance of the standard.

2.4.16 Plasma glucose

The concentration of glucose in blood plasma was determined using an enzyme/colour reagent in a commercial kit (Cat. No.124036; Boehringer Mannheim GmbH, Germany). The glucose was oxidized by glucose oxidase and, in the presence of peroxidase, the hydrogen peroxide formed the oxidized chromogen, Perid (2,2 Azino-di[3 ethyl-benzthiazoline sulphonate]). The colour intensity of the dye was proportional to the glucose concentration.

Reagents

1) Protein precipitating solution contained 1.6 g/l uranyl acetate and 9 g/l NaCl.

2) GOD-Perid reagent contained no less than 10 U/ml peroxidase and 1.0 mg chromogen/ml in 100 mM phosphate buffer, pH 7.0.

Procedure: Plasma (0.1 ml) was added to 1 ml uranyl acetate solution, mixed and centrifuged at 1500 g for 15 minutes. Supernatant (0.2 ml) was incubated in a test tube

with 5 ml GOD-Perid reagent at room temperature for 45 minutes. Blank determinations were performed on distilled water. The absorbance at 610 nm was read on a spectrophotometer and glucose concentration determined by reference to a standard solution containing 9.1 mg glucose/100 ml.

CHAPTER THREE

DETERMINATION OF THE FIRST-LIMITING AA FOR MILK PROTEIN PRODUCTION IN DAIRY COWS CONSUMING A DIET OF GRASS SILAGE AND A CEREAL-BASED SUPPLEMENT CONTAINING FEATHER MEAL

3.1 INTRODUCTION

Feather meal contains low concentrations of methionine, lysine, histidine and tryptophan (Goedeken *et al.*, 1990) and, furthermore, the concentrations of these AAs in blood plasma are low in dairy cows given feather meal (Chamberlain *et al.*, 1992). Intravenous infusion of these four AAs led to sizable increases of milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal (Choung and Chamberlain, 1995). Theoretical calculation of the supply of EAA and comparison with their outputs in milk suggested that the first-limiting AA was likely to be histidine with lysine and methionine likely to be second- and third-limiting respectively.

However, it was necessary to identify experimentally the first-limiting AA for milk production in dairy cows consuming this diet, and to examine the magnitude of the response of milk production to supplementation with the limiting AA. A series of four experiments was conducted. The first experiment was carried out to identify the first-limiting AA in the group of limiting AAs for milk production, omitting histidine and lysine in turn from an intravenously infused mixture of methionine, lysine, histidine and tryptophan. The second experiment was conducted to examine the effect of a single intravenous dose of the first-limiting AA on milk production. In the third and fourth experiments, responses to increasing intravenous doses of the first-limiting AA alone or to increasing intravenous doses of the first-limiting AA given with a fixed dose of the group

of next-limiting AAs were examined.

3.2 EXPERIMENTAL

3.2.1 Animals and their management

Twelve Friesian cows in their third or fourth lactation were used in Experiments 1, 2 and 4. The cows were 5-7, 10-12 and 8-10 weeks into their lactation at the start of Experiments 1, 2 and 4, respectively. In Experiment 3, six cows in their second or third lactation were used and the cows were 8-10 weeks into their lactation at the start of the experiment. Average body weights of the animals were approximately 565 (range 501-605; Experiments 1 and 2), 596 (range 523-645; Experiment 3) and 545 kg (range 515-606; Experiment 4). The animals were housed individually in metabolism stalls and milked each day at 06.00 and 15.00 h and food was provided in two equal meals at milking times.

The cows were given a basal diet consisting of *ad libitum* access to grass silage and 5 kg/day of a pelleted mixture of rolled barley, feather meal and citrus pulp (0.50: 0.25: 0.25, on a fresh weight basis) together with 2 kg/day rolled barley in all experiments. The amount of silage offered was adjusted to ensure a daily refusal of about 15% of that offered.

The silage of each experiment was made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth and ensiled, after addition of Add-Safe (ammonium tri-hydrogen tetraformate 550 g/l; ammonium tri-hydrogen tetra-propionate, 150 g/l; BP Chemicals Ltd, Hull, Humberside, UK) at 3 litres/tonne fresh grass in a bunker silo of 70-tonne capacity in Experiments 1, 2 and 3, and 9 litres/tonne in Experiment 4. The chemical composition of the silages is shown in Table 3.1 and that of the supplements in Table 3.2. The silages used in all experiments were well preserved. In Experiment 3, and especially in Experiment 4, the silage was of restricted fermentation with a low concentration of lactic acid. Although nominally of the same formulation, the feather meal cube used in

Table 3.1 The chemical composition (g/kg DM unless stated otherwise) of the silages used in the experiments

	Exp. 1 & 2	Exp. 3	Exp. 4
DM (g/kg)[1]	209	188	219
Organic matter	909	886	908
Total N	30.6	33.0	30.2
Ammonia-N (g/kg N)	138	150	134
NPN (g/kg N)	767	715	685
Neutral-detergent fibre	503	518	600
Acid-detergent fibre	307	360	349
pH	4.0	4.2	3.8
Water-soluble carbohydrate	26	2	86
Lactic acid	112	63	23
Acetic acid	20	27	12
Butyric acid	0	0	3
Ethanol	37	18	16

[1] By toluene distillation.

Table 3.2 The chemical composition (g/kg DM, unless stated otherwise) of the feather meal cubes (FMC) and rolled barley used in the experiments

	Exp. 1& 2		Exp. 3		Exp. 4	
	FMC	**Barley**	**FMC**	**Barley**	**FMC**	**Barley**
DM (g/kg)	882	848	856	845	880	852
Organic matter	906	972	917	979	912	977
Total N	41.4	21.0	42.1	19.9	49.8	20.2
Starch	281	654	125	625	161	599
Sugars	nd	nd	63	13	67	11
Neutral-detergent fibre	199	359	234	250	238	195
Acid-detergent fibre	65	97	122	55	128	58

nd, not determined.

Experiment 4 differed from those in the other experiments in its protein content by about 5 percentage units. The crude protein (N x 6.25) concentrations in the basal diet were approximately 203, 202, 212 and 214 g/kg dry matter (DM) for Experiments 1, 2, 3 and 4, respectively.

3.2.2 Experimental treatments and design

3.2.2.1 Experiment 1

Experiment 1 was designed as a 4 x 4 Latin square with 10-day periods. The four treatments were (1) the basal diet as described above (Basal); (2) Basal plus a continuous intravenous infusion supplying (g/day) 9.7 histidine, 9.1 methionine, 30.0 lysine and 2.6 tryptophan (4AA); (3) Basal plus infusion of the AA mixture without histidine (-His); and (4) Basal plus infusion of the AA mixture without lysine (-Lys).

All animals received the basal diet for at least 21 days before the start of the experiment. All infusions were dissolved in distilled water and infused via a volumetric infusion pump (Smith and Nephew Medicals Ltd, Hull, Humberside, UK) in a volume of 4 litres/day into a jugular vein, into which an indwelling polyethylene catheter (14g x 8 cm; Arrow International Inc, Reading, UK) had been inserted. The amount of AAs infused were the calculated approximate difference between the amounts absorbed from dietary inclusions of 1 kg/day feather meal and 1 kg/day fish meal, using the estimates of rumen-degradability and absorbability obtained previously (Chamberlain *et al.*, 1992).

Food intake and milk yield were recorded daily. The composition of milk was determined on a representative, composite sample from the last four consecutive milkings in each experimental period. Samples of blood were obtained from a jugular catheter at 09:45; 10:30; 11:00; 12:00; 13:00; 14:00 and 15:00 h on the last day of each period. Samples were taken into heparinized tubes and centrifuged immediately to separate plasma that was stored at –20°C prior to analysis. A bulked sample was analysed for AAs, glucose

and urea.

3.2.2.2 Experiment 2

Experiment 2 began 7 days after completion of Experiment 1, using the same four animals receiving the same diet as in Experiment 1. The experiment consisted of a 10-day treatment period during which the cows were given an infusion of histidine (9.7 g/day) between 10-day pre- and post-infusion control periods during which the cows received no infusion. All infusions were prepared and infused as described for Experiment 1.

Recording of food intake and milk yield and composition were as described for Experiment 1. Samples of blood were taken, treated and analysed as in Experiment 1.

3.2.2.3 Experiment 3

Experiment 3 was designed as a duplicated Williams Square with six animals, four 10-day periods and four treatments. The design is illustrated in Table 3.3. The four treatments were (1) basal diet (Basal), as described above, supplemented with intravenous infusions of histidine at (2) 3g/d; (3) 6 g/d and (4) 9 g/d. All animals received the basal diet for at least 21 days before the start of the experiment. All infusions were prepared and infused as described for Experiment 1.

Recording of food intake and milk yield and composition were as described for Experiment 1. Samples of blood were taken, treated and analysed as in Experiment 1.

3.2.2.4 Experiment 4

Experiment 4 was designed as an incomplete 5x5 Latin square (Youden square) with four animals and five 10-day periods. The five treatments were (1) basal diet (Basal) as described above; (2) Basal plus a continuous intravenous infusion supplying 0 g/d histidine plus the AA combination; (3) Basal plus infusion of 3 g/d histidine plus the AA

Table 3.3 The experimental design of Experiment 3: a duplicated Williams Square

	Cows			Cows		
Period	**1**	**2**	**3**	**4**	**5**	**6**
I	A	B	C	A	B	C
II	B	C	D	B	C	D
III	D	A	B	D	A	B
IV	C	D	A	C	D	A

Treatments: A, Basal diet; B, Basal + histidine (3 g/d); C, Basal + histidine (6 g/d); D, Basal + histidine (9 g/d).

combination; (4) Basal plus infusion of 6 g/d histidine plus the AA combination; and (5) Basal plus infusion of 9 g/d histidine plus the AA combination. The AA combination contained 8 g methionine, 28 g lysine and 2.5 g tryptophan. The amounts of AAs in the AA combination were determined on the basis of the amounts equivalent to 9 g histidine in the AA composition of the infusate in the 4AA treatment for Experiment 1. All animals received the basal diet for at least 21 days before the start of the experiment. All infusions were prepared and infused as described for Experiment 1.

Recording of food intake and milk yield and composition were as described for Experiment 1. Samples of blood were taken, treated and analysed as in Experiment 1.

3.2.3 Chemical analysis

Minced wet silage was analysed for DM by toluene distillation, total nitrogen, true protein, ammonia, lactic acid, ethanol, water-soluble carbohydrate, VFA and pH. Dried samples of silage were analysed for ash, NDF and ADF. Concentrate samples were analysed as appropriate for DM, total nitrogen, ash, NDF, ADF, sugars and starch. Blood plasma samples were analysed for urea, glucose and free AAs. Milk samples were analysed for fat, crude protein and lactose.

3.2.4 Statistical analysis

For statistical analysis, mean values for feed intake and milk yield were taken for the last 7 days of each experimental period. In Experiment 1, on three occasions, blockage of the jugular catheter prevented the withdrawal of blood samples while the AAs were being infused into the same catheter. Hence, for blood analysis, there were three missing plots in the statistical analysis. On examination of the results in Experiment 3, all the data for period 2 appeared grossly at odds with the rest of the experiment. Further investigation uncovered problems with the water supply to the cows during that period. Consequently all

the data for period 2 were removed before statistical analysis. Results of Experiment 1, 3 and 4 were analysed by ANOVA using the directives of Genstat 5 (Lawes Agricultural Trust, 1990). Results of Experiment 2 were analysed using a paired *t*-test using the directives of Genstat 5; responses to histidine were measured by comparison of observations made during the infusion period with the mean of those made in the pre- and post-infusion control periods.

All differences between pairs of means of treatments in Experiments 1, 3 and 4 were inspected by the least significant difference (LSD) method at the 5% level.

3.3 RESULTS AND DISCUSSION

3.3.1 Experiment 1

Silage intake and milk production are given in Table 3.4. There were no statistically significant effects of the treatments on the intake of silage. The yields of milk, milk protein and lactose were markedly increased by the 4AA and lysine-free treatments but were not affected when histidine was omitted from the infusion. The yields of milk protein were increased by 134 g/d (18%) and 111 g/d (11%) for the 4AA and lysine-free treatments relative to Basal, respectively. However, the concentration and the yield of milk fat showed a different response. The histidine-free treatment led to a pronounced increase in the concentration of fat relative to all other treatments whereas the yield of milk fat was increased ($P<0.05$) by all infusion treatments relative to Basal.

In addition to the expected effects on their respective concentrations in blood plasma, omitting histidine and lysine from the infusion reduced ($P<0.05$) the concentrations of the branched-chain AAs and arginine in blood plasma relative to the 4AA treatment (Table 3.5). The concentration of plasma glucose was increased ($P<0.05$) over Basal and the 4AA treatment by the histidine-free treatment whereas there was no statistically significant effect of the treatments on the concentration of urea in blood plasma by the treatments

Table 3.4 Effects of intravenous infusions of amino acids on food intake and milk production in Experiment 1

	Basal	4AA	- His	- Lys	SED[1]	P value[2]
DM intake						
Silage, kg/d	10.3	10.6	10.0	10.6	0.35	0.408
Supplement, kg/d	6.1	6.1	6.1	6.1	-	-
Milk yield, kg/d	25.9^{a}	28.4^{b}	25.2^{a}	28.0^{b}	0.64	0.017
Milk fat , g/kg	47.8^{a}	52.7^{a}	59.7^{b}	53.0^{a}	2.50	0.040
g/d	1231^{a}	1496^{b}	1508^{b}	1479^{b}	75.0	0.055
Milk protein, g/kg	28.7^{ab}	30.8^{b}	28.4^{a}	30.4^{ab}	0.99	0.165
g/d	740^{a}	874^{b}	715^{a}	851^{b}	32.8	0.019
Milk lactose, g/kg	47.3	47.7	45.6	46.8	0.93	0.287
g/d	1228^{b}	1354^{c}	1147^{a}	1310^{c}	27.8	0.006

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b,c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

Table 3.5 Effects of intravenous infusions of amino acids on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 1

	Basal	4AA	- His	- Lys	SED[1]	P value[2]
Histidine	33[a]	72[ab]	13[a]	97[b]	19.5	0.118
Threonine	138	137	112	124	16.7	0.518
Arginine	75[b]	75[b]	59[a]	59[a]	4.3	0.101
Tryptophan	49	63	55	57	6.5	0.385
Methionine	22	71	64	68	19.3	0.267
Valine	302[ab]	349[b]	275[a]	266[a]	18.4	0.111
Phenylalanine	54[b]	51[ab]	48[ab]	47[a]	2.3	0.211
Isoleucine	123[b]	154[c]	102[a]	105[ab]	6.4	0.034
Leucine	123[b]	118[b]	96[a]	93[a]	1.9	0.008
Lysine	69[a]	221[b]	192[b]	65[a]	34.5	0.084
Aspartic acid	7	7	4	5	1.9	0.485
Glutamic acid	97	94	81	89	9.1	0.416
Serine	157[b]	108[a]	106[a]	108[a]	12.7	0.117
Glycine	416	405	343	369	39.5	0.435
Alanine	124[b]	116[ab]	101[a]	114[ab]	5.6	0.150
Tyrosine	63[b]	47[a]	47[a]	48[ab]	5.1	0.181
Asparagine	91[b]	74[ab]	62[a]	71[ab]	8.6	0.215
Taurine	54[b]	42[a]	52[b]	53[b]	1.9	0.054
Glutamine	200	174	145	158	29.0	0.457
Ornithine	48[ab]	53[b]	37[a]	42[ab]	3.7	0.133
Total AA	2244	2430	1995	2039	175.5	0.290

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b,c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

Table 3.6 Effects of intravenous infusions of amino acids on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 1

	Basal	**4AA**	**- His**	**- Lys**	**SED**[1]	**P value**[2]
Glucose	636[a]	622[a]	694[b]	654[ab]	15.3	0.109
Urea	158	139	162	158	10.7	0.375

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test.

(Table 3.6).

The results of Experiment 1 confirmed that histidine was the first-limiting AA for the synthesis of milk protein. Furthermore, lysine was clearly not limiting since the supply of lysine from the basal diet was sufficient to allow the secretion of at least an extra 111 g/day of milk protein (Table 3.4). Taking these results together with those reported earlier (Choung and Chamberlain, 1995), indicated that histidine was first-limiting by a substantial margin over methionine and lysine. Although feather meal is low in tryptophan, calculations of supply and demand for milk synthesis suggested that it was unlikely to be limiting before methionine or lysine (Choung and Chamberlain, 1995). There was a suggestion of a link between AA balance and fat synthesis in that the histidine-free treatment increased the concentration and yield of milk fat. The synthesis of protein in milk was decreased by an unbalanced supply of AAs without the first-limiting AA, and was accompanied by an increase of fat secretion.

3.3.2 Experiment 2

There was no statistically significant effect of histidine infusion on the intake of silage or on milk production (Table 3.7). The concentrations of AAs in blood plasma are shown in Table 3.8. The concentrations of histidine and glutamic acid were increased by the infusion of histidine compared with Basal. But the concentrations of other AAs in blood plasma were unaffected by the histidine treatment. The histidine treatment did not affect the concentrations of plasma glucose and urea (Table 3.9).

3.3.3 Experiment 3

Silage intake and milk production are given in Table 3.10. The 3 g/d level of histidine administration increased ($P<0.05$) the silage intake relative to Basal and the 9 g/d level. All levels of histidine significantly increased milk yield over Basal but there was a

clearly defined maximum response at the 6g/d level. For the yield of milk protein, this pattern of response was especially marked; indeed, the small increases in the yield of protein for the 3 g/d and the 9 g/d levels were not statistically different from Basal, whereas the response to the 6 g/d level was a very considerable (17%) increase ($P<0.01$) in protein yield. Moreover, this maximum for milk protein yield was accompanied by minimum values for the concentration and yield of milk fat. The yield of milk lactose was increased by the 3 g/d and the 6 g/d levels but the 9 g/d level did not affect lactose output. The concentrations of milk protein and milk lactose were not affected by any of the treatments.

The concentrations of AAs in blood plasma are shown in Table 3.11. Statistically significant effects were seen for the infused AA, histidine, the concentration of which was increased by all three levels of infusion relative to the basal treatment and was markedly higher for the 9 g/d level than for the other levels. The concentrations of arginine, isoleucine and ornithine were increased ($P<0.05$) over Basal by the 6 g/d level. The 6 g/d level reduced the concentration of plasma glucose compared with Basal and the 3 g/d level (Table 3.12), reflecting the much increased output of lactose with this treatment. The concentrations of plasma urea were not affected by the increasing doses of histidine.

The results of this experiment provided further confirmation that histidine is the first-limiting AA with this basal diet and also indicated that very substantial increases of milk production are obtainable by supplementation with histidine alone. This is an important finding because, in many circumstances, supplementation with the first-limiting acid alone results in disappointingly small responses of milk production owing to rapidly emerging deficiencies in the supplies of the second and third limiting acids. The results also provided further evidence of the relationship between milk fat and AA balance where such imbalances can arise either from a surplus or from a deficit of histidine (or other AA).

Table 3.7 The effects of intravenous infusions of histidine on food intake and milk production in Experiment 2

	Basal	Histidine	SED[1]	P value
DM intake				
Silage, kg/d	11.6	11.9	0.20	0.127
Supplement, kg/d	6.1	6.1	-	-
Milk yield, kg/d	24.0	24.1	1.03	0.901
Milk fat , g/kg	45.6	47.3	2.44	0.507
g/d	1093	1143	92.6	0.607
Milk protein, g/kg	32.2	30.4	1.07	0.137
g/d	770	733	34.1	0.319
Milk lactose, g/kg	47.0	46.9	1.53	0.912
g/d	1130	1131	74.3	0.993

[1] SED, standard error of differences.

Table 3.8 Effects of intravenous infusion of histidine the concentrations (μmol/l) of amino acids in blood plasma in Experiment 2

	Basal	Histidine	SED[1]	P value
Histidine	13	83	12.0	0.001
Threonine	127	127	35.1	0.985
Arginine	73	69	10.6	0.780
Tryptophan	46	45	4.3	0.738
Methionine	12	13	1.3	0.633
Valine	333	349	60.9	0.804
Phenylalanine	50	55	5.2	0.385
Isoleucine	125	129	22.4	0.873
Leucine	116	117	16.9	0.934
Lysine	55	57	7.6	0.865
Aspartic acid	5	7	1.2	0.217
Glutamic acid	80	90	4.0	0.050
Serine	117	126	12.9	0.536
Glycine	409	393	47.0	0.749
Alanine	144	166	20.6	0.323
Tyrosine	48	50	3.6	0.650
Asparagine	80	86	6.1	0.402
Taurine	31	35	4.1	0.440
Glutamine	206	206	15.4	0.987
Ornithine	49	49	1.0	0.982
Total AA	2121	2202	219.1	0.723

[1] SED, standard error of differences.

Table 3.9 Effects of intravenous infusions of histidine on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 2

	Basal	**Histidine**	**SED**[1]	**P value**
Glucose	587	615	32.6	0.420
Urea	163	166	10.4	0.815

[1] SED, standard error of differences.

Table 3.10 The effects of intravenous infusions with various amounts of histidine on food intake and milk production in Experiment 3

	Histidine (g/d)					
	Basal	**3**	**6**	**9**	**SED[1]**	**P value[2]**
DM intake						
Silage, kg/d	11.5[a]	12.1[b]	12.0[ab]	11.5[a]	0.25	0.128
Supplement, kg/d	6.0	6.0	6.0	6.0	-	-
Milk yield, kg/d	22.8[a]	24.6[b]	26.2[c]	23.8[b]	0.39	0.002
Milk fat , g/kg	46.3[b]	45.6[b]	37.7[a]	44.4[b]	1.27	0.004
g/d	1059[ab]	1118[b]	999[a]	1047[a]	27.7	0.038
Milk protein, g/kg	29.4	28.7	29.8	29.5	0.83	0.628
g/d	670[a]	700[a]	781[b]	699[a]	22.2	0.018
Milk lactose, g/kg	45.9	47.4	47.2	46.1	0.91	0.375
g/d	1049[a]	1167[b]	1236[c]	1096[a]	28.1	0.005

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b,c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

Table 3.11 Effects of intravenous infusions with various amounts of histidine on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 3

	Histidine (g/d)					
	Basal	**3**	**6**	**9**	**SED[1]**	**P value[2]**
Histidine	12[a]	33[b]	37[b]	60[c]	7.9	0.018
Threonine	124	134	138	144	13.7	0.590
Arginine	70[a]	75[ab]	83[b]	71[a]	4.8	0.153
Tryptophan	42	43	45	45	2.3	0.508
Methionine	11	13	13	13	0.9	0.287
Valine	316[ab]	333[ab]	371[b]	301[a]	26.3	0.184
Phenylalanine	53	56	53	54	3.7	0.836
Isoleucine	128[a]	134[ab]	161[b]	125[a]	13.0	0.147
Leucine	119	126	134	111	10.7	0.308
Lysine	55	63	63	56	6.1	0.470
Aspartic acid	5	9	5	7	2.4	0.418
Glutamic acid	63	73	73	78	6.1	0.263
Serine	123	143	116	141	19.6	0.491
Glycine	328	398	394	367	42.2	0.419
Alanine	140[a]	173[ab]	167[ab]	180[b]	16.6	0.220
Tyrosine	52	52	53	53	3.9	0.980
Asparagine	81	93	96	100	12.4	0.523
Taurine	34	34	33	32	2.8	0.894
Glutamine	222	240	239	274	23.4	0.304
Ornithine	42[a]	60[b]	56[b]	48[ab]	5.8	0.116
Total AA	2020	2284	2329	2259	188.6	0.452

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b,c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

Table 3.12 Effects of intravenous infusions with various amounts of histidine on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 3

	Histidine (g/d)					
	Basal	**3**	**6**	**9**	**SED[1]**	**P value[2]**
Glucose	652[b]	647[b]	589[a]	629[ab]	17.9	0.077
Urea	201	215	216	214	13.4	0.677

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a,b] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

3.3.4 Experiment 4

Silage intake and milk production are shown in Table 3.13. The intake of silage was increased ($P<0.05$) over the histidine-free infusion treatment by the 6 g/d and 9 g/d levels of histidine infusion. Infusion of the AA mixture without histidine did not affect milk yield or the yield or concentration of protein, but this treatment did increase the concentration of milk fat markedly. Increasing doses of histidine produced linear increases in the yield of milk (i.e. increases of 3, 7 and 10% for the 3, 6 and 9 g/d levels of histidine infusion compared with the basal treatment, respectively). The concentration of milk protein was increased over Basal by all histidine treatments but, although increasing doses of histidine tended to linearly increase protein concentration, the values were not significantly different between the levels of histidine infusion ($P>0.05$). The yield of milk protein was increased by all histidine treatments relative to Basal and the histidine-free treatment, and was maximized at the highest level (164 g/d increase) showing a linear trend ($P<0.10$). The yield of lactose was significantly affected only at 9 g/d level.

The concentrations of AAs in blood plasma are shown in Table 3.14. The 6 and 9 g/d levels of histidine increased the concentration of histidine relative to the basal treatment. The concentrations of methionine and lysine were increased by all infusion treatments. Relative to the basal treatment, all histidine treatments were associated with higher concentrations of ornithine and the concentration of serine was decreased at 6 and 9 g/d levels of histidine. The concentration of glutamic acid at the 9 g/d level was increased relative to Basal and the histidine-free treatment. None of the treatments affected the concentrations of glucose and urea in blood plasma (Table 3.15).

It appeared that the adverse effects of histidine that were observed at the 9.7 g/d level used in Experiment 2 and at the 9.0 g/d level used in Experiment 3 may have been caused by an unbalanced AA supply, resulting in no increase of milk protein synthesis, and that this can be prevented by ensuring adequate supplies of the second- and third-limiting AAs.

When this is done, there is an uncomplicated linear response of the yield of milk protein to the increasing input of histidine. In addition to the clear effects on the yield of milk protein, it is interesting to note the effects on the yield and concentration of fat in the milk. The zero histidine treatment (i.e. the 3 AAs alone without histidine) would be expected to produce an unbalanced supply of AAs because it does not include the first-limiting AA. This treatment was associated with a marked increase in the concentration of milk fat, lending further support for the idea that AA supply can have a marked influence on the concentration of milk fat.

Table 3.13 Effects of intravenous infusions of amino acids with various amounts of histidine on food intake and milk production in Experiment 4

		Histidine (g/d)[1]					
	Basal	**0**	**3**	**6**	**9**	**SED[2]**	**P value[3]**
DM intake							
Silage, kg/d	11.3^{ab}	10.9^{a}	11.4^{ab}	11.7^{b}	11.8^{b}	0.33	0.137
Supplement, kg/d	6.1	6.1	6.1	6.1	6.1	-	-
Milk yield, kg/d	25.9^{a}	25.9^{a}	26.7^{b}	27.6^{c}	28.4^{d}	0.36	<.001
Milk fat , g/kg	40.8^{a}	46.3^{b}	39.0^{a}	39.5^{a}	39.3^{a}	1.94	0.027
g/d	1057^{a}	1204^{b}	1044^{a}	1088^{ab}	1116^{ab}	59.2	0.148
Milk protein, g/kg	27.5^{a}	28.1^{ab}	29.4^{bc}	30.0^{c}	31.0^{c}	0.79	0.011
g/d	715^{a}	727^{a}	785^{b}	826^{b}	879^{c}	18.6	<.001
Milk lactose, g/kg	48.4^{ab}	48.6^{b}	47.6^{ab}	47.4^{a}	47.5^{ab}	0.51	0.143
g/d	1252^{a}	1259^{a}	1269^{a}	1304^{ab}	1347^{b}	26.6	0.036

[1] All infusion treatments supplied, in addition to the histidine, 8 g methionine, 28 g lysine and 2.5 g tryptophan.

[2] SED, standard error of differences.

[3] Statistical significance of treatment effects by *F*-test.

[a,b,c,d] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test.

Table 3.14 Effects of intravenous infusions of amino acids with various amounts of histidine on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 4

		Histidine (g/d)[1]					
	Basal	**0**	**3**	**6**	**9**	**SED[2]**	**P value[3]**
Histidine	15^{a}	19^{a}	23^{ab}	29^{b}	47^{c}	4.1	<.001
Threonine	152	143	148	154	146	10.3	0.841
Arginine	74^{a}	77^{ab}	80^{ab}	86^{b}	82^{ab}	5.3	0.243
Tryptophan	44^{a}	50^{ab}	51^{b}	51^{b}	49^{ab}	3.2	0.181
Methionine	16^{a}	39^{b}	40^{b}	32^{b}	37^{b}	4.6	0.004
Valine	425	467	406	442	420	34.6	0.496
Phenylalanine	54^{b}	49^{ab}	48^{ab}	48^{ab}	43^{a}	3.2	0.090
Isoleucine	155	180	154	185	173	17.8	0.349
Leucine	153^{ab}	157^{b}	137^{ab}	151^{ab}	131^{a}	10.9	0.174
Lysine	60^{a}	111^{b}	127^{b}	104^{b}	103^{b}	15.0	0.020
Aspartic acid	5	4	5	5	5	0.6	0.719
Glutamic acid	47^{a}	46^{a}	49^{ab}	49^{ab}	51^{b}	1.5	0.037
Serine	135^{c}	118^{bc}	116^{bc}	99^{ab}	94^{a}	8.7	0.010
Glycine	385	378	347	399	343	31.1	0.374
Alanine	182	183	186	183	174	12.5	0.883
Tyrosine	61^{b}	55^{a}	55^{a}	55^{a}	54^{a}	2.9	0.157
Asparagine	123	122	124	127	130	9.0	0.893
Taurine	46^{ab}	39^{a}	56^{c}	52^{bc}	50^{bc}	3.9	0.020
Glutamine	282^{a}	350^{b}	315^{ab}	343^{ab}	357^{b}	27.2	0.120
Ornithine	54^{a}	65^{ab}	74^{b}	79^{b}	74^{b}	6.6	0.035
Total AA	2467	2651	2540	2668	2562	92.3	0.260

[1] All infusion treatments supplied, in addition to the histidine, 8 g methionine, 28 g lysine and 2.5 g tryptophan.

[2] SED, standard error of differences.

[3] Statistical significance of treatment effects by *F*-test.

[a,b,c] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test.

Table 3.15 Effects of intravenous infusions of amino acids with various amounts of histidine on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 4

		Histidine (g/d)[1]					
	Basal	**0**	**3**	**6**	**9**	**SED[2]**	**P value[3]**
Glucose	688	688	689	666	648	33.4	0.667
Urea	183	186	190	192	171	13.4	0.578

[1] All infusion treatments supplied, in addition to the histidine, 8 g methionine, 28 g lysine and 2.5 g tryptophan.

[2] SED, standard error of differences.

[3] Statistical significance of treatment effects by *F*-test.

CHAPTER FOUR

1. COMPARISION OF RESPONSES OF MILK PRODUCTION TO ADMINISTRATION OF HISTIDINE INTO THE ABOMASUM WITH THOSE OBTAINED TO INTRAVENOUS SUPPLEMENTATION AND 2. DIETARY ADDITION OF A SUPPLEMENT RICH IN HISTIDINE IN DAIRY COWS CONSUMING A DIET OF GRASS SILAGE AND A CEREAL-BASED SUPPLEMENT CONTAINING FEAHTER MEAL

4.1 INTRODUCTION

The implication of the results of experiments in Chapter 3, using intravenous infusion of AAs, was that an increase of milk production would be expected from an increased supply of histidine into the abomasum resulting from dietary addition of an effective rumen-protected form of the AA or from dietary addition of proteins rich in histidine and of low rumen degradability. These suggestions were tested in Experiments 5 and 6 in this chapter. The first experiment was carried out to compare responses of milk production to intravenous and intra-abomasal administration of two levels of histidine. The second experiment examined responses of milk production to progressively substituting avian blood meal, as a protein rich in histidine but poor in methionine, for part of the feather meal in the supplement.

4.2 EXPERIMENTAL

4.2.1 Animals and their management

A total of 13 Friesian cows in their third or fourth lactations were used. The cows were 10-12 (Experiment 5) or 12-16 (Experiment 6) weeks into their lactations at the start of the experiments. Average body weights of cows were approximately 526 (range 478-635) and 569 kg (range 509-608) in Experiments 5 and 6 respectively. They were housed individually in metabolism stalls and milked each day at 06:00 and 15:00 h and food was

provided in two equal meals at milking times.

For both experiments, the cows were given a basal diet consisting of *ad libitum* access to grass silage and 5 kg/day of pelleted mixture of rolled barley, feather meal and citrus pulp (0.50: 0.25: 0.25, on a fresh weight basis) together with 2 kg/day rolled barley.

The silage of each experiment was made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth and ensiled, after addition of Add-Safe (ammonium tri-hydrogen tetraformate 550 g/l; ammonium tri-hydrogen tetra-propionate, 150 g/l; BP Chemicals Ltd, Hull, UK) at 3.9 litres/tonne fresh grass in a bunker silo of 70-tonne capacity in Experiment 5 and 3 litres/tonne in Experiment 6. The chemical composition of the silage is shown in Table 4.1 and that of the supplements in Table 4.2. Both silages were well preserved with a low pH, moderate levels of ammonia and only very low concentrations of butyric acid. The feather meal cubes and rolled barley used in the two experiments had similar chemical compositions. The crude protein (N x 6.25) concentrations in the basal diet were approximately 198 and 206 g/kg DM for Experiments 5 and 6, respectively. As expected, the blood meal was much richer in histidine and lysine than was feather meal but contained a similar, low concentration of methionine (Table 4.3). Cystine was not determined because, in the absence of oxidation with performic acid before hydrolysis, cystine was destroyed. However, feather meal contains high concentrations of cystine, Buttery *et al.* (1978) reporting a value of 44 g/kg CP.

4.2.3 Experimental treatments and design

4.2.3.1 Experiment 5

Experiment 5 was designed as a 5 x 5 Latin square with five treatments and five 10-day periods. The five treatments were (1) the basal diet (Basal) supplemented with (2) 3 g/day of histidine into the abomasum, (3) 3 g/day of histidine intravenously, (4) 6 g/day of histidine into the abomasum and (5) 6 g/day of histidine intravenously.

Table 4.1 The chemical composition (g/kg DM unless stated otherwise) of the silages used in the experiments

	Exp. 5	**Exp. 6**
DM (g/kg)[1]	279	249
Organic matter	920	915
Total N	28.0	27.2
Ammonia-N (g/kg N)	164	136
NPN (g/kg N)	785	711
Neutral-detergent fibre	543	567
Acid-detergent fibre	375	314
pH	3.9	3.8
Water-soluble carbohydrate	6	8
Lactic acid	130	125
Acetic acid	32	28
Butyric acid	0	4
Ethanol	20	11

[1] By toluene distillation.

Table 4.2 The chemical composition (g/kg DM, unless stated otherwise) of the feather meal cubes (FMC), blood meal cubes (BMC) and rolled barley used in the experiments

	Exp. 5		Exp. 6		
	FMC	**Barley**	**FMC**	**BMC**	**Barley**
DM (g/kg)	883	877	892	906	871
Organic matter	897	979	904	907	973
Total N	44.0	18.9	50.0	54.1	20.6
Starch	171	492	171	204	494
Sugars	81	22	74	80	12
Neutral-detergent fibre	253	331	246	383	331
Acid-detergent fibre	122	62	115	86	63

Table 4.3 The amino acid composition (g/kg crude protein) of the feather meal and the blood meal used in Experiment 6

	Feather meal[1]	**Blood meal**[1]
Histidine	7	43
Threonine	42	45
Arginine	65	49
Methionine	7	9
Valine	75	67
Phenylalanine	49	59
Isoleucine	45	38
Leucine	79	97
Lysine	22	90
Aspartic acid	62	87
Glutamic acid	108	108
Serine	109	43
Glycine	76	34
Alanine	44	69
Tyrosine	36	38

[1] The feather meal and blood meal contained 842 and 879 g crude protein/ kg DM respectively.

All animals received the basal diet for at least 21 days before the start of the experiment. All infusions were dissolved in distilled water and infused in a volume of 4 l over a period of 23 h each day. For the intra-abomasal infusion, a single-channel peristaltic pump (Watson and Marlow 502S; Watson and Marlow Ltd, Falmouth, Cornwall, UK) was used. For the intravenous infusion, histidine was dissolved in distilled water, filtered and sterilized prior to infusion into a jugular vein using a volumetric infusion pump (Smith and Nephew Medicals Ltd, Hull, Humberside, UK).

Food intake and milk yield were recorded daily. The composition of milk was determined on a representative, composite sample from the last four consecutive milkings in each experimental period. Samples of blood were obtained from a jugular catheter at 09:45; 10:30; 11:00; 12:00; 13:00; 14:00 and 15:00 h on the last day of each period. Samples were taken into heparinized tubes and centrifuged immediately to separate plasma that was stored at –20°C prior to analysis. A bulked sample was analysed for AAs, plasma urea and glucose in blood plasma.

4.2.3.2 Experiment 6

The experimental design was a duplicated 4 x 4 Latin square with eight cows, four 21-day periods. The four treatments were (1) the basal diet as described above (Basal) and three treatments in which blood meal cubes were progressively substituted for equal weights of feather meal cubes; hence the treatments consisted of substitutions of (2) 0.5, (3) 1.0, and (4) 2.0 kg/day of blood meal cubes.

Recording of food intake and milk yield and composition were as described for Experiment 5. Samples of blood were taken, into heparinized containers, from a tail vein at 10:00 and 14:00 h on the last day of each period and stored and analysed.

4.2.4 Chemical analysis

Minced wet silage was analysed for DM by toluene distillation, total nitrogen, true protein, ammonia, lactic acid, ethanol, water-soluble carbohydrate, VFA and pH. Dried samples of silage were analysed for ash, NDF and ADF. Concentrate samples were analysed as appropriate for DM, total nitrogen, ash, NDF, ADF and starch. Blood plasma samples were analysed for urea, glucose and free AAs. Milk samples were analysed for fat, crude protein and lactose.

4.2.5 Statistical analysis

For statistical analysis, mean values for feed intake and milk yield were taken for the last 7 days of each experiment period. Results of both experiments were analysed by ANOVA using the directives of Genstat 5 (Lawes Agricultural Trust, 1990).

In Experiment 5 the treatment effects tested were 'Control' for a difference between the control and supplementation of histidine, 'Type' for the difference between infusion types, 'Level' for the differences between infused amounts of histidine and 'Type.Level' for a difference between levels in the shape of response interaction.

4.3 RESULTS AND DISCUSSION

4.3.1 Experiment 5

Silage intake was variable, with a tendency ($P<0.10$) for higher intakes with intravenous, compared with intra-abomasal, administration of histidine (Table 4.4). The concentration and yield of milk fat tended to be reduced ($P<0.10$) over Basal by the infusion treatments. None of treatments affected the yield of milk and the yields and concentrations of milk protein and milk lactose.

Concentrations of AAs in blood plasma are shown in Table 4.5. The concentration of histidine was increased ($P<0.001$) by the higher level of supplementation of histidine and by both routes of histidine administration compared with the basal treatment ($P=0.038$).

The intra-abomasal infusion of 6 g/d histidine significantly decreased the concentrations of valine and leucine relative to the 3 g/d level. There was a tendency ($P<0.10$) for the abomasal administration to be associated with increased plasma concentrations of methionine and phenylalanine compared with the intravenous administration. The concentrations of plasma glucose and urea were not affected either by the increasing doses of histidine or by the route of administration (Table 4.6).

The lack of response to histidine in this experiment is difficult to explain. The diet was similar to those used in previous experiments, and although it is possible that the unavoidable differences in the silages used in the various experiments may have influenced AA supply, there is nothing in the composition of the silage used in the present experiment to suggest that is a likely explanation.

Close examination of the milk records of individual animals throughout the experiment revealed some marked reductions of milk yield sometimes lasting several days. In this connexion, it is worth noting that all the cows used in this experiment were newly fitted with rumen cannulas. It may be that the recent operation for rumen fistulation, and the associated procedure of progressive stretching of the fistula to accommodate the large rumen cannula, together with the need for frequent catheterization of the jugular vein throughout the experiment imposed unusually high levels of experimental stress. It will be remembered that all the previous experiment in Chapter 3 used intravenous administration of histidine to cows that had not been surgically modified. Again, in the context of experimental stress, it is worth noting that the milk yields in this experiment were well below the level to be expected from the estimated ME intake (milk yields of around 23 kg/day would have been expected).

The choice of the amount of histidine supplied in this experiment was based on the fact that the administration of 6 g/d histidine alone in Experiment 3 showed the maximum response on milk protein synthesis and the infusion beyond this amount of histidine

Table 4.4 Effects of intravenous (IV) and intra-abomasal (IA) infusions of two levels of histidine on food intake and milk production in Experiment 5

		IA		IV			P value			
	Basal	3 g His	6 g His	3 g His	6 g His	SED[1]	Control	Type	Level	Type * level
DM intake										
Silage, kg/d	9.0	8.3	8.5	9.1	10.0	0.57	0.915	0.091	0.219	0.384
Supplement, kg/d	6.2	6.2	6.2	6.2	6.2	-	-	-	-	-
Milk yield, kg/d	16.9	17.5	17.5	17.5	17.8	0.73	0.282	0.743	0.714	0.779
Milk fat , g/kg	51.4	46.8	45.7	42.0	44.2	4.37	0.093	0.350	0.860	0.605
g/d	865	803	795	730	784	67.3	0.147	0.408	0.646	0.533
Milk protein, g/kg	30.7	31.1	30.0	30.3	30.6	0.99	0.807	0.878	0.547	0.371
g/d	519	539	544	527	537	17.6	0.253	0.475	0.567	0.846
Milk lactose, g/kg	45.7	44.2	43.7	44.9	43.4	1.54	0.227	0.903	0.390	0.670
g/d	772	777	767	787	772	59.4	0.946	0.856	0.782	0.961

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

Table 4.5 Effects of intravenous (IV) and intra-abomasal (IA) infusions of two levels of histidine on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 5

		IA		IV			P value[2]			
	Basal	3 g His	6 g His	3 g His	6 g His	SED[1]	Control	Type	Level	Type*Level
Histidine	15	14	24	17	30	3.4	0.038	0.117	<.001	0.538
Threonine	112	113	94	103	109	12.4	0.462	0.774	0.509	0.179
Arginine	78	81	73	73	78	8.0	0.799	0.796	0.844	0.248
Tryptophan	33	32	28	29	32	2.2	0.126	0.620	0.729	0.075
Methionine	12	13	12	11	12	0.8	0.444	0.076	0.803	0.148
Valine	307	341	265	299	298	20.6	0.684	0.781	0.022	0.025
Phenylalanine	49	52	48	46	47	2.7	0.693	0.063	0.579	0.237
Isoleucine	109	120	95	101	106	8.9	0.642	0.528	0.144	0.037
Leucine	111	115	93	101	99	6.5	0.093	0.398	0.022	0.040
Lysine	46	46	42	38	42	4.3	0.224	0.231	0.863	0.209
Aspartic acid	7	9	7	6	7	1.6	0.813	0.324	0.842	0.133
Glutamic acid	62	59	60	58	62	4.1	0.566	0.955	0.361	0.677
Serine	159	170	149	164	174	22.7	0.758	0.552	0.734	0.346
Glycine	474	502	465	483	479	41.3	0.801	0.932	0.495	0.579
Alanine	174	174	181	171	172	13.7	0.957	0.562	0.682	0.751
Tyrosine	50	55	47	51	51	3.4	0.686	0.974	0.158	0.092
Asparagine	36	34	33	34	36	2.9	0.558	0.353	0.857	0.459
Taurine	35	33	32	33	29	2.6	0.123	0.399	0.345	0.485
Glutamine	225	193	174	193	207	19.1	0.050	0.264	0.856	0.244
Ornithine	41	53	41	39	42	5.2	0.493	0.103	0.314	0.060
Total AA	2134	2163	1963	2047	2051	150.4	0.526	0.897	0.376	0.353

[1] SED, standard error of differences.
[2] Statistical significance of treatment effects by *F*-test.

Table 4.6 Effects of intravenous (IV) and intra-abomasal (IA) infusions of two levels of histidine on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 5

		IA		IV			P value[2]			
	Basal	**3 g His**	**6 g His**	**3 g His**	**6 g His**	**SED[1]**	**Control**	**Type**	**Level**	**Type*Level**
Glucose	685	702	709	692	692	23.5	0.466	0.421	0.836	0.836
Urea	225	200	201	207	213	29.6	0.422	0.655	0.882	0.918

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

apparently led to unbalanced supply for milk protein production. However, the results of Experiment 4 showed the linear tendency of milk protein increase by increasing doses of histidine with the addition of the next limiting AAs indicating the close relationship between histidine and the next limiting AAs on milk protein synthesis.

Therefore, it is concluded that although the lack of response may have been related to high levels of experimental stress in this experiment, a more likely cause of the absence of response to supplementation with histidine alone was to rapidly emerging deficiencies in the supplies of the second and third limiting acids with this basal diet.

4.3.2 Experiment 6

All three levels of blood meal inclusion increased the intake of silage DM relative to Basal (Table 4.7). The first level of inclusion of blood meal increased the yields of milk, milk protein and lactose and the concentration of milk protein but there was no further increase beyond the first level. The treatments did not affect the yield of milk fat but the highest level of inclusion of blood meal markedly reduced the concentration of fat.

The progressively increased intake of histidine with increasing intake of blood meal led to corresponding increases in the concentration of histidine in blood plasma (Table 4.8). However, despite its high concentration in blood meal, there was no such effect on the plasma concentration of lysine, which remained unaffected as the intake of blood meal increased. For the NEAA, falling intakes of serine and glycine, as the intake of feather meal was reduced, were accompanied by lowered concentrations of these AAs in plasma. The concentration of total AAs was decreased by the highest inclusion of blood meal. The lowest intake (10%) of blood meal increased ($P<0.05$) the concentration of plasma glucose compared with the higher levels of blood meal inclusion (Table 4.9). The concentrations of plasma urea were not affected by any of the treatments.

Although the results of Experiment 6 were as expected in that milk production was

Table 4.7 Effects of substituting blood meal for part of the feather meal in the supplement on food intake and milk production in Experiment 6

	Proportion of blood meal in mixture DM					
	0	**0.10**	**0.20**	**0.40**	**SED**[1]	**P value**[2]
DM intake						
Silage, kg/d	9.5[a]	10.2[b]	10.7[b]	10.6[b]	0.27	0.002
Supplement, kg/d	6.2	6.2	6.2	6.2	-	-
Milk yield, kg/d	20.3[a]	22.3[b]	22.3[b]	23.0[b]	0.64	0.005
Milk fat , g/kg	48.3[b]	43.4[ab]	42.8[ab]	38.5[a]	2.82	0.027
g/d	964	952	935	873	62.2	0.491
Milk protein, g/kg	29.3[a]	30.7[b]	30.8[b]	30.7[b]	0.46	0.013
g/d	593[a]	679[b]	681[b]	701[b]	25.2	0.003
Milk lactose, g/kg	46.6	47.1	45.9	46.9	0.92	0.619
g/d	948[a]	1049[b]	1029[b]	1082[b]	27.8	0.002

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a, b] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test.

Table 4.8 Effects of substituting blood meal for part of the feather meal in the supplement on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 6

	Proportion of blood meal in mixture DM					
	0	**0.10**	**0.20**	**0.40**	**SED[1]**	**P value[2]**
Histidine	14^{a}	21^{b}	25^{b}	36^{c}	2.7	<.001
Threonine	142^{b}	132^{b}	134^{b}	116^{a}	6.6	0.010
Arginine	73	76	74	69	3.7	0.319
Tryptophan	36	37	39	36	2.1	0.707
Methionine	15^{ab}	16^{b}	15^{ab}	14^{a}	0.7	0.048
Valine	296	306	294	253	18.3	0.051
Phenylalanine	50^{b}	50^{b}	53^{b}	45^{a}	2.7	0.057
Isoleucine	110^{ab}	115^{b}	116^{b}	101^{a}	6.3	0.122
Leucine	110^{ab}	118^{b}	117^{ab}	107^{a}	5.4	0.178
Lysine	46	50	53	53	3.4	0.934
Aspartic acid	9	10	9	8	1.1	0.680
Glutamic acid	58	57	57	53	3.1	0.489
Serine	131^{c}	109^{b}	100^{b}	83^{a}	6.7	<.001
Glycine	384^{c}	343^{b}	329^{ab}	294^{a}	17.6	0.001
Alanine	182	173	181	165	11.8	0.472
Tyrosine	53^{b}	53^{b}	51^{ab}	45^{a}	3.6	0.133
Asparagine	36^{b}	36^{b}	37^{b}	33^{a}	1.5	0.062
Taurine	33	35	34	31	2.3	0.386
Glutamine	246	230	244	215	18.1	0.325
Ornithine	43	45	43	45	3.4	0.934
Total AA	2058^{b}	2022^{b}	2004^{b}	1803^{a}	62.7	0.004

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a, b, c] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test.

Table 4.9 Effects of substituting blood meal for part of the feather meal in the supplement on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 6

	Proportion of blood meal in mixture DM					
	0	**0.10**	**0.20**	**0.40**	**SED[1]**	**P value[2]**
Glucose	641[ab]	660[b]	609[a]	611[a]	20.4	0.071
Urea	170	162	177	187	17.8	0.668

[1] SED, standard error of differences.

[2] Statistical significance of treatment effects by *F*-test.

[a, b] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test.

substantially increased by the inclusion of blood meal, they raise the question of why there was no further increase of milk production beyond the first level of inclusion. It is difficult to explain the lack of response in terms of AA supply because the inclusion of blood meal would be expected to increase the supply of all EAAs linearly with increasing level of inclusion. Hence, even if histidine were no longer first-limiting beyond the first level of inclusion of blood meal, the enhanced supply of all the other EAAs (Table 4.10) would be expected to fuel a further increase in the yield of milk protein. Particularly important in this regard would be the supplies of methionine and lysine, the putative next-limiting AAs. The calculated increase (Table 4.10) based on the AA composition of the basal diet (Table 4.11) in the supply of absorbable lysine was considerable (around 20 g/d for the highest level of blood meal) but the corresponding figure for methionine was very much lower (around 1 g/d). It is doubtful whether this amount of methionine would have been sufficient to allow a measurable increase in the synthesis of milk protein. Furthermore, although the high concentration of cystine in feather meal might contribute to the sulfur AA requirement of dairy cows, in fact, cystine of feather meal did not spare methionine for milk production (Pruekvimolphan *et al.*, 1997). Taken together with the results of Experiment 4, these results suggest that methionine was second-limiting for milk protein synthesis.

Table 4.10 The calculated increases (g/d), relative to the basal diet, in the supply of absorbable EAAs from progressively substituting blood meal for part of the feather meal in Experiment 6[1]

	Proportion of blood meal in mixture DM		
	0.10	**0.20**	**0.40**
Histidine	2.7	5.4	10.9
Threonine	1.0	2.0	4.0
Arginine	0.0	0.0	0.0
Methionine	0.3	0.5	1.1
Valine	0.7	1.5	3.1
Phenylalanine	1.6	3.2	6.5
Isoleucine	0.4	0.7	1.3
Leucine	2.8	5.5	11.0
Lysine	5.3	10.6	21.3
Tryptophan[2]	0.6	1.2	2.5

[1] Calculated with the following assumptions: ruminal degradability and intestinal digestibility of feather meal, 0.20 and 0.72 respectively (Chamberlain *et al.*, 1992); ruminal degradability and intestinal digestibility of blood meal, 0.15 (Palmquist *et al.*, 1993) and 0.80 (Johnson *et al.*, 1994) respectively.

[2] Tryptophan concentrations in feather meal and blood meal from Buttery *et al* (1978).

Table 4.11 Composition of EAA (g/ kg CP) of microbial protein, milk protein, silage and supplements used in the experiments

	Microbial protein[1]	**Milk protein**[2]	**Silage**[3]	**Feather meal**[4]	**Blood meal**[4]	**Barley**[5]
Histidine	17	26	25	7	43	26
Threonine	52	47	54	42	45	43
Arginine	49	36	53	65	49	57
Methionine	25	26	18	7	9	19
Valine	53	67	71	75	67	63
Phenylalanine	55	50	61	19	59	63
Isoleucine	54	60	59	45	38	42
Leucine	74	99	86	79	97	83
Lysine	81	83	41	22	90	44
Tryptophan[6]	16	13	14	4	12	12

[1] From Storm and Ørskov (1983).

[2] From Kaufmann (1980).

[3] From Chamberlain *et al.* (1982).

[4] From own analyses.

[5] From Buttery *et al.* (1978).

[6] Values for silage from Ohshima and McDonald (1979) and for feather meal and blood meal from Buttery *et al.* (1978).

CHAPTER FIVE

1. DETERMINATION OF THE SEQUENCE OF LIMITATION OF HISTIDINE, METHIONINE AND LYSINE AND 2. COMPARISON OF RESPONSES TO INTRAVENOUS INFUSION OF HISTIDINE ALONE, OR HISTIDINE IN COMBINATION WITH METHIONINE AND LYSINE WITH RESPONSES TO INTRAVENOUS INFUSION OF A MIXTURE OF ALL THE EAA IN COWS CONSUMING GRASS SILAGE AND A SUPPLEMENT CONTAINING FEATHER MEAL

5.1 INTRODUCTION

In relation to the future use of dietary supplements of histidine or histidine-rich proteins, it was important to establish how responses to enteral administration of histidine compared with those to intravenous administration. In the previous chapter, Experiment 5 examined responses to two levels of histidine given as infusion into the jugular vein or into the abomasum. However, the results of the experiment did not show any response on milk production for either route.

Apart from the effects of experimental stress mentioned in Chapter 4, there is an alternative, and perhaps more obvious, interpretation of the lack of response to histidine in Experiment 5 (and also in Experiment 2 in Chapter 3). Despite the demonstration, in earlier experiments, that histidine was first-limiting, for some reason, histidine may not have been first-limiting in these later experiments, or it may have been first-limiting by such a small margin that measurable increases in the yield of milk protein were prevented by a deficiency of the supply of the second-limiting AA.

It was therefore clear that the dietary 'model' needed to be characterized more fully in terms of the sequence of limitation of the AAs. In the first experiment, the responses of milk production were examined when each AA was omitted from a mixture of the group of possible next limiting AAs including methionine, lysine and tryptophan when histidine

was retained in all infusates. The second experiment compared responses of milk production to the intravenous infusions of 3 treatments which were of a mixture of EAA; a mixture of 3AA; and histidine alone, all treatments supplying the same amount of histidine. The aim of the second experiment was to determine how much of the milk production response to addition of all the EAA could be obtained by histidine alone or the group of 3 most-limiting AAs.

5.2 EXPERIMENTAL

5.2.1 Animals and their management

A total of 9 Friesian cows in their second to fourth lactations were used. The cows were 8-10 (Experiment 7) or 20-24 (Experiment 8) weeks into their lactations at the start of the experiment. Average body weights of animals were approximately 556 (range 504-607) and 585 kg (range 553-628) in Experiments 7 and 8, respectively. They were housed individually in metabolism stalls and milked each day at 06:00 and 15:00 h and food was provided in two equal meals at milking times.

The cows were given a basal diet consisting of *ad libitum* access to grass silage and 5 kg/day of a pelleted mixture of rolled barley, feather meal and citrus pulp (0.50: 0.25: 0.25, on a fresh weight basis) together with 2 kg/day rolled barley for Experiment 7. For Experiment 8, the cows were given a similar basal diet to that used for Experiment 7 but without the additional supplement of 2 kg/day rolled barley. The silage in each experiment was made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth and ensiled, after addition of Add-Safe at 12 litres/tonne in a bunker silo of 70 tonne capacity in Experiments 7 and 3 litres/tonne in Experiment 8. The chemical composition of the silage is shown in Table 5.1 and that of the supplements in Table 5.2. Both silages were well preserved with a low pH. The concentrations of lactic acid and residual water-soluble carbohydrate in the silages were very different. The feather meal cubes used in the two

Table 5.1 The chemical composition (g/kg DM unless stated otherwise) of the silages used in the experiments

	Exp. 7	**Exp. 8**
DM (g/kg)[1]	283	264
Organic matter	912	884
Total N	31.4	26.2
Ammonia-N (g/kg N)	107	161
NPN (g/kg N)	693	785
Neutral-detergent fibre	538	504
Acid-detergent fibre	307	314
pH	4.0	3.9
Water-soluble carbohydrate	119	44
Lactic acid	40	122
Acetic acid	12	26
Butyric acid	0	2
Ethanol	16	6

[1] By toluene distillation

Table 5.2 The chemical composition (g/kg DM, unless stated otherwise) of the feather meal cubes (FMC) and rolled barley used in the experiments

	Exp. 7		Exp. 8
	FMC	**Barley**	**FMC**
DM (g/kg)	878	861	869
Organic matter	901	977	898
Total N	46.1	17.9	49.1
Starch	252	623	297
Sugars	59	24	60
Neutral-detergent fibre	211	343	205
Acid-detergent fibre	114	73	113

experiments were of broadly similar composition. The crude protein (N x 6.25) concentrations in the basal diet were more similar, at approximately 212 and 198 g/d DM for Experiments 7 and 8, respectively.

5.2.3 Experimental treatments and design

5.2.3.1 Experiment 7

Experiment 7 was designed as a 5 x 5 Latin square with five treatments and five,10-day periods. The five treatments were (1) the basal diet as described above (Basal); (2) Basal plus a continuous intravenous infusion supplying (g/day) 9.0 histidine, 10.0 methionine, 25.5 lysine and 4.8 tryptophan (4AA); (3) Basal plus infusion of the AA mixture without methionine (-Met); (4) Basal plus infusion of the AA mixture without lysine (-Lys); and (5) Basal plus infusion of the AA mixture without tryptophan (-Trp).

All animals received the basal diet for at least 21 days before the start of the experiment. All infusions were dissolved in distilled water and infused, using a volumetric infusion pump (Smith and Nephew Medicals Ltd, Hull, Humberside, UK), in a volume of 4 litres/day into a jugular vein, into which an indwelling polyethylene catheter had been inserted before the start of the experiment. The amount of methionine, lysine and tryptophan infused were calculated as the approximate amounts supplied by the weight of casein containing 9 g histidine.

Food intake and milk yield were recorded daily. The composition of milk was determined on a representative, composite sample from the last four consecutive milkings in each experimental period. Samples of blood were obtained from a jugular catheter at 09:45; 11:00; 12:00; 13:00; 14:00 and 15:00 h on the last day of each period. Samples were taken into heparinized tubes and centrifuged immediately to separate plasma that was stored at –20°C prior to analysis. A bulked sample was analysed for AAs, glucose and urea.

5.2.3.2 Experiment 8

The experimental design was a 4x4 Latin square with four cows and four 10-day periods. The four treatments were (1) the basal diet as described above (Basal); (2) Basal plus a continuous intravenous infusion supplying a mixture of EAAs (EAA); (3) Basal plus infusion of a AA mixture of histidine, methionine, lysine (3AA); (4) Basal plus infusion of histidine alone (His). The amounts of AAs supplied by the infusion treatments in this experiment are shown in Table 5.3. The amount of AAs infused in the various treatments were calculated as the approximate amounts supplied by the weight of casein containing 6 g histidine.

Recording of food intake and milk yield and composition were as described for Experiment 7. Samples of blood were taken, treated and analysed as in Experiment 7.

5.2.4 Chemical analysis

Minced wet silage was analysed for dry matter by toluene distillation, total nitrogen, true protein, ammonia, lactic acid, ethanol, water-soluble carbohydrate, VFA and pH. Dried samples of silage were analysed for ash, NDF and ADF. Concentrate samples were analysed as appropriate for dry matter, total nitrogen, ash, NDF, ADF and starch. Blood plasma samples were analysed for urea, glucose and free amino acids. Milk samples were analysed for fat, crude protein and lactose.

5.2.5 Statistical analysis

For statistical analysis, mean values for feed intake and milk yield were taken for the last 7 days of each experimental period. Results of both experiments were analysed by ANOVA using the directives of Genstat 5 (Lawes Agricultural Trust, 1990). In Experiment 7, at the beginning of the experiment one of experimental cows was found to have lameness. She recovered well after the first period and completed the remaining periods.

Table 5.3 The amounts (g/day) of AAs supplied by the infusion treatments in Experiment 8

Amino acids	EAA	3 AA	His
Histidine	6.0	6.0	6.0
Methionine	7.1	7.1	-
Lysine	17.6	17.6	-
Tryptophan	3.6	-	-
Threonine	10.3	-	-
Valine	15.0	-	-
Phenylalanine	12.6	-	-
Isoleucine	11.8	-	-
Leucine	21.8	-	-
Arginine	8.6	-	-

Hence, for all analyses, there was one missing plot for the first period in the statistical analysis. All differences between pairs of means of treatments in each experiment were inspected by the LSD method at the 5% level.

5.3 RESULTS AND DISCUSSION

5.3.1 Experiment 7

Silage intake and milk production are given in Table 5.4. There was a tendency of increase of the intake of silage over Basal by the tryptophan-free treatment ($P<0.10$). The yield of milk was increased ($P<0.05$) over Basal and the lysine-free treatment by the tryptophan-free treatment. The yield and concentration of milk protein were increased by the 4AA and tryptophan-free treatments relative to Basal. The concentration of milk fat was increased ($P<0.05$) over the lysine-free treatment by the tryptophan-free treatment. None of treatments affected the concentrations and yield of milk lactose.

The concentrations of AAs in blood plasma are shown in Table 5.5. The concentration of histidine was increased over Basal by all infusion treatments. The methionine-free treatment increased the concentration of isoleucine relative to Basal and the 4AA treatment. The 4AA treatment increased the concentrations of all infused AAs and the tryptophan-free treatment decreased the concentrations of serine and glycine compared with Basal. The 4AA treatment decreased the concentration of glucose in blood plasma (Table 5.6). The methionine-free treatment increased ($P<0.05$) the concentration of plasma urea relative to Basal and the lysine-free treatment.

The amount of histidine infused was based on the results of Experiment 4 which showed the maximum response of milk protein synthesis at the 9 g/d level. Results for milk production and for the concentration of plasma tryptophan in the tryptophan-free treatment indicated that tryptophan was not limiting. No increase of the yield of milk protein by supplementation of AAs without methionine or without lysine indicated that

Table 5.4 Effects of intravenous infusions of amino acids on food intake and milk production in Experiment 7

	Basal	4AA	- Met	- Lys	- Trp	SED[1]	P value[2]
DM intake							
Silage, kg/d	12.0	12.1	12.5	12.4	13.0	0.43	0.250
Supplement, kg/d	6.1	6.1	6.1	6.1	6.1	-	-
Milk yield, kg/d	27.0^{a}	27.8^{ab}	27.5^{ab}	27.2^{a}	28.6^{b}	0.63	0.167
Milk fat , g/kg	39.2	39.0	38.7	38.2	38.0	1.71	0.986
g/d	1053^{ab}	1058^{ab}	1061^{ab}	1030^{a}	1101^{b}	30.0	0.271
Milk protein, g/kg	28.8^{a}	31.6^{b}	28.7^{a}	29.9^{a}	31.6^{b}	0.73	0.003
g/d	771^{a}	872^{bc}	784^{a}	812^{ab}	897^{c}	27.5	0.003
Milk lactose, g/kg	48.4	47.5	47.8	47.8	48.1	0.42	0.356
g/d	1305	1322	1312	1298	1376	37.2	0.294

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

[a, b, c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test

Table 5.5 Effects of intravenous infusions of amino acids on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 7

	Basal	4AA	- Met	- Lys	- Trp	SED[a]	P value[b]
Histidine	22[a]	43[b]	48[b]	52[b]	52[b]	7.3	0.010
Threonine	111[ab]	109[ab]	119[b]	115[b]	99[a]	7.2	0.148
Arginine	63[a]	69[ab]	74[b]	68[ab]	70[ab]	4.1	0.156
Tryptophan	42[a]	54[b]	52[b]	52[b]	40[a]	4.7	0.028
Methionine	14[a]	59[b]	15[a]	39[ab]	35[ab]	14.3	0.048
Valine	420[ab]	371[a]	469[b]	446[ab]	412[ab]	39.8	0.211
Phenylalanine	47[ab]	40[a]	50[b]	49[ab]	46[ab]	4.4	0.297
Isoleucine	156[a]	149[a]	186[b]	164[ab]	165[ab]	10.7	0.047
Leucine	148[ab]	132[a]	173[b]	158[ab]	151[ab]	14.6	0.164
Lysine	51[a]	147[b]	99[ab]	56[a]	101[ab]	23.0	0.009
Aspartic acid	5	5	5	5	5	0.4	0.295
Glutamic acid	50	49	51	56	53	3.7	0.379
Serine	124[c]	94[a]	118[bc]	99[ab]	79[a]	9.7	0.004
Glycine	418[b]	369[ab]	428[b]	376[b]	288[a]	39.2	0.031
Alanine	175	172	179	173	157	13.8	0.557
Tyrosine	54[b]	42[a]	54[b]	52[b]	45[ab]	4.5	0.080
Asparagine	39[ab]	40[ab]	43[b]	40[ab]	36[a]	2.4	0.094
Taurine	26[a]	30[ab]	34[ab]	30[ab]	35[b]	3.5	0.137
Glutamine	194	184	193	207	192	21.0	0.863
Ornithine	36[a]	43[ab]	47[b]	51[b]	47[b]	4.6	0.054
Total AA	2189[ab]	2201[ab]	2468[b]	2289[ab]	2107[a]	127.1	0.170

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

[a, b, c] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test

Table 5.6 Effects of intravenous infusions of amino acids on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 7

	Basal	**4AA**	**- Met**	**- Lys**	**- Trp**	**SED[1]**	**P value[2]**
Glucose	707[b]	602[a]	667[b]	686[b]	699[b]	29.5	0.029
Urea	167[a]	190[ab]	203[b]	172[a]	183[ab]	11.9	0.071

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

[a, b] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test

both AAs seemed to be co-limiting milk protein synthesis with histidine. Methionine was more likely to be the co-limiting AA or the very close second-limiting AA because the yield of milk protein in the methionine-free treatment was significantly different ($P<0.05$) from the 4AA treatment whilst for the lysine-free treatment it was not. However, the values for methionine-free and lysine-free treatments themselves were not significantly different.

5.3.2 Experiment 8

Silage intake and milk production are given in Table 5.7. There were no statistically significant effects of the treatments on the intake of silage. Compared with Basal, all infusion treatments increased the yields of milk, milk protein and lactose. Although there were no significant differences between treatments, the infusion of histidine alone tended to decrease ($P<0.10$) the yield of milk protein relative to the EAA and 3AA treatments. The high concentration of milk fat with Basal was reduced ($P<0.05$) by the 3AA treatment. The EAA and His treatments increased ($P<0.05$) the concentration of lactose compared with Basal.

Concentrations of AAs in blood plasma are shown in Table 5.8. The concentration of histidine was increased by all treatments compared with Basal. The concentrations of methionine and lysine were significantly increased by the EAA and 3AA treatments. The EAA infusion markedly increased the concentrations of the branched-chain AAs and phenylalanine. The concentration of tyrosine was greater for Basal than for the EAA and His treatments. The concentrations of plasma glucose and urea were not affected by any treatment (Table 5.9).

In Chapter 3, the intravenous administration of 6 g/d histidine alone significantly improved the synthesis of milk protein in Experiment 3. But, when histidine was supplied with the next limiting AAs, the response of milk protein synthesis was linearly increased by increasing doses of histidine (up to 9 g/d histidine) in Experiment 4. Experiment 8 was

carried out to compare the extent of responses to the histidine supplementation when it was supplied either alone, with the next limiting AAs or with the mixture of EAA. Histidine was infused at 6 g/d in all the treatments because the response was maximized at this level in Experiment 3 and also so that we could compare the responses between experiments. Tryptophan was removed from the 3AA treatment because the results of the last experiment indicated that it was clearly not limiting milk production.

The results of this experiment reconfirmed that histidine is the first-limiting AA for milk production of cows consuming the basal diet. However, the response of milk protein synthesis to the supplementation of total EAA was similar to that to the 3AA treatment (approximately 21% increase relative to Basal) whereas the His treatment increased protein yield by 12%.

Table 5.7 Effects of intravenous infusions of amino acids on food intake and milk production in Experiment 8

	Basal	EAA	3 AA	His	SED[1]	P value[2]
DM intake						
Silage, kg/d	13.4	12.5	12.9	12.5	0.41	0.204
Supplement, kg/d	4.4	4.4	4.4	4.4	-	-
Milk yield, kg/d	14.2[a]	17.1[b]	16.6[b]	16.5[b]	0.55	0.008
Milk fat , g/kg	58.5[b]	53.3[ab]	49.5[a]	50.6[ab]	3.69	0.170
g/d	800	891	814	817	53.9	0.399
Milk protein, g/kg	37.0	37.0	37.9	35.3	1.16	0.244
g/d	518[a]	625[b]	626[b]	578[b]	24.4	0.013
Milk lactose, g/kg	42.9[a]	44.3[b]	43.7[ab]	44.2[b]	0.46	0.081
g/d	617[a]	762[b]	730[b]	736[b]	29.8	0.011

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

[a, b] Means in the same row with unlike superscripts differ significantly ($P<0.05$) by *t*-test

Table 5.8 Effects of intravenous infusions of amino acids on the concentrations (μmol/l) of amino acids in blood plasma in Experiment 8

	Basal	EAA	3AA	His	SED[1]	P value[2]
Histidine	7[a]	17[b]	20[bc]	24[c]	2.2	0.001
Threonine	126[ab]	146[b]	111[a]	111[a]	13.9	0.122
Arginine	62	75	73	64	5.5	0.152
Tryptophan	35	37	35	34	2.2	0.528
Methionine	14[a]	21[b]	25[c]	12[a]	1.3	<.001
Valine	247[a]	327[b]	263[a]	258[a]	22.2	0.040
Phenylalanine	47[a]	59[b]	45[a]	45[a]	3.1	0.011
Isoleucine	96[a]	127[b]	109[ab]	102[a]	8.6	0.046
Leucine	94[a]	132[b]	91[a]	88[a]	8.2	0.005
Lysine	42[a]	63[b]	70[b]	41[a]	6.2	0.008
Aspartic acid	5	5	5	4	0.8	0.412
Glutamic acid	47[ab]	44[a]	51[b]	49[b]	2.0	0.073
Serine	134[b]	108[a]	108[a]	118[ab]	10.5	0.136
Glycine	282	263	297	278	28.3	0.712
Alanine	129[a]	131[a]	148[b]	132[a]	6.5	0.084
Tyrosine	49[b]	39[a]	44[ab]	39[a]	3.0	0.045
Asparagine	34[ab]	31[a]	36[b]	33[ab]	1.7	0.122
Taurine	19[ab]	18[ab]	20[b]	17[a]	1.1	0.140
Glutamine	150	160	158	171	19.5	0.765
Ornithine	36	42	43	37	4.5	0.467
Total AA	1655	1845	1749	1656	83.7	0.172

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

[a, b, c] Means in the same row with unlike superscripts differ significantly (P<0.05) by *t*-test

Table 5.9 Effects of intravenous infusions of amino acids on the concentrations (mg/l) of glucose and urea in blood plasma in Experiment 8

	Basal	**EAA**	**3AA**	**His**	**SED**[1]	**P value**[2]
Glucose	622	597	634	618	29.8	0.677
Urea	177	185	169	185	6.9	0.154

[1] SED, standard error of differences

[2] Statistical significance of treatment effects by *F*-test

CHAPTER SIX

GENERAL DISCUSSION

6.1 INTRODUCTION

The objectives of the project were to devise a diet for which the first-limiting AA could be clearly identified and with which sizable and reproducible increases in the yield of milk protein could be obtained when the first-limiting acid was infused intravenously or abomasally. This should then allow calculation of the efficiency with which the first-limiting AA is used for the secretion of milk protein, when it is supplied direct into the abomasum or into peripheral blood.

The results of Experiments 1, 3, 4 and 8 showed histidine to be the first-limiting AA for milk protein synthesis and also indicated that very substantial increases of milk production were obtained by supplementation with histidine alone. However, the responses to supplementation of histidine were not reproducible across all experiments.

The observations have been briefly discussed at the end of each experiment in Chapters 3, 4 and 5 but there remains a need for a more integrated discussion of the experiments and an examination of some aspects of the results, which have not previously been considered.

6.2 THE TECHNIQUE OF INTRAVENOUS ADMINISTRATION OF AAs

For the further refinement of the protein rationing schemes used in practice, knowledge of the efficiency of use of AAs for milk protein synthesis is required. It is difficult to estimate the efficiency by supplementation of AAs into the diet or the abomasum because with these techniques the exact quantity of AAs supplied is not known, owing to uncertainty around absorbability and the gut metabolism of AAs. The intravenous technique supplies AAs direct into the peripheral circulation via the jugular vein, thereby

eliminating or reducing the modifying effect of the gut tissues during absorption, allowing the efficiency of use of AAs to be estimated.

In terms of animal welfare, the intravenous technique is preferable to abomasal infusion because abomasal infusion needs a surgical preparation that may influence digestion and intake and, furthermore, may increase stress in the animal; in addition, a long recovery period is required after the operation. Even when the animals are fully recovered, they need special management for the rest of their lives. However, the intravenous technique requires no permanent surgical modification and also allows greater freedom of choice of experimental animals compared with the abomasal infusion technique because of the limited availability of fistulated cows. However, despite these advantages, the intravenous technique also requires special care to minimize the risk of infection. Particular care is needed in the preparation of solutions for infusion of AAs and in the maintenance of the jugular catheter.

When mixtures of AAs in the present experiments were infused to the jugular vein, the changes in milk yield occurred very rapidly; over 95% of the increase occurred within 24 h of the start of the infusion (Figure 6.1). Similarly, Metcalf *et al* (1996b) indicated that milk protein concentration increased by over 90% within 24 h when a mixture of EAA was intravenously infused. However, Choung and Chamberlain (unpublished) observed that animals did not reach their maximum level of response in milk yield until 5 days after the start of intra-abomasal infusion of casein. The intra-abomasal or duodenal infusion of AAs seems to produce slower responses. This faster response to intravenous infusion is a further advantage in that it permits the use of shorter experimental periods.

The intravenous technique has the drawback that it may be difficult to extrapolate to the response to be expected from dietary addition of AAs. Therefore, it was necessary to compare the response of milk production to the intravenous infusion of increasing doses of the first-limiting AA with the response to its intra-abomasal infusion (Experiment 5). If

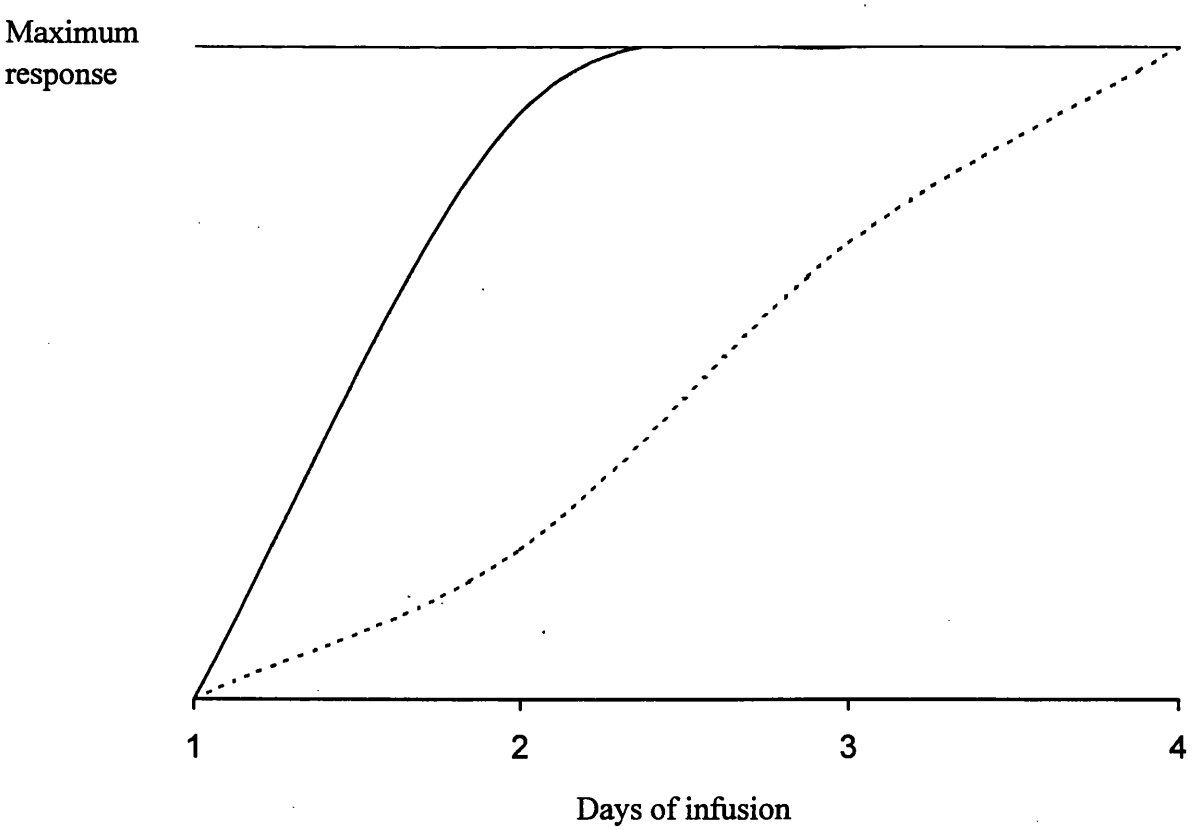

Figure 6.1 Time (days) to reach maximum response of milk yield to intravenous infusion of AAs (—; Experiments 1, 7 and 8) compared with abomasal infusion of casein (·····; Choung and Chamberlain, unpublished)

this experiment had been successful, it would have allowed a calculation of the net effects of absorbability and gut metabolism of histidine and an estimation of the efficiency of use of histidine supplied from the gut for protein output in milk.

6.3 GENERAL ASSESSMENT OF THE 'DIETARY MODEL'

In containing feather meal as the sole protein concentrate, the basal diet used in these experiments is unrepresentative of those used in practice. However, the dietary model based on feather meal provided a useful experimental diet characterized by a primary limitation of histidine. Furthermore, sizable responses of milk protein synthesis to histidine supplementation were obtained with this diet, although, in the later experiments, histidine supplementation did not always show positive responses of milk protein synthesis. This inconsistency first appeared in the results of Experiment 2. At that time, the favoured explanation was that the level of infusion of histidine alone was too high, such that it induced a severely unbalanced AA supply resulting in no response of milk protein yield. This explanation was consistent with subsequent observations in Experiments 3 and 4. However, as the series of experiments progressed it appeared that a more likely explanation was fluctuation of the supply of, or demand for, the next-limiting AAs, methionine and lysine.

6.3.1 Responses of milk protein production to supplementation of histidine

The results of Experiments 1, 3, 4 and 8 showed that histidine was the first-limiting AA for the synthesis of milk protein. When 6.0 g/d of histidine alone was supplemented (Experiment 3), the response of milk protein synthesis was maximized. In previous work, carried out by many researchers, responses to addition of the first-limiting AA alone have been relatively small because of the rapid appearance of deficiencies of the next-limiting AAs. For example, supplementations of methionine (Fisher, 1972) and lysine (Schwab *et*

al., 1976) into the dairy cow by intravenous and intra-abomasal infusions respectively increased milk protein yield by 30 and 18 g/d over the basal treatments (Table 6.1). Recent work in Finland (Huhtanen *et al.*, 1997; Vanhatalo *et al.*, 1997) showed that intra-abomasal infusion of 6.5 g/d of histidine alone increased milk protein yield by 3.5 and 4.5 % over the basal treatments in both experiments. In contrast with the responses of milk protein output in the Finnish experiments, the supplementation of 6.0 g/d of histidine alone into a jugular vein of dairy cows increased milk protein yield by 16.6 and 11.6 % (111 and 60 g/d) over the basal treatments in Experiments 3 and 8 respectively. Although part of the difference in response may relate to abomasal versus intravenous administration, the greater part of the difference probably relates to the different diets used.

Supplementation of histidine with the group of next limiting AAs containing methionine, lysine and tryptophan increased the yield of milk protein in Experiments 1, 4, 7 and 8 (Table 6.2) and the increases were considerable (ranging from 70 to 164 g/d) and reproducible. However, supplementation of 6 g/d of histidine alone into the jugular vein and abomasum in Experiment 5 did not increase milk protein yield. The results of Experiments 7 and 8 shed light on the likely cause of the lack of response in Experiment 5. Taken together, the results of Experiments 7 and 8 show that the response to histidine is dependent on the supplies of methionine and lysine, the next limiting AAs. It may be that fluctuations in the background supplies of methionine and lysine result in these acids becoming co-limiting with histidine, so preventing any measurable response to the addition of histidine alone. The likely magnitude of variations in the supplies of methionine, lysine and histidine is explored further in the following sections.

6.3.2 Variations in supply of microbial AAs

The ruminant receives 40 to 80% of its daily AA requirements from microbial protein flowing to the small intestine (Sniffen and Robinson, 1987). In the experiments in

Table 6.1 Increases in the yield of milk protein over the basal diet treatment with supplementation of AAs

Infusate	Supply of AA (g/d)	Increase of Milk protein yield		Infusion type	Reference
		g/d	%		
Methionine	11.2	30	6.1	Intravenous	Fisher (1972)
Lysine	27.8	18	2.1	Intra-abomasal	Schwab *et al.* (1976)
Histidine	6.5	24	3.5	Intra-abomasal	Huhtanen *et al.* (1997)
Histidine	6.5	29	4.2	Intra-abomasal	Vanhatalo *et al.* (1997)
Histidine	6.0	111	16.6	Intravenous	Experiment 3
Histidine	6.0	60	11.6	Intravenous	Experiment 8

Table 6.2 Increases of the yield of milk protein over the basal treatment to the intravenous infusions of mixtures of AAs including histidine in the experiments

Source of data	Supplementation of AAs (g/d)				Milk protein yield	
	His	Met	Lys	Trp	g/d	%
Exp 1	9.7	9.1	30.0	2.6	111	15.0
Exp 4	9.0	8.0	28.0	2.5	164	22.9
Exp 4	6.0	8.0	28.0	2.5	111	15.5
Exp 4	3.0	8.0	28.0	2.5	70	9.8
Exp 7	9.0	10.0	25.5	4.8	101	13.1
Exp 7	9.0	10.0	25.5	-	126	16.3
Exp 8	6.0	7.1	17.6	-	108	20.8

this thesis microbial AAs contributed 47 to 52% of total AA supply (1476 to 1726 g MCP/d; see Table 6.3), when the microbial protein flow to the duodenum of cows in the experiments was calculated assuming rates of microbial protein synthesis (MPS) of 11.0 g MCP/MJ FME (AFRC, 1992) and the microbial AA composition from Storm and Ørskov (1983). When the total AA flow and the flows of individual AAs to the duodenum were calculated and the milk protein score (i.e. concentration of AA relative to its concentration in milk protein) was estimated, histidine was the first-limiting AA in all the experiments irrespective of differences in MPS (Table 6.4). The calculated figures show that histidine is the first-limiting AA, although there were fluctuations in the supply of total AAs.

AFRC (1992) assumes an average microbial protein yield of 11.0 g MCP/MJ FME. However, in experiments in which they have been measured, microbial yields have been shown to vary considerably (Chamberlain and Thomas, 1979; Chamberlain and Thomas, 1980; Mathers and Miller, 1981; Goetsch and Owens, 1985; Robinson *et al.*, 1985; Merchen *et al.*, 1986; Madsen and Hvelplund, 1988) particularly when diets contain large proportions of grass silage (Greife *et al.*, 1985; Huhtanen, 1987; Rooke *et al.*, 1987; Chamberlain *et al.*, 1993). Indeed, in a review of published experiments in which various carbohydrate supplements were added to the rumen of animals consuming grass silage, Chamberlain and Choung (1995) calculated that the incremental increases of MPS varied from 7 to 43 g N/kg OMDR. Overall, the mean value for MPS with diets containing large proportions of grass silage (comparable to that in the experiments in this thesis) and cereal-based supplements is 8.6 g MCP/MJ FME (Thomas *et al.*, 1980; Chamberlain *et al.*, 1986; Chamberlain and Choung, 1995).

When the milk protein scores were calculated using the AA composition of microbial protein determined by Storm and Ørskov (1983) but with three different rates of microbial protein synthesis (i.e. 13.7, 11.0 and 8.6 g MCP/MJ FME), histidine remained the first-limiting AA for milk production on all the basal diets in the experiments in this thesis

Table 6.3 The calculated flow of microbial protein and EAAs (g/d) in the duodenum of cows fed the basal diet in the experiments[1]

	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
Histidine	20	22	21	21	19	19	22	20
Threonine	62	66	64	64	58	58	67	62
Arginine	58	62	61	60	54	55	63	58
Methionine	30	32	31	31	28	28	32	30
Valine	63	68	66	65	59	59	69	63
Phenylalanine	66	70	68	67	61	62	71	65
Isoleucine	64	69	67	66	60	60	70	64
Leucine	88	94	92	91	82	83	96	89
Lysine	96	103	100	99	90	91	105	96
Tryptophan	19	20	20	20	18	18	21	19
Total EAA	567	607	589	583	527	533	616	565
Total AA	1190	1274	1237	1224	1107	1119	1295	1187
MCP yield	1587	1699	1650	1633	1476	1492	1726	1583
ERDP/MJ FME	12.8	13.0	14.1	13.4	12.1	12.3	13.5	12.9

[1] Calculated according to AFRC (1992) and taking the composition of AAs in microorganisms in the rumen from Storm and Ørskov (1983).

Table 6.4 The calculated flow (g/d) of EAAs in the duodenum of cows fed the basal diet in the experiments[1]

	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
Histidine	38 (0.62)[2]	40 (0.63)	41 (0.62)	40 (0.60)	35 (0.60)	36 (0.58)	42 (0.62)	39 (0.60)
Threonine	114 (1.05)	121 (1.05)	126 (1.04)	126 (1.04)	108 (1.04)	115 (1.03)	128 (1.05)	121 (1.04)
Arginine	126 (1.51)	132 (1.50)	141 (1.52)	142 (1.53)	122 (1.52)	131 (1.54)	141 (1.51)	136 (1.53)
Methionine	43 (0.72)	46 (0.73)	47 (0.70)	46 (0.69)	40 (0.70)	42 (0.68)	48 (0.71)	45 (0.69)
Valine	144 (0.93)	152 (0.93)	162 (0.94)	163 (0.95)	139 (0.94)	150 (0.95)	163 (0.94)	157 (0.95)
Phenylalanine	127 (1.10)	135 (1.10)	141 (1.10)	140 (1.09)	121 (1.09)	128 (1.08)	142 (1.10)	135 (1.09)
Isoleucine	119 (0.86)	127 (0.87)	133 (0.86)	133 (0.86)	114 (0.86)	121 (0.85)	135 (0.87)	128 (0.86)
Leucine	180 (0.79)	191 (0.79)	201 (0.79)	201 (0.79)	172 (0.79)	184 (0.79)	202 (0.78)	193 (0.79)
Lysine	131 (0.68)	140 (0.69)	141 (0.66)	140 (0.65)	123 (0.67)	127 (0.65)	145 (0.67)	135 (0.65)
Tryptophan	28 (0.95)	31 (1.00)	31 (0.92)	30 (0.90)	26 (0.91)	27 (0.88)	32 (0.94)	29 (0.91)
Total EAA	1052	1116	1163	1161	1000	1062	1176	1117
Total AA	2315	2448	2569	2577	2218	2371	2592	2476

[1] Calculated according to AFRC (1992) and assuming a ruminal degradation of 0.20 for feather meal protein (Chamberlain *et al.*, 1992).

[2] Milk protein score (concentration of AA relative to its concentration in milk protein).

Table 6.5 The predicted concentrations (presented as the milk protein scores[1]) of histidine, methionine and lysine, and the flows of microbial AA and total AA (g/d) in whole duodenal digesta for animals consuming the basal diet in the experiments when microbial protein synthesis (g MCP/MJ FME) is varied from 8.6 to 13.7, but microbial composition is maintained constant[2]

MPS (g MCP/MJ FME)[3]		Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
13.7	Histidine	0.63	0.63	0.62	0.61	0.61	0.59	0.62	0.60
	Methionine	0.75	0.75	0.73	0.72	0.73	0.71	0.74	0.72
	Lysine	0.72	0.72	0.70	0.69	0.70	0.68	0.71	0.69
	Microbial AA (g/d)	1483	1582	1541	1521	1377	1397	1613	1480
	Total AA (g/d)	2607	2756	2873	2874	2488	2649	2909	2770
11.0	Histidine	0.62	0.63	0.62	0.60	0.60	0.58	0.62	0.60
	Methionine	0.72	0.73	0.70	0.69	0.70	0.68	0.71	0.69
	Lysine	0.68	0.69	0.66	0.65	0.67	0.65	0.67	0.65
	Microbial AA (g/d)	1190	1274	1237	1224	1107	1119	1295	1187
	Total AA (g/d)	2315	2448	2569	2577	2218	2371	2592	2476
8.6	Histidine	0.62	0.63	0.61	0.59	0.59	0.57	0.62	0.59
	Methionine	0.69	0.70	0.67	0.66	0.67	0.64	0.68	0.66
	Lysine	0.65	0.65	0.63	0.62	0.63	0.61	0.64	0.62
	Microbial AA (g/d)	931	993	968	955	864	877	1013	929
	Total AA (g/d)	2054	2167	2300	2308	1975	2129	2309	2219

[1] Concentration of AA relative to its concentration in milk protein.

[2] Fixed microbial composition from Storm and Ørskov (1983); 17, 25 and 81 g/kg DM for histidine, methionine and lysine respectively.

[3] The figures for microbial protein synthesis (MPS) from Chamberlain and Choung (1995): 13.7 (silage diet supplemented with intraruminal glucse and casein) from Rooke *et al.* (1987); 11.0 from AFRC (1992); and 8.6 (mean of the rates of MPS when diet contained grass silage plus cereal-based supplements) from Chamberlain and Choung (1995), Chamberlain *et al.* (1986) and Thomas *et al.* (1980).

(Table 6.5). It is particularly noticeable that, at the lowest rate of MPS, the differences between the scores of histidine and those of methionine and lysine are considerably smaller than at the higher rates of MPS. These calculations illustrate how a fall of around 20% in MPS, which is well within the range of values reported in the literature, can markedly alter the relative scores of the three limiting AAs. The more similar the scores of the three AAs become, the smaller the potential response to addition of histidine.

Recent reviews by Clark *et al.* (1992) and Rulquin and Vérité (1993) suggest that differences in AA composition of microbial protein can be large. Furthermore, it is noteworthy that, among AAs, the coefficients of variation of microbial AA composition were largest for histidine (21.3%) and methionine (25.6%) (Clark *et al.*, 1992). Thus large errors may occur in the estimation of the supply of individual AA passing to the small intestine if an average composition of ruminal bacteria is assumed. Although the diet containing feather meal is highly deficient in histidine, methionine and lysine, variations of composition and quantity of microbial AAs reaching the duodenum can decide the net effect on the order of limitation of AAs for milk protein synthesis. When the milk protein scores were calculated using the rate (11.0 g MCP/MJ FME) of MPS from AFRC (1992) but with five different AA compositions of microbial protein from Thomas and Chamberlain (1982), Storm and Ørskov (1983), Chamberlain *et al.* (1986), Clark *et al.* (1992) and Rulquin and Vérité, (1993) for the basal diet in the experiments in this thesis, the order of limitation of the three AAs varied (Table 6.6). So it would seem that, although similar diets were used in all the experiments in this thesis, if the AA composition of microbial protein varied, again within reported limits, the response of milk protein yield to supplementation of histidine alone would not be consistent because not only did the relative scores of the three AAs vary widely, but sometimes histidine was not first-limiting. For example, in Experiment 5 (Table 6.6), when the milk protein scores are calculated based on the microbial composition from Storm and Ørskov (1983), there would be scope

Table 6.6 The predicted concentrations (presented as the milk protein scores[1]) of histidine, methionine and lysine in whole duodenal digesta in animals consuming the basal diet in the experiments as affected by variations in the composition of microbial AA when microbial synthesis is constant [2]

Sources of microbial composition[3]	Composition of microbial AA (g/kg AA)		Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
A	Histidine	17	0.62	0.62	0.62	0.60	0.60	0.58	0.62	0.60
	Methionine	25	0.72	0.72	0.70	0.69	0.70	0.68	0.71	0.69
	Lysine	81	0.68	0.68	0.66	0.65	0.67	0.65	0.67	0.65
B	Histidine	20	0.68	0.68	0.67	0.65	0.66	0.64	0.68	0.65
	Methionine	22	0.66	0.66	0.65	0.63	0.64	0.62	0.66	0.64
	Lysine	83	0.70	0.70	0.67	0.66	0.68	0.66	0.69	0.67
C	Histidine	20	0.68	0.68	0.67	0.65	0.66	0.64	0.68	0.65
	Methionine	25	0.72	0.72	0.70	0.69	0.70	0.68	0.71	0.69
	Lysine	84	0.70	0.70	0.68	0.67	0.69	0.66	0.69	0.67
D	Histidine	20	0.68	0.68	0.67	0.66	0.66	0.64	0.68	0.65
	Methionine	26	0.74	0.74	0.72	0.71	0.72	0.69	0.73	0.71
	Lysine	79	0.67	0.67	0.65	0.64	0.66	0.63	0.66	0.64
E	Histidine	18	0.64	0.64	0.63	0.62	0.62	0.60	0.64	0.62
	Methionine	25	0.72	0.72	0.70	0.69	0.70	0.68	0.71	0.69
	Lysine	80	0.68	0.68	0.66	0.65	0.66	0.64	0.67	0.65

[1] Concentration of AA relative to its concentration in milk protein.

[2] Microbial protein synthesis from AFRC (1992); 11.0 g MCP/MJ FME.

[3] Sources of AA composition of microbial protein for A, B, C, D, and E respectively from Storm and Ørskov (data from sheep fed barley and straw; 1983); from Chamberlain *et al.* (data from sheep fed grass silage supplemented with barley; 1986); from Thomas and Chamberlain (data from sheep fed grass silage; 1982); from Clark *et al.* (data from animals receiving 61 dietary treatments in 35 experiments; 1992); and from Rulquin and Verite [data from 66 published data cited by Le Henaff (1991); 1993].

for an increased milk protein output in response to addition of extra histidine because histidine is more deficient (i.e. 0.60 for histidine vs. 0.70 and 0.67 for methionine and lysine). However, when the milk protein scores are calculated based on the figures from Chamberlain *et al.* (1986), methionine is first-limiting (i.e. 0.66, 0.64 and 0.68 for histidine, methionine and lysine) which would rule out a measurable response of milk protein yield to addition of histidine alone.

The overall effect of these variations in MPS and microbial composition on AA scores and the order of limitation is shown in Table 6.7. The clear implication is that the lack of response to histidine supplementation could be caused by variations of microbial AA composition and the rate of MPS.

6.3.3 Other sources of variation in supply of AAs

6.3.3.1 Silage

All the silages used in the experiments in the thesis were made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth. Add-Safe (ammonium tri-hydrogen tetraformate 550 g/l; ammonium tri-hydrogen tetra-propionate, 150 g/l; BP Chemicals Ltd, Hull, Humberside, UK) was used as an additive on all the silages but it was applied at different rates (3 l/tonne for the silages used in Experiments 1, 2, 3, 6 and 8, 3.9 l/tonne for the silage used in Experiment 5, 9 l/tonne for the silage used in Experiment 4 and 12 l/tonne for the silage used in Experiment 7). This led to some differences in concentrations of WSC and lactic acid which can influence rates of MPS (Jaakkola *et al.*, 1991, 1993; Miettinen, 1998). It is therefore possible that the silages themselves could have led to variations in MPS between the experiments but there is no obvious relationship between silage type and responses to AA supplements in these experiments.

6.3.3.2 UDP source

Table 6.7 The predicted concentrations (presented as the milk protein scores[1]) of histidine, methionine and lysine in whole duodenal digesta for the average composition of basal diet used in the experiments when values for microbial protein synthesis and the AA composition of microbial protein are varied.

MPS (g CP/MJ FME)[2]	Sources of microbial AA composition[3]	Apparent limiting amino acids		
		First	Second	Third
13.7	A	His (0.60)	Lys (0.69)	Met (0.72)
	B	Met (0.66)	His (0.67)	Lys (0.70)
	C	His (0.67)	Lys (0.71)	Met (0.72)
	D	His (0.67)	Lys (0.68)	Met (0.74)
	E	His (0.63)	Lys (0.68)	Met (0.72)
11.0	A	His (0.60)	Lys (0.67)	Met (0.69)
	B	Met (0.63)	His (0.65)	Lys (0.67)
	C	His (0.65)	Lys (0.68)	Met (0.69)
	D	His/Lys (0.65)		Met (0.71)
	E	His (0.62)	Lys (0.65)	Met (0.69)
8.6	A	His (0.60)	Lys (0.62)	Met (0.67)
	B	Met (0.61)	Lys (0.63)	Met (0.64)
	C	Lys (0.63)	His (0.64)	Met (0.66)
	D	Lys (0.61)	His (0.64)	Met (0.68)
	E	His/Lys (0.61)		Met (0.66)

[1] Concentration of AA relative to its concentration in milk protein.

[2] The figures for microbial protein synthesis (MPS) from Chamberlain and Choung (1995): 13.7 (silage diet supplemented with casein in the rumen) from Rooke *et al.* (1987); 11.0 from AFRC (1992); and 8.6 (mean of the rates of MPS when diet contained grass silage plus cereal-based supplements) from Chamberlain and Choung (1995), Chamberlain *et al.* (1986), Thomas *et al.* (1980).

[3] Sources of AA composition of microbial protein for A, B, C, D, and E from Storm and Ørskov (data from sheep fed barley and straw; 1983); from Chamberlain *et al.* (data from sheep fed grass silage supplemented with barley; 1986); from Thomas and Chamberlain (data from sheep fed grass silage; 1982); from Clark *et al.* (data from animals receiving 61 dietary treatments in 35 experiments; 1992); and from Rulquin and Verite [data from 66 published data cited by Le Henaff (1991); 1993].

Proteins of grass silage and supplementary barley are highly degradable in the rumen (AFRC, 1992). Thus most of the undegraded dietary protein (UDP) reaching the small intestine was supplied by feather meal. The protein quality of feather meal can be affected by processing conditions (Wang and Parsons, 1997) but variation due to processing would be expected to be small with the feather meal used in these experiments because it was supplied by one company (Prosper de Mulder Ltd, Doncaster, UK) using the same processing method. The feather meal cubes (FMC) were formulated as a mixture of rolled barley, feather meal and citrus pulp (0.50: 0.25: 0.25, on a fresh weight basis). However, the concentrations of protein in the FMC used in the experiments varied from 259 to 313 g CP/kg DM. This difference of the concentration of protein cannot be easily explained by the likely variations of protein contents of barley and citrus pulp [i.e. the protein concentrations of barley and citrus pulp range from 103 to 160 g/kg DM (average 128 g/kg DM) and from 71 to 72 g/kg DM (average 72 g/kg DM), respectively (MAFF, 1986)]. It is therefore assumed that the main source of the variation was in errors associated with mixing of the ingredients before cubing at the mill. Although the calculated duodenal flow of total EAA of the feather meal on the basal diets varied from 237 to 320 g/d in the experiments in this thesis, the effect on the calculated flows of histidine, methionine and lysine at the duodenum was small (Table 6.8).

6.3.4 Variations in demand for AAs

In Experiments 2 and 3, the supplementations of 9.7 and 9.0 g/d of histidine alone produced no response of milk production. It is assumed that beyond the 6.0 g/d level of supplementation histidine is no longer first-limiting. However, surprisingly the supplementation of 9.0 g/d of histidine alone in Experiment 3 actually decreased milk production compared with the 6.0 g/d of histidine infusion. Presumably, this depression occurred via a mechanism similar to that seen in growing animals where supplementing the

Table 6.8 The calculated flow of EAAs (g/d) from feather meal reaching the duodenum on the basal diets[1]

	Exp 1	**Exp 2**	**Exp 3**	**Exp 4**	**Exp 5**	**Exp 6**	**Exp 7**	**Exp 8**
Histidine	4	4	5	6	5	6	5	5
Threonine	25	25	31	34	28	34	30	33
Arginine	39	39	48	52	44	52	46	50
Methionine	4	4	5	6	5	6	5	5
Valine	45	45	55	61	50	61	53	58
Phenylalanine	30	30	36	40	33	40	35	38
Isoleucine	27	27	33	36	30	36	32	35
Leucine	48	48	58	64	53	64	56	61
Lysine	13	13	16	18	15	18	16	17
Tryptophan	2	2	3	3	3	3	3	3
Total EAA	237	237	290	320	266	320	281	305

[1] Calculated assuming the ruminal degradability of feather meal to be 0.20 (Chamberlain *et al.*, 1992).

[2] Tryptophan concentrations in feather meal from Buttery *et al.* (1978).

second-limiting AA can depress growth rate (Harper *et al.*, 1970). Thus supplementation of histidine beyond the level at which it is first-limiting could be detrimental. It is also possible that supplementation of excess histidine may increase the requirement of methionine because the final step in histidine catabolism requires tetrahydrofolate, the supply of which is modulated by methionine and its metabolites (Gifford *et al.*, 1986).

6.4 EFFICIENCY OF USE OF HISTIDINE FOR MILK PROTEIN PRODUCTION

The results of Experiment 4 allow the efficiency of use of histidine for milk protein synthesis to be estimated. A significant linear relation ($P<0.01$) was observed between the supplementation of histidine and the transfer of histidine to milk protein (Figure 6.2). The relation was described by the regression shown in Figure 6.2:

$Y= 0.431\ X\ (SE = 0.02) + 0.0702\ (P<0.01)$

where Y is the increase in histidine output in milk protein and X is the daily supply of histidine by intravenous infusion. Thus, the estimated efficiency of use of histidine infused into the jugular vein for milk protein production in Experiment 4 was 0.43.

In Experiment 3, in which histidine was infused alone, the response of milk protein production to the increasing dose of histidine was significant only at the 6.0 g/d level (111 g/d increase). The estimates of efficiency for the 3.0, 6.0 and 9.0 g/d levels of histidine supplementation were 0.26, 0.48 and 0.08, respectively. The maximum efficiency of 0.48 is similar to that of Experiment 4. The results of Experiment 3 illustrate the serious errors that can occur if efficiencies are calculated from 'spot' estimates involving only one level of supplementation. If the aim is to measure the maximum efficiency with which a limiting AA is used, the measurement must be based on a linear dose-response relationship. Again, in Experiment 8, 6.0 g/d of histidine alone significantly increased milk protein yield by 60 g/d. From this single observation in Experiment 8, the efficiency of use of histidine was 0.26 which is very much lower than that of the 6.0 g/d level in Experiments 3 and 4. The

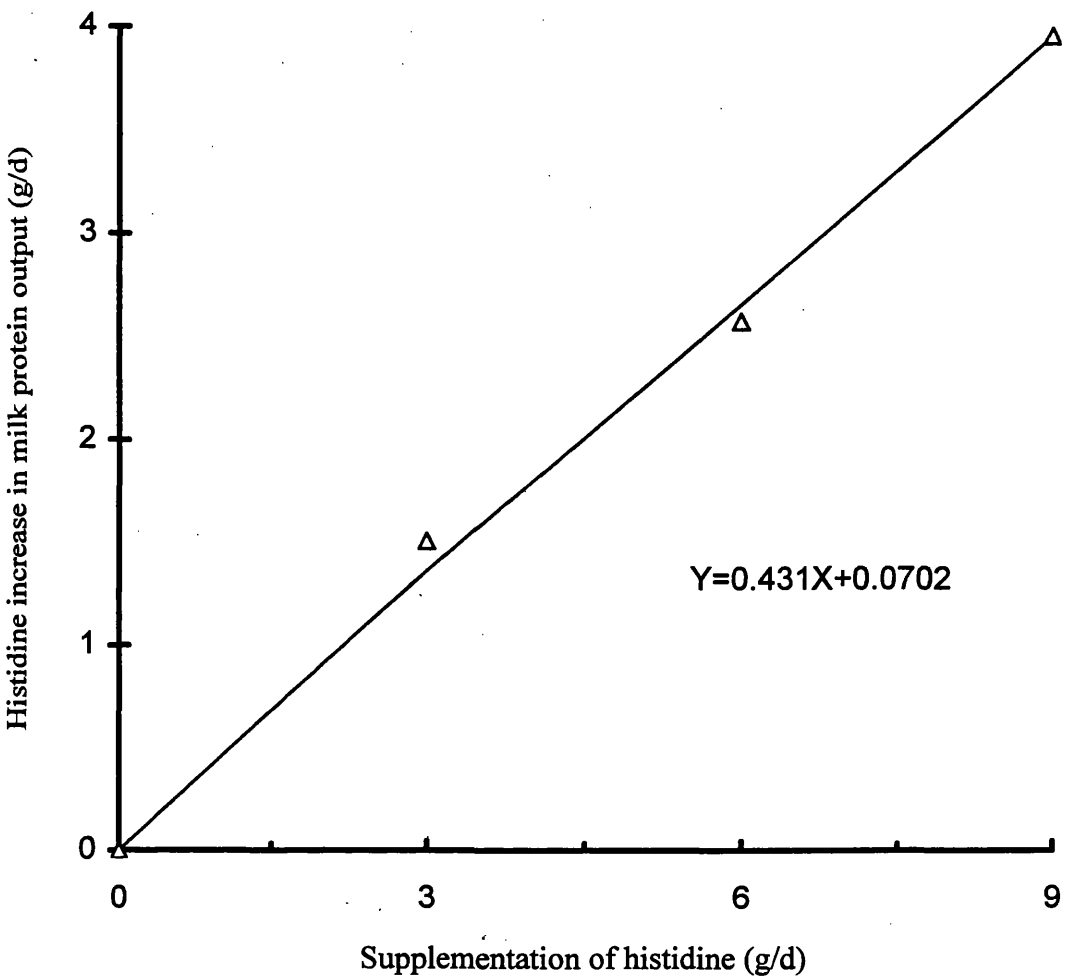

Figure 6.2 The relation between histidine increase in milk protein output and the intravenous supplementaton of increasing dose of histidine in Experiment 4
The regression equation was Y=0.431X+0.0702 ($P<0.01$)

results of Experiment 8 show that a dose-response relationship must be established in each separate experiment. Consistent with this view, efficiencies of use of lysine and histidine obtained from spot estimates from published results range from 0.05 to 0.40 (Schwab *et al.*, 1976, Schwab and Bozak, 1988ab) and from 0.10 to 0.26 (Huhtanen *et al.*, 1997; Vanhatalo *et al.*, 1997; Experiment 8), respectively (Table 6.9). It is evident from Table 6.9 that efficiencies of use of histidine, methionine and lysine calculated from the linear part of the dose-response relationship (i.e. when the acid is first-limiting) are higher than the calculations based on 'spot' estimates.

It is noticeable that efficiencies for methionine are markedly lower in all the experiments (Table 6.9). The efficiencies of use of methionine in the dose-response experiments of Socha *et al.* (1994) and Pisulewski *et al.* (1996) are surprisingly low. Further experimentation is needed to confirm these low values. Closer examination of the dose-response experiments shows that the ME supply in the experiment of Pisulewski *et al.* (1996) was around 5 MJ ME/d in excess of that needed for the observed milk production. Bearing in mind the errors associated with these calculations, it is possible that full expression of a milk response to addition of methionine may have been prevented by the supply of ME. However, this is not true of the experiment of Socha *et al.* (1994) where calculated supply of ME was in large excess (about 30 MJ/d) of that required for the observed milk production.

There is some evidence for a low efficiency of use of methionine for growth in beef steers (160 to 180 kg of live body weight), a value of 0.2 being reported by Campbell *et al.*, (1996, 1997). Again, results of experiments in pigs (Fuller *et al.*, 1989; Chung and Baker, 1992) indicated that utilization of methionine for protein accretion in the body appears to be lower (ranging from 0.77 to 0.80) than that of lysine (0.96) although the values are considerably higher than those reported for ruminants. There is clearly a need for further research but, based on the limited data available, methionine appears to be used

Table 6.9 Efficiency of use of the first-limiting amino acid for milk protein production of dairy cows from experiments using either a single dose of AA or a dose-response relationship

Supplementation of AA		Efficiency[1]	Infusion type	Reference
AA	g/d			
Spot estimates				
Lysine	25.0	0.19	Duodenal	Schwab and Bozak(1988b)
Lysine	27.8	0.05	Abomasal	Schwab *et al.* (1976)
Lysine	30.0	0.40	Duodenal	Schwab and Bozak (1988a)
Methionine	11.2	0.07	Intravenous	Fisher (1972)
Methionine	12.0	0.06	Abomasal	Rogers *et al.* (1979)
Histidine	6.0	0.26	Intravenous	Experiment 8
Histidine	6.5	0.10	Abomasal	Huhtanen *et al.* (1997)
Histidine	6.5	0.12	Abomasal	Vanhatalo *et al.* (1997)
Dose-response estimates[2]				
Lysine	0 to 20.0	0.83	Abomasal	Schwab *et al.* (1992b)
Histidine	0 to 9.0	0.43	Intravenous	Experiment 4
Methionine	0 to 7.0	0.15	Duodenal	Socha *et al.* (1994)
Methionine	0 to 24.0	0.09	Abomasal	Pisulewski *et al.* (1996)

[1] Efficiency of use = output of AA in milk protein/infused AA into the duodenum, abomasum or jugular vein.

[2] From the linear part of the dose-response relationship.

for milk protein secretion in dairy cows with a lower efficiency than is lysine. Corresponding figures for histidine from the experiments in this thesis are higher than the values reported for methionine but are still substantially below those reported for lysine (Table 6.9).

Differences between the efficiencies of use of histidine, methionine and lysine might be expected from biochemical considerations. Lysine is utilized mostly for protein synthesis. In contrast, in addition to incorporation into protein, methionine is involved in a multitude of pathways leading to synthesis of specialized compounds, such as phospholipids, carnitine, creatine, and the polyamines. At the same time, methionine provides methyl groups for a number of transmethylation reactions involved in the regulation of DNA activity and oncogene status and sulphur groups for the synthesis of cyst(e)ine (transsulphuration). Methionine also provides methylgroups for the synthesis of choline and phosphatidylcholine (Colin-Schoellen *et al.*, 1995). These two compounds, constituents of lipoproteins, take part in the blood transport of lipids and in the supply of fatty acids to the udder cells (Emmanuel and Kennelly, 1984). Therefore, it is reasonable to expect that the availability of methionine for protein synthesis could be markedly affected by the competing demands of its alternative uses.

Histidine is a major component of haemoglobin in blood (haemoglobin contains approximately 8% of histidine) and of carnosine (a dipeptide containing histidine and β-alanine) in muscle and brain. Both compounds are a major body reservoir for histidine (Easter and Baker, 1977; Cho *et al.*, 1984). Cho *et al.* (1984) indicated that inclusion of histidine in the diet is necessary for the long-term maintenance of nitrogen balance and haemoglobin concentration in young men fed a low nitrogen diet. Thus haemoglobin and carnosine can serve as sources of histidine in animals during deficiency of histidine (du Vigneaud *et al.*, 1937; Kopple and Swendseid, 1975; Clemens *et al.*, 1978; Cho *et al.*, 1984) and protein (Waterlow, 1969; Fisher *et al.*, 1975). In this connexion, it is interesting

to note that the haemoglobin concentration averaged 96 g/l blood (from 17 observations) of cows fed grass silage and the cereal-based supplement containing feather meal in Experiments 3, 4, 5 and 6 compared with an average of 125 g/l in cows fed a diet of grass silage and cereal-soya supplement (C-H Kim, J-J Choung and DG Chamberlain, unpublished observation). This raises the incidental question of whether the haemoglobin concentration in blood might be a useful index for assessing the histidine status of dairy cows, and whether the milk production response to histidine supplementation is influenced by the haemoglobin or carnosine status of the cow. Regrettably, the short-term change-over design experiments used in this thesis do not allow this question to be addressed.

Given the different biochemical uses of individual AAs in the body, it might be expected that the efficiency of use of an AA for milk protein secretion would depend on the physiological and nutritional conditions of the experimental animals.

6.5 IMPLICATIONS FOR PROTEIN RATIONING SCHEMES

The modern protein evaluation systems [AAT (Madsen, 1985; Hvelplud and Madsen, 1990), AP (NRC, 1985), PDI (Vérité *et al.*, 1987), ADPLS (SCA, 1990), CNCPS (Fox *et al.*, 1992) and MP (AFRC, 1992, 1993)] assume fixed values for the efficiency of utilization of absorbed AAs for milk protein production (ranging from 0.64 to 0.75). The Dutch DVE system (Tamminga *et al.*, 1994; Subnel *et al.*, 1994) proposed a variable efficiency after analysing a number of feeding trials which showed efficiency to vary from 0.5 to 0.7 throughout lactation, being influenced by effects of DVE (MP): NEL (ME) ratio and level of milk production. The UK MP system (AFRC, 1992, 1993) assumes that MP intake is converted to milk protein with an efficiency of 0.68. In practice, however, the marginal response to increasing MP is far below any of the conventional estimates of efficiency of use of MP for milk protein synthesis e.g. the marginal efficiency of MP was around from 0.2 to 0.4 for the response of milk protein production (Webster, 1992;

Oldham, 1994; Newbold *et al.*, 1994; Metcalf *et al.*, 1997; Christie *et al.*, 1998). Recent work from Mansbridge *et al.* (1999) shows that marginal efficiency for MP is 0.625 when ME and MP supplies are co-limiting but, again, the efficiency falls quite sharply as MP intake increases which agrees with Webster (1992) and Oldham (1994). It should be noted that data from experiments in this thesis and also most data in the literature refer to marginal responses to AA when MP supply is at or above requirement.

Oldham (1994) and Subnel *et al.* (1994) suggested that if the increment of MP was used both to supply the ME required for extra milk synthesis as well as the AAs required to secrete extra milk protein, then the efficiency of use of even an ideal AA mixture at the margin would be low. In addition to the possible impact of ME:MP ratio on AA use in dairy cows, there is also the possibility that where the metabolizable AA supply increases with no change in the supply of other energy-yielding nutrients, so that any extra glucose demand to maintain a constant milk protein:lactose (and possibly also fat) ratio comes from AA catabolism, then the maximum rate of conversion of metabolizable AA (metabolizable protein) to milk protein would again be low. Thus, Oldham (1996) suggested that if absorbed AAs are used as general purpose energy (ME), an efficiency of utilization of 0.33 can be calculated, whereas if used to synthesize lactose, the value would be about 0.28. Again, the mobilization or retention of body protein might influence the AA utilization for milk protein synthesis (NRC, 1985; Tamminga *et al.*, 1994). For example, absorbed AAs might be utilized for body tissue deposition or replacement of carnosine and haemoglobin *etc*. Thus, it is should be recognized that the overall efficiency of use may be much higher. For example, this is seen particularly clearly in the work reported by Whitelaw *et al.* (1986) where response in milk nitrogen was low but a combination of milk nitrogen plus body nitrogen retention (productive nitrogen) indicates the overall efficiency of utilization was much higher.

Therefore, the assumption of a high and constant efficiency of utilization of absorbed

AAs in protein rationing schemes demands serious questioning. High efficiencies may be observed only when the AA profile of the absorbed protein is ideal or close to it, and AA supply is limiting. It may be argued that the net efficiency with which absorbed AAs are used for milk protein production should be regarded not as a constant but as a variable to be predicted within the feeding system from attributes both of diet (balance of AAs supplied) and the cow (efficiency of use of ideal AA mixture) (BBSRC, 1998). Again, among the protein evaluation systems, only the Dutch DVE system (Tamminga *et al.*, 1994; Subnel *et al.*, 1994) has adopted the concept of a nutritional response into its protein requirements model. It must be remembered that the systems that exist at present were designed to allow the calculation of protein needs for a defined level of performance. They were not designed to allow the prediction of performance from a defined allowance – that calls for a system of nutrient partition to be included (BBSRC, 1998).

It should also be remembered that the efficiency of use of MP for lactation from the MP system (AFRC, 1992, 1993) and in the other systems [except CNCPS (O'Connor *et al.*, 1993)] referred to, does not refer to individual AAs, only total AA. An attempt has been made (O'Connor *et al.*, 1993) to incorporate different values for the efficiency of use of individual EAA for lactation, ranging from 0.42 for arginine to 1.0 for phenylalanine (although the values for the other EAAs all lie between 0.8 and 1.0). However, the values adopted have been selected from a range of published and unpublished observations, in which the available estimates for some of the EAAs varied widely. Indeed, the authors themselves recognize the provisional nature of the estimates and draw attention to the need for research to generate more reliable values (O'Connor *et al.*, 1993). The need to consider individual AAs will be brought more sharply into focus if very substantial differences in the efficiency of individual AAs emerge from further research.

6.6 FUTURE STUDIES

The obvious weakness of the dietary model used in the experiments in this thesis is that responses to histidine given alone were variable, or even non-existent as in Experiments 2 and 5. It is suggested that the most likely explanation for these variable responses is background variation in the supplies of methionine and lysine, the two next-limiting AAs. Therefore, to serve as a basis for future studies, the dietary model would need to be modified. Although it is conceivable that alterations to the basal diet, such as standardizing ERDP/FME ratios and standardizing as closely as possible the type of silage could be made, it would seem unlikely that the degree of control would be sufficient to eliminate background variation in microbial AA composition since the factors controlling the latter are not known. A better approach would be to ensure adequate supplies of methionine and lysine via intravenous infusion on the basal treatment, along the lines of Experiments 4 and 7. Using this approach, the model could be adapted to estimate the efficiencies of use of methionine and lysine by making each one first-limiting on the basal treatment by infusing the other two acids from the group of three (histidine, methionine and lysine). Using such a technique, it should be possible to move away from the feather meal-based diet on to any basal diet of choice, although it would be necessary to characterize the likely order of limitation of the AA on whatever basal diet was selected.

One question remaining from the experiments in the thesis is how responses to intravenously infused histidine compare with those to histidine given into the abomasum. This experiment could be repeated using the amended basal treatment and the investigation extended in subsequent experiments to compare responses to intravenous and intra-abomasal administration of methionine and lysine.

The amended model would lend itself to a wider investigation of nutritional and physiological factors influencing the efficiency of use of AAs for milk production. For example the effects of varying the background supply (via infusion into the gut or blood, as appropriate) of glucogenic precursors (propionate, starch and NEAA) or long-chain fatty

acids could be examined. Similarly, the effect of stage of lactation on efficiency use of AAs could be investigated. For example, particularly in early lactation, there can be a shortage of glucose or glucogenic precursors and in such cases AAs can be used as glucogenic precursors so losing their aminogenic properties (Bergman *et al.*, 1968; Mepham, 1982; Lomax and Baird, 1983; Hvelplund and Madsen, 1990; Lobley, 1992). Moreover it is not clear to what extent the amount of AAs available from mobilization of body reserves in early lactation are used for milk protein production or to what degree AAs are withdrawn from possible use for milk protein production later in lactation by restoring body reserves. Hence, many factors can influence the net efficiency of utilization of AAs for milk protein production and there are many potential interactions due to the fact that many influences can be present simultaneously. More research is necessary to quantify the effect of each of the influences and the interactions between them on the utilization of AAs for milk protein production. More specifically, in relation to histidine, its efficiency of use for milk protein synthesis might be influenced by carnosine and haemoglobin status. Therefore, it would be interesting to examine effects of haemoglobin status on responses to histidine. One way of doing this would be to run down haemoglobin levels during extended periods on the feather meal-containing diet to defined values before measuring milk protein responses to supplementary histidine.

REFERENCES

Addison JM, Burston D and Matthews DM. (1972). Evidence for active transport of the dipeptide glycylsarcosine by hamster jejunum *in vitro*. *Clinical Science* **43**, 907-911.

Addison JM, Burston D, Payne JW, Wilkinson S and Matthews DM. (1975). Evidence for active transport of tripeptides by hamster jejunum *in vitro*. *Clinical Science and Molecular Medicine* **49**, 305-312.

Adibi SA. (1971). Intestinal transport of dipeptides in man: Relative importance of hydrolysis and intact absorption. *Journal of Clinical Investigation* **50**, 2266.

Adibi SA. (1969). The influence of molecular structure of neutral amino acids on their absorption kinetics in the jejunum and ileum of human intestine *in vivo*. *Gastroenterology* **56**, 903-913.

Adibi SA and Phillips E. (1968). Evidence for greater absorption of amino acids from peptide than from free form in human intestine. *Clinical Research* **16**, 446.

Adibi SA and Gray SJ. (1967). Intestinal absorption of essential amino acids in man. *Gastroenterology* **52**, 837-845.

Adibi SA, Gray SJ and Menden E. (1967). The kinetics of amino acid absorption and alteration of plasma composition of free amino acids after intestinal perfusion of amino acid mixtures. *American Journal of Clinical Nutrition* **20**, 24-33.

Agricultural and Food Research Council. (1993). Energy and protein requirements of ruminants. *An advisory manual prepared by the AFRC Technical committee of responses to nutrients*. Wallingford, UK: CAB international.

Agricultural and Food Research Council. (1992). Nutritive requirements of ruminant animals: Protein. Technical committee on responses to nutrients. Report No. 9. *Nutrition Abstracts and Reviews (Series B)* **62**, 787-835.

Agricultural Research Council. (1984). *The nutrient requirements of ruminant livestock*, Supplement No. 1. Report of protein group of the ARC Working Party. Farnham Royal, Slough, UK.

Agricultural Research Council (1980). *The nutrient requirements of ruminant livestock.* Commonwealth Agricultural Bureaux, Farnham Royal, Slough, UK.

Aguilar TS, Harper AE and Benevenga NJ. (1972). Efficiency of utilization of indispensable amino acids for growth by the rat. *Journal of Nutrition* **102**, 1199-1208.

Alpers DH. (1986). Uptake and fate of absorbed amino acids and peptides in the mammalian intestine. *Federation Proceedings* **45**, 2261-2267.

Andrew SM, Waldo DR and Erdman RA. (1994). Direct analysis of body composition of dairy cows at three physiological stage. *Journal of Dairy Science* **77**, 3022-3033.

Annison EF. (1956). Nitrogen metabolism in the sheep. Protein digestion in the rumen. *Biochemistry Journal* **64**, 705-714.

Armstrong DG and Hutton K. (1975). Fate of nitrogenous compounds entering the small intestine. In *Digestion and metabolism in the ruminant*, pp. 432-447 [McDonald IW and Warner ACI, editors]. USA: The University of New England publishing unit.

Armstrong DG, Savage GP and Harrison DG. (1977). Digestion of nitrogenous substances entering the small intestine with particular reference to amino acids in ruminant livestock. In *Proceedings of 2 nd internatinal symposium on protein metabolism and nutrition*, pp. 55-60. The Netherlands.

Association of Officical Analytical Chemists. (1975). *Official methods of analysis* Arlington, Virginia, USA.

Backwell FRC. (1994). Use of selective ion monitoring to demonstrate peptide utilization by tissues. *Proceedings of the Nutritional Society* **53**, 457-464.

Backwell FRC, Hipolito-Reis M, Wilson D, Bruce LA, Buchan V and MacRae JC. (1997). Quantification of circulating peptides and assessment of peptide uptake across the gastrointestinal tract of sheep. *Journal of Animal Science* **75**, 3315-3322.

Backwell FRC, Bequette BJ, Wilson D, Metcalf JA, Franklin MF, Beever DE, Lobley GE and MacRae JC. (1996). Evidence for the utilization of peptides for milk protein synthesis in the lactating dairy goat *in vivo*. *American Journal of Physiology* **271**, R955-R960.

Backwell FRC, Bequette BJ, Wilson D, Calder AG, Wray-Cahen D, Metalf JA, MacRae JC, Beever DE, and Lobley GE. (1994). Utilization of dipeptides by the caprine mammary gland for milk protein synthesis. *American Journal of Pysiology* **267**, R1-R6.

Baker RD and George MJ. (1971). Pattern of neutral amino acid uptake along rat small intestine. *Biochimica et Biophysica Acta* **225**, 315-325.

Ballmer PE, McNurlan MA, Milne E, Heys SD, Buchan V, Calder AG and Garlick PJ. (1990). Measurement of albumin synthesis in humans: A new approach employing stable isotopes. *American Journal of Physiology* **259**, E797-E803.

Baracos VE, Brun-Bellut J and Marie M. (1991). Tissue protein synthesis in lactating and dry goats. *British Journal of Nutrition* **66**, 451-465.

Barnard EA. (1969). Biological functions of pancreatic ribonuclease. *Nature* **221**, 340-344.

Bartley EE. (1976). Bovine saliva: Production and function. In *Buffers in ruminant physiology and metabolism*, pp. 61-81 [Weinberg MS and Sheffner AL, editors]. New York, USA: Church and Dwight Company.

Bartley EE, Avery TB, Watt BR, Davidovitch A, Galitzer S and Lassman B. (1981). Ammonia toxicity in cattle: V. Ammonia concentration of lymph, and portal, carotid and jugular blood after ingestion of urea. *Journal of Animal Science* **53**, 494-498.

Bartley EE, Davidovitch AD, Barr GW, Griffel GW, Dayton AD, Deyde CW and Bechtle RM. (1976). Ammonia toxicity in cattle. I. Rumen and blood changes associated with toxicity and treatment methods. *Journal of Animal Science* **43**, 835-841.

Beever DE and Cottrill BR. (1993). Protein systems for feeding ruminant livestock: A European assessment. *Journal of Dairy Science* **77**, 2031-2043.

Bender DA. (1985). *Amino acid metabolism*. New York, USA: John Wiley and Sons.

Ben-Ghedalia D, Tagari H and Bondi A. (1976). The ileum of the sheep as a site of protein digestion. *British Journal of Nutrition* **36**, 211-217.

Bequette BJ and Backwell FRC. (1997). Amino acid supply and metabolism by the ruminant mammry gland. *Proceedings of the Nutrition Society* **56**, 593-605.

Bequette BJ, Backwell FRC and Crompton LA. (1998). Current concept of amino acid and protein metabolism in the mammary gland of the lactating ruminant. *Journal of Dairy Science* **81**, 2540-2559.

Bequette BJ, Backwell FRC, Calder AG, Metcalf JA, Beever DE, MacRae JC and Lobley GE. (1997). Application of a U-carbon-13-labelled amino acid tracer in lactating dairy goats for simultaneous measurements of the flux of amino acids in plasma and the partition of amino acids to the mammary gland. *Journal of Dairy Science* **80**, 2842-2853.

Bequett BJ, Metcalf JA, Wray-Cahen D, Backwell FRC, Sutton JD, Lomax MA, MacRae JC and Lobley GE. (1996). Leucine and protein metabolism in the lactating dairy cow mammary gland: Responses to supplemental dietary crude protein intake. *Journal of Dairy Research* **63**, 209-222.

Bergen WG. (1978). Digestion and absorption of nitrogenous components. *Federation Proceedings* **37**, 1223-1227.

Bergen WG, Purser DB and Cline JH. (1968). Effects of ration on the nutritive quality of rumen microbial protein. *Journal of Animal Science* **27**, 1497-1501.

Bergmann EN. (1989). Splanchnic and peripheral uptake of amino acids in relation to the gut. *Federation Proceedings* **45**, 2277-2282

Bergman EN and Peel JM. (1984). Integration of amino acid metabolism in the ruminant. In *Herbivore nutrition in the subtropics and tropics*, pp. 613-628 [Gilchrist FMC and MacKie RI, editors]. Craighall, Johannesburg, South Africa: Science Press.

Bergman EN and Heitmann RN. (1978). Metabolism of amino acids by the gut, liver, kidneys and peripheral tissues. *Federation Proceedings* **37**, 1228-1232.

Bergman EN and Wolff JE. (1971). Metabolism of volatile fatty acids by liver and portal drained viscera in sheep. *American Journal of Physiology* **221**, 586-592.

Bergman EN, Katz ML and Kaufman CF. (1970). Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. *American Journal of Physiology* **219**, 785-793.

Bergman EN, Starr DJ and Reulein SS. (1968). Glycerol metabolism and gluconeogenesis in the normal and hypoglycemic sheep. *American Journal of Physiology* **215**, 874-880.

Bickerstaffe R, Annison EF and Linzell JL. (1974). The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. *Journal of Agricultural Science* **82**, 71-85.

Binder HJ. (1970). Amino acid absorption in the mammalian colon. *Biochimica et Biophysica Acta* **219**, 503-506.

Biotechnology and Biological Science Research Council. (1998). *Responses in the yield of milk constituenst to the intake of nutrients by dairy cows.* BBSRC Technical committee on responses to nutrients, Report No. 11. Wallingford, UK: CAB Interantional.

Bird PR. (1973). Sulphur metabolism and excretion studies in ruminants: XII. Nitrogen and sulphur composition of ruminal bacteria. *Australian Journal of Biological Science* **26**, 1429-1434.

Bird PR and Moir RJ. (1972). Sulphur metabolism and excretion studies in ruminants: VIII. Methionine degradation and utilization in sheep when infused into the rumen or abomasum. *Australian Journal of Biological Science* **25**, 835-848.

Black AL, Anand RS, Bruss ML, Brown CA and Nakagiri JA. (1990). Partitioning of amino acids in lactating cows: Oxidation to carbon dioxide. *Journal of Nutrition* **120**, 700-710.

Block RJ and Bolling D. (1951). *The amino acid composition of proteins and foods.* Chapter X, p. 487. Springfield, USA: Charles C Thomas Publisher.

Bondi AA. (1987). *Animal Nutrition.* New York, USA: John Wiley and Sons.

Botts RI, Hemken RW and Bull LS. (1978). Protein reserves in the lactating dairy cow.

Journal of Dairy Science **62**, 433-440.

Bouchard R and Conrad HR. (1973). Determination of the most limiting amino acid for dairy cows using plasma amino acid concentrations. *Federation Proceedings* **32**, 905 (Abstract).

Brent BE and Bartley EE. (1984). Thiamin and niacin in the rumen. *Journal of Animal Science* **59**, 813-822.

British Standard Institution. (1969). *Gerber method for the determination of fat in milk and milk products,* BS696 Part2: Methods.

Broderick GA, Satter LD and Harper AE. (1974). Use of plasma amino acid concentration to identify limiting amino acids for milk production. *Journal of Dairy Science* **57**, 1015-1023.

Bruckental I, Ascarelli I, Yosif B and Alumot E. (1991). Effect of duodenal proline infusion on milk production and composition in dairy cows. *Animal Produciton* **53**, 299-303.

Bryant MP. (1973). Nutritional requirements of the predominant rumen cellulolytic bacteria. *Federation Proceedings* **32**, 1809-1813.

Burrin DG, Ferrell CL, Eisemann JH and Britton RA. (1991). Level of nutrition and splanchnic metabolite flux in young lambs. *Journal of Animal Science* **69**, 1082-1092.

Burston D, Addison JM and Matthews DM. (1972). Uptake of dipeptides containing basic and acidic amino acids by rat small intestine *in vitro*. *Clinical Science* **43**, 823-837.

Buttery PJ and Foulds AN. (1985). Amino acid requirement of ruminants. In *Recent advances in animal nutrition-1985,* pp. 257-271 [Haresign W and Cole DJA, editors]. London, UK: Butterworths.

Buttery PJ, Bozon D and Soar JB. (1978). Amino acid composition of feedstuffs. In *Recent advances in animal nutrition-1978*, pp. 185-187. [Haresign W and Lewis D, editors]. London, UK: Butterworths.

Campbell CG, Titgemeyer EC and St-Jean G. (1997). Sulphur amino acid utilization by growing steers. *Journal of Animal Science* **75**, 230-238.

Campbell CG, Titgemeyer EC and St-Jean G. (1996). Efficiency of D- vs L-methionine utilization by growing steers. *Journal of Animal Science* **74**, 2482-2487.

Casper DP, Schingothe DJ, Yang CM and Mueller CR. (1987). Protected methionine supplementation with extruded blend of soybeans and soybean meal for dairy cows. *Journal of Dairy Science* **70**, 321-330.

Chalupa W. (1978). Digestion and absorption of nitrogenous compounds in ruminants, p. 211. *Procedings of the 3rd World Congress on Animal Feeding*, Mardrid, Spain.

Chamberlain DG and Choung J-J. (1995). The importance of rate of ruminal fermentation of energy sources in diets for dairy cows. In *Recent advances in animal nutrition-1995*, pp. 3-27 [Garnsworthy PC, Wiseman J and Haresign W, editors]. Nottingham, UK: Nottingham University Press.

Chamberlain DG and Thomas PC. (1982). Effect of intravenous supplements of L-methionine on milk yield and composition in cows given silage-cereal diets. *Journal of Dairy Research* **49**, 25-28.

Chamberlain DG and Thomas PC. (1980). The effects of urea and artificial saliva on rumen bacterial protein synthesis in sheep receiving a high cereal diet. *Journal of the Scienc of Food and Agriculture* **31**, 432-439.

Chamberlain DG and Thomas PC. (1979). Ruminal nitrogen metabolism and the passage of amino acids to the duodenum in sheep reciving diets containg hay and concentrates in various proportions. *Journal of the Science of Food and Agriculture* **30**, 677-686.

Chamberlain DG, Robertson S and Choung J-J. (1993). Sugars versus starch as supplements to grass silage: Effects on ruminal fermentation and the supply of microbial protein to the small intestine, estimated from the urinary excertion of purine derivatives, in sheep. *Journal of the Science of Food and Agriculture* **63**, 189-194.

Chamberlain DG, Choung J-J and Robertson S. (1992). Protein nutrition of dairy cows receiving grass silage diets: Effects of feeding a protein supplement of unbalanced amino acid composition. *Journal of the Scienc of Food and Agriculture* **60**, 425-430.

Chamberlain DG, Thomas PC and Quig J. (1986). Utilization of silage nitrogen in sheep and cows: Amino acid composition of duodenal digesta and rumen microbes. *Grass and Forage Science* **41**, 31-38.

Champredon C and Pion R. (1979). Intravenous infusion of amino acids in the lactating goat. *Annales de Recherches Veterinaires*. **10**, 379-381.

Champredon C, Debras E, Mirand PP and Arnal M. (1990). Methionine flux and tissue protein synthesis in lactating and dry goats. *Journal of Nutrition* **120**, 1006-1015.

Chang TW and Goldberg AL. (1978). The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. *Journal of Biological Chemistry* **253**,3685-3695.

Cheng B, Navab F, Lis NT, Miller TN and Matthews DM. (1971). Mechanisms of dipeptide uptake by rat small intestine *in vitro*. *Clinical Science* **40**, 247-259.

Cheng K-J and Wallace RJ. (1979). The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea in the urea flux. *British Journal of Nutrition* **42**, 553-557.

Cho ES, Anderson HL, Wixom RL, Hanson KC and Krause GF. (1984). Long-term effects of low histidine intake on men. *Journal of Nutrition* **114**, 369-384.

Choung J-J and Chamberlain DG. (1995). The effect of intravenous supplements of amino acids on the milk production of dairy cows consuming grass silage and a supplement containging feather meal. *Journal of the Sciece of Food and Agriculture* **68**, 265-270.

Choung J-J and Chamberlain DG. (1993). The effects of abomasal infusions of casein or soya protein isolate on the milk production of dairy cows in mid lactation. *British Journal of Nutrition* **69**, 103-115.

Choung J-J and Chamberlain DG. (1992a). Protein nutrition of dairy cows receiving grass silage diets. Effects on silage intake and milk production of postruminal supplements of casein or soya-protein isolate and the effects of intravenous infusions of a mixture of methionine, phenylalanine and tryptophan. *Journal of the Science of Food and Agriculture* **58**, 307-314.

Choung J-J and Chamberlain DG. (1992b). Effects of abomasal infusions of sodium caseinate, soya-protein isolate and a water extract of fish-meal on the utilization of nitriogen by dairy cows. *Journal of the Science of Food and Agriculture* **60**, 131-133.

Christiansen ML and Webb KE Jr. (1990a). Nitrogen utilization and digestibility of amino acids by lambs fed a high-concentrate diet with limestone or magnesium oxide. *Journal of Animal Science* **68**, 2095-2104.

Christiansen ML and Webb KE Jr. (1990b). Intestinal acid flow, dry matter, starch and protein digestibility and amino acid absorption in beef cattle fed a high concentrate diet with defluorinated rock phosphate, limestone or magnesium oxide. *Journal of Animal Science* **68**, 2105-2118.

Christie H, Emmans GE, Oldham JD and Roberts DJ. (1998). The effects of energy intake on responses to metabolisable protein in dairy cows. *Proceedings of the British Society of Animal Science* **1998**, 20 (Abstract). Penicuik, UK: BSAS.

Chung TK and Baker DH. (1992). Efficiency of dietary methionine utilization by young pigs. *Journal of Nutrition* **122**, 1862-1869.

Clark JH, Klusmeyer TH and Cameron MR. (1992). Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *Journal of Dairy Scienc* **75**, 2304-2323.

Clark JH, Spires HR, Derrig RG and Bennink MR. (1977). Milk production, nitrogen utilization and glucose synthesis in lactating cows infused postruminally with sodium caseinate and glucose. *Journal of Nutrition* **107**, 631-644.

Clarke EMW, Ellinger GM and Phillipson AT. (1966). The influence of the diet on the nitrogenous components passing to the duodenum and through the lower ileum of sheep. *Proceedings of the Royal Society (Series B)* **166**, 63-79.

Clemens RA, Kopple JD and Swendseid ME. (1978). Metabolic effects of histidine-free diets in rats. *Federation Proceedings*. **37**, 263.

Coelho da Silva JR, Seeley RC, Thomson DJ, Beever DE and Armstrong DG. (1972a). The effect in sheep of physical form on the sites of digestion of a dried lucerne diet. 2. Sites of nitrogen digestion. *British Journal of Nutrition* **28**, 43-61.

Coelho da Silva JR, Seeley RC, Beever DE, Prescott JHD and Armstrong DG. (1972b). The effect in sheep of physical forms and stage of growth on the sites of digestion of a dried grass. 2. Sites of nitrogen digestion. *British Journal of Nutrition* **28**, 357-371.

Cohen LL and Huang KC. (1964). Intestinal transport of tryptophan and its derivatives, *American Journal of Physiology* **206**, 647-652.

Colin-Schoellen O, Laurent F, Vignon B, Robert JC and Sloan B. (1995). Interactions of ruminally protected methionine and lysine with protein source or energy level in the diets of cows. *Journal of Dairy Science* **78**, 2807-2818.

Connell A, Calder AG, Anderson SE and Lobley GE. (1997). Hepatic protein synthesis in the sheep: Effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. *British Journal of Nutrition* **77**, 255-271.

Cook RM, Brown RE and Davis CL. (1965). Protein metabolism in the rumen. I. Absorption of glycine and other amino acids. *Jouranl of Dairy Science* **48**, 475-483.

Cottyn BG and Boucque GN. (1968). Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agriculture and Food Chemistry* **16**, 105-107.

Craft IL, Geddes D, Hyde CW, Wise IJ and Matthews DM. (1968). Absorption and malabsorption of glycine and glycine peptides in man. *Gut* **9**, 425.

Dado RG, Mertens DR and Shook GE. (1993). Metabolizable energy and absorbed protein requirements for milk component production. *Journal of Dairy Science* **76**, 1575-1588.

Danfær A, Tetens V and Agergaard N. (1995). Review and an experimental study on the physiological and quantitative aspects of gluconeogenesis in lactating ruminants. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology* **111B**, 201-210.

Daniel H, Morse EL and Adibi SA. (1991). The high and low affinity transport systmes for dipeptides in kidney brush border membrane respond differently to alterations in pH gradient and membrane potential. *Journal of Biological Chemistry* **266**, 19917-

19924.

Daugaard J. (1978). *Undersogelser over methionintilskud til malkekoer*. Ph D Thesis. Institue of Animal Science. RVAU, Copenhagen, Denmark.

Demaux G, LeBars H, Molle J, Rérat A and Simmonet H. (1961). *Academie Veterinaire de France*, Bulletin **34**, 85-88.

DePeters EJ and Cant JP. (1992). Nutritional factors influencing the nitrogen composition of bovine milk: A review. *Journal of Dairy Science* **75**, 2043-2070.

Derrig RG, Clark JH and Davis CL. (1974). Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization and amino acid nutrition of the dairy cow. *Journal of Nutrition* **104**, 151-159.

Dewar WA and McDonald P. (1961). Determination of dry matter in silage by distillation with toluene. *Journal of the Science of Food and Agriculture* **12**, 790-795.

DiRienzo DB. (1990). *Free and peptide amino acid fluxes across the mesenteric and non-mesenteric viscera of sheep and calves*. Ph D Dissertation. Blacksburg, Virginia Polytechnic Institute and State University

Dixon RM and Nolan JV. (1986). Nitrogen and carbon flows between the caecum, blood and rumen in sheep given chopped lucerne (*Medicago sativa*) hay. *British Journal of Nutrition* **55**, 313-332.

Dixon RM and Nolan JV. (1982). Studies of the large intestine of sheep. *British Journal of Nutrition* **47**, 289-300.

Durand M and Kawashima R. (1980). Influence of minerals in rumen microbial digestion. In *Digestive physiology and metabolism in ruminants*, pp. 375-408 [Ruckebusche Y and Thivend P, editors]. Lancaster, UK: MTP Press.

du Vigneaud V, Sifferd RH and Irving GW Jr. (1937). The utilization of L-carnosine by animals on a histidine-deficient diet. *Journal of Biological Chemistry* **117**, 589-597.

Easter RA and Baker DH. (1977). Nitrogen metabolism, tissue carnosine concentration and blood chemistry of gravid swine fed graded levels of histidine. *Journal of Nutrition* **107**, 120-125.

Egan AR, MacRae JC and Lamb CS. (1983). Threonine metabolism in sheep. 1. Threonine catabolism and gluconeogenesis in mature Blackface wethers given poor quality hill herbage. *British Journal of Nutrition* **49**, 373-383.

Eisemann JH and Nienaber JA. (1990). Tissue and whole-body oxygen uptake in fed and fasted steers. *British Journal of Nutrition* **64**, 399-411.

Elsden SR and Gibson QH. (1954). The estimation of lactic acid using ceric sulphate.

Biochemical Journal **58**, 154-158.

Emmanuel B and Kennelly JJ. (1984). Kinetics of methionine and choline and their incorporation into plasma lipids and milk components in lactating goats. *Journal of Dairy Science* **67**, 1912-1918.

Engelhardt W v, Hinderer S and Wipper E. (1978). Factors affecting the endogenous urea-N secretion and utilization in the gastrointestinal tract. In *Ruminant Digestion and Feed Evaluation*, pp. 4.1-4.12 [Osbourn DF, Beever DE and Thomson DJ, editors]. London, UK: Agricultural Research Council.

Erfle JD and Fisher LJ. (1977). The effects of intravenous infusion of lysine, lysine plus methionine or carnitine on plasma amino acids and milk production of dairy cows. *Canadian Journal of Animal Science* **57**, 101-109.

Fei Y-J, Kanai Y, Nussberger S, Ganapathy V, Leibach FH, Romero MF, Singh SK, Boron WF and Hediger MA. (1994). Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368**, 563-566.

Fejes J, Faixova Z, Varady J and Cibula M. (1991). Transfer of amino acids through mucous rumen membrane of sheep *in vitro*. *Veterinarni Medicina* **36**, 551-559.

Fisher H, Konlande J and Strumeyer D. (1975). Levels of histidine and histidine derivatives in breast muscle of protein-depleted and repleted adult cockerels. *Nutrition and Metabolism* **18**, 120-126.

Fisher LJ. (1972). Response of lactating cows to the intravenous infusion of amino acids. *Canadian Journal of Animal Science* **52**, 377-384.

Fisher LJ. (1969). Effect of methionine infusion on milk production and free amino acids in plasma of cows. *Journal of Dairy Science* **52** (Supplement 1), 943 (Abstract).

Ford AL and Milligan LP. (1970). Tracer studies of urea recycling in sheep. *Canadian Journal of Animal Science* **50**, 129-135.

Fox DG, Sniffen CJ, O'Connor JD, Russell JB and Van Soest PJ. (1992). A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *Journal of Dairy Science* **70**, 3578-3596.

Fraser DL, Ørskov ER, Whitelaw FG and Franklin MF. (1991). Limiting amino acids in dairy cows given casein as the sole source of protein. *Livestock Production Science* **28**, 235-252.

Fujita M, Ohta H, Kawai K, Matsui H and Nakao M. (1972). Differential isolation of microvillous and basolateral plasma membranes from intestinal mucosa: Mutually exclusive ditribution of digestive enzyme and ouabain-sensitive ATPase. *Biochimica et Biophysica Acta* **274**, 336-347.

Fuller MF, McWilliam R, Wang TC and Giles LR. (1989). The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. *British Journal of Nutrition* **62**, 255-267.

Ganapathy V and Leibach FH. (1983). Role of pH gradient and membrane-potential in dipeptide transport in intestinal and renal brush-border membrane-vesicles from the rabbit – Studies with L-carnosine and glycyl-L-proline. *Journal of Biological Chemistry* **258**, 14189-14192.

Gardner MLG. (1998). Transmucosal passage of intact peptides. In *Peptide in mammalian protein metabolism-tissue utilization and clinical targeting*, pp. 11-28 [Grimble GK and Backwell FRC, editors]. London, UK: Portland Press.

Gardner MLG. (1984). Intestinal assimilation of intact peptides and proteins from the diet – A neglected field. *Biological Reviews of the Cambridge Philosophica Society* **59**, 289-331.

Gardner MLG and Wood D. (1988). Transport of peptides across the gastrointestinal tract. *Biochemical Society Transactions* **17**, 934-937.

Gibb MJ, Ivings WE, Dhanoa MS and Sutton JD. (1992). Changes in body components of autumn calving Holstein-Friesian cows over the first 29 weeks of lactation. *Animal Production* **55**, 339-360.

Gifford C, Dodds SJ, Gustafson JM and Mercer LP. (1986). The relationship between serum histidine and methionine as a function of dietary-protein or amino acid mix. *Federation Proceedings* **45**, 1028 (Abstract).

Goedeken FK, Klopfenstein TJ, Stock RA, Britton RA and Sindt MH. (1990). Protein value of feather meal for ruminants as affected by blood additions. *Journal of Animal Science* **68**, 2936-2944.

Goering HK and Van Soest PJ. (1970). *Forage Fibre Anaylsis in Agricultural Handbook* No. 379.

Goetsch AL and Owens FN. (1985). Effects of calcium source and level on site of digestion and calcium levels in the digestive tract of cattle fed high-concentrate diets. *Journal of Animal Science* **61**, 995-1003.

Grant RJ and Haddad SG. (1998). Effect of a mixture of feather and blood meals on lactational performance of dairy cows. *Journal of Dairy Science* **81**, 1358-1363.

Greife HA, Rooke JA and Armstrong DG. (1985). The digestion by cattle of silage-containing diets fed at two dry matter intakes. 2 Digestion of total amino acids and of D-alanine and D-glutamic acid. *British Journal of Nutrition* **54**, 483-492.

Grimbleby FM. (1956). The determination of lactose in milk. *Journal of Dairy Research* **23**, 229-237.

Guerino F, Huntington GB and Erdman RA. (1991). The net portal and hepatic flux of metabolites and oxygen consumption in growing beef steers given postruminal casein. *Journal of Animal Science* **69**, 387-395.

Guillaume B, Otterby DE, Stern MD, Linn JG and Johnson DG. (1991). Raw or extruded soybeans and rumen-protected methionine and lysine in alfalfa-based diets for dairy cows. *Journal of Dairy Science* **74**, 1912-1922.

Guinard J and Rulquin H. (1995). Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. *Journal of Dairy Science* **78**, 2196-2207.

Guinard J and Rulquin H. (1994). Effect of graded levels of duodenal infusion of casein on mammary uptake in lactating cows. 2. Individual amino acid. *Journal of Dairy Science* **77**, 3304-3315.

Guinard J, Rulquin H and Vérité R. (1994). Effect of graded levels of duodenal infusions of casein on mammary uptake in lactating cows. 1. Major nutrients. *Journal of Dairy Science* **77**, 2221-2231.

Halfpenny AF, Rook JAF and Smith GH. (1969). Variations with energy nutrition in the concentrations of amino acids of the blood plasma in the dairy cow. *British Journal of Nutrition* **23**, 547-557.

Harmon DL. (1991). Volatile fatty acid and glucose utilisation by the portal-drained viscera in ruminants. In *Proceedings of the 12th EAAP Symposium on energy metabolism in farm animals* (EAAP Publication No. 58) [Wenk C and Boessinger M, editors]. Zürich, Switzerland: ETH-Zentrum.

Harmon DL, Krikemeier KK and Gross KL. (1993). Influence of addition of monesin to an alfalfa hay diet on net portal and hepatic nutrient flux in steers. *Journal of Animal Science* **71**, 218-225.

Harmon DL, Gross KL, Krehbiel CR, Kreikemeier KK, Coffey KP, Avery TB and Klindt J. (1991). Effects of feeding endophyte-intected fescue hay on portal and hepatic nutrient flux in steers. *Journal of Animal Science* **69**, 1223-1231.

Harmon DL, Avery TB, Huntington GB and Reynolds PJ. (1988). Influence of ionophore addition to roughage and high-concentrate diets on portal blood flow and net nutrient flux in cattle. *Canadian Journal of Animal Science* **68**, 419-429.

Harper AE, Benevenga NJ and Wohlhueter RM. (1970). Effects of ingestion of disproportionate amounts of amino acids. *Physiological Review* **50**, 428-557.

Hecker JF. (1971). Metabolism of nitrogenous compounds in the large intestine of sheep. *British Journal of Nutrition* **25**, 85-95.

Heger J and Frydrych Z. (1989). Efficiency of utilization of amino aicds. In *Absorption*

and utilization of amino acids, Vol. 1, pp. 31-56, [Friedman M, editor]. Boca Ration, USA: CRC Press Inc.

Heitmann RN and Bergman EN. (1978). Glutamine metabolism, interogan transport and glycogenicity in the sheep. *American Journal of Physiology* **234**, 197-203.

Hespell RB. (1984). Influence of ammonia assimilation pathways and survival strategy on rumen microbial growth. In *Herbivore Nutrition*, pp.346-358 [Gilchrist FMC and MacKie RI, editors]. Craighall, South Africa: The Science Press.

Hogan JP. (1960). The absorption of ammonia through the rumen of the sheep. *Autsralian Journal of Biological Science* **14**, 448-460.

Hopfer U. (1987). Membrane transport mechanisms for hexoses and amino acids in the small intestine. In *Physiology of the gastrointestinal tract (2nd Ed)*, pp. 1499-1526 [Johnson LR, editor]. New York, USA: Raven Press.

Horn DB and Squire CR. (1967). An improved method for the estimation of ammonia in blood plasma. *Clinica Chimica Acta* **17**, 99-105.

Huhtanen P. (1987). The effects of intraruminal infusions of sucrose and xylose on nitrogen and fibre digestion in the rumen and intestines of cattle receiving diets of grass silage and barley. *Journal of Agricultural Science in Finland* **59**, 405-424.

Huhtanen P, Vanhatalo A and Varvikko T. (1997). Effects of abomasal infusions of histidine, leucine and glucose on milk production in cows given a grass silage based diet. *Journal of Dairy Science* **80** (Supplement 1), 404 (Abstract).

Huida L. (1982). Gas chromatographic determination of water and ethanol in silage by internal standard method. *Journal of Agricultural Society in Finland* **54**, 137-143.

Huntington GB. (1990). Energy metabolism in the digestive tract and liver of cattle: Influence of physiological state and nutrition. *Reproduction Nutrition Development* **30**, 35-47.

Huntington GB. (1989). Hepatic urea synthesis and site and rate of urea removal for blood of beef steers fed alfalfa hay or a high concentrate diet. *Canadian Journal of Animal Science* **69**, 215-223.

Huntington GB. (1986). Uptake and transport of nitrogenous compounds by the ruminant gastrointestinal tract. *Federation Proceedings* **45**, 2260.

Huntington GB and Eisemann JH. (1988). Regulation of nutrient supply by gut and liver tissues. *Journal of Animal Science* **66** (Supplement 3), 35-48.

Huntington GB, Varga GA, Glenn BP and Waldo DR. (1988). Net absorption and oxygen consumption by Holstein steers fed alfalfa or orchardgrass silage at two equalized intakes. *Journal of Animal Science* **66**, 1292-1302.

Hvelplund T and Madsen J. (1990). *A study of the quantitative nitrogen metabolism in the gastro-intestinal tract, and the resultant new protein evaluation system for ruminants. The AAT-PBV system.* Ph D thesis. Institute of Animal Science. The Royal Veterinary and Agricultural University. Copenhagen, Denmark.

Illg DJ, Sommerfeldt JL and Schingoethe DJ. (1987). Lactational and systemic responses of high producing dairy cows to the addition of protected methionine in soybean meal diets. *Journal of Dairy Science* **70**, 620-629.

INRA. (1978). *Alimentation des ruminants*. INRA, Versailles, France.

Irwin MI and Hegsted DM. (1971). A conspectus of research of amino acid requirements of man. *Journal of Nutrition* **101**, 539-566.

Jaakkola S, Huhtanen P and Kaunisto V. (1993). VFA proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. In *Proceedings of 10th International Conference of Silage Research*, pp. 139-140 [O'Kiely P, O'Connell M and Murphy J, editors]. Dublin, Ireland.

Jaakkola S, Huhtanen P and Hissa K. (1991). The effect of cell wall degrading enzymes or formic acid on fermentation quality and on digestion of grass silage by cattle. *Grass and Forage Science* **46**, 75-87.

Jarrige R. (1989). *Ruminant nutrition: Recommended allowances and feed tables*. INRA, Paris, France.

Johns JT and Bergen WG. (1973). Studies on amino acid uptake by ovine small intestine. *Journal of Nutrition* **103,** 1581-1586.

Johnson TR, Cecava MJ, Sheis EB and Cunningham KD. (1994). Addition of ruminally degradable crude protein and branched-chain volatile fatty acids to diet containing hydrolysed feather meal and blood meal for lactating cows. *Journal of Dairy Science* 77, 3676-3682.

Jones WT, Lyttleton JW and Mangan JL. (1978). Interactions between fraction I leaf protein and other surfactants involved in the bloat syndrome. 1. Foam stabilising materials. *New Zealand Journal of Agricultural Research* **21**, 401-407.

Jorgensen GN and Larson BL. (1968). Conversion of phenylalanine to tyrosine in the bovine mammary secretory cell. *Biochemica et Biophysica Acta* **165**, 121-126.

Jungas RL, Halperin ML and Brosnan JT. (1992). Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiological Review* **72**, 419-448.

Karasov WH and Diamond JM. (1987). Adaptation of intestinal nutrient transport. In *Physiology of the Gastrointestinal Tract-2nd Edition*, pp. 1489-1497 [Johnson LR, editor]. New York, USA: Raven Press.

Kaufmann W. (1980). Protein degradation and synthesis within the reticulo-rumen in relation to milk protein synthesis. In *Factors affecting the yields and contents of milk constituents of milk constituents of commercial importance* (Document 125), pp 152-158. Brussels, Belgium: International Dairy Federation.

King KJ, Bergen WG, Sniffen CJ, Grant AL and Grieve DB. (1991). An assessment of abosorbable lysine requirements in lactating cows. *Journal of Dairy Science* **74**, 2530-2539.

Kopple JD and Swendseid ME. (1975). Evidence that histidine is an essential amino acid in normal and chronically uremic man. *Journal of Clinical Investigation* **55**, 881-891.

König BA, Oldham JD and Parker DS. (1984). The effect of abomasal infusion of casein on acetate, palmitate and glucose kinetics in cows during early lactation. *British Journal of Nutrition* **52**, 319-328.

Krehbiel CR, Harmon DL and Schneider JE. (1992). Effects of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. *Journal of Animal Science* **70**, 904-914.

Larsen PR, Ross JE and Tapley DF. (1964). Transport of neutral, dibasic and N-methyl-substituted amino acids by rat intestine. *Biochimca et Biophysica Acta* **88**, 570-577.

Lawes Agricultural Trust. (1990). *Genstat 5*, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK.

Lee J, Treloar BP, Sinclair BR, Prosser CP, Davis SR and Harris PM. (1997). Utilization of methionine by the mammary gland of the lactating goat. *Proceedings of the New Zealand Society of Animal Production* **56**, 53-57.

Le Henaff L. (1991). Thése de Docteur No 253, Université de Rennes I: Section Science Biologiques. France.

Leibholz J. (1971). The absorption of amino acids from the rumen of the sheep. II. The transfer of histidine, glycine, and ammonia across the rumen epithelium *in vitro*. *Australian Journal of Agricultural Research* **22**, 647-653.

Leng RA and Nolan JV. (1984). Symposium: Protein nutrition of the lactating dairy cow. Nitrogen metabolism in lactating cows. *Journal of Dairy Science* **73**, 3208-3216.

Lescoat P, Sauvant D and Danfær A. (1996). Quantitative aspects of blood and amino acid flows in cattle. *Reproduction Nutrition Development* **36**, 137-174.

Lobley GE. (1992). Control of the metabolic fate of amino acids in ruminants: A review. *Journal of Animal Science* **70**, 3264-3275.

Lobley GE. (1991). The importance of interactions on protein and amino acid metabolism. In *Proceedings of the 6th EAAP international symposium on protein metabolism and nutrition*, pp. 66-79 [Eggum BO, Boisen S, Børsting C, Danfær DA and Hvelplund

T, editors]. National Institute of Animal Science, Foulum, Denmark.

Lobley GE and Milano GD. (1997). Regulation of hepatic nitrogen metabolism in ruminants. *Proceedings of the Nutrition Scociety* **56**, 547-563.

Lobley GE, Weijs PJM, Connell A, Calder AG, Brown DS and Milne E. (1996). The fate of absorbed and exogenous ammonia as influenced by forage or forage-concetnrate diets in growing sheep. *British Journal of Nutrition* **76**, 231-248.

Lobley GE, Connell A, Lomax MA, Brown DS, Milne E, Calder AG and Farningham DAH. (1995). Hepatic detoxification of ammonia in the ovine liver: Possible consequences for amino acid catabolism. *British Journal of Nutrition* **73**, 667-685.

Lobley GE, Milne V, Lovie JM, Reeds PJ and Pennie K. (1980). Whole-body and tissue protein synthesis in cattle. *British Journal of Nutrition* **43**, 491-502.

Lomax MA and Baird GD. (1983). Blood flow and nutrient exchange across the liver and gut of the dairy cow. *British Journal of Nutrition* **49**, 481-496.

Mabjeesh SJ, Kyle CE, MacRae JC and Bequette BJ. (1998). Responses in mammary gland metabolism of lysine in lactating goats infused with lysine and methionine. *Journal of Animal Science* **76** (Supplement 1)/*Journal of Dairy Science* **81**(Supplement 1), 813 (Abstract).

MacRae JC and Ulyatt MJ. (1974). Quantitative digestion of fresh herbage by sheep. 11. The sites of digestion of some nitrogenous constituents. *Journal of Agricultural Science* **82**, 309-319.

MacRae JC, Bruce LA, Brown DS and Calder AG. (1997). Amino acid use by the gastrointestinal tract of sheep given lucerne forage. *American Journal of Physiology-Gastrointestinal and Liver physiology* **36**, G1200-G1207.

MacRae JC, Backwell FRC, Bequette BJ and Lobley GE. (1995). Protein metabolism in specific organs. In *Proceedings of the 7th European association for animal production symposium on protein metabolism and nutrition,* European association for animal production No. 81, pp. 297-308 [Nunes AF, Portugal AV, Costa JP and Ribeiro JR, editors]. Vale de Santarem, Portugal: Estaçâo Zootécnica Nacional.

MacRae JC, Lobley GE, Bruce LA, Luo Q, Calder AG and Farningham DAH. (1993). Leucine kinetics across the portal drained viscera of sheep. *Journal of Animal Science* **71** (Supplement 1), 279 (Abstract).

Madsen J. (1985). The basis of the proposed Nordic protein evaluation system for ruminants. The AAT-PBV system. *Acta Agriculturae Scandinavica* **25** (Supplement), 9-20.

Madsen J and Hvelplund T. (1988). The influence of different protein supply and feeding level on pH, ammonia concentration and microbial protein synthesis in the rumen of

cows. *Acta Agriculturae Scandinavica* **38**, 115-125.

Mahadevan S, Erfle JD and Sauer FD. (1980). Degradation of soluble and insoluble proteins by *Bacteroides amylophilus* protease and by rumen microorganisms. *Journal of Animal Science* **50**, 723-728.

Maltby SA. (1993). *Nutritional Regulation of Splanchnic Nitrogen and Energy Metabolism*. PhD thesis, University of Reading, UK.

Mansbridge RJ, Metcalf JA, Oldham JD, Blake JS and Newbold JR. (1999). The response of net milk protein output to metabolisable protein supply in dairy cows. *Proceedings of the British Society of Animal Science* **1999**, 19 (Abstract). Penicuik, UK: BSAS.

Mathers JC and Miller EL. (1981). Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped lucerne and rolled barley. *British Journal of Nutrition* **45**, 587-604.

Matthews DM. (1972). Rates of peptide uptake by small intestine. In *CIBA Symposium-peptide transport in bacteria and mammalian gut*, pp. 71-88 [Elliot K and O'Connor M, editors]. Amsterdam, The Netherlands: Associated Scientific Publishers.

Matthews JC and Webb KE Jr. (1995). Absorption of L-carnosine, L-methionine, and L-methionylglycine by isolated sheep ruminal and omasal epithelial tissue. *Journal of Animal Science* **73**, 3464-3475.

Matthews DM and Laster L. (1965). Competition for intestinal transport among five neutral amino acids. *American Journal of Physiology* **208**, 601-606.

Matthews DM, Addison JM and Burston D. (1974). Evidence for active transport of the dipeptide carnosine (β-alanyl-L-histidine) by hamster jejunum *in vitro*. *Clinical Science and Molecular Medicine* **46**, 693-705.

Matthews JC, Wong EA, Bender PK, Bloomquist JR and Webb KE Jr. (1996a). Demonstration and characterization of dipeptide transport system activity in sheep omasal epithelium by expression of mRNA in *Xenoupus laevis* Oocytes. *Journal of Animal Science* **74**, 1720-1727.

Matthews JC, Pan YL, Wang S, McCollum MQ and Webb KE Jr. (1996b). Characterization of gastrointestinal amino acid and peptide transport proteins and the utilization of peptides as amino acid substrates by cultured cells (myogenic and mammary) and mammary tissue explants. *In Nutrient management of food animals to enhance the environment* [Kornegay ET, editor]. Boca Raton, USA: CRC Press.

Mepham TB. (1982). Amino acid utilization by lactating mammary gland. *Journal of Dairy Science* **65**, 287-298.

Mepham TB. (1979). Nitrogen metabolism in the mammary gland. In *Protein metabolism in the ruminant*, pp. 4.1-4.12 [Buttery PJ, editor]. London, UK: Agricultural

Research Council.

Mepham TB and Linzell JL. (1966). A quantitative assessment of the contribution of individual plasma amino acids to the synthesis of milk proteins by the goat mammary gland. *Biochemical Journal* **101**, 76-83.

Meijer GAL, van der Meulen J and van Vuuren AM. (1992). In *EAAP/ASAS Workshop on the biology of lactation in farm animals*, 24 (Abstract). Madrid, Spain.

Meijer GAL, de Visser H, van der Meulen J, van der Koelen CJ, and Klop A. (1995). Effect of glutamine or propionate infused into the abomasum on milk yield, milk composition, nitrogen retention and net flux of amino acids across the udder of high yielding dairy cows. In *Proceedings of the 7th European association for animal production symposium on protein metabolism and nutrition*, European association for animal production No. 81, pp. 157-160 [Nunes AF, Portugal AV, Costa JP and Ribeiro JR, editors]. Vale de Santarem, Portugal: Estaçâo Zootécnica Nacional.

Merchen NR, Firkins JL and Berger LL. (1986). Effect of intake and forage level on ruminal turnover rates, bacterial protein synthesis and duodenal amino acid flows in sheep. *Journal of Animal Science* **62**, 216-225.

Metcalf JA, Mansbridge RJ, Blake JS, Newbold JR and Oldham JD. (1997). Examining the metabolisable protein system at ideal protein:energy ratios in dairy cows. *Proceedings of the British Society of Animal Science* **1997**, 83 (Abstract). Penicuik, UK: BSAS.

Metcalf JA, Wray-Cahen D, Chettle EE, Sutton JD, Beever DE, Crompton LA, MacRae JC, Bequette BJ and Backwell FRC. (1996a). The effect of dietary crude protein as protected soybean meal on mammary metabolism in the lactating dairy cow. *Journal of Dairy Science* **79**, 603-611.

Metcalf JA, Crompton LA, Wray-Cahen D, Lomax MA, Sutton JD, Beever DE, MacRae JC, Bequette BJ, Backwell FRC and Lobley GE. (1996b). Responses in milk constituents to intravascular administration of two mixtures of amino acids to dairy cows. *Journal of Dairy Science* **79**, 1425-1429.

Metcalf JA, Beever DE, Sutton JD, Wray-Cahen D, Evans RT, Humphries DJ, Backwell FRC, Bequette B and MacRae JC. (1994). The effect of supplementary protein on *in vivo* metabolism of the mammary gland in lactating dairy cow. *Journal of Dairy Science* **79**,1816-1827.

Michael V, Fonty G, Millet L, Bonnernoy F and Gouet P. (1993). *In vitro* study of the proteolytic activity of rumen anaerobic fungi. *FEMS Microbiological Letters* **110**, 5-11.

Miettinen H. (1998). *Effects of nutrient supply, especially volatile fatty acids, on blood metabolites, mammary nutrient metabolism and milk production in dairy cows.* Ph D thesis. University of Helsinki, Finland.

Ministry of Agriculture, Fisheries and Food. (1986). *Feed composition*: UK tables of feed composition and nutritive value for ruminants. Buckinghamshire, UK: Chalcombe publications.

Miyamoto Y, Ganapathy V and Leibach FH. (1985). Proton gradient-coupled uphill transport of glycylsarcosine in rabbit renal brush-border membrane vesicles. *Biochemical and Biophysical Research Communications* **132**, 946-953.

Mnilk B, Harris CI and Fuller MF. (1996). Lysine utilization by growing pigs: Simultaneous measurement of protein accretion and lysine oxidation. *British Journal of Nutrition* **75**, 57-67.

Molenaar AJ, Davis SR and Wilkins RJ. (1992). Expression of α-$_{S1}$-Casein, and lactoferrin genes is heterogeneous in sheep and cattle mammary tissue. *Journal of Histochemistry and Cytochemistry* **40**, 611-618.

Munck BG. (1966). Amino acid transport by the small intestine of the rat. The existence and specificity of the transport mechanism of amino acid and its relation to the transport of glycine. *Biochimica et Biophysica Acta* **120**, 97-103.

National Research Council. (1985). *Ruminant Nitrogen Usage*. Washington DC, USA: National Academy Press.

Newbold JR, Cottrill BR, Mansbridge RJ and Blake JS. (1994). The effect of metabolizable protein on intake of grass silage and milk protein yield in dairy cows. *Animal Production* **58**, 455A.

Nocek J and Russell JB. (1988). Protein and carbohydrate on an integrated system: Relationship of ruminal availability to microbial contribution and milk production. *Journal of Dairy Science* **71**, 2070-2107.

Nolan JV and Leng RA. (1972). Dynamic aspects of ammonia and urea metabolism in sheep. *British Journal of Nutrition* **27**, 177-194.

Norton BW, MacKintosh JB and Armstrong DG. (1982). Urea Synthesis and degradation in sheep given pelleted grass diets containing flaked barley. *British Journal of Nutrition* **48**, 249-264.

Nugent JHA and Mangan JL. (1981). Characteristics of the rumen proteolysis of fractions I (18S) leaf protein from lucerne (*Medicago sativa* L). *British Journal of Nutrition* **46**, 39-58.

Nugent JHA, Jones WT, Jordan DJ and Mangan JL. (1983). Rates of proteolysis in the rumen of the soluble proteins casein, fraction I (18S) leaf protein, bovine serum albumin, and bovine submaxillary mucoprotein. *British Journal of Nutrition* **50**, 357-368.

Nutzenadel W and Scriver CR. (1976). Uptake and metabolism of β-alanine and L-

carnosine by rat tissues *in vitro*: Role in nutrition. *American Journal of Physiology* **230**, 646-651.

Obara Y and Shimbayashi K. (1988). Quantitative aspects of appearance of recycled urea in the digestive tract of goats. *Japan Agricultural Research Quality* **21**, 284-290.

Obara Y and Shimbayashi K. (1987). The appearance of recycled urea in the digestive tract of goats fed a high-protein ration. *Japanese Journal of Zootechnology Science* **58**, 611-617.

Obara Y and Shimbayashi K. (1980). The appearance of recycled urea in the digestive tract of goats during the final third of a once-daily feeding of a low-protein ration. *British Journal of Nutrition* **44**, 295-305.

Obara Y and Shimbayashi K. (1979). Intra-ruminal injection of urea and changes in secretion of parotid saliva in sheep. *British Journal of Nutrition* **42**, 497-505.

Obara Y, Dellow DW and Nolan JV. (1991). The influence of energy-rich supplements on nitrogen kinetics in ruminants. In *Physiological aspects of digestion and metabolism in ruminants*, pp. 515-539 [Tsuda T, Sasaki Y and Kawashima R, editors]. Tokyo, Japan: Academic Press.

O'Connor JD, Sniffen CJ, Fox DG and Chalupa W. (1993). A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *Journal of Animal Science* **71**, 1298-1311.

Oddy VH, Lindsay DB and Fleet IR. (1988). Protein synthesis and degradation in the mammary gland of lactating goats. *Journal of Dairy Research* **55**, 143-154.

Ohshima M and McDonald P. (1979). Changes during ensilage in the nitrogenous components of fresh and additive-treated ryegrass and lucerne. *Journal of the Science of Food and Agriculture* **30**, 97-106.

Oldham JD. (1996). Will responses to protein become more predictable? Proceedings of Society of Chemical Industry, Agriculture and Environment Group Symposium: The scope for the future development of the UK Metabolisable Protein System. *Journal of Science of Food and Agriculture*, SCI Lecture Paper Series, Paper No. 0012.

Oldham JD. (1994). Amino acid nutrition of the dairy cow. In *Amino acids in farm animal nutrition*, pp. 351-375 [D'Mello JPF, editor]. Wallingford, UK: CAB international.

Oldham JD. (1984). Protein-energy interrelationships in dairy cows. *Journal of Dairy Science* **67**, 1090-1114.

Oldham JD. (1980). Amino acid requirements for lactation in high-yielding dairy cows. In *Recent advances in animal nutrition - 1980*, pp. 33-65 [Haresign W, editor]. London, UK: Butterworth.

Oldham JD and Tamminga S. (1980). Amino acid utilization by dairy cows. I. Methods of varying amino acid supply. *Livestock Production Science* **7**, 437-452.

Oldham JD, Bines JA and MacRae JC. (1984). Milk production in cows infused abomasally with casein, glucose or aspartic and glutamic acids early in lactation. *Proceedings of Nutrition Society* **43**, 65A.

Oldham JD, Hart IC and Bines JA. (1982). Formaldehyde-treated proteins for dairy cows – effects on blood hormone concentrations. *British Journal of Nutrition* **48**, 543-547.

Oldham JD, Hart IC and Bines JA. (1978). Effect of abomasal infusions of casein, arginine, methionine or phenylalanine on growth hormone, insulin, prolactin, thyroxine and some metabolites in blood from lactating goats. *Proceedings of Nutrtion Society* **37**, 9A.

Ørskov ER, Grubb DA and Kay RNB. (1977). Effect of postruminal glucose or protein supplementation on milk yield and composition in Friesian cows in early lactation. *British Journal of Nutrition* **38**, 397-405.

Ørskov ER, Fraser C and MacDonald I. (1971). Digestion of concentrates in sheep. 3. Effects of rumen fermentation of barley and maize diets on protein digestion. *British Journal of Nutrition* **26**, 477-486.

Owens FN and Zinn R. (1988). Protein metabolism of ruminant animals. In *The ruminant animal: Digestive physiology and nutrition,* pp. 227-249 [Church DC, editor]. Rentic-Hall, Englewood Cliffs, NJ, USA: A Reston book.

Owens FN and Bergen WG. (1983). Nitrogen metabolism of ruminant animals: Historical perspective, current understanding and future implications. *Journal of Animal Science* **57** (Supplement 2), 498-518.

Palmquist DL, Weisbjerg MR and Hvelplund T. (1993). Ruminal, intestinal, and total digestibities of nutrients in cows fed diets high in fat and undergradable protein. *Journal of Dairy Science* **76**, 1353-1364.

Papas A, Sniffen CJ and Muscato TV. (1984a). Effectiveness of rumen-protected methionine for delivering methionine postruminally in dairy cows. *Journal of Dairy Science* **67**,545-552.

Papas AM, Vicini JL, Clark JH and Sander SP. (1984b). Effect of rumen-protected methionine on plasma free amino acids and production by dairy cows. *Journal of Nutrition* **114**, 2221-2227.

Phillips WA, Webb KE Jr and Fontenot JP. (1979). Characteristics of threonine, valine and methionine absorption in the jejunum and ileum of sheep. *Journal of Animal Science* **48**, 926-933.

Phillips WA, Webb KE Jr and Fontenot JP. (1976). *In vitro* absorption of amino acids by

the small intestine of sheep. *Journal of Animal Science* **42,** 201-207.

Piccioli Cappelli F, Seal CJ and Parker DS. (1993). [l-^{13}C]Leucine uptake into the portal vein of sheep receiving exogenous glucose intravascularly of intraduodenally. *Proceedings of the Nutrition Society* **52**,192A.

Piepenbrink MS and Schingoethe DJ. (1998). Ruminal degradation, amino acid composition, and estimated intestinal digestibilities of four protein supplements. *Journal of Dairy Science* **81**, 454-461.

Piepenbrink MS, Overton TR and Clark JH. (1996). Response of cows fed a low crude protein diet to ruminally protected methionine and lysine. *Journal of Dairy Science* **79**, 1638-1646.

Pisulewski PM, Rulquin H, Peyraud JL and Vérité R. (1996). Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. *Journal of Dairy Science* **79**,1781-1791.

Potthast V, Prigge H and Pfeffer E. (1977). Untersuchungen aur dynamik der N-umsetzungen beim schaf. *Z. tierernahr. Futtermittlekd* **38**, 338-339.

Prins RA, Hungate RE and Prast ER. (1972). Function of the omasum in several ruminant species. *Comparison of Biochemical Physiology* **43**, 155-163.

Prior RL, Clifford AJ, Hogue DE and Visek WJ. (1970). Enzymes and metabolites of intermediary metabolism in urea fed sheep. *Journal of Nutrition* **100**, 438-444.

Pruekvimolphan S, Grummer RR and Bertics SJ. (1997). Effects of feather meal on lactation performance of dairy cows fed methionine deficient diets. *Journal of Dairy Science* **80** (Supplement 1), P408.

Purser DB and Buechler SM. (1966). Amino acid composition of rumen organisms. *Journal of Dairy Science* **49**, 81-84.

Quigley JP and Gotterer GS. (1969). Distribution of (Na+K)-stimulated ATPase activity in rat intestinal mucosa. *Biochimica et Biophysica Acta* **173**, 456-468.

Reeds PJ, Burrin DG, Davis TA and Fiorotto ML. (1993). Postnatal growth of gut and muscle: competitors or collaborators? *Proceedings of the Nutrition Society* **52**, 57-67.

Reiser S and Christiansen PA. (1971). Stimulation of basic amino acid uptake by certain neutral amino acids in isolated intestinal epithelial cells. *Biochimica et Biophysica Acta* **241**, 102-113.

Reiser S and Christiansen PA. (1969). A cross-inhibition of basic amino acid transport by neutral amino acids. *Biochimica et Biophysica Acta* **183**, 611-624.

Reynolds CK. (1992). Metabolism of nitrogenous compounds by ruminant liver. *Journal of*

Nutrition **122**, 850-854.

Reynolds CK and Tyrell HF. (1991). Effect of mesenteric vein L-alanine infusion on liver metabolism in beef heifers fed on diets differing in forage:concentrate ratio. *British Journal of Nutrition* **66**, 437-450.

Reynolds CK and Huntington GB. (1988). Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* **60**, 539-551.

Reynolds CK, Tyrrell HF and Reynolds PJ. (1993). Effects of diet forage-to-concentrate ratio and intake on net visceral metabolism of VFA in growing beef heifers. *Journal of Dairy Science* **76** (Supplement 1), 283 (Abstract).

Reynolds CK, Casper DP, Harmon DL and Milton CT. (1992a). Effect of CP and ME intake on visceral nutrient metabolism in beef steers. *Journal of Animal Science* **70** (Supplement 1), 315 (Abstract).

Reynolds CK, Lapierre H, Tyrrell HF, Elsasser TH, Staples RC, Gaudreau P and Brazeau P. (1992b). Effects of growth-hormone releasing factor and feed intake on energy metabolism in growing beef steers: Net nutrient metabolism by the portal-drained viscera and liver. *Journal of Animal Science* **70**, 752-763.

Reynolds CK, Tyrrell HF and Armentano LE. (1992c). Effects of mesenteric vein *n*-butyrate infusion on liver metabolism by beef steers. *Journal of Animal Science* **70**, 2250-2261.

Reynolds CK, Tyrrell HF and Harmon DL. (1991a). Effects of diet forage-to-concentrate ratio and mesenteric vein L-alanine infusion on visceral metabolism of volatile fatty acids in beef heifers. *Journal of Dairy Science* **74** (Supplement 1), 257 (Abstract).

Reynolds CK, Tyrrell HF and Reynolds PJ. (1991b). Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: Whole body energy and nitrogen balance and visceral heat production. *Journal of Nutrition* **121**, 994-1003.

Reynolds CK, Tyrell HF and Reynolds PJ. (1991c). Effects of diet forage-to-concentrate ration and intake on energy metabolism in growing beef heifers: Net nutrient metabolism by visceral tissues. *Journal of Nutrition* **121**, 1004-1015.

Reynolds CK, Huntington GB, Tyrrell HF and Reynolds PJ. (1988). Net metabolism of volatile fatty acids, D-β-hydroxybutyrate, nonesterified fatty acids, and blood gases by portal-drained viscera and liver of lactating Holstein cows. *Journal of Dairy Science* **71**, 2395-2405.

Richardson CR and Hatfield EE. (1978). The limiting amino acids in growing cattle. *Journal of Animal Science* **46**, 740-745.

Robert JC, Sloan B and Bourdeau K. (1994). The effect of supplementation of corn silage plus soybean meal diets with rumen-protected methionine on the lactational performance of dairy cows in early lactation. *Journal of Dairy Science* **77** (Supplement 1), 92 (Abstract).

Robinson PH, Chalupa W, Sniffen CJ, Julen WE, Sato H, Watanabe K, Fujieda T and Suzuki H. (1998). Ruminally protected lysine or lysine and methionine for lactating dairy cows fed a ration designed to meet requirements for microbial and postruminal protein. *Journal of Dairy Science* **81**, 1364-1373.

Robinson PH, Sniffen CJ and Van Soest PJ. (1985). Influence of level of feed intake on digestion and bacterial yield in the forestomachs of dairy cattle. *Canadian Journal of Animal Science* **65**, 437-444.

Roets E, Massart-Leën AM, Peeters G, Verbeke R. (1983). Metabolism of leucine by the isolated perfused udder. *Journal of Dairy Research* **50**, 413-424.

Roets E, Massart-Leën AM, Verbeke R and Peeters G. (1979). Metabolism of [U-^{14}C; 2,3-^{3}H]-L-valine by the isolated perfused udder. *Journal of Dairy Research* **46**, 47-57.

Roets E, Verbeke R, Massart-Leën AM, Peeters G. (1974). Metabolism of [^{14}C] citrulline in the perfused sheep and goat udder. *Biochemical Journal* **144**, 435-446.

Rogers GL and McLeay LM. (1977). Response of lactating cows to abomasal infusions of casein, methionine, and glucose. *Proceedings of New Zealand Society of Animal Production* **37**, 46-49.

Rogers GL, Pierce-Sandner SB, Papas AM, Polan CE, Sniffen CJ, Muscato TV, Staples CR and Clark JH. (1989). Production responses of dairy cows fed various amounts of rumen-protected methionine and lysine. *Journal of Dairy Science* **72**, 1800-1817.

Rogers JA, Krishnamoorthy U and Sniffen CJ. (1987). Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. *Journal of Dairy Science* **70**, 789-798.

Rooke JA, Lee NH and Armstrong DG. (1987). The effects of intraruminal infusions of urea, casein, glucose syrup and a mixture of casein and glucose syrup on nitrogen digestion in the rumen of cattle receiving grass silage and continuous intraruminal infusions of sucrose. *British Journal of Nutrition* **57**, 89-98.

Roth FX and Kirchgessner M. (1980). Contribution of dietary nucleic acids to the N metabolism. Paper 9. *Procedings of 3rd EAAP Symposium on protein metabolism and nutrition.* Baunschweig, Germany.

Rubino A, Field M and Shwachman H. (1971). Intestinal transport of amino acid residues of dipeptides. 1. Influx of the glycine residue of glycyl-L-proline across mucosal border. *Journal of Biological Chemistry* **246**, 3542-3548.

Rulquin H. (1987). Identification of some limiting amino acids in the dairy cow by postruminal administrations. *Reproduction Nutrition Development* **27**, 299-300.

Rulquin H. (1986). Influence of the amino acid composition of 3 duodenally infused proteins on milk secretion in the dairy cow. *Reproduction Nutrition Development* **26**, 347-348.

Rulquin H and Delaby L. (1997). Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. *Journal of Dairy Science* **80**, 2513-2522.

Rulquin H and Delaby L. (1994a). Effects of energy status on lactational responses of dairy cows to rumen-protected methionine. *Journal of Dairy Science* **77** (Supplement 1), 91 (Abstract).

Rulquin H and Delaby L. (1994b). Lactational response of dairy cows to graded amounts of rumen-protected methionine. *Journal of Dairy Science* **77** (Supplement 1), 91 (Abstract).

Rulquin H and Journet M. (1987). Efficiency of utilization of amino acids for lactation in ruminants. In *Feeding evaluation and protein requirement systems for ruminants*, pp. 171-186 [Jarrige R and Alderman G, editors]. Brussels, Belgium: EUR 10657EN, EEC.

Rulquin H and Vérité R. (1993). Amino acid nutrition of dairy cows: Productive effects and animal requirements. In *Recent advances in animal nutrition*, pp. 55-77 [Garnsworthy PC and Cole DJA, editors]. Nottingham, UK: Nottingham Uiversity Press.

Russell JB, Sniffen CJ and Van Soest PJ. (1983). Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *Journal of Dairy Science* **66**, 763-775.

Russell JB, O'Connor JD, Fox DG, Van Soest PJ and Sniffen CJ. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science* **70**, 3551-3561.

Saito H and Inui K-I. (1993). Dipeptide transporters in apical and basolateral membranes of the human intestinal cell line Caco-2. *American Journal of Physiology.* **265**, G289-G294.

Sakata T and Tamate H. (1978). Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. *Journal of Dairy Science* **61**, 1109-1113.

Salter DN and Smith RH. (1977). Digestibilities of nitrogen compounds in rumen bacteria and in other components of digesta in the small intestine of the young steer. *British Journal of Nutrition* **38**, 207-216.

SCA. (1990). *Feeding standards for Australian livestock.* Standing Committee of

Agriculture, Ruminants sub-committee. Melbourne, Australia: CSIRO Publications.

Schingoethe DJ. (1996). Balancing the dairy cow amino acid needs. *Animal Feed Science and Technology* **60**,153-160.

Schingoethe DJ, Casper DP, Yang C, Illg DJ, Sommerfeldt JL and Meuller CR. (1988). Lactational response to soybean meal, heated soybean meal, and extruded soybeans with ruminally protected methionine. *Journal of Dairy Science* **71**, 173-180.

Schultz SG, Yu-Tu L and Strecker CK. (1972). Influx of neutral amino acids across the brush border of rabbit ileum. Stereospecificity and the roles of the alpha amino and alpha-carboxylate groups. *Biochemica et Biophysica Acta* **288**, 367-379.

Schwab CG. (1996). Amino acid nutrition of the dairy cow: current status. In *Proceedings of 1996 Cornell Nutrition conference for Feed Manufacturers*, pp.184-207.

Schwab CG and Bozak CK. (1988a). Production response to duodenal infusion of methionine and lysine at peak lactation. *Journal of Dairy Science* **71** (Supplement 1), 160 (Abstract).

Schwab CG and Bozak CK. (1988b). Performance of cows in mid-lactation receiving lysine and methionine by duodenal infusion. *Journal of Dairy Science* **71** (Supplement 1), 290 (Abstract).

Schwab CG, Satter LD and Clay AB. (1976). Response of lactating dairy cows to abomasal infusion of amino acids. *Journal of Dairy Science* **59**, 1254-1270.

Schwab CG, Bozak CK, Whitehouse NL and Mesbah MMA. (1992a). Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. *Journal of Dairy Science* **75**, 3486-3502.

Schwab CG, Bozak CK, Whitehouse NL and Olson VM. (1992b). Amino acid limitation and flow to duodenum at four stages of lactation. 2. Extent of lysine limitation. *Journal of Dairy Science* **75**, 3503-3518.

Seal CJ and Parker DS. (1996). Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal-drained viscera in growing steers fed a forage diet: II. Ammonia, urea, amino acid and peptides. *Journal of Animal Science* **74**, 245-256.

Seal CJ and Parker DS. (1992). Isolation and characterization of circulating low-molecular-weight peptides in steer, sheep and rat portal and peripheral-blood. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular biology* **99B**, 679-685.

Seal CJ and Parker DS. (1991). Increase plasma free amino acid concentrations and net absorption of amino acids into portal and mesenteric veins with intra-ruminal propionate infusion into forage-fed steers. In *Proceedings of the EAAP 6th*

international symposium on protein metabolism and nutrition, pp. 184-186 [Eggum BO, Boisen S, Borsting C, Danfær A and Hvelplund T, editors]. Herning, Denmark: National Institute of Animal Science.

Seal CJ and Reynolds CK. (1993). Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutrition Research Reviews* **6**, 185-208.

Seal CJ, Parker DS, Balcells J and Mole JL. (1993). Nitrogen digestion in forage-fed sheep with and without intraruminal propionate infusion. *Journal of Agricultural Science* **120**, 107-114.

Sepúlveda FV and Smith MW. (1979). Different mechanisms for neutral amino acid uptake by newborn pig colon. *Journal of Physiology* **286**, 479-490.

Sepúlveda FV and Smith MW. (1978). Discrimination between different entry mechanisms for neutral amino acids in rabbit ileal mucosa. *Journal of Physiology* **282**, 73-90.

Shennan DB and McNeillie SA. (1994). Characteristics of alpha-aminoisobutyric-acid transport by lactating rat mammary-gland. *Journal of Dairy Research* **61**, 9-19.

Shennan DB, Calvert DT, Backwell FRC and Boyd CAR. (1998). Peptide aminonitrogen transport by the lactating rat mammary gland. *Biochimica et Biophysica Acta-Biomembranes* **1373**, 252-260.

Siddons RC, Nolan JV, Beever DE and MacRae JC. (1985). Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of nitrogen. *British Journal of Nutrition* **54**, 175-187.

Silk DBA, Fairclough PD, Clark ML, Hegarty JE, Marrs TC, Addison JM, Burston D, Clegg KM and Matthews DM. (1980). Use of a peptide rather than free amino acid nitrogen source in chemical defined 'elemental' diets. *Journal of Parenteral and Enteral Nutrition* **4**, 548-553.

Sloan BK. (1997). Developments in amino acid nutrition of dairy cows. In *Recent advances in animal nutrition-1997*, pp. 167-198 [Garnsworthy PC and Cole DJA, editors]. Nottingham, UK: Nottingham Uiversity Press.

Smith RH. (1984). Essential amino acid requirements and rationing systems for ruminants. In *Proceedings of VIth international symposium on amino acids*, pp. 319-329 [Zebrowska T, Buraczeska L, Buraczewski S, Kowalczyk J and Pastuszewska DB, editors]. Warsaw, Poland: Polish Scientific Publisher.

Smith RH. (1979). Synthesis of microbial nitrogen compounds in the rumen and their subsequent digestion. *Journal of Animal Science* **49**, 1604-1614.

Smith RH. (1975). Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. In *Digestion and metabolism in the ruminant*, pp. 399-415. [McDonald IW and Warner ACI, editors]. Armidale,

Australia: University of New England Publishing Unit.

Somogyi M. (1945). Determination of blood sugar. *Journal of Biological Chemistry* **160**, 69-73.

Sniffen CJ and Robinson PH. (1987). Microbial growth and flow as influenced by dietary manipulations. *Journal of Dairy Science* **70**, 425-441.

Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG and Russell JB. (1992). A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. *Journal of Animal Science* **70**, 3562-3577.

Socha MT, Schwab CG, Putnam DE, Whitehouse NL, Kierstead NA and Garthwaite BD. (1994). Determining methionine requirements of dairy cows during early lactation by postruminally infusing incremental amounts of methionine. *Journal of Animal Science* **72** (Supplement 1), 65 (Abstract).

Somers M. (1961). Factors influencing the secretion of nitrogen in sheep saliva. I. The distribution of nitrogen in the mixed and parotid saliva of sheep. *Australian Journal of Experimental Biology and Medical Science* **39**, 111-122.

Spencer RP and Samiy AH. (1961). Intestinal absorption of L-phenylalanine *in vitro*. *American Journal of Physiology* **200**, 501-504.

Spencer RP, Bow TM and Markulis MA. (1962). Amino group requirement for *in vitro* intestinal transport of amino acids. *American Journal of Physiology* **202**, 171-173.

Spires HR, Clark JH, Derrig RG and Davis CL. (1975). Milk production and nitrogen utilization in response to postruminal infusion of sodium caseinate in lactating cows. *Journal of Nutrition* **105**, 1111-1121.

Stern MD, Hoover WH, Sniffen CJ, Crooker BA and Knowlton PH. (1978). Effects of nonstructural carbohydrate, urea and soluble protein levels on microbial protein synthesis in continuous culture of rumen contents. *Journal of Animal Science* **47**, 944-956.

Storm E and Ørskov ER. (1983). The nutritive value of rumen micro-organisms in ruminants. 1. Large scale isolation and chemical composition of rumen micro-organisms. *British Journal of Nutrition* **50**, 463-470.

Storm E and Ørskov ER. (1982). Biological value and digestibility of rumen microbial protein in lamb small-intestine. *Proceedings of the Nutritional Society* **41**, 78A.

Storm E, Ørskov ER and Smart R. (1983a). The nutritive value of rumen micro-organisms in Ruminants. 2. The apparent digestibility and net utilization of microbial N for growing lambs. *British Journal of Nutrition* **50**, 471-478.

Storm E, Brown DS and Ørskov ER. (1983b). The nutritive value of rumen micro-

organisms in ruminants. 3. The digestion of microbial amino and nucleic acids in, and losses of endogenous nitrogen from, the small intestine of sheep. *British Journal of Nutrition* **50**, 479-485.

Subnel APJ, Meijer RGM, van Straalen WM and Tamminga S. (1994). Efficiency of milk protein production in the DVE protein evaluation system. *Livestock Production Science* **40**, 215-224.

Tagari H and Bergman EN. (1978). Intestinal disappearance and portal blood appearance of amino acids in sheep. *Journal of Nutrition* **108**, 790-803.

Takuwa N, Shimada T, Matsumoto H and Hoshi T. (1985). Proton-coupled transport of glyclyglycine in rabbit renal brush-border membrane vesicles. *Biochimica et Biophysica Acta* **814**, 186-190.

Tamminga S. (1992). Nitrogen metabolism in the rumen and amino acid supply. In *Developments and future perspectives in farm animal nutrition,* pp. 3-18 [Findlayson HJ, editor]. University of Newcastle upon Tyne, UK.

Tamminga S and Oldham JD. (1980). Amino acid utilization by dairy cows. II. Concept of amino acid requirements. *Livestock Production Science* **7**, 453-463.

Tamminga S, Van Straalen WM, Subnel APJ, Meijer RGM, Steg A, Wever C and Blok MC. (1994). The Dutch Protein evaluation System: The DVE/OEB-system. *Livestock Production Science* **40**, 139-155.

Taniguchi K, Matthews JC and Dirienzo DG. (1995). Net nutrient flux by visceral tissues of beef steers given abomasal and ruminal infusion of casein and starch. *Journal of Animal Science* **73**, 236-249.

Tas MV, Evans RA and Axford RFE. (1981). The digestibility of amino acids in the small intestine of the sheep. *British Journal of Nutrition* **45**, 167-174.

Teleni E. (1993). Catabolism and synthesis of amino acids in skeletal muscle: their significance in monogastric mammals and ruminants. *Australian Journal of Agricultural Research* **44**, 443-461.

Thomas PC and Chamberlain DG. (1982). The utilization of silage nitrogen. In *Forage protein conservation and utilization*, pp. 121-145 [Griffiths TW and Maguire MF, editors]. Luxembourg, Luxembourg: Commission of the European Communities.

Thomas PC and Chamberlain DG. (1981). The protein rationing of farm ruminants: Progress and problems. In *Hannah Research Institute: Report 1981*, 97-113.

Thomas PC, Chamberlain DG, Kelly NC and Wait MK. (1980). The nutritive value of silage. Digestion of nitrogenous constituents in sheep receiving diets of grass silage and grass silage and barley. *British Journal of Nutrition* **43**, 469-479.

Thornton RF, Bird PR, Sommers M and Moir RJ. (1970). Urea excretion in ruminants. 3. The role of the hind-gut (caecum and colon). *Australian Journal of Agricultural Research* **21**, 345-354.

Tyrrell HF, Moe PW and Flatt WP. (1970). Influence of excess protein intake on energy metabolism of the dairy cow. In *Proceedings of the 5th Symposium on energy metabolism of farm animals (EAAP publication No. 13)*, pp. 69-72. [Schurch A and Wenk C, editors]. Zürich, Switzerland: Juris Druck-Verlag.

Umagat H, Kucera P and Wen LF. (1982). Total amino acid analysis using preculumn fluorescence derivatization. *Journal of Chromatography* **239**, 463-474.

Vanhatalo A, Huhtanen P and Varvikko T. (1997). Response of dairy cows fed grass silage based diets to the abomasal infusion of histidine alone or in combinations with methionine and lysine. *Journal of Dairy Science* **80** (Supplement 1), 441 (Abstract).

Van Roth C. (1939). *Vorratspflege und Lebensmittelforschung* **2**, 22.

Van Soest PJ. (1987). *Nutritional Ecology of the Ruminant.* Ithaca, USA: Comstock Publishing Associates.

Van Straalen WM, Salaun C, Veen WAG, Rijpkema YS, Hof G and Boxem Tj. (1994). Validation of protein evaluation systems by means of milk production experiments with dairy cows. *Netherlands Journal of Agricultural Science* **42**, 89-104.

Van't Klooster AT and Boekholt HA. (1972). Protein digestion in the stomachs and intestines of the cow. *Netherland Journal of Agricultural Science* **20**, 272-284.

Verbeke R, Peeters G, Massart-Leën AM and Cocquyt G. (1968). Incorporation of DL-[2-^{14}C]arginine in milk constituents by the isolated lactating sheep udder. *Biochemical Journal* **106**, 719-724.

Vérité R, Michalet-Doreau B, Chapoutot P, Peyraud JL and Poncet C. (1987). Revision du systeme des proteines digestibles dans l'intestine (PDI). *Bulletin Technique C.R.Z.V.* Theix, INRA, **70**, 19-34.

Wallace RJ. (1994). Amino acid and protein synthesis, turnover, and breakdown by ruminal microorganisms. In *Principles of protein nutrition of ruminants*, pp. 72-111 [Asplund JM, editor]. Boca Raton, FL, USA: CRC Press.

Wallace RJ and Cotta MA. (1988). Metabolism of nitrogen containing compounds. In *The rumen microbial ecosystem,* pp. 217-249 [Hobson PN, editor]. London, UK: Elsevier Applied Science.

Wallace RJ and Brammall ML. (1985). The role of different species of bacteria in the hydrolysis of protein in the rumen. *Journal of General Microbiology* **131**, 821-832.

Wallace RJ and Joblin KN. (1985). Proteolytic activity of rumen anaerobic fungus. *FEMS*

Microbiological Letters **29**, 19-25.

Wang X and Parsons CM. (1997). Effect of processing systems on protein quality of feather meals and hog hair meals. *Poultry Science* **76**, 491-496.

Warner ACI. (1956). Proteolysis by rumen microorganisms. *Journal of General Microbiology* **14**, 749-762.

Waterlow JC. (1969). The assessment of protein nutrition and metabolism in the whole animal, with special reference to man. In *Mammalian protein metabolism*, pp 326-390 [Munro HN, editor]. New York, USA: Academic Press.

Webb KE Jr. (1990). Intestinal absorption of protein hydrolysate products. A review. *Journal of Animal Science* **68**, 3011-3022.

Webb KE Jr and Matthews JC. (1998). Peptide absorption and its significance in ruminant protein metabolism. In *Peptides in mammalian protein metabolism*, pp. 1-10 [Grimble GK and Backwell FRC, editors]. London, UK: Portland Press.

Webb KE Jr and Bergman EN. (1991). Amino acid and peptide absorption and transport across the intestine. In *Physiological aspects of digestion and metabolism in ruminants*, pp. 111-128 [Tsuda T, Sasaki Y and Kawashima R, editors]. Tokyo, Japan: Academic Press.

Webb KE Jr, Matthews JC and DiRienzo DB. (1992). Peptide absorption. A review of current concepts and future perspectives. *Journal of Animal Science* **70**, 3248-3257.

Webster AJF. (1992). The metabolizable protein system for ruminants. In *Recent advances in animal nutrition-1992,* pp. 93-110 [Garnsworthy PC, Haresign W and Cole DJA, editors]. London, UK: Butterworths.

Webster AJF, Kitcherside MA, Keirby JR and Hall PA. (1986). Evaluation of protein foods for dairy cows. *Animal Production* **38**, 549(Abstract).

Weichselbaum TE, Hagerty JC and Mark HB Jr. (1969). A reaction rate method for ammonia and blood urea nitrogen utilizing pentracyanonitrosyloferrate catalyzed Bethelot reaction. *Analatical Chemistry* **41**, 848-850.

Weller RA. (1957). The amino acid composition of hydrolysates of microbial preparations from the rumen of sheep. *Australian Journal of Biological Science* **10**, 384.

Wessels RH and Titgemeyer EC. (1998). Effect of abomasal infusion of aspartate on nitrogen balance and plasma amino acids in Holstein steers. *Reproduction Nutrition Development* **38**, 309-313.

Whitelaw RG, Milne JS, Ørskov ER and Smith JS. (1986). The nitrogen and energy metabolism of lactating cows given abomasal infusions of casein. *British Journal of Nutrition* **55**, 537-556.

Whitelaw FG, Hyldgaard-Jensen J, Reid RS and Kay MG. (1970). Volatile fatty acid production in the rumen of cattle given an all-concentrate diet. *British Journal of Nutrition* **24**, 179-195.

Wilde CJ, Addey CVP and Knight CH. (1989). Regulation of intracellular casein degradation by secreted milk proteins. *Biochimica et Biophysica Acta* **992**, 315-319.

Williams VJ. (1969). The relative rates of absorption of amino acids from the small intestine of the sheep. *Comparative Biochemistry and Physiology*. **29**, 865-870.

Wilson JW and Webb KE Jr. (1990). Lysine and methionine transport by bovine jejunal and ileal brush border membrane vesicles. *Journal of Animal Science* **68**, 504-514.

Wilson PN and Strachan PJ. (1981). The contribution of undegraded protein to the protein requirements of dairy cows. In *Recent developments in ruminant nutrition-1981*, pp. 228-247 [Haresign W and Cole DJA, editors]. London, UK: Butterworth.

Windmueller HG. (1982). Glutamine utilization by the small intestine. *Advances in Enzymology and Related Areas of Molecular Biology* **53**, 201-237.

Windmueller HG and Spaeth AE. (1980). Respiratory fuels and nitrogen metabolism *in vivo* in small intestine of fed rats. Quantitative importance of glutamine, glutamate and aspartate. *Journal of Biological Chemistry* **255**, 107-112.

Windmueller HG and Spaeth AE. (1975). Intestinal metabolism of glutamate and glutamine from the lumen as compared to glutamine from blood. *Archives of Biochemistry and Biophysics* **171**, 662-672.

Windmueller HG and Spaeth AE. (1974). Uptake and metabolism of plasma glutamine by the small intestine. *Journal of Biological Chemistry* **249**, 5070-5079.

Wohlt JE, Clark JH, Derrig RG and Davis CL. (1977). Valine, leucine, and isoleucine metabolism by lactating bovine mammary tissue. *Journal of Dairy Science* **60**, 1875-1882.

Wolff JE and Bergman EN. (1972). Gluconeogenesis from plasma amino acids in fed sheep. *American Journal of Physiology* **223**, 455-460.

Wong D I-J. (1984). *The effects of supplementary methionine on milk secretion in the dairy cow.* Ph D Thesis. University of Glasgow, UK.

Wrong OM, Edwards CJ and Chadwick VS. (1981). *The large intestine: Its role in mammalian nutrition and homeostasis*. New York, USA: John Wiley and Sons.

Xu S, Harrison JH, Chalupa W, Sniffen C, Julien W, Sato H, Fujeda T, Watanabe K, Ueda T and Suzuki H. (1998). The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *Journal of Dairy Science* **81**, 1062-1077.

Yang CMJ, Schingoethe DJ and Caspar DP. (1986). Protected methionine and heat-treated soybean meal for high producing dairy cows. *Journal of Dairy Science* **69**, 2348-2357.

Zinn RA and Owens FN. (1982). Predicting net uptake of non-ammonia N from the small intestine. In *Protein requirements of cattle: Proceedings of international symposium on ruminant physiology*, p. 133 [Owens FN, editor]. Stillwater, Oklahoma, USA: Oklahoma State University.